Isotopic turnover rates in tissues of American Alligators (*Alligator mississippiensis*)

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Introduction

Stable isotope analysis is an important tool in ecology because it can be used to determine the trophic level and origins of nutrient resources for individual consumers. This tool also can be useful in identifying migratory routes and seasonal feeding grounds that are of conservation value for a particular species. Of particular value to studies of threatened and endangered species is the fact that different tissues incorporate the isotopic signature of the diet over different timescales, allowing inferences to be drawn about both short- and long-term diets with a single non-destructive sampling event. However, the timescale of tissue turnover varies across broad taxonomic groups. Turnover rates are known for many types of tissues in many species of birds, mammals, and fish, but this knowledge is severely lacking for reptiles and completely absent for crocodilians.

The goal of this study was to determine the isotopic turnover rates and discrimination values of blood plasma, red blood cells (RBC), and scutes of American alligators to provide rates and values that can be used in studies of wild alligators as well as other crocodilians. The study provides the first such data for crocodilians and significantly advances our ability to use isotopic techniques to understand spatial and temporal variation in diets and feeding locations which are necessary for drafting appropriate conservation measures for these animals. Though the turnover rates and discrimination values determined in this study are specific to American alligators, the turnover rates and discrimination values can be used as estimates for other crocodilians because most crocodilians exhibit similar metabolic rates. Furthermore, gathering isotope samples is usually less invasive and destructive than other techniques for studying crocodilian feeding habits, so making stable isotope analysis more accurate and effective should encourage researchers to use them and cause less stress for the animals under study.

Methods

The study took place at the St. Augustine Alligator Farm in St. Augustine, FL, USA between May 2010 and May 2011. Before the experiment began, scute (~1cm^2), RBC, and plasma (~5ml of blood total) samples were taken from 14 juvenile male alligators to provide baseline carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values. Scute samples were taken from the end of the tail of each individual using surgical scissors and blood was taken from the dorsal cervical sinus using 18 gauge syringes. Blood was immediately separated into its RBC and plasma components using a centrifuge spun at 3000 rpm for 20 seconds. All samples were frozen at -4°C and transported to the lab. All alligators were also weighed and measured.

The alligators were then split into two groups (experimental and control) of seven individuals each. The seven alligators in the experimental group had their diets switched from the normal captive crocodilian pellet diet to an experimental diet of catfish that differed in its stable isotope values from the pellet diet. The seven alligators in the control group did not have their diet switched and remained on the commercial pellet diet. All alligators were fed comparable

amounts of food (~0.5 kg/week). Fish and pellet samples were collected throughout the experiment and analyzed to ensure that the diet within each group did not vary isotopically during the study.

After the switch, blood samples were taken after two weeks, four weeks, eight weeks, 16 weeks, 32 weeks, and one year. Scute samples were taken after 8 weeks, 32 weeks and one year. During each sampling period, all alligators were also weighed and measured. All isotope samples were processed following standard techniques at the Florida International University Stable Isotope Laboratory. Turnover rates were analyzed using an exponential decay model and discrimination values were calculated by subtracting the isotope values of the pellet diet from the isotope values of the tissues sampled from the control group.

Results

In the experimental group, all three tissues showed clear shifts away from the control diet and toward the experimental diet for both $\delta^{13}C$ and $\delta^{15}N$. The exponential decay functions fit the data very well for all three tissues. For plasma, RBCs, and scutes the $\delta^{13}C$ half-lives were 63.0, 141.5, and 147.5 days, respectively, and the $\delta^{15}N$ half-lives were 62.4, 277.3, and 103.5 days, respectively. The estimated $\delta^{13}C$ complete turnover times (ie four half-lives) for plasma, RBCs, and scutes were 252.0, 566.0, and 590.0 days, respectively, and the estimated $\delta^{15}N$ complete turnover times were 249.6, 1,109.2, and 414.0 days, respectively.

The mean $\delta^{15}N$ discrimination values ($\Delta\delta^{15}N$) for all control alligators were positive but of lesser magnitude than traditionally assumed for all tissues (plasma, +0.35‰ ± 0.04‰ SE; RBCs, +0.95‰ ± 0.05‰ SE; scutes, +1.22‰ ±0.08‰ SE). The $\delta^{13}C$ discrimination values ($\Delta\delta^{13}C$) were relatively small for each tissue (plasma, -0.04‰ ± 0.07‰ SE; RBCs, +0.03‰ ± 0.07‰ SE; scutes, +0.61‰ ± 0.12‰ SE).

Conclusions

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. While alligators exhibited this pattern of tissue turnover rates for $\delta^{13}C$, $\delta^{15}N$ turnover rates deviated from this pattern. The $\delta^{15}N$ turnover rate for RBCs was by far the slowest rate of all three tissues and almost twice as slow as the $\delta^{13}C$ rate for RBCs. This result can partially be explained by the fact that reptilian RBCs are nucleated and therefore have longer life spans than the same cells in species that have non-nucleated RBCs (e.g., mammals). Indeed, alligator RBCs display exceptionally long life spans, reaching 1320 days under some conditions, while mammalian RBCs can survive only 36-120 days. Also, the $\delta^{15}N$ turnover rate may be much slower than the $\delta^{13}C$ 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 CO_2 as the RBCs carry the molecule to the lungs to be exhaled. 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.

Ectotherms generally exhibit slower metabolic rates than do endotherms; thus, we would expect ectotherm tissues to be characterized by isotope turnover rates slower than those for endotherm tissues. Indeed, all measured endotherm complete turnover rates are faster than 160 days, In contrast, reptile plasma and RBC tissues can display short estimated complete turnover rates (eg 19 days for *Caretta caretta*) but also much longer rates (eg 1109 d for *A. mississippiensis*) 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 days. 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 days - are among the slowest recorded for any animal, despite their growth during the study (mean increase in body mass, 41% ± 21% SD).

Consistent with the general trend across taxa the $\Delta\delta^{13}C$ values of alligators were small and therefore should closely reflect dietary sources in the wild. Alligator $\Delta\delta^{15}N$ values were less than the values found for the same tissues in every non-reptilian species studied to date. Previous studies of $\Delta\delta^{15}N$ values for three different reptile species using the same tissues that we used found $\Delta\delta^{15}N$ values ranging from +0.16‰ (juvenile *C. caretta*) to +2.92‰ (juvenile *Chelonia mydas*) despite using comparably sized growing juveniles and similarly carnivorous diets. This broad range of $\Delta\delta^{15}N$ values highlights the difficulties in using $\delta^{15}N$ 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.

Additionally, when our alligator data are compared with data currently available for large endothermic carnivores, the results suggest that $\Delta\delta^{15}N$ values are not conserved within broadly similar trophic guilds (i.e., mobile large-bodied top predators). For example, alligator plasma $\Delta\delta^{15}N$ values are much smaller than those of endothermic large top predators such as seals. Though extensive data on $\Delta\delta^{15}N$ 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 $\Delta\delta^{15}N$ 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.

In conclusion, the observed variation in the quantified isotope parameters from our study, along with studies of other reptiles and non-reptiles, 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 crocodilian species and should be useful for elucidating the roles of alligators and closely related crocodilians 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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