

## Using Stable Isotopes to Study Seasonal Diet and Trophic Niche Variation in a Desert Small Mammal Community

### **Experimental Plan**

We will utilize bulk stable carbon and nitrogen isotope analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of plant tissues, invertebrates (whole-body), and small mammal tissues (red blood cells (RBC) and plasma) and the Bayesian mixing model framework, MixSIAR, to estimate small mammal diet composition across seasons at the Sevilleta National Wildlife Refuge (Stock and Semmens 2013). Additionally, using Stable Isotope Bayesian Ellipses in R (SIBER), we will create standard ellipses of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data for specific small mammal sample sets (e.g. functional groups or species for a particular season) as a proxy for trophic niche width and various community metrics (Jackson et al. 2011). Using these methods, we aim to gain a better understanding of how small mammal diets and trophic niches vary seasonally in a desert ecosystem and how well seasonal diet shifts may or may not reflect the availability of food resources on the landscape (e.g.  $\text{C}_3$  forbs and shrubs or  $\text{C}_4$  grasses).

Our study site is located within the Sevilleta National Wildlife Refuge in the northern Chihuahuan Desert. This is an ideal location to answer our questions because there are two trapping webs already established in a mixed shrubland-grassland ecosystem and relevant data is routinely collected and made available by the Sevilleta Long Term Ecological Research Site. This data includes temperature and precipitation measurements and aboveground net primary production (ANPP) and  $\text{C}_3$  versus  $\text{C}_4$  abundance estimates. Typically,  $\text{C}_3$  plants (e.g. Creosote (*Larrea tridentata*), winter fat (*Krascheninnikovia lanata*) and numerous forb species) have a competitive advantage during the winter, while  $\text{C}_4$  plants (e.g. the gramma grasses (*Bouteloua eriopoda* and *B. gracilis*)) have a competitive advantage during the summer. We collected plant in September 2017. Invertebrate samples will be collected in September and October 2017. Small mammals are live-trapped and tagged and non-lethal samples, including hair and blood, are taken monthly from March through October each year. Samples will be analyzed for their %C, %N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  values using a Costech Elemental Analyzer connected to a Delta V Plus Mass Spectrometer via a Conflo Interface in the University of New Mexico Center for Stable Isotopes (UNM CSI). For our study, we only analyze data from 2017.

We will utilize the Bayesian mixing model framework, MixSIAR, to estimate diet composition of the small mammals. MixSIAR is advantageous over other common Bayesian mixing models for numerous reasons, including its ability to account for small sample sizes and uncertainty in discrimination values and concentration dependence (Stock and Semmens 2013). We will use the median and 95% Bayesian credible intervals to interpret the contributions of specific food sources to a consumer's diet. The SIBER package will allow us to evaluate the isotopic niches (a proxy for trophic niches) of the small mammals by generating standard ellipses in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bivariate plot. We will compare isotopic niches across species and seasons to study trophic niche/food resource partitioning between species and seasonal trophic niche variation within species.

### **Methods**

**Study System.** Our study will be conducted at the Sevilleta National Wildlife Refuge, located in the northern Chihuahuan Desert. We have two trapping webs containing 145 traps each located in a mixed shrubland-grassland transition zone.

**Sample Collection, Chemical Preparation, and Stable Isotope Analysis.** Plant samples were collected from both webs on September 11, 2017. At least five samples of each dominant species were collected at each web. Plant samples will be dried, ground to a fine powder using liquid nitrogen and a mortar and pestle, and weighed into tin capsules for bulk %C, %N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  analysis. Invertebrate samples will be collected using pitfall traps (Laub et al. 2009). We will set one pitfall trap 10 m away from the end of each line on each web (12 pitfall traps per web, 24 total). These traps will be set and checked

September 22 - 24, 2017 and October 20 - 22, 2017. Invertebrates collected will be transported back to the laboratory, where they will be dried, lipid-extracted via a series of sonications with a 2:1 Chloroform-Methanol mixture, rinsed with DDI water five times, dried again, ground to a fine powder using liquid nitrogen and a mortar and pestle, and weighed into tin capsules for bulk %C, %N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  analysis. Small mammals will be live-trapped September 22 - 25, 2017 and October 20 - 23, 2017. We will collect blood samples from each small mammal captured and will separate the plasma (turnover time of approximately two to three weeks) from the RBC (turnover time on the order of months) via centrifugation. Plasma will be pipetted into pre-weighed tin capsules, left to dry, and weighed in its capsules before bulk %C, %N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  analysis. All samples will be analyzed using a Costech Elemental Analyzer connected to a Delta V Plus Mass Spectrometer via a ConFlo Interface in the UNM CSI.

**Seasonal Trophic Niche Comparisons.** The different trophic niches of the small mammal species caught in the webs will be determined using an analysis of their isotope values throughout the different seasons. The  $\delta^{15}\text{N}$  values of the small mammals will be an indication of their trophic level because nitrogen isotopes fractionate significantly between diet to consumer tissue (Post 2002). The  $\delta^{13}\text{C}$  values will be used to trace food chains from the plants and invertebrates to the small mammals (Perkins, 2014). The  $\delta^{13}\text{C}$  values in plants can also be used to trace the seasonal variation in  $\text{C}_3$  and  $\text{C}_4$  plants in relation to small mammal diet. In higher temperatures, usually in the spring and summer seasons,  $\text{C}_4$  abundance increases with the growth of grasses and dicots. In cooler temperatures, around the fall and winter,  $\text{C}_3$  plants dominate the landscape. By analyzing the diet of small mammals using the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of plasma and RBC across seasons, we can determine if the abundance or depletion of certain plants causes a shift in the preferred food source of the small mammals. This can also provide insight into the different food preferences between species or individuals occupying the same niche. We can use SIBER to compare the isotopic niches and thus, trophic niche widths, found in this ecosystem (Jackson et al. 2011). SIBER will use our isotope data to create standard ellipses, taking into account any uncertainties and variances within the samples (Jackson et al. 2011). The standard ellipses act as a proxy for trophic niche width (i.e. larger standard ellipses indicate larger trophic niches).

**MixSIAR and Diet Composition.** We will use the %C, %N,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  values of our plant and invertebrate samples as food sources in our MixSIAR models (Stock and Semmens 2013). We will use plasma and RBC  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values as consumer values across 2017 trapping events to study how diet composition varies over time. Additionally, we will look at the diets of individuals to see how much intraspecies variation there is in diet composition. We will use the median and 95% Bayesian credible intervals to interpret the contributions of specific food sources to a consumer's diet.

## References

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