The listing of the New Mexico meadow jumping mouse (*Zapus hudsonius luteus*) under the U.S. Endangered Species Act ignited a host of multi-agency efforts aimed at habitat recovery[1]. The subspecies occupies and hibernates in richly vegetated riparian zones in the southwestern United States. However, these areas comprise less than one percent of Arizona's ecosystems so they are especially sensitive to livestock grazing and human recreation[2].

Jumping mice are at high risk of extinction in the next decade so knowledge of community-level associations such as diet and microbial interactions can be critical for their conservation. First, knowledge of diet can be used to restore habitat. Foraging behavior was only described for a single site where tracked individuals fed upon the canopies of tall herbaceous plants[3]. While we know they are herbivorous, resource managers in multiple states require taxonomic and regionally-specific diet surveys to protect them. Second, microbiomes are integral to the physiology of animals. In populations, microbiomes reflect host health, tolerance to environmental perturbations, fragmentation, and inbreeding[4]. In this sense, microbiomes can function as fine-scale bioindicators to identify populations of focal conservation.

Sampling Design: As part of a major study effort in summer 2016, we obtained 212 genetic samples (fecal, buccal, dorsal fur) from 50 live-captured jumping mice in two montane regions of the U.S. southwest (eastern Arizona and northern New Mexico). We based our sample collection on previous microbiome studies using trap-collected feces[5]. Next summer we will sample southern New Mexico and Colorado for their southern and northern-most distributions.

Aim I: Identification of herbeal diet: A large-scale genetic survey of jumping mouse feces across sites will identify common and site-specific dietary taxa and reveal any signs of specialization. I suspect that jumping mice consume a variety of plants that permit their canopy foraging behavior. In support of this, preliminary data from metagenomic analysis performed by our lab in 2015 indicated that mice were consuming diverse plant taxa. I recently developed a DNA metabarcode assay that produces faster, cheaper, and higher-throughput data than metagenomics and returns more informative results. This optimizes diet discovery for conservation efforts with limited funding. Instead of whole genome components, the ITS2 plant barcode is targeted in the feces and sequenced in parallel within a high-throughput platform (Illumina MiSeq). Our laboratory actively uses these sequencers to identify bat species and microbiomes from guano. Using the ITS2 as a metabarcode, I identified diet items in the feces of 14 jumping mice captured in 2015 (Figure 1A: two examples). Taxonomies are assigned with a DNA reference library I built from publically available data with the same taxa as site vegetation surveys. This system should allow me to characterize jumping mouse diet in all major distributions within two years.

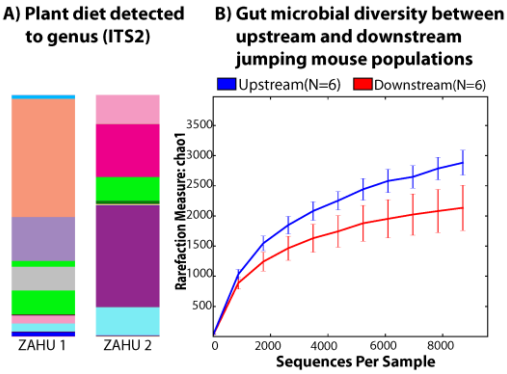

Figure 1. (A) Plant genera detected in the feces of two jumping mice from different trapping sites are depicted by different colors. While diet is diverse for both individuals, they are mostly foraging for varied, site-specific taxa. (B) Jumping mouse populations living downstream from human recreation zones exhibited lower gut microbial diversity than another population 5 km upstream.

Aim II: Microbiome signals: I will use 16sRNA amplicon sequencing on all sample types to identify the resident microflora of jumping mice and their habitat-associated patterns. This will

involve characterization of taxonomic associations, community structure, and diversity using standard quantitative pipelines[6]. Using these approaches, I have already detected lower microbial diversity in populations with interface to zones of human recreation (Figure 1B). Lower gut diversity could result from gastrointestinal illness or be a sign of younger mice downstream; therefore, microbiome signals like these can identify focal habitat for conservation managers. Along these lines, I will explore the relationships of host microbiota to those in water sources near capture areas. This will involve enteric-selective culturing and genotype assays for pathogenic strains of *Escherichia coli* and *Streptococcus faecalis* to detect contamination from recreational and agricultural practices that can cause disease in mouse populations. I operate in a pathogen and microbiome institute that facilitates infectious disease surveillance.

Intellectual Merit: This work introduces a minimally-invasive means to monitor herbeal feeding for a scientifically-challenging species. Genetic approaches allow for a high-throughput, reproducible framework to identify diet far beyond the limits of conventional microscopic and field observation. This knowledge is critical for developing informed and regionally-specific revegetation strategies. The use of ITS2 to identify plant taxa in mixed environmental samples has not been previously explored but shows consistent identification of dietary taxa; other metabarcode approaches require multiple gene targets as well as custom, publically unavailable reference libraries to achieve the same results.

To date, microbiome analysis for meadow jumping mice has not been performed and is seldom applied to imperiled species[4]. This technology has been frequently used to study human health and offers new tools for studying wildlife populations and habitat connectivity. Microbiome signals could potentially be used to identify conservation hotspots.

Broader Impacts: This work will allow me to continue mentoring an undergraduate researcher who began working with me in January 2016. She is learning current techniques in molecular ecology and has already presented a poster on jumping mouse diet in a research symposium. I will share results with federal, state, private and tribal agencies (White Mountain Apache and Southern Ute) through management meetings and quarterly reports. I will also present at national and international conferences. Public outreach for the work will be disseminated through local news outlets such as KNAU (NPR), NAU-TV and AZ Game and Fish Department, and will be shared through our team's existing social media platform.

References

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