Tissue-Carbon Incorporation Rates in Lizards: Implications for Ecological Studies Using Stable Isotopes in Terrestrial Ectotherms

Robin W. Warne*
Casey A. Gilman†
Blair O. Wolf‡
Biology Department, MSC03 2020, 1 University of New Mexico, Albuquerque, New Mexico 87131-0001

Accepted 2/1/2010; Electronically Published 5/4/2010

ABSTRACT

Carbon stable isotope ($\delta^{13}C$) analysis can be used to infer the origin and to estimate the flow of nutrient resources through animals and across ecological compartments. These applications require knowledge of the rates at which carbon is incorporated into animal tissues and diet-to-tissue discrimination factors ($\Delta^{13}C$). Studies of carbon dynamics in terrestrial vertebrates to date have focused almost solely on endothermic animals; ectotherms such as reptiles have received little attention. Here we determined carbon incorporation rates and $\Delta^{13}C$ in tissues of prairie lizards (*Sceloporus undulatus consobrinus*) and collared lizards (*Crotaphytus collaris*). The smaller lizard, *S. undulatus*, had carbon retention times of 25 and 61 d in plasma and red blood cells (RBC), respectively, compared with 44 and 311 d for the larger *C. collaris*. Liver, muscle, and skin carbon retention times for *S. undulatus* were 21, 81, and 94 d. Growth contributed 9%–19% of the carbon incorporated into these tissues. This contribution is similar to endotherms measured at comparable developmental stages. Mean $\Delta^{13}C$ for plasma ($-0.2‰ \pm 0.4‰$ Vienna Pee Dee Belemnite Standard) and RBCs ($-1.3‰ \pm 0.8‰$) were similar to values reported for other vertebrates. Carbon incorporation rates for these ectotherms, however, are seven times slower than in similarly sized adult endotherms. Although a limited comparison with data for warm-water fishes suggests comparable incorporation rates between aquatic and terrestrial ectotherms, this study highlights the lack of experimental data for isotope dynamics in ectotherms across a range of temperatures, body sizes, and developmental stages.

* Corresponding author; e-mail: rwarne@unm.edu.
† E-mail: cgilman@unm.edu.
‡ E-mail: wolf@unm.edu.

Physiological and Biochemical Zoology 83(4):608–617. 2010. 1522-2152/2010/8304-9035$15.00
DOI: 10.1086/651585

Introduction

The analysis of carbon stable isotope ratios ($\delta^{13}C$) in the tissues of consumers is a powerful method for determining the origin of nutrient resources and estimating their flow through animals (O'Brien et al. 2000; Gauthier et al. 2003) and across ecological compartments (Fry et al. 1978; Hobson et al. 1994; Wolf et al. 2002; Warne et al. 2010). Because tissue-carbon turnover rates and biological processes vary across taxa, knowledge of taxon-specific isotope incorporation rates (Tieszen et al. 1983; Carleton and Martínez del Rio 2005) and diet-to-tissue discrimination factors (DeNiro and Epstein 1978, 1981) are necessary to interpret field data. Numerous controlled experiments have investigated isotope discrimination and incorporation rates in the tissues of endothermic animals such as mammals and birds (see references in Kelly 2000; Vanderklift and Ponsard 2003), aquatic ectotherms such as fish (see references in MacAvoy et al. 2001; Suzuki et al. 2005; and Tarboush et al. 2006), and invertebrates (see references in Spence and Rosenheim 2005). To our knowledge, only a few published studies have quantified carbon and nitrogen incorporation and discrimination in terrestrial ectothermic vertebrates. Seminoff et al.'s (2006) study of freshwater turtles provided important insight into the nitrogen-isotope dynamics in reptiles; however, the experiment was of too short a duration to allow for the carbon in target tissues to reach isotopic equilibration after the diet switch. The importance of growth in the carbon incorporation and discrimination in hatchling reptiles has been the subject of a study by Reich et al. (2008) and Fisk et al. (2009), who examined loggerhead turtles and snakes. Although these studies are very informative, it is unlikely that the isotopic incorporation values measured can be extended to reptiles of other age classes (Martínez del Rio et al. 2009). These same cautions hold true for the larger number of studies that examine carbon and nitrogen dynamics in rapidly growing larval and juvenile fish (Suzuki et al. 2005; Tarboush et al. 2006; Martínez del Rio et al. 2009). As a consequence, the isotopic data derived from an increasing number of field studies of reptiles lacks the information on tissue-carbon turnover rates and discrimination factors needed for robust interpretation (Godley et al. 1998; Magnusson et al. 2001; Hatase et al. 2002; Struck et al. 2002; Biasatti 2004; Seminoff et al. 2006; Wallace et al. 2006; Warne et al. 2010).

In this study, we used a diet-switch experiment to determine the carbon incorporation rates and diet-to-tissue discrimination factors at isotopic equilibrium for skin, liver, muscle, plasma, and red blood cells (RBC) of adult prairie lizards *Sceloporus undulatus consobrinus* and plasma and RBC of adult collared lizards *Crotaphytus collaris*. We chose these animals because they are commonly used for ecological studies and

represent a fivefold difference in body mass. This study is the first to provide carbon incorporation rates and discrimination factors for lizards and among the first to provide these data for terrestrial ectothermic vertebrates. These data inform our current understanding of how tissue and isotope turnover patterns differ among aquatic and terrestrial ectothermic vertebrates and endotherms across a range of temperatures, body sizes, and developmental stages.

Material and Methods

Lizard Capture and Maintenance

Nine adult male collared lizards (*Crotaphytus collaris*) and 27 adult, male prairie lizards (*Sceloporus undulatus consobrinus*) were wild caught and maintained in a room at the biology department of the University of New Mexico. Lizards were captured by hand using noose poles on Bureau of Land Management lands near Albuquerque, New Mexico. Each lizard was toe clipped for permanent identification and measurements of snout-to-vent length (SVL) and body mass were recorded monthly during the course of this study. All lizards were captured and maintained under the approval of the Institutional Animal Care and Use Committee (UNM-IACUC 07UNM007). Lizards were housed in individual glass terrariums (20 gal. for *Sceloporus undulatus* and 60 gal. for *C. collaris*) and were provided a sand substrate as well as perch and shelter spaces constructed from stacked pieces of plywood and rock. Lizards were kept on a 12L : 12D photoperiod, and a heat lamp (100 W for *S. undulatus* and 150 W for *C. collaris*) placed at one end of the terrarium and focused on the wood perch site provided a thermal gradient that ranged from 39° ± 1.7°C at the perch to 26° ± 0.8°C at the cool end of the tank. Resulting mean daytime body temperatures were 36.3° ± 6.2°C. Lizards were also provided with an ultraviolet-B fluorescent light (ZooMed UVB 10.0 fluorescent) to supply UVB light for vitamin D synthesis.

Diet Shift Experiment and Stable Isotope Analysis

Lizards used in this study were captured from field sites in which their diet was largely composed of C_3 plant-derived carbon. Essential to this study is the observation that differences in photosynthetic biochemistry inherent in C_3 and C_4 plants produces distinct differences in the $\delta^{13}C$ of their tissues, which can then be used to trace the movement of nutrients through consumers (Fry et al. 1978; Ambrose and DeNiro 1986; Gannes et al. 1997). Lizards from C_3-dominated environments were shifted to a diet composed of crickets raised on dog food with a C_4 plant composition. By switching lizards to a C_4-based diet, we were able to estimate carbon incorporation rates as their tissues shifted away from C_3-baseline values.

Lizards were switched to a C_4-based insect diet on the day of their capture and subsequent to the initial blood sampling (see below). Lizards were fed crickets ($\delta^{13}C = -16.2‰ \pm 0.1‰$ Vienna Pee Dee Belemnite Standard [VPDB], $n = 35$) raised on a C_4-based dog food (Iams Smart Puppy Large Breed Formula; $\delta^{13}C = -15.8‰ \pm 0.5‰$ VPDB, $n = 4$ batches). For stable isotope analysis we obtained a blood sample from each lizard at capture, just before the diet switch (day 0); we also euthanized two *S. undulatus* (see below) to obtain baseline $\delta^{13}C$ values for liver, skin, and muscle. To calculate carbon incorporation rates for plasma and RBCs, blood samples were taken from three lizards of each species on days 2, 4, 6, 9, 12, 19, 26, 33, 40, 47, 54, 61, 68, 83, 104, 131, 158, 173, 194, 215, 236, 250, 300, and 350 after the diet switch. Nine lizards of each species were used for this portion of the experiment, and individuals were randomly split into three permanent sampling groups that were sampled alternately over the duration of the experiment. Blood samples were obtained by slipping a microcapillary tube (Fisherbrand heparinized 50-μL microhematocrit capillary tubes; Fisher Scientific) ventral and posterior to the eyeball to puncture the retro-orbital sinus. Before this procedure, a local anesthetic (0.5% tetracaine hydrochloride ophthalmic solution; Akorn) was applied to the eye. Blood samples were stored in a cooler at 4°C and centrifuged within 24 h of collection to separate plasma and RBCs. A 15-μL sample of plasma was pipetted into a precleaned tin capsule and air dried for isotope analysis. We also placed a 0.5-mg sample of air dried RBCs into a second tin capsule for analysis.

The $\delta^{13}C$ of liver, skin, and thigh muscle tissues were measured in 18 *S. undulatus*. A single individual was euthanized on days 0, 7, 21, 30, 60, 93, 123, 153, 186, 228, 275, 318, and 360 after the diet switch. Two or more individuals were sampled on days 0, 286, and 360 to gauge variation. Lizards were euthanized via an intraperitoneal injection of sodium pentobarbital (using a dose of 60 mg kg^{-1}). Tissue samples were collected and freeze dried, and a 0.5-mg sample was placed into a precleaned tin capsule for isotope analysis.

Measurements of $\delta^{13}C$ were conducted on a continuous-flow isotope ratio mass spectrometer (Thermo-Finnigan IRMS Delta Plus) with samples combusted in a Costech ECS 4010 Elemental Analyzer in the University of New Mexico Earth and Planetary Sciences Mass Spectrometry lab. The precision of these analyses was ± 0.1‰ SD for $\delta^{13}C$ based on long-term variation of the laboratory standard. A laboratory standard calibrated against international standards (valine $\delta^{13}C$ −26.3‰ VPDB) was included on each run in order to make corrections to raw values obtained from the mass spectrometer.

Stable isotope ratios are expressed using standard delta notation (δ) in parts per thousand (‰) as $\delta X = (R_{sample}/R_{standard} - 1) \times 1{,}000$. Here R_{sample} and $R_{standard}$ are the molar ratios of $^{13}C/^{12}C$ of the sample and the reference standard, respectively.

Statistical Analysis: Carbon Incorporation Models

The incorporation of a new dietary isotopic composition into an animal's tissue has often been approximated using one-compartment models that exhibit first-order kinetics. Cerling et al. (2007) recently challenged the use of single compartment models and has shown that multicompartment models sometimes provide more robust estimates of isotope incorporation

rates in some animal tissues. This work was extended by Martínez del Rio and Anderson-Sprecher (2008) by proposing the use of information theoretic criteria to choose the "best" model. To select the most robust model for each tissue, we first conducted preliminary diagnoses of the need for either one- or two-compartment models using the reaction-progress variable method (RPV) of Cerling et al. (2007) and then determined the best-fit model using the statistical approach recommended by Martínez del Rio and Anderson-Sprecher (2008). Briefly, the RPV method (see Cerling et al. (2007 for detailed methods) describes a turnover experiment as the fractional approach to equilibrium:

$$\frac{\delta_t - \delta_{\text{eq}}}{\delta_{\text{eq}} - \delta_{\text{init}}} = (1 - F), \tag{1}$$

where δ_{eq} is the data-derived isotopic equilibrium value, δ_{init} is the initial isotope value, $F = 0$ is day 0 of the diet switch, and $F = 1$ at equilibrium ($t =$ infinity). This variable normalizes isotopic exchange rates by scaling the difference between initial and equilibrium values to 1 and can be log transformed to represent a straight line for one-compartment systems. The intercept of this line represents the fractional contribution of a compartment to isotope exchange (e.g., intercept of 0 = 100%). Graphical analysis of this linearized RPV can then be used to determine whether there is a delay (intercept > 0) or multiple compartments (intercept < 0) driving isotope incorporation.

Dietary isotope incorporation rates were then calculated by iterative curve-fitting routines in SigmaPlot 8.0 using both a one-compartment model,

$$\delta_t = \delta_{\text{eq}} - (\delta_{\text{eq}} - \delta_{\text{init}})e^{-T/\tau}, \tag{2}$$

and a two-compartment model,

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

Here δ_{init} and δ_{eq} are initial and equilibrium isotopic values, T is time in days, τ is the residence time for a carbon element, and p is the fractional contribution of each compartment in a two-compartment model. The use of τ as a measure of isotope incorporation rate differs from most previous isotopic turnover studies, which have used the fractional rate of incorporation ($\lambda = 1/\tau$) in order to estimate the half-life ($t_{1/2} = -\tau \ln(2) = -\ln(2)/\lambda$) of an element in a tissue (Hobson and Clark 1992; Carleton and Martínez del Rio 2005; Cerling et al. 2007). In our use of τ, we follow the recommendation of Martínez del Rio and Anderson-Sprecher (2008) for two reasons: (1) τ has a clear intuitive interpretation as the average retention (or residence) time of an element, and (2) the nonlinear routines used to fit equations (2) and (3) give SE estimates for all its parameters, including average τ. We then used the Akaike Information Criterion corrected for small sample sizes (AICc) to determine whether a one- or two-compartment model was more appropriate for our data. Burnham and Anderson (2002) suggest that if the difference in AICc ($\Delta i = \text{AICc}_1 - \text{AICc}_2$) is negative, then model 1 is supported; whereas model 2 has stronger support if Δi is positive. If AICc supported a two-compartment model, we estimated average retention time as $\tau_{\text{ave}} = p\tau_1 + (1 - p)\tau_2$.

Although the above models account for tissue turnover resulting from catabolism, they do not account for the addition of new material in growing animals (reviewed in Martinez del Rio et al. 2009). Hesslein et al. (1993) proposed that the fractional rate of isotopic incorporation (λ) was the sum of growth (k) and catabolism (c) of a tissue and that the contribution of growth and catabolism could be determined by $\lambda = k + c$. Assuming a one-compartment system and substituting λ for $1/\tau$, equation (1) becomes

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

Here we use the Hesslein et al. (1993) model because *S. undulatus* are indeterminate growers, and the adult males grew during the course of this study. To estimate k, we fit nonlinear curves to the change in mass of *S. undulatus* over the course of this study (Kaufmann 1981). The Hesslein et al. (1993) model assumes that animals are growing exponentially; in our experiments, however, the growth of *S. undulatus* was best described by a power function ($y = ax^b$). Because *S. undulatus* growth was not exponential and their specific growth rate was thus not constant (Kaufmann 1981), partitioning λ into growth and catabolism is not straightforward (Martínez del Rio et al. 2009). To approximate the influence of growth, we used the approach of MacAvoy et al. (2005), who examined turnover in maturing mice that also exhibited growth best described by a power function (see also McIntyre and Flecker 2006; Tarboush et al. 2006). We assumed that the mean of individual specific growth rates ($k = \ln(\text{mass}_T/\text{mass}_0)/T$), as defined by Hesslein et al. (1993), for the measurement period that approximately matched the retention time (τ) of any given tissue also reflects the contribution of growth to tissue turnover. For example, we measured the mass of *S. undulatus* at ~30-d intervals; if a tissue had a $\tau = 63$ d, then we used the mean k for mass change over the 1–60-d interval. We then calculated the contribution of growth to tissue turnover as $\lambda = k + c$. To graphically assess the incorporation patterns that would occur if growth were the only factor contributing to tissue turnover, we set $c = 0$ in equation (4).

All δ^{13}C estimates are reported as mean ± SE‰ VPDB. Diet-to-tissue discrimination ($\Delta^{13}C_{\text{tissue-diet}}$) values were calculated as the difference between lizard tissue δ^{13}C values at equilibrium and δ^{13}C of the feeder crickets. Differences between cricket and lizard δ^{13}C tissue values were compared using t-tests.

Results

Initial (day 0) plasma δ^{13}C values of wild-caught *Sceloporus undulatus consobrinus* had a mean of −25.5‰ ± 0.2‰, which was close to values reported for local C_3 plants (−27.3‰ ±

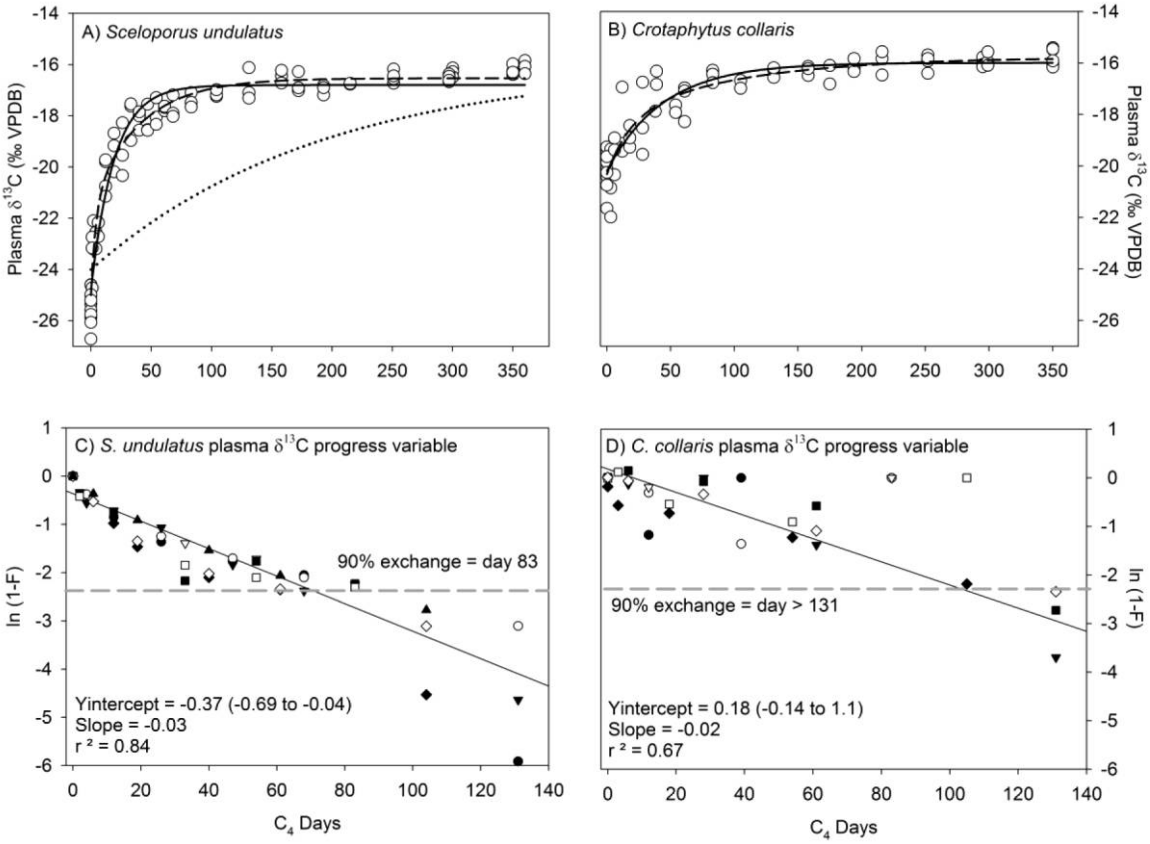

Figure 1. Changes in the δ^{13}C values of *Sceloporus undulatus consobrinus* (*A*, *C*) and *Crotaphytus collaris* (*B*, *D*) plasma during a 350-d diet-switch experiment. Animals taken from the wild, where they fed on a C_3-based food web, were switched to a C_4 cricket diet at day 0. Fitted curves are for one-compartment (*solid curve*) and two-compartment (*dashed curve*) models. The dotted curve (*A*) shows the expected incorporation rate only due to growth (rate constant $k = 0.0042$ d^{-1}) in *S. undulatus*. Note that *C. collaris* did not grow during this study. Examination of the intercept of reaction-progress variable plots (*C*, *D*) was used to determine whether one- or two-compartment models would best fit the data. Differing symbols in plots *C* and *D* represent individual lizards over time.

0.04‰ VPDB, mean ± SE, 34 spp.) and 11 species of lizards (−21.1‰ ± 0.8‰) in a C_3-dominated grassland-shrubland in the Chihuahuan Desert (Warne et al. 2010). Immediately after capture, *Crotaphytus collaris* had plasma δ^{13}C values of −20.2‰ ± 0.2‰ VPDB, which were close to average C_3 plant values. The plasma values of *S. undulatus* were initially more negative than the *C. collaris* values because of differences in the plant communities at the collection sites. Both species had initial plasma δ^{13}C values that were very different from the experimental diet of C_4-raised crickets (−16.2‰ ± 0.1‰ VPDB) used for the diet switch.

Both *S. undulatus* and *C. collaris* readily ate the C_4 feeder crickets. All *S. undulatus* exhibited mass gains that were best described by a power function (mean r^2 ± SD, 0.76 ± 0.18). In AICc comparisons with linear models, the power models were supported by Δi values that ranged from −1.8 to −6. Specific growth rate constants steadily decreased from $k = 0.0042$ d^{-1} for the day 1–30 interval to $k = 0.002$ d^{-1} for the day 195 interval to $k = 0.001$ d^{-1} for the day 1–200 interval and $k = 0.0006$ d^{-1} for the day 1–300 interval. The larger-bodied *C. collaris*, in contrast, had generally stable masses over the course of this study. One experimental *C. collaris* was euthanized at day 31 after being found in the cage with a hernia.

Carbon Incorporation Rates

Assessment of the appropriate use of the one- or two-compartment models of carbon incorporation by the RPV method ($\ln(1 - F)$) was in accordance with AICc comparisons. The intercepts of the linearized RPV for the tissues of both species had wide confidence intervals (range = −0.9 to 1.1) that overlapped the origin and generally supported the use of one-compartment models (Figs. 1*C*, 1*D*, 2*C*, 2*D*, 3*D*–3*F*). Similarly, negative Δi between model AICc values showed support for using the simpler and less parameterized one-compartment models for both species (Table 1). One tissue was exceptional: the incorporation of carbon into plasma of *S. undulatus* was best described by a two-compartment model as shown by a negative RPV intercept (Fig. 1*C*) and a positive Δi (Table 1). Although the negative RPV intercept of *S. undulatus* liver (Fig. 3*F*) also suggested the need for a two-compartment model, AICc comparisons supported a one-compartment model; we

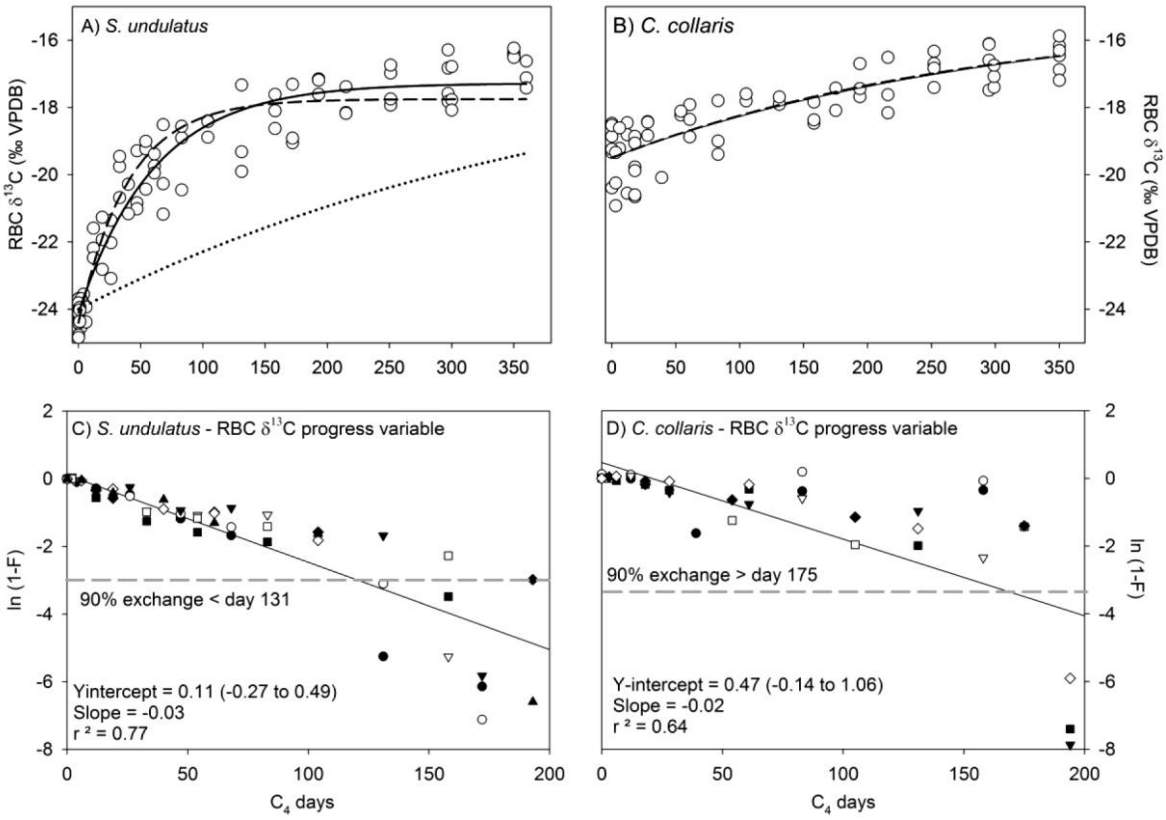

Figure 2. Changes in the $\delta^{13}C$ values of *Sceloporus undulatus consobrinus* (*A*, *C*) and *Crotaphytus collaris* (*B*, *D*) red blood cells during a 350-d diet-switch experiment. Animals taken from the wild, where they fed on a C_3-based food web, were switched to a C_4 cricket diet at day 0. See Figure 1 for a description of the differing curves and symbols. The dotted curve (*A*) shows the expected incorporation rate only due to growth (rate constant $k = 0.0023\ d^{-1}$) in *S. undulatus*. At most, two-compartment models were needed to fit the data as determined by reaction-progress variable plots (*C*, *D*).

thus selected the simpler, less parameterized one-compartment model for *S. undulatus* liver.

Mean (±SE) carbon retention time (τ) in *C. collaris* plasma was 44.4 ± 6.4 d and 311.4 ± 170 d in RBCs (Table 1). Carbon retention time in *S. undulatus* plasma was 25 ± 4.1 d and 60.7 ± 5 d in RBCs. Carbon retention time for *S. undulatus* somatic tissues ranged from 21.3 ± 2.8 d in liver to 80.9 ± 13.4 d in muscle and 94.3 ± 16.6 d in skin (Fig. 4).

Growth contributed minimally to tissue-carbon turnover in *S. undulatus* (Figs. 1*A*, 2*A*, 3, *dotted curves*). The relative contribution of growth to carbon turnover ranged from 9% to 11% in liver and plasma to 14% to 19% in RBCs, muscle, and skin (Table 1).

$\Delta^{13}C$ Diet-to-Tissue Discrimination

Diet-to-tissue discrimination ($\Delta^{13}C_{tissue\text{-}diet}$) differed between lizard species and among tissues (Table 2). The $\Delta^{13}C$ for RBC of *S. undulatus* (−1.6‰ ± 0.8‰) and *C. collaris* (−1.0‰ ± 0.6‰) indicated that this tissue was significantly depleted relative to the diet (−16.2‰ ± 0.1‰; one-sample *t*-test, $t < -4$, $P < 0.005$; Table 2). Plasma $\Delta^{13}C$ for *S. undulatus* (−0.5‰ ± 0.3‰) also showed a significant depletion relative to the diet ($t < -4$, $P < 0.005$; Table 2). *Crotaphytus collaris* plasma $\Delta^{13}C$ (0.2‰ ± 0.3‰), in contrast, was not significantly different from that of the diet (one-sample $t = 2$, $P > 0.05$; Table 2). The $\Delta^{13}C$ for *S. undulatus* liver (−0.6‰ ± 0.2‰; $t < -4$, $P < 0.005$), skin (−1.0‰ ± 0.5‰; $t < -4$, $P < 0.005$), and muscle (−1.8‰ ± 0.2‰; $t < -4$, $P < 0.005$) showed these tissues to be depleted in $\delta^{13}C$ relative to the diet (Table 2). It should be noted, however, that the mean $\delta^{13}C_{eq}$ for RBC of individual *C. collaris* indicated that >98% isotope exchange was −17.2‰ ± 0.6‰ VPDB (Fig. 2*D*), while the one-compartment model estimate for $\delta^{13}C_{eq}$ of this tissue was −15‰ ± 1.5‰ (Table 1). These differences suggest that the RBC of *C. collaris* may not have reached equilibrium with the diet (Fig. 2*B*).

Discussion

Carbon incorporation rates and diet-to-tissue discrimination ($\Delta^{13}C$) varied among tissues and between lizard species. One- and two-compartment models both provided robust fits to our incorporation data (Figs. 1–3). However, one-compartment models were consistently better ($\Delta i < 0$; Table 1) across all tissues and species with the exception of *Sceloporus undulatus consobrinus* plasma (Fig. 1*A*). Most tissues in both lizard species,

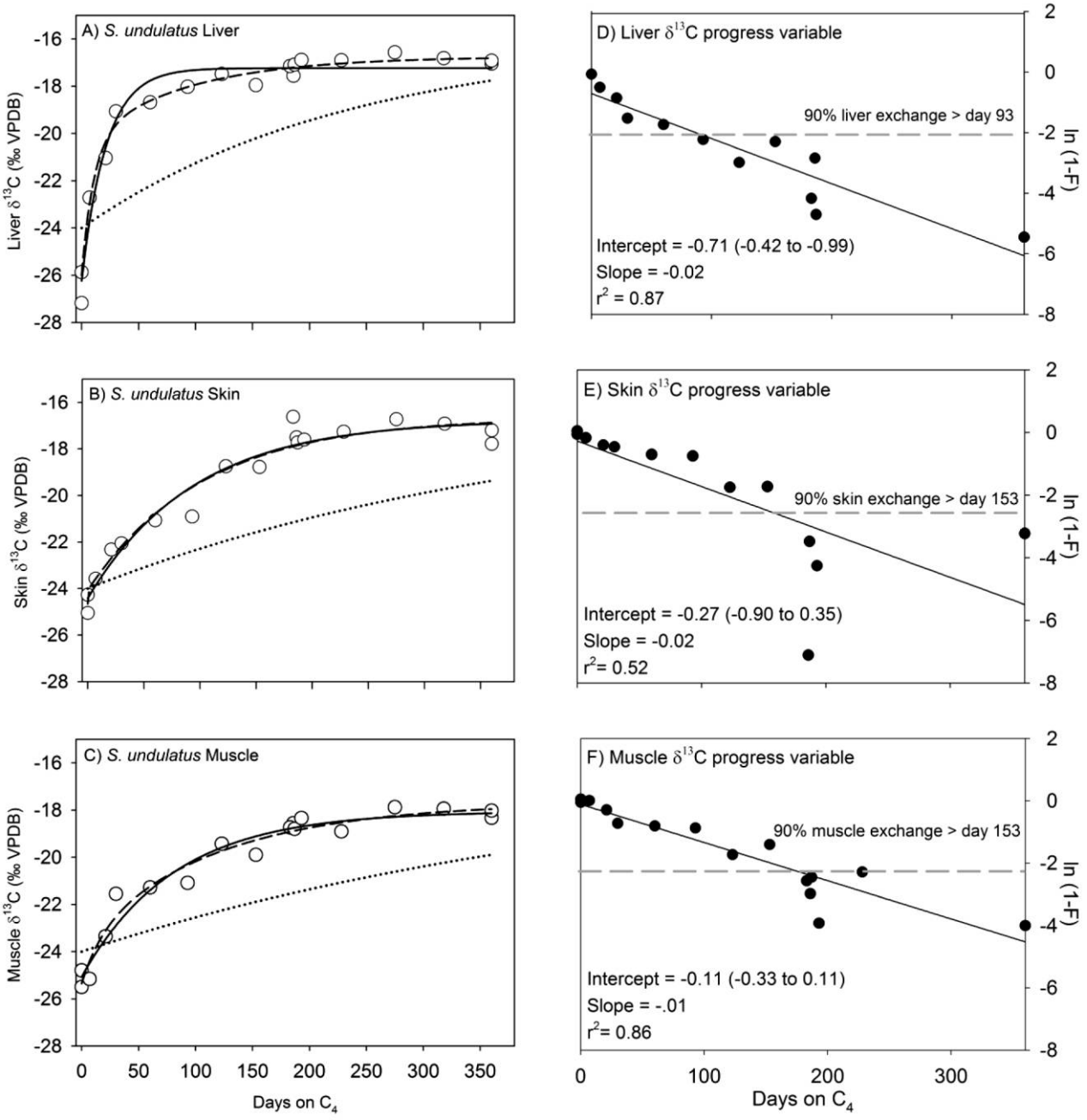

Figure 3. Changes in the $\delta^{13}C$ values of liver (*A*, *D*), skin (*B*, *E*), and muscle (*C*, *F*) of *Sceloporus undulatus consobrinus* during a 350-d diet-switch experiment. Animals taken from the wild, where they fed on a C_3-based food web, were switched to a C_4 cricket diet at day 0. See Figure 1 for a description of the differing curves and symbols. The specific growth rate constants used to plot the expected carbon incorporation only due to growth (*dotted curves*) were $k = 0.0042$ for liver (*A*) and $k = 0.002$ for skin (*B*) and muscle (*C*). At most, two-compartment models were needed to fit the data as determined by reaction-progress variable plots (*D–F*).

with the exception of *Crotaphytus collaris* RBCs, reached equilibrium with the experimental diet (Figs. 1–3). The smaller *S. undulatus* showed significantly faster carbon incorporation rates for both blood tissues relative to the larger *C. collaris* (Table 1). In *S. undulatus*, liver and plasma showed significantly faster carbon incorporation rates relative to RBCs, muscle, and skin (Fig. 4). Comparisons of the two species indicated that the larger *C. collaris* exhibited a higher degree of variation in carbon incorporation rates (Figs. 1*B*, 2*B*) and $\Delta^{13}C$ (Table 2). The greater variation observed in *C. collaris* could have been because of greater variance in their body condition, because only one of the eight *C. collaris* gained mass after its capture. In the following discussion, we explore the implications of our results for understanding the influence of body size, growth, and metabolism on carbon incorporation dynamics in terrestrial ectothermic vertebrates.

Table 1: Carbon incorporation in lizard tissues as described by the best-fit one- or two-compartment model

Tissue	Equation	One-Compartment AICc	Two-Compartment AICc	Δi	% Growth (k/λ)[a]	τ
Crotaphytus collaris:						
Plasma	$-16 - 4.2e^{-T/44.4}$	106.6	109.9	−3.3		44.4 ± 6.4
RBC	$-15 - 4.5e^{-T/311.4}$	103	107.8	−4.8		311.4 ± 170
Sceloporus undulatus consobrinus:						
Plasma	$-16.8 - 8(.42e^{-T/4.1} + .58e^{T/40})$	141.8	129.2	**12.7**	.11	25 ± 4.1[b]
RBC	$-17.3 - 6.8e^{-T/60.7}$	142.1	154.9	−12.8	.14	60.7 ± 5.0
Liver	$-17.2 - 9e^{T/21.3}$	33.9	38.1	−4.2	.09	21.3 ± 2.8
Skin	$-16.8 - 7.7e^{T/94.3}$	34.3	42.5	−8.1	.19	94.3 ± 16.6
Muscle	$-18 - 7e^{T/81.9}$	32.5	39.8	−7.2	.16	80.9 ± 13.4

Note. Best fit was determined by comparisons (Δi) of Akaike information criterion estimates (AICc). Boldface denotes Δi support for a two-compartment model. Percent contribution of growth to carbon incorporation was only estimated in the tissues of *Sceloporus undulatus consobrinus*, but not in *Crotaphytus collaris* because this species did not grow. Retention time of carbon (τ) is given as the mean (±SE) days for a tissue.

[a] k was estimated by mean specific growth rate for the measurement period (mass at 30-d intervals) that corresponded to τ, and $\lambda = 1/\tau$.

[b] Retention time is given by τ_{ave} for a two-compartment model.

Body Size and Carbon Incorporation

A recent study by Carleton and Martínez del Rio (2005) showed that the fractional rate of isotopic incorporation (λ) in the blood of eight bird species declined with body mass to approximately the −1/4 power. This result supported their prediction derived from the metabolic theory of ecology (Brown et al. 2004), which states that many physiological and ecological traits scale with body size, temperature, and metabolic strategy. The slower rate of isotope incorporation that we found in the blood of the larger-bodied *C. collaris* (47 g) relative to *S. undulatus* (12 g) is suggestive of a negative allometric trend similar to that found in birds. Indeed, these lizard species, which exhibit a fourfold difference in body mass, also show an ~30% difference in λ for plasma (~70% for RBC), which is close to the relationship suggested by the −1/4 scaling exponent. A −1/4 power scaling exponent suggests that animals that differ in mass by a factor of 2 should have a 20% difference in λ (Martínez del Rio et al. 2009). Although a sample size of only two species precludes any further speculation, our results do suggest that an expanded data set of incorporation rates for lizards with a wide range of body sizes could be an interesting line of inquiry in the future.

Growth and Carbon Incorporation

Another factor that influences carbon incorporation rates is growth, or the addition of new material to a tissue. Studies have found that growth can account for 10% of the carbon incorporated in adult-sized animals that are still maturing and up to 100% in rapidly growing larva or juveniles (reviewed in Martínez del Rio et al. 2009). We found that growth contributed between 9% and 19% of the carbon incorporated into the tissues of *S. undulatus* (Table 1). Although these lizards were all adults at the start of the study, they are indeterminate growers that responded positively to an ad lib. diet. Our results are similar to an ~26% contribution of growth to carbon incorporation found in adult zebra fish (Tarboush et al. 2006) and ~10% in subadult mice (MacAvoy et al. 2005). These combined results support the hypothesis put forward by Martinez del Rio et al. (2009) that the relative contribution of growth to isotopic incorporation is roughly the same in ectotherms and endotherms that are measured at comparable developmental stages.

Carbon Dynamics in Ectotherms

Ectothermic vertebrates are known to have field metabolic rates that are an order of magnitude or more lower than those of similar-sized endotherms (Nagy et al. 1999). As a consequence, it might be expected that ectotherms should also exhibit slower carbon incorporation rates (reviewed in Martínez del Rio et al. 2009). This is because isotope turnover is thought to be a function of tissue-specific rates of protein turnover (Carleton and Martínez del Rio 2005). Although numerous studies have examined the isotope incorporation dynamics of aquatic ec-

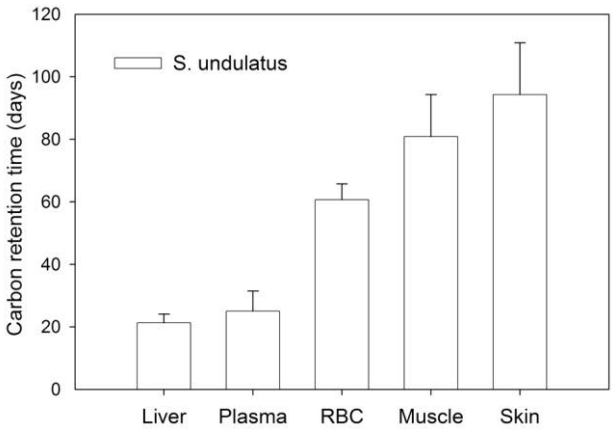

Figure 4. Carbon retention time in the tissues of *Sceloporus undulatus consobrinus*. Values are mean (±SE) estimates from fitted models (see Table 1). The order of retention times between tissues was generally consistent with previous studies for birds and mammals but on the order of 20 times slower than in endotherms.

Table 2: Mean (±SE) $\delta^{13}C$ values at equilibrium and diet-to-tissue discrimination ($\Delta^{13}C_{tissue\text{-}diet}$) for *Sceloporus undulatus consobrinus* and *Crotaphytus collaris* tissues

	Model $\delta^{13}C_{eq}$	$\Delta^{13}C_{tissue\text{-}diet}$
Cricket	−16.2 ± .4	…
C. collaris:		
Plasma	−16.0 ± .1	.2 ± .3
RBC	−15.0 ± 1.5	1.2 ± .6
S. undulatus:		
Plasma	−16.5 ± .1	−.5 ± .3
RBC	−17.3 ± .2	−1.1 ± .8
Liver	−17.2 ± .2	−1.0 ± .2
Skin	−16.8 ± .4	−.8 ± .5
Muscle	−18.1 ± .3	−1.9 ± .2

Note. Tissue $\delta^{13}C$ equilibrium estimates were derived from fitted models (see Table 1).

totherms (Fry and Arnold 1982; McIntyre and Flecker 2006), most of these studies have focused on rapidly growing fish larvae and juveniles (see references in MacAvoy et al. 2001; Suzuki et al. 2005; and Tarboush et al. 2006). As a consequence, comparisons of these data with incorporation dynamics of adult animals in other taxa such as mammals and birds are problematic. Our data, which focus on adult reptiles, allow a limited comparison with currently available endotherm data. Comparing the carbon incorporation rates of the lizards from this study with data on mammals and birds indicates that lizards show incorporation rates that are approximately 20 times slower than mammals and birds of a similar body size (Carleton and Martínez del Rio 2005; MacAvoy et al. 2006; Carleton et al. 2008). Hobson and Clark (1993), for example, reported a carbon half-life in American crow blood plasma of 2.9 d, a rate that is 4–10 times faster than that of our lizards ($t_{1/2}$ = 11 and 35 d for *S. undulatus* and *C. collaris*, respectively) even given the large differences in body mass among species (12 and 47 g for the lizards and 428 g for the crows). Extending these comparisons to other experiments is difficult because most studies of mammals and birds report values for whole blood (plasma + RBC). However, a rough comparison of our lizard RBC data, which represents the primary contributor to whole blood incorporation rates, to mammal and bird whole blood incorporation rates from the literature suggests that lizard RBCs have carbon incorporation rates ($t_{1/2}$ = 35–257 d; Fig. 2) that range from 7 to 20 times slower than those reported for similar-sized birds ($t_{1/2}$ = 5–11 d; Carleton and Martínez del Rio 2005) and mammals ($t_{1/2}$ = 17 d; MacAvoy et al. 2006).

In addition to body size and metabolism, environmental temperature is an important determinant of carbon turnover in ectotherms (Fry and Arnold 1982; Hesslein et al. 1993; Bosley et al. 2002). With the exception of a recent study by Bosley et al. (2002), the available data preclude in-depth, experimentally informed estimates of the direct effects of temperature on carbon incorporation rates. Existing data sets tend to be focused on rapidly growing larva or juvenile fishes, which make it difficult to isolate the effects of developmental stage from those of temperature when comparing rates of carbon incorporation across studies. Furthermore, current data sets reflect a strong interest in cold-water fishes, and few studies examine these processes over a range of environmental temperatures (see Hesslein et al. 1993; MacAvoy et al. 2001; Bosley et al. 2002; Tarboush et al. 2006). Tarboush et al.'s (2006) study of the warm-water zebra fish provides some insight, however, into how our data compare with those of warm-water fishes. They found that the muscle carbon of adult-sized zebra fish (~0.3g) had a half-life of 53 d (maintained at 28.5°C), which is very similar to the half-life of 56 d that we observed in muscle of the much larger *S. undulatus* (12 g) with active body temperatures of ~37°C and inactive temperatures of ~22°C. In comparison, the reported half-life of muscle carbon in cold-water fish range from 2 to 17 d in rapidly growing juveniles (Bosley et al. 2002) to over a year in adults (Hesslein et al. 1993). Although these comparisons suggest that environmental temperatures do produce strong differences in the carbon turnover rates of ectotherms, caution in comparing data for aquatic versus terrestrial taxa may be warranted because of characteristic differences in their respective thermal environments. Terrestrial vertebrate ectotherms are likely to experience a greater diel body temperature range and higher maximum body temperatures than aquatic animals because of the opportunity for basking under radiant heat sources (Shine 2005). Because of the nonlinear affects of temperature on biological rates (Gillooly et al. 2002), the average body temperature and carbon turnover of an aquatic may not be directly comparable with a terrestrial ectotherm with access to a radiant heat source. Studies that partition the effects of growth and temperature on carbon incorporation rates in the tissues of a range of ectotherms are clearly needed to fill these gaps in knowledge.

Our measurements of carbon discrimination ($\Delta^{13}C$; Table 2), in contrast, show no differences compared with those values observed in endotherms or other ectotherms (Ogden et al. 2004; Carleton and Martínez del Rio 2005). This observation is not surprising, however, because diet-to-tissue discrimination is not strongly tied to biological rates and temperature, as are tissue-carbon incorporation rates, but is the result of chemical and physical discrimination associated biological processes. Biochemical processes such as the Kreb's cycle, nutrient catabolism, and tissue synthesis are conserved across taxa (Carleton and Martínez del Rio 2005), suggesting that discrimination values may also be conserved to some degree. These results combined with the carbon incorporation differences between the large *C. collaris* and the smaller *S. undulatus* support the suggestion by Carleton and Martínez del Rio (2005) that isotopic incorporation rates, not $\Delta^{13}C$ factors, are strongly influenced by size-dependent and tissue-specific metabolic and protein turnover rates.

In conclusion, our data provide insight into carbon incorporation rates in ectothermic vertebrates of differing sizes and inform our understanding of how these turnover rates differ among endotherms and ectotherms. We found that lizards have carbon incorporation rates that are dramatically slower than

those of endothermic vertebrates but show diet-to-tissue discrimination values that are similar. The limited comparison of our lizard data with that of warm-water fishes suggest comparable tissue incorporation rates between aquatic and terrestrial ectotherms. This research also highlights the lack of experimental data that examines isotope incorporation rates in ectotherms across a range of temperatures, diets, body sizes, and developmental stages. Given this deficit in knowledge, we second the call of Martínez del Rio et al. (2009) and others for extending this research so that we can better our understanding of the processes that underlie isotopic distributions and dynamics in animal tissues.

Acknowledgments

We thank D. Garcia for his diligent assistance in maintaining our experimental lizards and G. Spintzyk for her enthusiastic help in capturing the lizards. We also thank T. E. Cerling, D. DeNardo, and J. H. Brown for their helpful comments on the manuscript. We further thank T. Turner for the loan of his aquariums and V. Atudori and Z. Sharp for providing access to and training on the University of New Mexico Earth and Planetary Sciences isotope ratio mass spectrometer as well as ZooMed reptile supply manufacturer for their generous support with lighting supplies. Research support was provided by a National Science Foundation (NSF) Doctoral Dissertation Improvement Grant (DEB-0710128) and by an NSF GK-12 fellowship (DGE-0538396) to R.W.W. Research support was also provided by NSF grants (DEB-0213659 and IOB-0426764) to B.O.W. and to the Sevilleta Long Term Ecological Research (DEB-0217774).

Literature Cited

Ambrose S. and M. DeNiro. 1986. The isotopic ecology of East African mammals. Oecologia 69:395–406.

Biasatti D. 2004. Stable carbon isotopic profiles of sea turtle humeri: implications for ecology and physiology. Palaeogeogr Palaeoclimatol Palaeoecol 206:203–216.

Bosley K., D. Witting, R. Chambers, and S. Wainright. 2002. Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. Mar Ecol Prog Ser 236:233–240.

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.

Burnham K. and D. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer, New York.

Carleton S.A., L. Kelly, R. Anderson-Sprecher, and C. Martínez del Rio. 2008. Should we use one-, or multi-compartment models to describe ^{13}C incorporation into animal tissues? Rapid Commun Mass Spectrom 22:3008–3014.

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.

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.

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

———. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351.

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.

Fry B. and C. Arnold. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia 54: 200–204.

Fry B., A. Joern, and P.L. Parker. 1978. Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. Ecology 59:498–506.

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.

Gauthier G., J. Bety, and K.A. Hobson. 2003. Are greater snow geese capital breeders? new evidence from a stable-isotope model. Ecology 84:3250–3264.

Gillooly J.F., E.L. Charnov, G.B. West, V.M. Savage, and J.H. Brown. 2002. Effects of size and temperature on development time. Nature 417:70–73.

Godley B., D. Thompson, S. Waldron, and R. Furness. 1998. The trophic status of marine turtles as determined by stable isotope analysis. Mar Ecol Prog Ser 166:277–284.

Hatase H., N. Takai, Y. Matsuzawa, W. Sakamoto, K. Omuta, K. Goto, N. Arai, and T. Fujiwara. 2002. Size-related differences in feeding habitat use of adult female loggerhead turtles *Caretta caretta* around Japan determined by stable isotope analyses and satellite telemetry. Mar Ecol Prog Ser 233:273–281.

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 $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Can J Fish Aquat Sci 50:2071–2076.

Hobson K.A. and R.G. Clark. 1992. Assessing avian diets using stable isotopes. I. Turnover of ^{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.

Hobson K.A., J.F. Piatt, and J. Pitocchelli. 1994. Using stable isotopes to determine seabird trophic relationships. J Anim Ecol 63:786–798.

Kaufmann K. 1981. Fitting and using growth curves. Oecologia 49:293–299.

Kelly J. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool 78:1–27.

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.

MacAvoy S.E., S.A. Macko, and G.C. Garman. 2001. Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey. Can J Fish Aquat Sci 58:923–932.

Magnusson W.E., A.P. Lima, A.S. Faria, R.L. Victoria, and L.A. Martinelli. 2001. Size and carbon acquisition in lizards from Amazonian savanna: evidence from isotope analysis. Ecology 82:1772–1780.

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. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111.

McIntyre P. and A. Flecker. 2006. Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. Oecologia 148:12–21.

Nagy K.A., I.A. Girard, and T.K. Brown. 1999. Energetics of free-ranging mammals, reptiles, and birds. Annu Rev Nutr 19:247–277.

O'Brien D.M., D.P. Schrag, and C.M. del Rio. 2000. Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. Ecology 81:2822–2831.

Ogden L., K. Hobson, and D. Lank. 2004. Blood isotopic (δ^{13}C and δ^{15}N) turnover and diet-tissue fractionation factors in captive dunlin (*Caldris alpina pacifica*). Auk 121:170–177.

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.

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

Shine R. 2005. Life history evolution in reptiles. Annu Rev Ecol Syst 36:23–46.

Spence K.O. and J.A. Rosenheim. 2005. Isotopic enrichment in herbivorous insects: a comparative field-based study of variation. Oecologia 146:89–97.

Struck U., A. Altenbach, M. Gaulke, and F. Glaw. 2002. Tracing the diet of the monitor lizard *Varanus mabitang* by stable isotope analyses (δ^{15}N, δ^{13}C). Naturwissenschaften 89:470–473.

Suzuki K., A. Kasai, K. Nakayama, and M. Tanaka. 2005. Differential isotopic enrichment and half-life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: implications for analyzing migration. Can J Fish Aquat Sci 62:671–678.

Tarboush R., S. MacAvoy, S. Macko, and V. Connaughton. 2006. Contribution of catabolic tissue replacement to the turnover of stable isotopes in *Danio rerio*. Can J Zool 84:1453–1460.

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 δ^{13}C analysis of diet. Oecologia 57:32–37.

Vanderklift M. and S. Ponsard. 2003. Sources of variation in consumer-diet delta ^{15}N enrichment: a meta-analysis. Stable Isot Ecol 136:169–182.

Wallace B., J. Seminoff, S. Kilham, J. Spotila, and P. Dutton. 2006. Leatherback turtles as oceanographic indicators: stable isotope analyses reveal a trophic dichotomy between ocean basins. Mar Biol 149:953–960.

Warne R.W., A.D. Pershall, and B.O. Wolf. 2010. Linking precipitation and C_3-C_4 plant production to resource dynamics in higher trophic level consumers. Ecology 91:1628–1638.

Wolf B.O., C. Martínez del Rio, and J.Y. Babson. 2002. Stable isotopes reveal that saguaro fruit provides different resources to two desert dove species. Ecology 83:1286–1293.