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# Slow Isotope Turnover Rates and Low Discrimination Values in the American Alligator: Implications for Interpretation of Ectotherm Stable Isotope Data

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## ABSTRACT

Stable isotope analysis has become a standard ecological tool for elucidating feeding relationships of organisms and determining food web structure and connectivity. There remain important questions concerning rates at which stable isotope values are incorporated into tissues (turnover rates) and the change in isotope value between a tissue and a food source (discrimination values). These gaps in our understanding necessitate experimental studies to adequately interpret field data. Tissue turnover rates and discrimination values vary among species and have been investigated in a broad array of taxa. However, little attention has been paid to ectothermic top predators in this regard. We quantified the turnover rates and discrimination values for three tissues (scutes, red blood cells, and plasma) in American alligators (*Alligator mississippiensis*). Plasma turned over faster than scutes or red blood cells, but turnover rates of all three tissues were very slow in comparison to those in endothermic species. Alligator  $\delta^{15}\text{N}$  discrimination values were surprisingly low in comparison to those of other top predators and varied between experimental and control alligators. The variability of  $\delta^{15}\text{N}$  discrimination values highlights the difficulties in using  $\delta^{15}\text{N}$  to assign absolute and possibly even relative trophic levels in field studies. Our results suggest that interpreting stable isotope data based on parameter estimates from other species can be problematic and that large ectothermic tetrapod tissues may be characterized by unique stable isotope dynamics relative to species occupying lower trophic levels and endothermic tetrapods.

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## Introduction

Over the past 30 years, stable isotope analysis (SIA) has become a common tool for helping elucidate trophic interactions and food web structure. SIA has been used to study temporal and spatial variation in food web structure (e.g., Fry 1991; Hobson and Welch 1992), interspecific niche partitioning (e.g., Stewart et al. 2003), habitat connectivity (e.g., Anderson and Polis 1998; Rosenblatt and Heithaus 2011), and individual specialization (e.g., Bearhop et al. 2006; Newsome et al. 2009; Matich et al. 2011), among other applications. The most commonly used elements in ecological SIA are carbon (C) and nitrogen (N; Fry 2006). The ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  (expressed in standard delta notation as  $\delta^{13}\text{C}$ ) is altered only slightly as C moves up the food chain (typically between  $-1\text{‰}$  and  $+1\text{‰}$ ), while the ratio of  $^{15}\text{N}$  to  $^{14}\text{N}$  ( $\delta^{15}\text{N}$ ) typically increases as the amount of  $^{15}\text{N}$  in consumer tissues increases (between  $+2\text{‰}$  and  $+6\text{‰}$  per trophic level) as N moves up the food chain (DeNiro and Epstein 1978, 1981; Minigawa and Wada 1984; Peterson and Fry 1987; Post 2002; Caut et al. 2009). Thus,  $\delta^{13}\text{C}$  can be used to track the original source(s) of a consumer's nutrients, and  $\delta^{15}\text{N}$  can be used to estimate a consumer's relative trophic position (i.e., higher  $\delta^{15}\text{N}$  indicates higher trophic position; Fry 2006). Despite the prevalence of SIA in ecological studies, however, there remain important questions concerning the dynamics of isotopes as they move through the food web that necessitate controlled studies to adequately interpret field data.

Of particular importance are the changes in  $\delta$  ratios with each trophic transfer ("discrimination," or  $\Delta$  values) and the time required for tissues, especially metabolically active ones, to incorporate the  $\delta$  values of their diets ("turnover rates"). It is well known that discrimination values and turnover rates can vary considerably among species and tissue types because of variable metabolic rates and pathways (Gannes et al. 1997; Post 2002; Caut et al. 2009). Selection of appropriate discrimination values and turnover rates, therefore, is critical for assessing trophic interactions, trophic positions, and patterns of specialization of consumers (e.g., Caut et al. 2009; Hussey et al. 2010; Bond and Diamond 2011).

Discrimination values and turnover rates have been experimentally determined for many tissue types in many species of animals, but there is a high degree of variation among taxa in the number of studies. For example, a literature search using

Web of Science and combinations of the search terms “isotope,” “turnover,” “discrimination,” and “fractionation” returns C or N isotope discrimination values or turnover rates for at least one tissue from 62 fishes, 41 invertebrates, 30 birds, and 25 mammals. In contrast, isotope parameters are available for only one species of amphibian (McIntyre and Flecker 2006) and eight species of reptile (Seminoff et al. 2006, 2007, 2009; Reich et al. 2008; Fisk et al. 2009; Warne et al. 2010; Murray and Wolf 2012). This lack of stable isotope parameters for ectothermic tetrapods limits our overall understanding of stable isotope dynamics, in particular possible differences between large ectothermic and endothermic top predators. Elucidating these differences is important because large ectothermic top predators, particularly crocodylians, have been dominant predators in tropical aquatic systems for millions of years and likely exert variable degrees of control over aquatic and terrestrial ecosystem dynamics. Currently, many of the extant crocodylian species are endangered or threatened (Martin 2008), yet their functional roles in tropical ecosystems are still largely unknown. Accurate application of SIA to these types of animals could lead to greater understanding of their roles in food webs and improved management and conservation strategies.

In this study we quantified discrimination values and turnover rates for the American alligator (*Alligator mississippiensis* Daudin), an ectothermic top predator that inhabits the southeastern United States (Mazzotti and Brandt 1994). We hypothesized that isotopic turnover rates for alligators would be slower than those for most other vertebrates previously studied because of their slow metabolism, but we had no a priori predictions about how  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  discrimination values in *A. mississippiensis* might compare to those in other vertebrates. Our overarching goal was to elucidate the isotope parameters of a large reptilian top predator and investigate how stable isotope dynamics might vary between ectotherms and endotherms in general, among large carnivores, and among species of reptiles.

## Material and Methods

### Experimental Design

All procedures were carried out under a permit (09-015) from Florida International University’s Institutional Animal Care and Use Committee. The study was conducted between May 2010 and May 2011 at the St. Augustine Alligator Farm (SAAF) in St. Augustine, Florida, using 14 captive-born and captive-raised juvenile American alligators. Each individual was identified using previously implanted passive integrated transponder tags (Avid Identification Systems, Norco, CA). Each alligator was measured for total length, snout-vent length, head length, and tail girth to the nearest 0.1 cm and mass to the nearest 0.5 kg before the study began. Body condition was calculated using Fulton’s condition factor formula ( $M/\text{SVL}^3$ )  $\times 10^5$ , where  $M$  is body mass and SVL is snout-vent length (Fujisaki et al. 2009). At the beginning of the experiment, the alligators ranged in age from 3.3 to 8.4 yr (mean,  $5.7 \pm 1.2$  SD) and in total length from 78.6 to 114.8 cm (mean,

$93.4 \pm 13.4$  SD). All individuals were immature males (size at maturity, 1.5–1.8 m; Abercrombie 1989; Dalrymple 1996), which minimized the possible confounding effect of variation in metabolism between genders and life stages. Also, because juvenile alligators grow at similar rates until maturity (i.e., growth rates vary little across ages and sizes of juvenile alligators; Jacobsen and Kushlan 1989), it is unlikely that variation in growth rates would confound results.

To assess isotope turnover rates of three tissues easily collected during field studies (scutes, red blood cells [RBCs], and blood plasma), we carried out a diet-switch experiment in which the alligators were split into two groups. The control group ( $n = 7$ ) was housed in a fenced-in outdoor unroofed pen (6 m  $\times$  6 m, with a 0.5-m-deep pool), and the experimental group ( $n = 7$ ) was housed in an enclosed, roofed concrete pen (4 m  $\times$  4 m, with a 0.5-m-deep pool) to limit the possibility of small birds and mammals accidentally becoming prey for the experimental group and shifting the isotope values of their tissues. Both groups were composed of randomly selected individuals. The two groups did not differ in length, weight, or body condition at the beginning of the experiment ( $t$ -test:  $t_{12} = -0.37$ ,  $P = 0.72$ ;  $t_{12} = -0.66$ ,  $P = 0.52$ ;  $t_{12} = -1.65$ ,  $P = 0.13$ , respectively). For approximately 3 yr before the study began, all of the alligators were predominantly fed a diet of homogenized pork-based food pellets (protein, 45.0%; fat, 9.5%; Mazuri, Richmond, IN), manufactured specifically for captive crocodylians. Rarely, their diet was supplemented with mice and rats. When the experiment began, the alligators in the control group continued to be fed the pellet diet, while the alligators in the experimental group were switched to a diet of channel catfish (*Ictalurus punctatus*; protein, 16.4%–17.5%; fat, 10.3%–13.2%; Grant and Robinette 1992; Silva and Ammerman 1993). All of the catfish were farm raised (Carolina Classics Catfish, Ayden, NC) on a diet that consisted mainly of soy, corn, and wheat. The catfish were all harvested in one batch to minimize isotopic variability and were frozen whole and shipped to SAAF, where they were stored in a normal freezer. Before being fed to the alligators in the experimental group, the catfish were thawed and cut into small chunks. Each group of alligators was fed equal amounts of food approximately two times per week, and efforts were made to ensure that each of the study animals was fed equally during each feeding, though occasionally during feedings some individuals consumed slightly more than others. Isotopes from 14 random samples each of the catfish and pellet diet were analyzed at the beginning of the study to determine the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the two diets and to assess their consistency. We performed SIA on diet samples only at the beginning of the study because stable isotope ratios are unaffected by storing tissues in normal freezers (Bosley and Wainright 1999; Barrow et al. 2008; Bugoni et al. 2008). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the pellet diet were  $-17.55\text{‰} \pm 0.14\text{‰}$  SE and  $5.97\text{‰} \pm 0.03\text{‰}$  SE, respectively, while the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the catfish diet were  $-23.19\text{‰} \pm 0.58\text{‰}$  SE and  $9.69\text{‰} \pm 0.70\text{‰}$  SE, respectively. The differences in  $\delta$  values between the two diets (5.64‰ for  $\delta^{13}\text{C}$  and 3.72‰ for  $\delta^{15}\text{N}$ ) are similar in magnitude to the spread

of isotope values found in wild alligator populations (e.g., Rosenblatt and Heithaus 2011) and thus represent real isotopic shifts that could naturally occur. Other candidate foods for the experimental diet (*Rattus rattus*, *Oncorhynchus mykiss*, *Gallus gallus domesticus*, *Mugilidae* sp.) were tested, but isotopic values were not sufficiently different from the control diet to provide insights into discrimination values and turnover rates.

Before the diet switch, small samples (~1 cm<sup>2</sup>) of scutes (raised scales on the back and tail) were collected from the terminal tail scutes of each alligator using surgical scissors. Also, a small amount of blood (3–4 mL) was collected from the dorsal cervical sinus using an 18-gauge, 3.8-cm needle and a 5-mL syringe (Owens and Ruiz 1980). Blood samples were immediately separated into their RBC and plasma components using a centrifuge spun at 3000 rpm for 30 s. All samples were frozen and transported to the lab, where they were stored at –4°C. These initial samples served as baseline isotope measurements for each group. After the diet switch, blood samples were collected from each alligator in both groups after 2, 4, 8, 16, and 32 wk and 1 yr. Because we predicted slower isotope turnover rates in scute tissue, we collected scute samples only after 8 and 32 wk and 1 yr. During each sampling period, all alligators were weighed and measured. The experiment had to be terminated after 1 yr because of space limitations at SAAF.

Once in the lab, scute samples were washed with deionized water and then transferred, along with the plasma and RBC samples, to an oven and dried at 60°C for at least 72 h. All samples were then powdered using a mortar and pestle, and between 0.4 and 0.7 mg of sample was placed in individual 3 × 5-mm tin cups for analysis. Crocodylian scutes are not homogenous tissues but instead are composed of a keratin surface layer and a collagen core (Radloff et al. 2012). We analyzed them whole instead of separating them into their constituent parts because when the two tissues are sampled from wild alligators, they do not significantly differ in their isotope values (J. Nifong, unpublished data), though they may differ in their isotope turnover rates and discrimination values. Isotopic analyses were performed at Florida International University's Stable Isotope Laboratory using standard elemental analyzer isotope ratio mass spectrometer procedures (Fry 2006). Seven scute samples, 10 plasma samples, and 20 RBC samples were analyzed in duplicate, and the mean error attributable to the equipment was 0.05‰ ± 0.006‰ SE for δ<sup>15</sup>N and 0.09‰ ± 0.01‰ SE for δ<sup>13</sup>C. The standard deviations of an internal standard (glycine), based on 12 within-run samples during each of eight runs, were 0.06‰ for δ<sup>15</sup>N and 0.08‰ for δ<sup>13</sup>C.

Lipid content of isotope samples is a potential confounding factor in SIA because lipids generally are depleted in <sup>13</sup>C in comparison to carbohydrates and proteins and therefore exhibit more negative δ<sup>13</sup>C values (DeNiro and Epstein 1977; Post et al. 2007). Therefore, tissue samples characterized by high lipid content could appear to have lower δ<sup>13</sup>C values than low-lipid tissues when in fact they may just contain different fractions of biochemical components. As a result, lipid-influenced δ<sup>13</sup>C values could alter estimates of discrimination values. Further-

more, the different biochemical components of the diet can be subject to “isotopic routing,” meaning ingested nutrients may not be used equally to build and maintain different consumer tissues (Gannes et al. 1997). For animals that consume high-protein diets, such as the alligators fed the pellet diet in our study, dietary protein is most likely exclusively used for tissue synthesis, while carbohydrates and lipids are catabolized (Gannes et al. 1997). Therefore, in our study, alligator tissues and diets that exhibited high lipid content needed to be normalized through lipid extraction for proper analysis of the δ<sup>13</sup>C discrimination values.

First, we analyzed all of the samples without extracting any lipids because lipid extraction procedures carry the possibility of altering the δ<sup>15</sup>N value of the tissues (Logan et al. 2008). Then, we identified whether tissues from either group of alligators or the pellet diet exhibited C : N ratios >3.5 because this threshold indicates the potential presence of a large fraction of lipids that could affect δ<sup>13</sup>C analyses (Post et al. 2007). A subset of tissue and diet samples characterized by high C : N ratios then were reanalyzed after lipids had been extracted using the following procedure: approximately 50 mg of each sample was weighed on filter paper (Whatman, Buckinghamshire, UK) and then folded up inside the filter paper, secured with a sterile paper clip, and placed in a vial. Each vial was then filled with 4 mL of 2 : 1 dichloromethane : methanol solvent, which is as effective at removing lipids as chloroform but does not remove as much protein (Erickson 1993; Cequier-Sanchez et al. 2008). Vials were then capped and placed in a refrigerator for 15 h. The solvent was then drained and 3 mL of fresh solvent added for 3 h, followed by 2 mL of fresh solvent for another 3 h. Samples were then removed from the vials, redried for at least 72 h, weighed into tin cups, and analyzed using the previously described procedure.

#### Analyses

To determine the isotope turnover rates for both δ<sup>13</sup>C and δ<sup>15</sup>N for all three tissues, we fitted exponential decay curves to the isotope data gathered from the experimental group. We used the exponential decay equation  $y = a + be^{ct}$ , where  $y$  is the δ<sup>13</sup>C or δ<sup>15</sup>N value at time  $t$  (days since diet switch);  $a$  is the value of the asymptote being approached by the curve;  $b$  is the total change in δ<sup>13</sup>C or δ<sup>15</sup>N value after the diet switch; and  $c$ , the parameter that was solved for, is the fractional turnover value (Hobson and Clark 1992a; Seminoff et al. 2007). We then used the fractional turnover value ( $c$ ) to calculate the isotopic half-life ( $t_{1/2}$ ) using the equation  $t_{1/2} = \ln(0.5)/c$ , where  $t_{1/2}$  represents the amount of time (in days) it takes for half of the isotopes to be exchanged in a tissue and 0.5 indicates that 50% of the isotopes were exchanged (Seminoff et al. 2007). Complete isotopic turnover is reached in roughly four half-lives, so we multiplied each  $t_{1/2}$  value by 4 to estimate the complete turnover rate for each isotope for each tissue (Seminoff et al. 2007; Vander Zanden et al. 2010).

Diet-tissue discrimination values ( $\Delta$ ) for δ<sup>13</sup>C and δ<sup>15</sup>N for each tissue were calculated using the equation  $\Delta = \delta_{\text{tissue}} -$

$\delta_{\text{diet}}$ , where  $\delta_{\text{tissue}}$  represents the mean  $\delta$  values of each tissue sampled from the control group for the duration of the study and  $\delta_{\text{diet}}$  represents the mean  $\delta$  value of the pellet diet (Hobson and Clark 1992b). We averaged the  $\delta$  values of each tissue over the duration of the study for control-group individuals because the control group had been fed on the same diet for at least 4 yr (3 yr before the study plus 1 yr during the study); thus, we assumed that all three tissues had reached isotopic equilibrium with the diet. If the C : N ratio of a tissue or the pellet diet was  $>3.5$ , then we calculated  $\Delta$  for  $\delta^{13}\text{C}$  using the  $\delta^{13}\text{C}$  values from the lipid-extracted samples. All analyses were carried out using SigmaPlot 11 (Systat, Chicago, IL).

## Results

### Growth

Alligators in both control and experimental groups grew during the experiment (average SVL growth,  $3.2 \text{ cm} \pm 2.4 \text{ SD}$  [6.6% of initial SVL  $\pm 4.5 \text{ SD}$ ]; average weight gain,  $1.0 \text{ kg} \pm 0.9 \text{ SD}$  [28.7% of initial body mass  $\pm 20.4 \text{ SD}$ ]), but there were no significant differences in growth between treatments (*t*-test:  $t_{11} = 0.7$ ,  $P = 0.5$ ;  $t_{11} = 1.3$ ,  $P = 0.2$ , respectively). There was no difference in body condition of individuals between groups at the start (see "Material and Methods") or conclusion of the experiment (control group,  $\bar{x} = 2.9 \pm 0.4 \text{ SD}$ ; experimental group,  $\bar{x} = 2.8 \pm 0.2 \text{ SD}$ ; Mann-Whitney rank-sum test:  $T = 43.0$ ,  $P = 0.9$ ).

### Turnover Rates

We did not detect any significant differences in either  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  between different sampling events for the control-group tissues (ANOVA: all  $P > 0.27$  except scutes  $\delta^{13}\text{C}$ , where  $P = 0.06$ ), suggesting that isotope values for all tissues in the control group were at isotopic equilibrium (fig. 1). In contrast, in the experimental group, all three tissues showed clear shifts away from the control diet and toward the experimental diet for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (fig. 2). However, for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  only plasma appeared to equilibrate with the experimental diet after 1 yr (fig. 2). Despite this result, the exponential decay functions applied to the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values significantly fitted the data for plasma and RBCs (all  $P < 0.001$ ), and the fits for the scute  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were marginally nonsignificant ( $P = 0.06$  and  $0.05$ , respectively), most likely because of the use of only four data points (fig. 2). For plasma, RBCs, and scutes the  $\delta^{13}\text{C}$  half-lives were 63.0, 141.5, and 147.5 d, respectively, and the  $\delta^{15}\text{N}$  half-lives were 62.4, 277.3, and 103.5 d, respectively. The estimated  $\delta^{13}\text{C}$  complete turnover times (i.e., four half-lives) for plasma, RBCs, and scutes were 252.0, 566.0, and 590.0 d, respectively, and the estimated  $\delta^{15}\text{N}$  complete turnover times were 249.6, 1,109.2, and 414.0 d, respectively.

### Discrimination Values

The mean C : N ratios of the plasma, RBC, and scute samples from the control group were  $3.65 \pm 0.02$ ,  $3.17 \pm 0.009$ , and

$3.09 \pm 0.02 \text{ SE}$ , respectively, and the C : N ratio of the pellet diet was  $5.92 \pm 0.07 \text{ SE}$ . The mean C : N ratios of the plasma, RBC, and scute samples from the experimental group were  $3.66 \pm 0.03$ ,  $3.19 \pm 0.01$ , and  $3.08 \pm 0.01 \text{ SE}$ , respectively. Therefore, we extracted lipids only from the pellet diet and plasma samples from each group because their C : N ratios were  $>3.5$  (Post et al. 2007). The mean C : N ratios of the pellet diet and plasma samples from the control and experimental groups after lipid extraction were  $5.00 \pm 0.06$ ,  $3.43 \pm 0.02$ , and  $3.61 \pm 0.02 \text{ SE}$ , respectively. The  $\delta^{13}\text{C}$  values of the pellet diet and plasma samples from the control and experimental groups before lipid extraction were  $-17.52\text{‰} \pm 0.15\text{‰}$ ,  $-17.60\text{‰} \pm 0.07\text{‰}$ , and  $-19.42\text{‰} \pm 0.23\text{‰} \text{ SE}$ , respectively, and after lipid extraction the values were  $-17.30\text{‰} \pm 0.17$ ,  $-17.54\text{‰} \pm 0.07\text{‰}$ , and  $-19.23\text{‰} \pm 0.24\text{‰} \text{ SE}$ , respectively. These shifts in  $\delta$  ratios were not statistically significant (Mann-Whitney rank-sum test:  $T = 208.0$ ,  $P = 0.1$  for diet;  $T = 922.0$ ,  $P = 0.3$  for control plasma;  $T = 740.0$ ,  $P = 0.4$  for experimental plasma); therefore, we used the nonlipid extracted  $\delta^{13}\text{C}$  values for all subsequent analyses. We also compared the lipid-extracted plasma  $\delta^{13}\text{C}$  values to the expected plasma  $\delta^{13}\text{C}$  values generated by Post et al.'s (2007) lipid correction equation for aquatic animals. We found that the  $\delta^{13}\text{C}$  values produced by the lipid correction equation (mean,  $-17.29 \pm 0.07 \text{ SE}$ ) were significantly higher than the lipid-extracted  $\delta^{13}\text{C}$  values (Mann-Whitney rank-sum test:  $T = 642.0$ ,  $P < 0.001$ ) but only by  $0.25\text{‰}$ , which is not a large enough difference to be ecologically meaningful.

The mean  $\Delta\delta^{15}\text{N}$  values for all control alligators were positive but of lesser magnitude than traditionally assumed for all tissues (plasma,  $+0.35\text{‰} \pm 0.04\text{‰} \text{ SE}$ ; RBCs,  $+0.95\text{‰} \pm 0.05\text{‰} \text{ SE}$ ; scutes,  $+1.22\text{‰} \pm 0.08\text{‰} \text{ SE}$ ; fig. 2). The  $\Delta\delta^{13}\text{C}$  values were relatively small for each tissue (plasma,  $-0.04\text{‰} \pm 0.07\text{‰} \text{ SE}$ ; RBCs,  $+0.03\text{‰} \pm 0.07\text{‰} \text{ SE}$ ; scutes,  $+0.61\text{‰} \pm 0.12\text{‰} \text{ SE}$ ). For comparison, we also calculated the approximate  $\Delta$  values for each tissue from the individuals in the experimental group by using the estimated complete turnover times as the *t* parameters in the exponential decay equations and solving for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ . We then subtracted these estimated tissue isotope equilibrium values from the isotope values of the catfish diet. The C : N ratio of the catfish diet was  $6.77 \pm 0.51 \text{ SE}$ , so we used a lipid correction equation for aquatic animals ( $\delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + (0.99 \times \text{C} : \text{N})$ ) to normalize the catfish  $\delta^{13}\text{C}$  values (Post et al. 2007). The  $\Delta\delta^{13}\text{C}$  values calculated from the alligators in the experimental group were different from those of the control group but were still relatively small (table 1). In contrast, there was an important difference between the two groups concerning the  $\Delta\delta^{15}\text{N}$  values. All  $\Delta\delta^{15}\text{N}$  values were negative for the experimental group (table 1).

## Discussion

Quantifying species- and tissue-specific stable isotope discrimination values and turnover rates is essential for proper analysis and interpretation of field data. Using a diet-switch experiment,



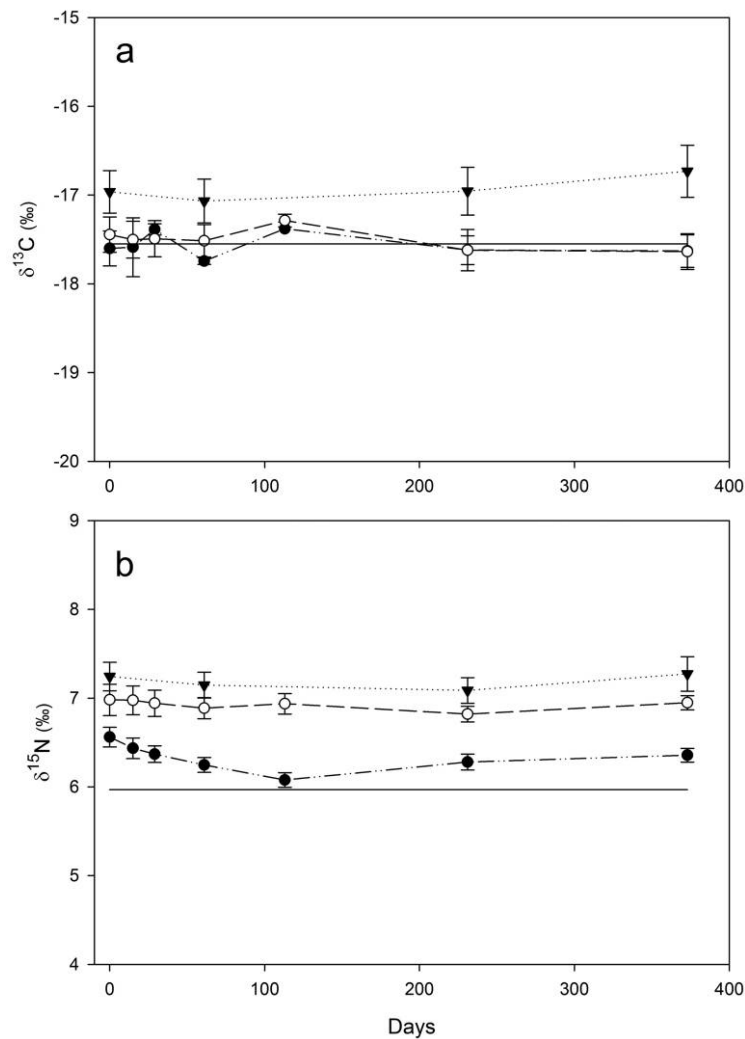


Figure 1. The  $\delta^{13}\text{C}$  stable isotope values (a) and the  $\delta^{15}\text{N}$  stable isotope values (b) from three American alligator tissues sampled from the control group over 1 yr. The control group did not undergo a diet switch before tissue collection. Filled circles and the dash-dot line represent blood plasma, open circles and the dashed line represent red blood cells, and triangles and the dotted line represent scutes. Solid lines represent the mean isotope value of the control diet. Error bars are  $\pm$  SE.

we provide the first data on isotope turnover rates and discrimination values of a crocodylian. We found that isotope turnover rates of American alligators were considerably slower than those of most other taxa studied, especially for RBCs, and that  $\Delta\delta^{15}\text{N}$  values were much smaller than often is assumed. These results underscore important differences in isotope dynamics between different reptilian species and between endothermic and ectothermic taxa.

Across taxa, there is relatively predictable variation in relative turnover times across tissue types. Plasma tends to turn over most rapidly, skin the slowest, and RBCs at an intermediate rate (reviewed in Dalerum and Angerbjorn 2005). While alligators exhibited this pattern of tissue turnover rates for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  turnover rates deviated from this pattern. The  $\delta^{15}\text{N}$  turnover rate for RBCs was by far the slowest rate of all three tissues and almost twice as slow as the  $\delta^{13}\text{C}$  rate for RBCs. This result

can partially be explained by the fact that reptilian RBCs are nucleated (Dessauer 1970) and therefore have longer life spans than the same cells in species that have nonnucleated RBCs (e.g., mammals). Indeed, alligator RBCs display exceptionally long life spans, reaching 1,320 d under some conditions (Cline and Waldmann 1962), while mammalian RBCs can survive only 36–120 d (reviewed in Rodnan et al. 1957). Also, the  $\delta^{15}\text{N}$  turnover rate may be much slower than the  $\delta^{13}\text{C}$  rate in RBCs because N is a crucial component of the hemoglobin molecule that makes up much of the mass of long-lived alligator RBCs, whereas metabolically generated C is transported into and out of alligator RBCs in the form of  $\text{CO}_2$  as the RBCs carry the molecule to the lungs to be exhaled (Jensen et al. 1998). Therefore, hemoglobin-linked N may remain in an RBC for the entire life span of the cell, while C may turn over relatively more quickly as part of respiration.

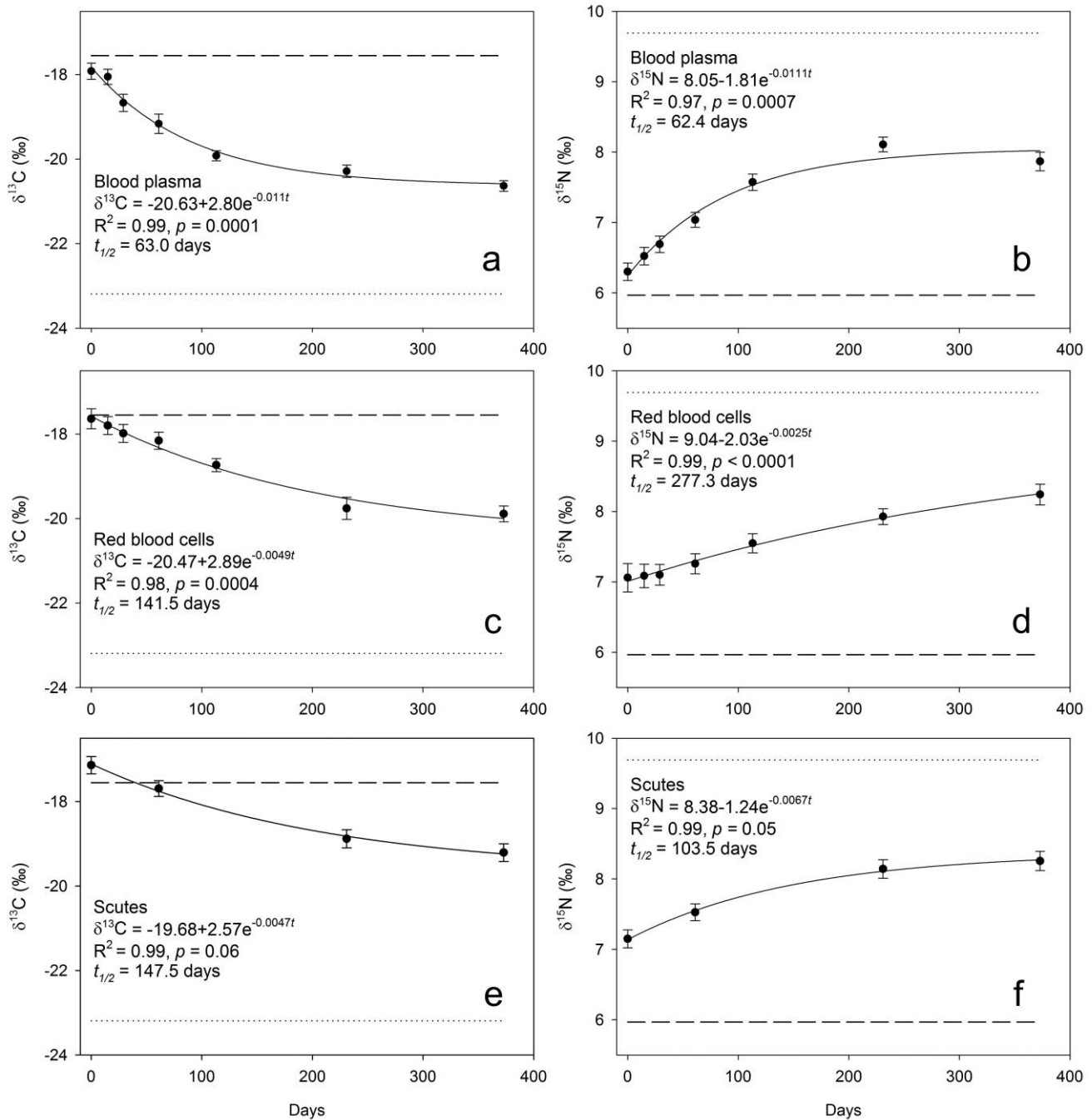


Figure 2. Stable  $\delta^{13}\text{C}$  isotope values from blood plasma (a), red blood cells (c), and scutes (e) and stable  $\delta^{15}\text{N}$  isotope values from blood plasma (b), red blood cells (d), and scutes (f) from American alligators (*Alligator mississippiensis*) in the experimental group collected over 1 yr following a diet switch. The curved line on each graph represents the exponential decay curve ( $y = a + be^{-ct}$ ; see text for definitions of each parameter) used to model each set of isotope turnover parameters. The parameter  $t_{1/2}$  represents the time it takes (in days) for 50% of the isotopes in each tissue to turn over and was determined using the equation  $t_{1/2} = \ln(0.5)/c$ . Dashed lines represent the mean isotope value of the control diet, and dotted lines represent the mean isotope value of the experimental diet. Error bars are  $\pm$  SE.

Ectotherms generally exhibit slower metabolic rates than do endotherms (Hulbert and Else 2004); thus, we would expect ectotherm tissues to be characterized by isotope turnover rates slower than those for endotherm tissues. Dalerum and An-

gerbjorn (2005), in a review of mammal and bird isotope studies, reported no estimated complete turnover rates ( $t_{1/2} \times 4$ ) for plasma or RBCs greater than 160 d, with all but two rates less than 20 d. More recent studies have also found relatively

Table 1: Approximate discrimination values ( $\Delta$ ) calculated from estimated isotope values at tissue equilibrium from alligators in the experimental group

Isotope and tissue	Estimated isotope value at tissue equilibrium (‰)	Isotope value of catfish diet (‰)	Approximate $\Delta$ value at equilibrium (‰)	$\Delta$ value from control group
$\delta^{13}\text{C}$ :				
Plasma	-20.45	-19.80	-.65	-.04
Red blood cells	-20.29	-19.80	-.49	+.03
Scutes	-19.52	-19.80	+.28	+.61
$\delta^{15}\text{N}$ :				
Plasma	7.94	9.69	-1.75	+.35
Red blood cells	8.91	9.69	-.78	+.95
Scutes	8.30	9.69	-1.39	+1.22

Note. The  $\Delta$  values were calculated using the equation  $\Delta = \delta_{\text{tissue}} - \delta_{\text{diet}}$ , and the  $\delta^{13}\text{C}$  value of the catfish diet was corrected for lipid content using the equation  $\delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + (0.99 \times \text{C:N})$  (Post et al. 2007). The  $\Delta$  values from the control group are provided for comparison.

short estimated complete turnover rates for plasma and RBCs in Pallas's long-tongued bat (*Glossophaga soricina*; estimated complete turnover, 97–158 d; Mirón et al. 2006) and the arctic fox (*Vulpes lagopus*; estimated complete turnover, 16–172 d; Lecomte et al. 2011). In contrast, reptile plasma and RBC tissues can display short estimated complete turnover rates (e.g., 19 d for *Caretta caretta*; table 2) but also much longer rates (e.g., 1,109 d for *Alligator mississippiensis*; table 2) that have never been found in endotherms. Other ectotherms display patterns similar to those in reptiles in terms of estimated complete isotope turnover rates for RBCs and plasma, with fishes (including sharks) displaying widely varying rates that range from 11 to 432 d (Buchheister and Latour 2010; German and Miles 2010; Logan and Lutcavage 2010; Kim et al. 2012). These trends suggest that isotope turnover rates in ectotherms can be relatively fast in some species and even comparable to rates observed in endotherms but that isotope turnover rates in other ectotherm species can also be orders of magnitude slower than those in endotherms. The mechanisms responsible for differences in turnover rates among ectotherms are not clear, but potential factors include variation in body size, activity levels, diet type and quality, growth rates, and species-specific physiology.

The estimated complete turnover rates found for juvenile alligators in this study—which ranged from 250 to 1,109 d—are among the slowest recorded for any animal, despite their growth during the study (mean increase in body mass,  $41\% \pm 21\%$  SD). Fisk et al. (2009) reported slower estimated complete  $\delta^{15}\text{N}$  turnover rates for whole blood and muscle (1,664 and 2,496 days, respectively) in corn snakes (*Elaphe guttata guttata*) but only for those individuals fed an “uptake” diet (i.e., a diet that was enriched in  $^{15}\text{N}$  isotopes in relation to the previous diet). In contrast, snakes fed on an “elimination” diet (i.e., the diet was depleted in  $^{15}\text{N}$  isotopes relative to the initial control diet) exhibited much faster estimated complete turnover rates of only 300 and 454 d for whole blood and muscle, respectively. In our study, the experimental group of alligators was also fed an uptake diet in terms of  $\delta^{15}\text{N}$  values but an

elimination diet in terms of  $\delta^{13}\text{C}$  values. Boecklen et al. (2011) conducted a meta-analysis on the effects of diet-switch directionality on isotope turnover rates and did not find support across taxa for the pattern reported by Fisk et al. (2009), but because of small sample size, they concluded that the effect of diet-switch directionality on isotope turnover rates remains an open question.

Isotope turnover rates are composed of two components: turnover due to growth and turnover due to normal tissue maintenance (catabolic turnover; Hesslein et al. 1993; Reich et al. 2008). In our study, we used juvenile alligators, capable of relatively rapid growth in comparison to adult alligators (Chabreck and Joanen 1979). Thus, the turnover rates quantified in our study are some combination of growth turnover and catabolic turnover and may be faster than the turnover rates of adult alligators, which, though they grow indeterminately (Jacobsen and Kushlan 1989), grow more slowly than juveniles. Both Reich et al. (2008) and Murray and Wolf (2012) were able to partition isotope turnover rates into their growth and catabolic turnover components using exponential growth models based on changes in body mass. Unfortunately, in our study we were unable to accurately partition isotope turnover rates into their growth and catabolic turnover components because of the low number of sampling events ( $n = 7$ ), the slow growth of the alligators in terms of body mass (mean, 0.95 kg/yr), and the lack of accuracy in our body mass measurements (0.5-kg increments). For loggerhead turtles (*C. caretta*), Reich et al. (2008) found that, depending on the tissue type, growth was responsible for 15%–52% of the turnover rates. Murray and Wolf (2012) reported that growth was responsible for 13%–50% of carbon turnover in multiple tissues of juvenile desert tortoises (*Gopherus agassizii*). We would expect turnover rates in juvenile alligators to follow a similar pattern, and thus it is very likely that adult alligators actually display slower turnover rates than the ones we found in this study (e.g., Sun et al. 2012).

Consistent with the general trend across taxa (reviewed in Caut et al. 2009), the  $\Delta\delta^{13}\text{C}$  values of alligators (range,  $-0.04\%$



Table 2: Known discrimination values and turnover rates for reptile plasma, red blood cells, and scutes

Species and tissue	Discrimination value (‰)		Half-life (d)		Estimated complete turnover rate (d)		Reference
	$\Delta \delta^{13}\text{C}$	$\Delta \delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
<i>Chelonia mydas</i> :							
Plasma	-.12	+2.92					Seminoff et al. 2006
Red blood cells	-1.11	+.22					Seminoff et al. 2006
<i>Trachemys scripta</i> :							
Plasma		+3.80	35.6		142.4 <sup>a</sup>		Seminoff et al. 2007
Red blood cells		+1.90					Seminoff et al. 2007
<i>Caretta caretta</i> (hatchling):							
Plasma	+.29	+.32			20.0	18.5	Reich et al. 2008
Red blood cells	-.64	-.25			76.9	71.4	Reich et al. 2008
<i>C. caretta</i> (juvenile):							
Plasma	-.38	+1.50			20.0	18.5	Reich et al. 2008
Red blood cells	+1.53	+.16			76.9	71.4	Reich et al. 2008
<i>Dermochelys coriacea</i> :							
Plasma	-.58	+2.86					Seminoff et al. 2009
Red blood cells	+.46	+1.49					Seminoff et al. 2009
<i>Crotaphytus collaris</i> :							
Plasma	+.20				44.4		Warne et al. 2010
Red blood cells	+1.20				311.4		Warne et al. 2010
<i>Sceloporus undulatus consobrinus</i> :							
Plasma	-.50				25.0		Warne et al. 2010
Red blood cells	-1.10				60.7		Warne et al. 2010
<i>Gopherus agassizii</i> :							
Plasma	+1.00–1.60				32.9		Murray and Wolf 2012
Red blood cells	+.20–.80				126.7		Murray and Wolf 2012
<i>Alligator mississippiensis</i> :							
Plasma	-.04	+.35	63.0	62.4	252.0 <sup>a</sup>	249.6 <sup>a</sup>	This study
Red blood cells	+.03	+.95	141.5	277.3	566.0 <sup>a</sup>	1,109.2 <sup>a</sup>	This study
Whole scutes	+.61	+1.22	147.5	103.5	590.0 <sup>a</sup>	414.0 <sup>a</sup>	This study

Note. The  $\Delta$  values for alligators were taken from the calculations using the control group.

<sup>a</sup>Estimated complete turnover rate values calculated by multiplying  $t_{1/2}$  values by 4. Calculation methods for the other turnover rates can be found within the given source material.

to +0.61‰; table 1) were small and therefore should closely reflect dietary sources in the wild. Alligator  $\Delta\delta^{15}\text{N}$  values (range, -1.75‰ to +1.22‰; table 2) were less than the values found for the same tissues in every nonreptilian species studied to date (+1.23‰ to +6.30‰; reviewed in Caut et al. 2009) and considerably below the +3.40‰ value often applied to calculations of isotopic trophic levels (Post 2002). Indeed, the approximate  $\Delta\delta^{15}\text{N}$  values from the experimental group were actually negative, suggesting that even the assumption that  $\delta^{15}\text{N}$  values increase with each trophic step may not hold for all species and all diet types. Previous studies of  $\Delta\delta^{15}\text{N}$  values for three different reptile species using the same tissues that we used found  $\Delta\delta^{15}\text{N}$  values ranging from +0.16‰ (juvenile *C. caretta*) to +2.92‰ (juvenile *Chelonia mydas*) despite using comparably sized growing juveniles and similarly carnivorous diets (Seminoff et al. 2006, 2009; Reich et al. 2008). This broad range of  $\Delta\delta^{15}\text{N}$  values highlights the difficulties in using  $\delta^{15}\text{N}$  to assign absolute and possibly even relative trophic levels in

field studies. Observed differences among species could have been caused by a number of factors, including differing activity levels, species-specific physiology, and diet quality (Caut et al. 2009). Given the broad similarities between the studies, we hypothesize that the variation in  $\Delta\delta^{15}\text{N}$  values between the reptile species is caused by some combination of different species-specific growth patterns, isotopic routing pathways, and patterns of protein synthesis. Identifying the specific causes of these differences is difficult because of the lack of understanding about isotope dynamics at the molecular level.

Additionally, when our alligator data are compared with data currently available for large endothermic carnivores, the results suggest that  $\Delta\delta^{15}\text{N}$  values are not conserved within broadly similar trophic guilds (i.e., mobile large-bodied top predators). For example, alligator plasma  $\Delta\delta^{15}\text{N}$  values are much smaller than those of endothermic large top predators such as seals (e.g., harbor seal *Phoca vitulina*, +2.7‰ to +3.2‰; gray seal *Halichoerus grypus*, +2.9‰ to +3.3‰; harp seal *Phoca groen-*

*landica*, +3.6‰; northern fur seal *Callorhinus ursinus*, +5.2‰; Lesage et al. 2002; Kurle 2002). Though extensive data on  $\Delta\delta^{15}\text{N}$  values across tissues of both large carnivorous endotherms and ectotherms are lacking, these initial studies may indicate that, in general, large carnivorous ectotherms are characterized by  $\Delta\delta^{15}\text{N}$  values lower than those in large carnivorous endotherms, and thus generalized isotope parameters should not be applied across such varied groups because it could lead to the assignment of incorrect trophic levels.

Both  $\Delta\delta^{15}\text{N}$  and  $\Delta\delta^{13}\text{C}$  values vary with diet type and quality (Robbins et al. 2005, 2010; Mirón et al. 2006; Caut et al. 2009, 2010; Hill and McQuaid 2009; Dennis et al. 2010). A review of isotope data from 82 different species from many disparate groups revealed a pattern wherein  $\Delta\delta^{15}\text{N}$  and  $\Delta\delta^{13}\text{C}$  values tend to decrease as the isotope values of the diet increase (i.e., discrimination values are lower at higher  $\delta^{15}\text{N}$  and less negative  $\delta^{13}\text{C}$ ; Caut et al. 2009). Despite some disagreement in the literature (Auerswald et al. 2010; Perga and Grey 2010), this pattern has been further supported by recent laboratory experiments (Caut et al. 2008; Dennis et al. 2010) and data reanalysis (Caut et al. 2010). Although our results for  $\Delta\delta^{15}\text{N}$  values were consistent with this pattern, our results for  $\Delta\delta^{13}\text{C}$  values were not. Although we used only two different diets during the experiment and the  $\Delta$  values derived from the experimental group are somewhat rough estimates, our results still imply that alligator  $\Delta$  values can vary considerably depending on the type of diet being consumed.

Last, our findings concerning tissue-specific turnover rates in alligators have implications for the use of stable isotopes from ectotherms for the reconstruction of diet histories and measures of individual specialization. Over the past decade SIA has been promoted as an important tool for answering questions of individual specialization (e.g., Bolnick et al. 2002; Matthews and Mazumder 2004; Urton and Hobson 2005; Newsome et al. 2009). One way SIA can be used to elucidate patterns of individual specialization is to compare isotope values between multiple tissues that turn over at different rates (e.g., Bearhop et al. 2006; Matich et al. 2011). For example, if three tissues with different turnover rates (e.g., 10, 30, and 90 d) all displayed similar isotope values (allowing for differential discrimination values) for one individual, then that individual could be considered a specialist since its isotope values were constant across different temporal scales. However, the applicability of this method may be limited in species such as alligators because tissues that turn over quickly in other species (e.g., plasma) turn over comparatively slowly in alligators. Thus, isotope information gathered from alligator plasma would be unable to resolve questions concerning daily, weekly, or even monthly diet variability, and therefore some specialization metrics (e.g., Bearhop et al. 2006; Matich et al. 2011) could overestimate specialization in alligators since any short-term diet variability would be obscured by the turnover rate of the tissue. Other metrics for understanding specialization, however, may be facilitated by long turnover rates in tissues. For example, the spread between individual isotope values within a population (Layman et al. 2007) can be an indicator of long-term differ-

ences in diets among individuals on the timescale reflected by the tissue being used. Therefore, although alligator tissues may not be amenable for understanding stability of diets over relatively short time periods, even a single tissue type may provide information on within-population variation in trophic interactions (e.g., Burkholder et al. 2011).

In conclusion, the observed variation in the quantified isotope parameters from our study, along with studies of other reptiles and nonreptiles, underscores the need for species- and tissue-specific values to be used in the interpretation and analysis of any field-based isotope study. The values derived in our study are the first isotope parameters described for any crocodylian species and should be useful for elucidating the roles of alligators and closely related crocodylians in food web and community dynamics. Yet, many important questions regarding discrimination values and turnover rates remain. For example, how do diet quality, body size, and variation in growth and metabolic rates between individuals of the same age class and/or gender influence discrimination values and turnover rates? Answering these questions and elucidating isotope dynamics in a wider array of species will more fully enable an understanding of the complexities of SIA, including its proper applications and limitations.

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