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ABSTRACT

When attempting to determine the diet of wild animals, a limited number of techniques currently exist. Often, biologists look at the stomach contents or feces of an animal, if they cannot observe what it is eating directly. However, these techniques often cannot be used with reptiles because they may not eat often or may have an empty stomach when the contents of their stomach are examined. Many ecologists have begun to use stable isotopes of carbon and nitrogen to determine what an animal has eaten. Stable isotopes are useful because unlike radioactive isotopes, stable isotopes do not decay and thus can be used as a better tracer through different trophic levels. Obtaining stable isotopes can be accomplished by analyzing a small tissue sample from the animal and comparing its carbon and nitrogen stable isotope levels to those of several of its potential prey. Since variation in carbon and nitrogen isotopes exists in all living things and is maintained with increases in trophic level, these chemical signatures can be good indicators of an animal's current diet. Our central question is to test the assumption that there is a direct correlation between the stable isotope signatures found in Side-blotched Lizards (*Uta stansburiana*) and some of their insect prey in the lab, where we could control their diet. We predicted that there would be a direct correlation between the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) levels of the lizards and those of their prey. We found that, although $\delta^{13}C$ and $\delta^{15}N$ values of lizards generally matched their diet, diet complexity did not significantly alter lizard toe tissue stable isotope ratios. This method has the potential to be more effective at determining wild reptile diet than other techniques currently used.

INTRODUCTION

In the last decade, stable isotope analysis has frequently been used to estimate the diets of wild animals and to track energy and nutrients through food webs and trophic levels. Stable isotopes are attractive tools for ecologists because they have the potential to augment existing data sets and allow for direct, quantitative comparisons among multiple species. Two isotopes of the same element have the same number of protons but varying numbers of neutrons. The most common isotope of an element is usually a stable isotope, whereas less common isotopes can be either stable or unstable (radioactive). Stable isotopes differ from unstable isotopes mainly due to the fact that they do not decay into other isotopes or elements. Since they do not decay, they can persist for a long time in the tissues of animals. Ecologists can use this property to measure what an organism eats using the stable isotope composition of its body. Stable isotope ratios do not differ by large amounts, so delta notation is used to make small value differences appear larger for more accurate data analysis. Delta notation for carbon and nitrogen isotope ratios in this paper will be represented by the following symbols respectively: $\delta^{13}C$ (ratio of ^{13}C:^{12}C) and $\delta^{15}N$ (ratio of ^{15}N:^{14}N). Research suggests that stable isotope ratios can help us learn more about the physical and biological processes that produce the varying isotope signatures in living organisms (Gannes et al. 1997).

When ecologists are tracking and capturing animals in the wild to try and identify what they are eating, there are a limited number of ways that they can successfully determine an animal's diet. Current practices include looking at the stomach contents of the animal or analyzing the feces. This can be problematic because the food they have eaten may be unrecognizable or may be incorrectly identified, or the stomach may be empty (Durso and Mullin 2017). Stable isotope analysis is a method that has been used before, but laboratory validations of the technique are

rare (Gannes et al. 1997). Our experiment allowed us to manipulate the diet of captive side-blotched lizards to see if the relationship between what an organism eats and its stable carbon and nitrogen ratios is predictable.

MATERIALS AND METHODS

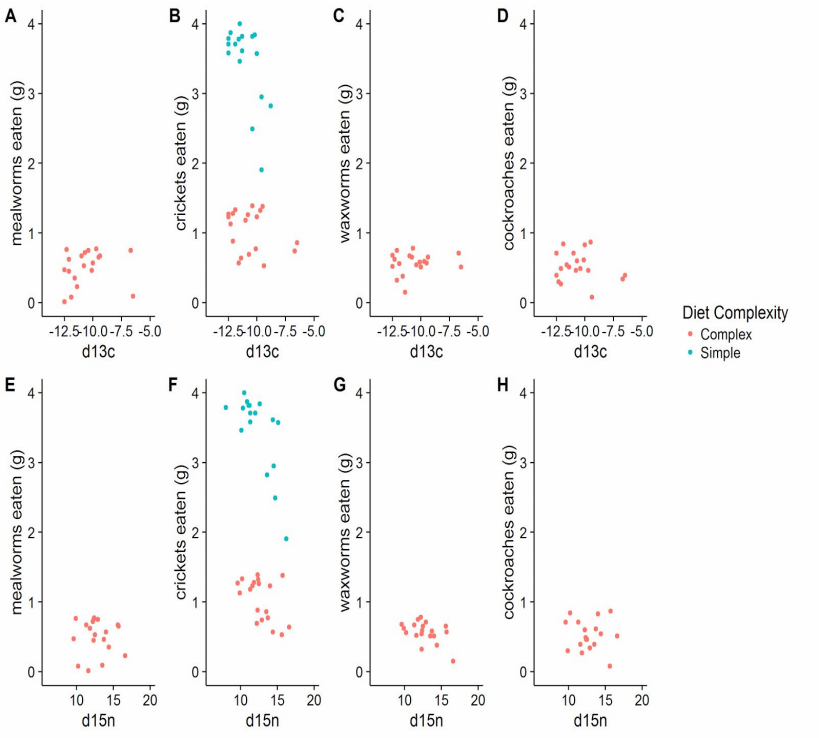
We collected 44 male Side-blotched Lizards (*Uta stansburiana*) from a single site in St. George, Utah in the spring of 2014. This species of lizard is about 50 mm in snout-vent length, weighs about 5 g, and is commonly found in the western regions of the United States (Jones and Lovich, 2009). The lizards were collected under the authority of the Utah State Department of Wildlife Resources protocol COR75 #1COLL8382 and handled in accordance with USU IACUC protocol #2068. Following an 18 day acclimation period, initial measurements were taken and we began phase one of the experiment, in which the lizards were offered between 0.2 and 0.4 g of farm raised crickets (*Acheta domestica*, Fluker's ®, Port Allen, Louisiana, USA) once every other day or every day for eight days. For phase two of the experiment, the lizards were put on varying food treatments and we collected data analyzing how many insects they ate and the total mass of the insects consumed. Half of the lizards were restricted to eating only crickets, and the other half were allowed to eat as many crickets as they wanted *ad libitum* for a total period of 32 days. For phase three of the experiment, each of the two groups were randomly split up again into subgroups and were fed either a simple or complex diet. The simple diet consisted of feeder crickets and the complex diet consisted of a mixture of crickets, cockroaches (*Blaptica dubia*), waxworms (*Galleria mellonella*), and mealworms (*Tenebrio molitor*). The insects used in the study are insects that are similar to insects eaten by side-blotched lizards in the wild.

After the diet complexity testing, tail and toe tissue samples were collected from most of the specimens involved in the study. The samples were separated and labeled and then put in a

drying oven until ready to be analyzed. Later, we weighed out approximately 1.0 mg of each sample and around 1.5 mg of the prey samples and wrapped them in tin capsules. All samples were analyzed by a mass spectrometer at the USU Stable Isotope Laboratory, to measure the relative carbon and nitrogen stable isotope ratios.

RESULTS

After going through the data and comparing the difference between the toe and tail samples, we discovered that there is clearly a difference in the delta values between the three different experiments performed on the lizards when comparing the toe and tail tissue samples. The figure to the right shows the differing delta values compared to what the lizard was eating. We found that the average delta values increased faster with the tail tissue than with the toe tissue supporting the idea that the toe tissue is a good indicator of long-term diet and the tail tissue is a better indicator for short-term diet for the lizard.



Statistical Analysis

Using an ANOVA in R (package car), we compared the carbon and nitrogen values to each other to see their variance. We initially hypothesized that the lizards who were fed the diet consisting of only crickets would have carbon and nitrogen levels that matched the markers for

the crickets. We used a two level explanatory factor for diet complexity, where the levels were simple and complex. For the test of the diet complexity we found that there was not any major significance between the two different diet types ($F_{1,31}$ = 1.0001; p = 0.33).

We then performed a two level explanatory factor for the varying types of food eaten by the lizards and we received similar statistics. We also included a covariate for the lizard mass and it was marginally nonsignificant. There was probably a small effect of the size of the lizard but we did not see it in the data. When we performed the same type of test with the nitrogen values, the data again was very similar. We conclude that no interactions were significant between any of the explanatory variables.

DISCUSSION

Our data initially showed that there was not a strong correlation between the $\delta^{13}C$ and $\delta^{15}N$ values obtained from the lizard tissue samples and the insects. We believe that tail growth was a better indicator of how the lizards' stable carbon and nitrogen values changed because the tail was completely grown after the change in diet whereas the toe samples may not have had a chance to grow quickly enough to be used as an accurate indicator of diet.

A follow-up study needs to be done in order to look at different aspects of the study including the need to see if the turnover rate in this type of lizard is the similar as the one done in Lattanzio and Miles (2016) to verify the assumption that tail tissue is a better indicator than toe tissue.

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