New Mexico meadow jumping mouse Project Report for 2018-2021

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Executive Summary

1. Between 2018 and 2020, we captured 108 New Mexico meadow jumping mice (*Zapus hudsonius luteus*) at 25 sites in Arizona (Apache-Sitgreaves National Forest, Arizona Game & Fish Department lands), Colorado (tribal and Bureau of Reclamation lands managed by Colorado Parks & Wildlife), and New Mexico (Santa Fe National Forest).
2. We identified home ranges for 70 jumping mice (29 females, 41 males) in Arizona, Colorado, and New Mexico from 2017, 2018, 2019, and 2020. Animals were tracked between June and October. Home ranges calculated using Minimum Convex Polygon (MCP) and 95% Kernel Probability (KD) for 70 jumping mice averaged 3.02 ± 0.46 ha and 2.80 ± 0.64 ha, respectively. Home ranges for males (MCP = 3.70 ± 0.71 ha, KD = 3.79 ± 1.04 ha) were larger than females (MCP = 2.06 ± 0.45 ha, KD = 1.40 ± 0.29 ha). Animals moved an average of 11 ± 2 m from streams and maximum distance from stream averaged 41 ± 5 m. The furthest distance we located a jumping mouse from stream was 169 m. Mean and maximum distance moved from last location averaged 62 ± 5 m and 273 ± 27 m, respectively. Average distance from centroid was 85 ± 10 m.
3. Home ranges were larger in June and July (post emergence) for males than females. Female home ranges were of consistent size through the active period. Home ranges were similar for both sexes in August and September. Home ranges for male jumping mice (n = 41) calculated using 95% Kernel Probability (KD) averaged 8.0 ± 1.4 ha during the first half of the summer (days 165 [14 Jun] – 199 [18 Jul]) and 1.1 ± 0.16 ha during the second half of the active season (days 200 [19 Jul] – 285 [11 Oct]). Female home ranges during the first (days 175 [23 Jun] – 199 [18 Jul]) and second (days 200 [19 Jul] – 285 [11 Oct]) of the summer using 95% Kernel Probability (KD) averaged 1.4 + 0.2 ha and 1.4 + 0.2 ha, respectively.
4. We confirmed 3 hibernacula for 3 individuals (1 female in Arizona and 2 male jumping mice [1 in Arizona, 1 in Colorado]). These hibernacula ranged from 3 to 100 m (median = 5.5; mean ± SE: 24 ± 19) from streams and were underground. Animals entered hibernation between 23 Aug and 5 Oct. In 2019, one female (believed to be a subadult) had not entered hibernation by 16 Oct when we removed her collar.
5. In June 2020, we excavated the hibernaculum we located in 2019 for the female on the Apache-Sitgreaves National Forests and discovered it was shallow with a V-shaped entrance that was plugged during hibernation. The entrance led to a chamber ~15 cm (6 in) below ground. A bolus

nest composed of dried vegetation was in the chamber. Depth from ground level to top of the hibernation chamber was 6.5 cm (2.6 in). The female that used this hibernaculum was recaptured 1 July 2020 and her radio collar removed, indicating she overwintered successfully in this hibernaculum. In June 2021, we excavated the male hibernaculum in Arizona; it was ~33 cm (13 in) below ground and contained a bolus nest. Work on hibernacula is being continued under a new CEAP agreement by Jennifer Zahratka, PhD student. To date, she has confirmed 10 hibernacula.

6. We demonstrated feasibility of using environmental DNA (eDNA) isolated from vegetation to detect the jumping mouse by developing a species-specific assay for quantitative real-time PCR, then testing the long-term (e.g., 4 months) persistence of jumping mouse eDNA on plant material. We conducted a field trial at 6 locations along 2 occupied streams to evaluate our assay's capability to detect present-day eDNA. We also opportunistically swabbed plants following visual observation of jumping mice. We determined that fewer than eight copies of target DNA within a sample were needed for a positive detection at 95% confidence. From field trial samples, we successfully detected the species from randomly swabbed vegetation and from vegetation recently used by individuals. Our study demonstrated that mammalian eDNA can persist on vegetation long after the animal was present, highlighting the promise of using eDNA from plants to detect this species.
7. We used DNA metabarcoding to identify dietary taxonomies of the known food types for New Mexico meadow jumping mice. We found a varied diet most frequently of graminoids, forbs, and lepidoptera. Detecting up to 9 dietary taxa in individuals, jumping mice consumed more and different combinations of plant taxa as they approached the known hibernation window. This emphasizes the importance of forbs and graminoids as the foundation of the jumping mouse diet and as habitat for insect prey. The breadth of different diet items they consume suggests plasticity in resource use, potentially accommodating diverse patterns of seed production through their active period. A peak in seed availability in late summer, just prior to hibernation, could play a role in their accumulation of pre-hibernation fat stores.
8. Using microsatellite (nuDNA) and mitochondrial (mtDNA), we examined the hypothesis of hydrologically mediated gene flow (via riparian networks) by assessing structure, diversity, and the possibility of overland dispersal among disconnected watersheds in the White Mountains, Arizona. Both a priori and de novo structure supported stream-mediated gene flow. However, inferred clusters crossed watershed boundaries, and migration rates and dispersers suggest infrequent use of overland paths at nearby sub-drainages among each watershed. Analysis of mtDNA indicated that this was likely a long-term phenomenon, also suggesting that one of the watersheds (Little Colorado River) may have been more historically diverse than its contemporary snapshot (using nuDNA). Our analysis led us to suspect fragmentation in its eastern fork, an area most impacted by human development. We conclude that the boundaries of drainages affect the White Mountains population structure but short, unknown, overland paths among them likely play a role in replenishing genetic diversity.
9. For outreach, created a video describing results of telemetry work, gave 10 presentations on research results at meetings for resource managers and scientists, and are preparing manuscripts from our work. Peer-reviewed publications provide a sound scientific basis for management decisions. The manuscript on the track plating method was published in the Wildlife Society Bulletin (Harrow et al. 2018). Two other manuscripts are submitted and currently in review. We anticipate ≥ 6 additional publications from this project. We have drafted a Wildlife Habitat Evaluation Guide, that will be completed with newly generated data from the geomorphology study completed in 2021.

10. We acquired additional funding from Fish and Wildlife Service, Forest Service, and Arizona Game and Fish Department to survey for and examine diet of New Mexico meadow jumping mice.

Justification

The New Mexico meadow jumping mouse (*Zapus hudsonius luteus*) is considered a riparian obligate that uses tall, dense herbaceous vegetation along perennial flowing water such as streams, ditches, and wet meadows. Although jumping mice are found in riparian areas with moist soils, they also use adjacent dry upland areas beyond the floodplain to nest, bear and raise young, and hibernate. Jumping mice need high quality food sources prior to hibernation to accumulate fat reserves; seeds are thought to provide these reserves. The diet of the jumping mouse is not clearly defined but observations from other subspecies and from a small sample in New Mexico indicated they may shift from a dominance on insects shortly after emergence from hibernation in spring to seeds just before entering hibernation in fall. Surveys identified 8 geographic areas in Arizona, New Mexico, and Colorado where jumping mice occurred, but some areas remain to be surveyed. Livestock grazing is thought to affect jumping mouse habitat. To develop recommendations for grazing (timing, frequency) and allow continuation of livestock grazing while promoting habitat for the jumping mouse, this study identifies home range sizes and habitat requirements, hibernacula and their proximity to water, diet, and develops an environmental DNA (eDNA) test to detect the species.

Objectives

1. Conduct field surveys for jumping mice to continue to survey for and identify areas of suitable habitat for the endangered New Mexico jumping mouse.
2. Generate information on home range and habitat use, including hibernacula.
3. Develop an environmental DNA (eDNA) test to non-invasively identify presence of this species.
4. Identify diet through genetic analyses.

Overview

2018

Our research focus for 2018 was on identifying home ranges and movements for the New Mexico meadow jumping mouse across the range of the species (Arizona, Colorado, New Mexico). We collected fecal samples for dietary analysis and live-trapped or track plated at new locations (Gila National Forest) considered potential habitat. Because we wanted to sample areas known to support good jumping mouse populations (to increase capture probability for radio telemetry), we captured animals along streams that had been previously sampled on the Apache-Sitgreaves (AZ) and Santa Fe National Forests (NM).

For field work conducted from June to September 2018, we hired a crew leader and 3 technicians to capture small mammals. We trained field crew personnel in small mammal capture, identification, and radio telemetry techniques. Data for track plating, trapping, and telemetry were entered and summarized.

The manuscript on the track plating procedure that we developed was published in the Wildlife Society Bulletin in 2018. On 6-7 March, we presented our research results to the New Mexico Meadow Jumping Mouse (NMMJM) Science Update Meeting in Durango, Colorado. Our presentations included:

1. Vegetation structure and composition in Occupied and Non-Occupied NMMJM sites
2. Home Ranges, Nest Structures, and Vegetative Composition within Home Ranges of NMMJM
3. Diet determination using Metagenomics/Stable Isotopes and Metabarcoding
4. Non-Invasive Track Plating Detection Method for NMMJM
5. NMMJM occupancy model for the Apache-Sitgreaves National Forests

2019

During 2019, we continued to focus on radio telemetry to increase number of home ranges identified for New Mexico meadow jumping mice across the range of the species in Arizona, Colorado, and New Mexico, determine whether we could track animals into hibernation and identify hibernacula, further define diet, collect fecal samples for analysis of gene flow, and test an eDNA method for non-invasive monitoring. We also resurveyed areas on the Gila National Forest (New Mexico) using track plating to try and detect the jumping mouse.

Using fecal samples collected during 2019 and previous field seasons, Dan Sanchez analyzed diet of 132 unique jumping mice for presence of insects. We detected the presence of insect DNA in 89% of samples genetically confirmed to be from jumping mice. Taxa belonged to 11 orders and potentially 71 families. We detected Lepidoptera in 68% of the individuals; we also detected Hemiptera (47%), Diptera (39%), Coleoptera (18%), and Hymenoptera (14%). The most common Lepidopteran family was Noctuidae (moths). Aphididae (aphids) was common for Hemipterans and Braconidae and Ichneumonidae were common among Hymenoptera. These insects are parasitoid wasps that feed on aphids and other insects. Insect consumption appeared common, particularly for insects that are associated with above-ground plant biomass (e.g., stem, leaf, seed). This could suggest either opportunistic or accidental insectivory while foraging on plants. Insects did not appear to be a dominant food source.

Dan also used fecal samples to test for genetic isolation of jumping mouse populations in 3 disconnected watersheds on the Apache-Sitgreaves National Forests (Black, Little Colorado, San Francisco Rivers) and found moderate to subtle genetic differentiation among individuals occurring in different watersheds, suggesting animals move within but not between watersheds.

We also developed a highly sensitive species-specific, non-invasive environmental DNA (eDNA) assay to detect DNA of New Mexico meadow jumping mouse. We sampled eDNA from 3 sources: abandoned day nests (n = 4), plants (n = 69), and water (n = 6). We identified DNA of jumping mice in 3 of 4 nests, 4 of 69 plant swabs, and none of the water samples. The plant swab method held promise, and we want to further test this method.

We hired a crew leader and field crew (4 technicians) to capture small mammals. Field crew personnel were trained in small mammal capture, identification, and radio telemetry. We surveyed for jumping mice and collected home range data from June to October 2019, following 2 animals into hibernation. All data were entered and summarized.

We created a 9-minute video describing home range sizes for the jumping mouse ("A Space in Time: Movements and Home Ranges of the New Mexico Meadow Jumping Mouse" [https://www.youtube.com/watch?v=V62PNsP7WXk]). On 14 and 19 November, we presented results from our field season to the Fish & Wildlife Service and Forest Service biologists meeting, respectively, in Albuquerque.

2020

Despite the onset of the COVID-19 pandemic, we were able to conduct field work in 2020. The focus of our fieldwork was to increase number of home ranges identified for New Mexico meadow jumping mice in June (just after immergence), track animals into hibernation and describe hibernacula, collect fecal samples to start a project that will examine fecal cortisol metabolites, and measure stream geomorphological characteristics for streams that were occupied vs unoccupied by jumping mice.

Two graduate students working on jumping mouse projects (Jennifer Zahratka, Charlotte Rozanski) and José Martínez-Fonseca served as crew leads for these projects and I hired 4 technicians. Dan Sanchez continued to work on his analysis of diet and population genetics of jumping mice. We submitted 2 papers for publication (eDNA method, report of predation of a jumping mouse by a western terrestrial garter snake); one (garter snake predation event) was published.

Methods

Study Area

From 2018 to 2020, we live-trapped or track plated 44 sites across the range of the jumping mouse (Arizona, Colorado, New Mexico) between 7 June and 24 October. We used radio telemetry for home range estimation and to track animals into hibernation. We used track plating to identify presence of jumping mice (Table 1). Some information (home range data, hibernacula) from this work conducted in Colorado on the Southern Ute Indian Reservation (SUIR, in collaboration with the Tribe), is also included in this report.

We surveyed along perennial streams that included aquatic vegetation near the stream, mesic meadows, dry meadows, ponderosa pine (*Pinus ponderosa*) and mixed conifer forest. Riparian areas were typically dominated by sedges (*Carex* spp.), rushes (*Juncus* spp.), grasses (*Poa* spp.), and forbs (Patton and Judd 1970). Ponderosa pine with Gambel oak (*Quercus gambelii*), alligator juniper (*Juniperus deppeana*), and New Mexico locust (*Robinia neomexicana*) dominated at elevations closer to 2,000 m. Higher elevation areas included white fir (*Abies concolor*) and Douglas-fir (*Pseudotsuga menziesii*) with scattered spruce (*Picea* spp.).

Trapping, Home Ranges, and Telemetry

We set 80 to 240 Sherman traps (LNA 7.6 x 8.9 x 22.9 cm) per night at each site for 1 to 5 nights (120 to 1200 trap nights per site) to capture jumping mice. When necessary (i.e., high capture rates), we limited data collection of non-target species to reduce risk to jumping mice and collected only genus for some taxa (e.g., *Microtus*, *Sorex*). Traps were set along a riparian transect at each site, alternating traps to target habitat on both sides of streams. When we captured jumping mice for radio collaring, we limited handling stress by taking only sex, reproductive condition, and mass of animals. If we did not radio collar a jumping mouse, we collected morphological data. For those jumping mice that were radio collared, we used 0.47 g BD-2XC or BD-2XCT (temperature sensitive for determining hibernation) transmitters with titanium antennae (Holohil Systems Ltd, Ontario, Canada) fitted with a 23 to 27 mm TYGON sleeve to cover the antenna wire and prevent abrasion to the neck of the animal. We wrapped the antenna twice through the collar and crimped the collar around the animal's neck. Total mass of the BD-2XC collar including sleeve and crimp was ~0.6 g. To minimize effects to the animal we limited mass of the collar to <5% of its body mass (i.e., a 0.6 g collar should be placed on an animal >12 g); we only collared animals ≥15 g. In 2020, we also inserted a Passive Integrated Transponder (PIT) tag (Biomark, Boise, ID) into the interscapular region of the dorsum of each jumping mouse.

Temperature sensitive transmitters indicate the skin temperature of the animal through pulse interval changes. Technicians record the number of pulse intervals during a given period. This pulse interval count is plotted on a graph supplied with each temperature sensitive transmitter for conversion to a temperature. These transmitters can identify changes in skin temperature that may indicate hibernation or mortality.

For home ranges, we collected ≤2 observations per day and ≥3 observations per night per animal. We approached each animal to ≤10 m, using red light and minimizing talking to avoid disturbing the animal. At each location we recorded GPS location, observations about the habitat (vegetation, distance to stream), and, if we saw the animal, observations about its actions. We separated locations by ≥1 hour and collected data throughout the night (dusk to dawn). Our goal was to acquire ≥30 locations over ≥7 days for each individual. When collecting day locations, we attempted to locate and describe nest structure, if it was observed, on each day following capture and collaring. Once we met our goals at the end of the tracking session, we attempted to recapture animals using a butterfly net at their day nest to remove the collar. If we successfully recaptured the jumping mouse, we removed the collar and weighed and inspected the animal for injury or abrasions from the collar before releasing it at

its capture site. To track jumping mice into hibernation, we also captured animals at day nests, but then fit them with new collars while they were still active to continue tracking.

In 2020, to compare home ranges of montane voles (*Microtus montanus*), a potential habitat competitor and dominant species in riparian zones, with jumping mice, we radio collared 4 individuals. We used the same tracking procedures as for jumping mice, except we distributed the collection of observations more equally throughout the 24-hour period each day, since voles are active day and night.

We used ArcGIS to map data for home range analysis. We calculated Minimum Convex Polygon (MCP) and Kernel Density Probabilities (KD; 95%) for each animal. We calculated average and maximum distance between successive nocturnal locations for each jumping mouse. We also calculated maximum distance from the centroid by finding the centroid of all the locations for an animal and calculating the distance from that centroid. These measurements might indicate how far a jumping mouse was willing to travel.

We mapped stream locations using National Hydrology Dataset (NHD) Flowline data, the best general stream dataset available to us. We manually revised the flowlines when we knew an error existed. We calculated the distance from stream as the distance, in meters, to the closest point on the nearest flowline. We calculated maximum distance from flowline to determine furthest distance each animal moved from streams during our surveillance period as well as average distance and standard deviation of average distance moved from streams based on all locations.

Track Plating

We used track plates to survey for jumping mice on the Gila National Forest (Harrow et al. 2018). Track plates (80 per site) were placed 3 to 5 m apart along riparian areas, alternating placement of traps so both sides of the stream were surveyed (same method as with Sherman live traps). We placed bait along the top or edge of the felt pad of the enclosure to attract rodents to the boxes. Track plates were checked for 2 to 4 days and scored for presence or absence of jumping mice tracks by 2 independent reviewers. The identification of ≥1 confirmed track at a site by both reviewers indicated presence of jumping mice.

Hibernacula

In 2019 and 2020, we captured New Mexico meadow jumping mice at 2 sites on the Apache-Sitgreaves National Forests. In August and September 2019, we sampled within an elk and cattle (2.5 m tall) exclosure on the San Francisco River (33.863026°, -109.176607°), and in August 2020, we sampled the East Fork Little Colorado River by Gabaldon Horse Camp (33.930351°, -109.489153°).

We identified dates and times when animals appeared to enter hibernation. We suspected animals were hibernating when the transmitter signal was underground, and the animal remained in same location for >3 successive fixes during a 24-hr period late in the active season. We continued to monitor animals 1 to 4 times per day and concluded animals were in hibernation after documenting no movement from the transmitter signal for >7 consecutive days.

We confirmed a 2019 hibernaculum on the Apache-Sitgreaves National Forests by excavating it after emergence of the jumping mouse in 2020 and with approval of USFWS and US Forest Service. Beginning 2020, we used 2 techniques to confirm hibernacula: 1) use of PIT tags coupled with use of a HPR Plus Reader with a BP Plus Portable Antenna (Biomark, Boise, ID) with increased readability underground and 2) use of temperature-sensitive radio collars to monitor skin temperature of underground animals.

We also buried data loggers (Onset Computer Corporation, Bourne, MA) to measure soil temperatures at 3 depths (10, 30, and 50 cm) placed 3 m from each of the 2020 hibernacula along a random bearing. We buried data loggers to measure soil and ambient air temperatures, as well as light intensity at a randomly located reference site in 2020 at each of the two hibernation sites – the Florida

River in Colorado and the East Fork of the Little Colorado River in Arizona. Data loggers were retrieved and downloaded in 2021 once animals had emerged from hibernation.

We measured characteristics of the hibernaculum (depth, dimensions). At each confirmed hibernaculum we also collected aspect (downslope cardinal direction with a calibrated compass), slope (rise/run or elevation change as 0-360 degrees), horizontal cover (% of vegetation causing a visual obstruction) measured with a Robel pole in all four cardinal directions from the hibernaculum, and canopy cover (%) measured with a densiometer in all four cardinal directions from the hibernaculum.

Diet Analysis

We live-captured 187 New Mexico meadow jumping mice at 31 sites in the summers of 2016, 2017, 2018, 2019, and 2020. We collected fecal samples from live traps after release of captured jumping mice. Our goal was to produce metabarcoding data that targeted plants, arthropods, and fungi as potential diet items. We also generated metabarcoding data for rodents to verify the species of a sample and its purity. We prepared amplicon libraries from feces, bait, mock communities, and non-template controls (NTCs) in two PCR steps. In the first PCR step, we prepared multiplex cocktails that included two plant markers (ITS2, rbcL), an arthropod marker (COI), a fungal marker (ITS2), and a rodent marker (cyt B). We implemented the multiplex PCR to lower the cost and time of library preparation. This was also to make the process more amenable for regular, expedited updates on the diet and monitoring efforts in the future. We implemented a suite of filtering procedures to avoid field contaminants, sequencing error, non-target taxa, and features found in bait or NTCs. For each marker, we only retained a feature if it was observed at least twice in the dataset (i.e., singleton removal). We also performed relative abundance filtering, such that we discarded features within a sample if it was represented in less than 1.1% of a sample's reads. This was to avoid variants that resulted from field contamination. We used R package vegan (Dixon 2003) to evaluate compositional variation among months. We calculated Jaccard dissimilarity for a binary (presence-absence) table and Bray-Curtis dissimilarity for a read abundance table. We first tested for homogeneity among group dispersions using the betadisp function. We then conducted a PERMANOVA using the adonis function with 999 permutations. We ran post-hoc tests using the EcolUtils package (Salazar 2021) with sequential Bonferroni corrections of the P-values. For graphical summary, we reduced dimensionality with non-metric multi-dimensional scaling (NMDS) using the metaMDS function. We ran the NMDS from two to five dimensions to evaluate stress. We chose an NMDS configuration that resulted in stress lower than 0.2 but with no more than three dimensions. We rotated the points in ordination space by month in the first two axes. To investigate sex differences in diet and whether arthropod consumption was temporally correlated, vectors for the sex of an animal and presence of an arthropod group were fit along the month-rotated axes. Significance of correlation was tested against 999 permutations. A goodness of fit test was also conducted among the month groupings in ordination space.

For more details of sample collection and analysis see Sanchez (2021).

Population Genetics

We captured 147 jumping mice from June-September for six years (2015-2020) at 22 sites. We define a site as any 250 to 350 m linear tract spanning a stream bank. We collected feces of jumping mice from traps using tweezers, which were bleach-wiped and flame-sterilized before and after collection. We subsampled 6 to 10 fecal pellets into a single vial containing 0.5 mL sterile RNAlater (Ambion, Austin, TX, USA). On most occasions, we filled 2 to 6 vials per individual, depending on the amount of fecal deposition. Vials were frozen at -80°C within 168 hours, depending on accessibility from the field site to the laboratory. In addition to feces collected in the field, we were loaned 19 historical specimens from the Museum of Southwestern Biology. Most (n = 17) of the specimen DNA was already extracted and arrived in 2 ng/uL concentrations. These specimens were originally collected in 1979 (n =

13) and 1991 (n = 4) from 3 sites on the Black River (BR) and a single locality in the Little Colorado River (LCR; n = 2). Collected in 1963, the LCR tissues arrived as small excisions of skin and pelage from the midline.

DNA was extracted using different protocols for feces, contemporary tissue, and historical skin clips. For fecal samples, we removed RNAlater from each vial and extracted genomic DNA using the QiaAmp Fast Stool Mini Kit (Qiagen, Valencia, CA), following the human DNA analysis protocol. We used microsatellite markers (9 loci).

We pre-defined populations based on watershed membership to examine a priori genetic diversity and structure. We tested for variation within, between, and among watersheds in an analysis of molecular variance. We evaluated pairwise differentiation based on two differentiation statistics: FST and its significance using FSTAT 2.9.4 at 999 permutations (Goudet 2003) and G'ST (Hedrick 2005) using mmod R package (Winter 2012). To visualize the degree of differentiation among the assumed populations, we conducted a discriminant analysis of principal components (DAPC) in adegenet (Jombart et al. 2010). For the final model, we selected the minimum number of PCs where the alpha score is maximized. To explore change through space and time, we also conducted a variation of the DAPC analysis in which the historical samples (1963-1991) were partitioned from the contemporary watersheds (time-and-space variation). These historic samples overwhelmingly included specimens from the northwestern drainages of the BR watershed. R analyses were conducted in R 4.0.3 (R Core Team 2020). We used program STRUCTURE (Pritchard et al. 2000) to identify population clusters under Hardy-Weinberg equilibrium and genotypic disequilibrium. We investigated rates of contemporary gene flow and assignment of dispersers among the three watersheds. Assignment tests examine the probability that each individual was born in the population in which it was sampled. For these analyses we only considered individuals that were captured between 2015 and 2020.

For more details of sample collection and analysis see Sanchez (2021).

eDNA

We compiled cytB sequence data from NCBI GenBank for New Mexico meadow jumping mice and 43 non-target rodent and mammal species. Non-target rodent sequences were compiled from expert opinion and include co-occurring small mammals that are not range-specific to New Mexico meadow jumping mice. Sequences for distantly related mammal species were selected to represent potential but unlikely sources of contamination from environmental samples in New Mexico meadow jumping mouse habitat. We aligned target sequences for New Mexico meadow jumping mouse (n = 3) and non-target species sequences (n = 97) in MEGA7 (Kumar et al. 2016) and trimmed to the length of the target sequences. We used PrimerProspector (Walters et al. 2011) to develop de novo, species-specific primers for the New Mexico meadow jumping mouse that excluded non-target sequences.

We tested amplification performance of each candidate primer set using DNA isolated from liver and fecal samples of a jumping mouse museum specimen (MSB:Mamm:324198). In this panel, we included four trap fecal samples from New Mexico meadow jumping mice, one tissue sample from western jumping mouse *(Zapus princeps)*, and fecal samples from four non-target species that commonly co-occurred (*Peromyscus*, *Microtus*, *Sorex*, and *Neotoma*). DNA was extracted from samples using modified Qiagen protocols (Walker et al. 2016). We visualized PCR products on 2% agarose gels. Included on each PCR plate was a negative template control consisting of master mix with PCR grade water substituted for template. We narrowed assays based on the observation of specific bands and band intensity on agarose gels.

We developed a two-step criterion to distinguish false positive amplifications from potential true positives. This criterion was used to ensure that true positive detections were validated and any false positive amplifications were properly interpreted (Edmunds and Burrows 2020).

We used occupancy modeling to generate occupancy information and further evaluate potential false negative and false positive errors from sample collection in the field or from laboratory tests. We applied a Bayesian framework, developed by Griffin et al. (2020) and executed in an Rshiny app (Diana et al. 2021, Griffin et al. 2020), in which the probability of presence or absence of the species is set randomly as a covariate by the model. This enables determination of the probability at a site of: species presence (ψ), a sample from an occupied site containing the target species' DNA (θ_{11}), a sample from an unoccupied site containing the target species' DNA (θ_{10}), a qPCR replicate of a sample that contains DNA of the target species is positive (p_{11}), and a qPCR replicate of a sample that does not contain DNA of the target species is positive (p_{10}). False negative probabilities in the field and lab are 1- θ_{11} and 1- p_{11}, respectively. We used default settings of posterior means for all model parameters, as suggested by the authors (Diana et al. 2021).

In fall 2017, we collected 4 nests of New Mexico meadow jumping mice 3 to 6 months after radio telemetry identified these as actively used during summer. Because jumping mice clipped vegetation, constructed the nest, then spent up to 15 hrs/day (dawn to twilight) in the nest, they may leave substantial amounts of DNA behind. Day nests were in cooler locations and protected from full sun much of the day, so DNA may not degrade as quickly, particularly since the day nests were at high elevation (2100 to 2400 m). Following collection, nests were stored in individual dry containers at 20°C. In December 2017, we collected 5 to 8 subsamples from distinct regions of each nest (interior, exterior, and sides). The subsamples (1 cm^3) were stored in 2 mL centrifuge tubes and kept at -20°C until they were extracted using the Qiagen DNeasy Plant Mini Kit in March of 2018 to test for vegetation composition (100 µL elutions). We then tested these DNA samples with our short fragment qPCR assay in triplicate using the reaction and cycling conditions listed above.

Just prior to our eDNA sample collection, we captured 15 New Mexico meadow jumping mice at 2 sites on the Santa Fe National Forest in New Mexico, during a 2-week trapping period in June 2019 (1204 trap nights [TN] where 1 TN = 1 trap open for 1 night). We swabbed herbaceous vegetation in areas used by jumping mice as determined by radio telemetry and track plating. We used the stream as a transect and swabbed vegetation at 6 locations every 20 m along the transect, for a total of 100 m. At each location we used 5 sterile cotton swabs (n = 60 swabs). With each swab we sampled a 0.50 m^2 area which included vegetation such as forbs, grasses, sedges, rushes, moss, and live and dead woody vegetation. An individual plant was swabbed along stem, leaf, and flower or seed regions. We sampled vegetation that represent common diet items for the New Mexico meadow jumping mouse (Wright and Frey 2014, Sanchez 2021), and swabbed regions of the plant an animal could have contacted, such as when moving through the stem region for cover or resting on upper leaves and flowers to consume seeds. Each swab was placed in sterile RNAlater solution to affix and preserve potential eDNA (Ambion, Austin, TX, USA). We also opportunistically swabbed 9 locations immediately following the visual observation of a radio-collared jumping mouse (e.g., abandoned day nests and grasses or forbs where animals were visually observed). DNA was extracted from the swabs using the Qiagen DNeasy Blood & Tissue Kit following manufacturer protocols for the 96-well setup (100 µL elutions) and each sample was analyzed in quadruplicate according to the qPCR conditions above.

Results

Trapping and Home Ranges

In Arizona and New Mexico, we captured 2384 animals during 11,306 trap nights from 2017 to 2020. Deer mice (*Peromyscus maniculatus*) and voles (*Microtus* spp.) accounted for most captures; jumping mice accounted for 8.4% of captures (Table 2).

Across all years (2017-2020) and all states within the range of the jumping mouse (Arizona, Colorado, New Mexico), home ranges (n = 70) averaged 3.0 ± 0.5 ha for MCP and 2.8 ± 0.6 ha for KD (Table 3). However, we noted differences in home ranges for males versus females. Male home ranges

(MCP = 3.70 ± 0.71 ha, KD = 3.79 ± 1.04 ha) were approximately twice as large as those for females (MCP = 2.06 ± 0.45 ha, KD = 1.40 ± 0.29 ha). Home ranges for males were larger and more variable in June and July (14 Jun – 19 Jul [days 165-200]: MCP = 6.2 ± 0.9 ha, KD = 8.0 ± 1.4 ha) than August through October (20 Jul – 11 Oct [days 201-284]: MCP = 2.1 ± 0.4 ha, KD = 1.1 ± 0.2 ha) (Figure 1). Home ranges for females were less variable throughout the active period (24 Jun – 19 Jul [days 175-200]: MCP = 1.9 ± 0.4 ha, KD = 1.4 ± 0.2 ha; 20 Jul – 3 Oct [days 201-277]: MCP = 2.2 ± 0.5 ha, KD = 1.4 ± 0.3 ha) (Figure 1). Males used larger home ranges between emergence in late May or early June and 19 July (Day 200). Their home ranges were much smaller after 19 July until immergence (Figure 2).

During the 4-year study period on the ASNF, we documented predation of jumping mice by long-tailed weasels (*Mustela frenata*; n = 2 documented events), terrestrial garter snakes (*Thamnophis elegans vagrans*; n = 2 events and another event in Colorado [Zahratka et al. 2020]), and an unknown owl (n = 1 event). We suspect other causes of predation include feral horses and domestic cats.

Track Plating

We did not detect jumping mice at any sites on the Gila National Forest (GNF, Table 1). At 3 sites we detected no tracks of any rodent. Because we had no detections of jumping mice on the GNF, we did not live-trap, sample eDNA, vegetation, or diet. Flooding at 4 sites during the monsoon season (San Francisco River in 2018, Dry Blue sites in 2019) interrupted our ability to check track plates during the session.

Hibernacula

2019 Hibernacula - Arizona

In 2019, we radio collared 2 jumping mice on the San Francisco River and followed jumping mice into hibernation. We tracked a female and male from September to mid-October. Animals entered hibernation in early October (Table 4). They remained in the same location until collars failed on 16 Oct. We identified 2 likely hibernacula. Both animals increased mass during the tracking period (Table 4).

We radio collared a female (frequency 150.380, 150.299) on 15 Sep and monitored her until 16 Oct. During the night of 30 Sep – 1 Oct, she appeared to select a hibernaculum. She was active at 21:47 on 30 Sep but had located to the site we later identified as her hibernacula by 00:22 on 1 Oct (WGS84 12S 667777, 3748330). She did not move from this site during the remaining period that we monitored her collar. She selected a stream bank outside the San Francisco River exclosure, ~5.5 m from the stream (Table 4, Figure 3). The jumping mouse constructed a nest of grasses and Gambel oak (*Quercus gambelii*) leaves within ~10.5 cm diameter oblong cavity. Depth from ground level to top of the nest material in the hibernaculum chamber and to bottom of the nest was 6.5 cm and 15 cm, respectively. The distance from entrance to the underground nest was 21 cm (from duff to nest). The tunnel appeared to be constructed along an old root and had a sharp, v-shaped bend. The chamber was topped on one side by a tree root with the space below forming a small cavity (Figure 4).

We radio collared a male (frequency 148.787, 150. 059) on 8 Sep on the San Francisco River and continued to monitor him until 16 Oct. We identified his hibernacula on 5 October along a stream bank within the San Francisco River exclosure, ~3 m from the stream (17:06, WGS84 12S 668525, 3748534; Table 4). He did not move from this site during the remaining period that we monitored his collar. We were unable to locate and confirm his location in 2020.

2020 Hibernacula - Arizona

We radio collared a male (frequency 151.619) with a temperature-sensitive transmitter on 23 Aug on the East Fork Little Colorado River and monitored him until 10 Sep. He was located underground on 27 Aug (08:32, WGS84 12S 0639604, 3755227) and did not move from this site during the remaining period that we monitored him. Based on preliminary analysis of the data, arousal bouts, which typically

interrupt hibernation (i.e., extended torpor periods) occurred several times during our monitoring. He used a site outside exclosures, in grasses, 100 m from the stream (Table 4, Figure 5).

2020 Hibernaculum - Colorado

We radio collared a male (frequency 148.689, 150.306) on 31 Aug on the Florida River and monitored him until 10 Oct. He was located in an underground site on 28 Sep and did not move from this site during the remaining period that we monitored him. He selected a site 8 m from the stream (Table 4, Figure 6).

Movements and Designated Critical Habitat

We noticed no clear pattern among hibernacula in relation to vegetative cover or previously used locations used by jumping mice. Although 2 of 3 hibernacula were <10 m from streams, one was almost 100 m from stream, on the border of designated critical habitat (Figures 7-10).

Diet Analysis

Using 4 dietary markers, we surveyed dietary breadth and seasonal diversity from the feces of 161 sequenced individuals. Among 5 sequencing runs, we generated 48,258,691 raw paired-end reads prior to filtering. Following marker separation, denoising, and filtering, we retained 18,418,346 (51%) of the overall reads for analysis of taxonomy and diversity. After filtering, we verified that 95.7% of the individuals were from New Mexico meadow jumping mouse and were not contaminated or mislabeled with feces of another co-occurring species. This allowed us to proceed with the dietary analysis of 154 verified New Mexico meadow jumping mouse individuals, 145 of which we successfully acquired dietary information.

Taxonomic analysis revealed vast taxonomic breadth despite stringent filtering of features and ground-truthed cross-referencing of the taxa classified (Table 5). Of the focal dietary groups considered, we were able to classify dietary features to 5 phyla, 9 classes, 41 orders, 117 families, 161 genera, and 84 species. Ground-truth cross-referencing also indicated an ability to classify genera for both plant markers. After cross-referencing, we retained 43% of classified species (out of 133 candidate species) but 92% of classified genera (out of 187 candidate genera). The rest were collapsed to next highest level in taxonomy. Among the plant taxonomies, 95% were classified to genus and 41% were classified to species.

Forb, graminoid, and lepidopteran species were most consistent among individual diets (Figure 11, Table 6). In terms of available vegetation, capture localities were often dominated by graminoids such as sedge (*Carex* spp.), rush (*Juncus* spp.); members of Poaceae such as Agrostis spp., Poa spp., Bromus spp., *Glyceria* spp., and Phalaris spp.; forbs such as *Epilobium* spp., *Rudbeckia lancinata*, *Geum macrophyllum*, *Potentilla* spp., and *Hymenoxys* spp.; and scrub-shrub species of *Salix* spp., *Rosa* spp., and *Alnus* spp. Almost all of these taxa were detected in the diet of jumping mice, except for *Agrostis* spp., *Hymenoxys* spp., and *Rosa* spp. The most commonly detected graminoids in the diet were grasses, rushes, and sedges: *Triticum* sp., *Poa* sp., *Phalaris* sp., *Juncus* sp., *Eleocharis* sp., and *Carex* sp. The most common forbs in the diet were *Geranium* sp., *Rudbeckia laciniata*, *Geum macrophyllum*, *Ranunculus* sp., *Tragopogon dubius*, and *Persicaria lapathifolia*. Resources from trees and shrubs (*Alnus* sp., *Pinus* sp., *Picea* sp., *Humulus lupulus*, *Salix* sp.) were less frequently consumed (<10 % of individuals). Of all plant species detected in the diet, most (74%) were native and perennial (81%) growth types. Although we detected multiple arthropod orders in the feces of jumping mice, lepidopteran species were detected in almost half of the individuals. Examination of the taxonomies for data derived from intestinal contents revealed that arthropod diet items were likely ingested and not a result of exogenous contamination. We detected lepidopterans in all specimens. In one of the specimens, we only detected Lepidoptera and not plants or fungi. Half of the arthropod classifications to family or lower levels were of various species of moth (48%). Most (80%) arthropod taxa were phytophagous. Less frequently detected arthropod taxa included flies, parasitoid wasps, mites, beetles, and spiders (< 25% individuals). Hypogeous fungi (false-

truffles) were the most uncommon of the taxonomic groups detected among jumping mice. We detected at least five species from three families of false-truffles. Six of the seven detections occurred later in the season in August or September. The other was detected in the feces of a single individual in June.

We found variation in dietary diversity from June to September both in the number of plant taxa consumed and the composition of diets among individuals. We detected up to nine plant features per individual, with a median of four. Variation among alpha diversity metrics among months was significant. Among the study duration and two core study areas (Jemez Mountains, NM and White Mountains, AZ), dietary richness ($\chi 2$= 11.09, df = 3, p = 0.01) and diversity ($\chi 2$= 11.32, df = 3, p = 0.01) increased by at least August (Figure 12). We found no difference in evenness among months ($\chi 2$= 4.27, df = 3, p = 0.23). Compositional dissimilarity of the diets among the month groupings also differed (Figure 13). Homogeneity of dispersion was non-significant (p > 0.05) and the PERMANOVA showed differences among months for both binary and abundance weighted feature tables (p = 0.001). The post-hoc test further revealed that all pairs of months differed for both binary and abundance weighted feature tables (p < 0.05). For NMDS analyses (Figure 13) with both weightings, we selected ordination models with three axes (stress < 0.2) but only considered the first two axes for analysis. A goodness of fit among months on the rotated NMDS axis showed significant differences among the month groupings of binary (R^2 = 0.38, p = 0.001) and abundance-weighted features (R^2 = 0.36, p = 0.001). We also found weak but significant association among sexes along the rotated axis. Male diets were correlated with June diets, whereas females were correlated with August and September diets (R^2 = 0.1, p < 0.05 for both weightings). Females were slightly correlated with the appearance of more similar (tightly grouped) diets. Male diets were more correlated along an axis with more dissimilar diets. We did not detect seasonal correlation in arthropod or fungal consumption along the NMDS axes. However, this might be an effect of not sampling at the earliest and latest portions of the active season, where the effect would be predicted to occur.

For more details see Sanchez (2021).

Population Genetics

We selected 9 markers that performed well in initial screening for our final multiplex panels (Table 7). Of the 147 samples screened, 17.7% did not yield data for at least 8 markers and were thus excluded from the dataset. The 8 markers used in this study were highly discriminating for individual identity.

Measures of genetic diversity (expected, observed, allelic richness) in nuclear markers was moderate for all populations, highest for BR, trended lower for LCR and San Francisco River (SF), and was lowest for SF (Table 8). LCR and SF exhibited lower estimates for observed heterozygosity (HO) than expected (HE). SF also exhibited the lowest rarified allelic richness (AR, p = 0.11), whereas BR and LCR exhibited similar magnitudes. The inbreeding coefficient (FIS) was low overall (0.09) but estimates were elevated for LCR (0.12) and SF (0.14) relative to BR (-0.03).

A priori evaluations of differentiation showed weak but discernable structure among watersheds. FST values ranged from 0.02 to 0.04 (Table 9) and variation among watersheds (Table 10) explained ~7% of the total variation. Despite low estimates, pairwise comparisons (p < 0.05) and variation among watersheds (p = 0.001) were significant. LCR gave approximately the same magnitude of differentiation between the other two watersheds. The most pronounced difference was between BR and SF, which was twice as differentiated as the other pairwise comparisons. In the time-and-space model (Figure 14), historic BR fell intermediate to contemporary LCR and BR. The SF group was more dissimilar to the contemporary BR grouping.

For contemporary samples (2015-2020), we only found low but significant migration from LCR to SF (Table 11, Figure 15). All inferred origins were from either BR or LCR. SF only received dispersers (n =

7) from other watersheds (Figure 15). Of two localities in the SF watershed, only one locality received dispersers from other watersheds. All localities that received dispersers were at terminal portions of the headwaters, most adjacent to the headwaters of other watersheds. Females (n = 6) and males (n = 5) appeared to be evenly represented among all inferred dispersal events.

Analysis of mtDNA revealed a different pattern of diversity among the watersheds than nuDNA. The final analysis included the cytB sequences (929 bp) of 89 individuals. For samples that required sequencing (largely from fecal DNA), we successfully sequenced 74 specimens at all 4 over-lapping regions. Samples that failed sequencing (n = 27) generally failed at a single region due to messy chromatograms and were therefore unable to be assembled. We found seven haplotypes throughout the study area (Table 12). Two low frequency haplotypes were private to the BR watershed (III and IV), and two low frequency haplotypes were private to the LCR (V and VII). A network of the haplotypes (Figure 16) revealed two haplogroups separated by a hamming distance of 4. However, the haplogroups were common to either LCR (LCR-haplogroup) or BR (BR-haplogroup), but not SF. That is, the LCR-haplogroup (II, V, VI, VII) included haplotypes that were always detected in LCR. The BR-haplogroup (I, III, IV) included haplotypes that were always detected in BR. Haplotype I was most common in the BR haplogroup whereas haplotype II was most common in the LCR haplogroup. Whereas nuDNA revealed highest diversity (Table 8) for BR, LCR was higher in all mtDNA diversity metrics (except segregating sites). Similar to the nuDNA, individuals from the SF watershed had the lowest mtDNA diversity metrics. We note that most (11) of the individuals we grouped into BR came from a single locality but the rest of the individuals from BR were distributed among six other localities.

For more details see Sanchez (2021).

eDNA

Two qPCR assays for eDNA detection of New Mexico meadow jumping mouse were highly sensitive and specific. Primer combination 97F and 219R (long fragment assay) produced a 161 bp amplicon. Primer combination 97F and 166R (short fragment assay) produced a 110 bp amplicon (Table 13). A standard curve analysis resulted in long fragment assay efficiency = 95.2 % (R^2 = 0.99, y-intercept = 32.8, and slope = -3.4) and short fragment assay efficiency = 101.8% (R^2 = 0.99, y-intercept = 32.3, and slope = -3.3).

Both short and long fragment assays amplified New Mexico meadow jumping mouse DNA over non-target DNA, and both the short and long fragment assays provided comparably low rates of non-target amplification. Among technical replicates of New Mexico meadow jumping mouse samples, we found that 100% (111/111) and 99.1% (110/111) amplified for the short and long fragment assays, respectively. All 37 jumping mouse samples amplified when considering a detection threshold of at least two of three technical replicates amplifying. For technical replicates of non-target samples, 0% of amplifications passed the selection criteria for the long fragment assay and only 2.13% (3/141) passed for the short fragment assay. The limits of detection for both assays were less than 8 copies. We report < 8 copies because all 6 of the replicates of our lowest dilution in the standard curve amplified.

We found that the probability of occurrence (ψ) was 0.32, which was lower than the model's expected value set as 0.5. The true-positive probability for each sample (θ_{11}) and each qPCR replicate (p_{11}) were 0.275 and 0.669, respectively, both values smaller than the model's set expected value of 0.9 for each. The false-positive probability for each sample (θ_{10}) and each qPCR replicate (p_{10}) were 0.008 and 0.003, respectively; both values smaller than the model's set expected values of 0.1 for each (Table 14).

The short fragment assay detected eDNA of the New Mexico meadow jumping mouse in subsamples from 3 of 4 abandoned day nests. Amplifications were determined true for *Zapus* if both Ct and melting temperature corresponded to the selection criteria. For each nest where eDNA was detected, the mean technical replicate success was at least 2 of 3.

We detected jumping mice from 3 samples (3/9, 33%) that were opportunistically swabbed following a visual sighting of the target species. Two detections were from grasses and one from an abandoned day nest. For these samples, 1 to 2 technical replicates amplified. Detections were confirmed true according to the stepwise selection criteria if the Ct fell within the LOD and the sample Tm concurred with the positive control. We observed 1 detection along the tested transect from a single technical replicate. This sample also reamplified as a single replicate in a subsequent qPCR run. This swab was from a grassy patch without previous observation of New Mexico meadow jumping mouse presence. Using the long fragment assay, we detected jumping mice from the same 3 opportunistic swabs that were positive with the short fragment assay. Additionally, we detected jumping mice from 1 additional transect sample and 1 additional informed grass sample. However, the Ct of amplifications resulting from the long fragment assay fell outside of the criteria for a positive detection, even though the melting temperature corresponded to the positive control. Although these findings technically do not pass our stepwise selection criteria, we still found it important to report them to show the potential field efficacy of our assay.

Discussion

Home Ranges and Hibernacula

Our home range data indicated that males made larger movements and had larger home ranges in June and July than August through October. Presumably males were seeking females for mating after emergence and once breeding season was completed, they reduced home range size. Female home ranges varied less throughout the active season, although home ranges for 5 females (4 from SFNF in 2018 and 1 from AGFD in 2019) were smallest (range 0.20 to 0.78 ha) from 15 to 27 August. Adult females may be giving birth to and tending pups during this time (confirmed for 2 of these adult females on SFNF in 2018) and remaining close to maternity nests. Alternatively, males may be more flexible in habitat use and diet than females and range more widely to acquire resources. Regardless of sex, jumping mice remained close to streams, indicating their reliance on riparian areas and vegetation.

Sites used for hibernacula were unremarkable in appearance and highly variable in vegetation composition. These characteristics make them more concealed to a human eye and potentially for predators that use visual cues to identify habitat for hunting. Hibernacula varied in distance to water and vegetative cover, but most were close to streams, and all were outside the flood plain.

Entry into hibernation (immergence) might be influenced by mass of the animal (heavier animals entered hibernation earlier), age class (adult or juvenile; we believe the animals tracked in 2019 were young of the year, that the animals tracked in Arizona in 2020 were adults, and the animal tracked in Colorado in 2020 was likely young of the year), elevation (animals enter hibernation at higher elevations earlier than those at lower elevation), or other factors.

We monitored animals for >7 days during the day and night after they shifted to hibernacula; none moved. We detected fluctuations in skin temperatures for one male in a hibernaculum indicating this animal was practicing torpor and likely entering hibernation. The animal remained underground but appeared to plug soil at the hibernaculum entrance on one occasion prior to documenting any arousals (i.e., skin temperature increases). Brem et al. (2020) recently monitored arousal from torpor by one female *Zapus hudsonius* in a lab setting and found torpor bouts ranged from 2.1 to 12.8 days (mean 7.7 days). Our data are currently being analyzed with 2021 data (n = 10 hibernacula confirmed as of 2021) to determine possible torpor bout duration.

The male (151.619) on the East Fork Little Colorado River hibernated 100 m from the river, the furthest distance from stream of any of the hibernacula. His hibernaculum was north facing, in open (grassland), but within 1 m of conifer trees. This site might receive a thermal boost (i.e., warmer soil temperatures) earlier in the spring than sites along the stream and trigger earlier emergence than females or males with lower masses. Earlier emergence could allow males to forage, gain weight, and

better prepare for breeding than males emerging later. This jumping mouse was the largest (31.6 g) in our sample of hibernating animals and could represent a mature, more experienced animal. This, however, is speculative given our small sample size.

Our data continue to contribute to our understanding of how jumping mice use riparian areas, especially during hibernation, a critical component of their life history. The hibernacula that we documented suggest variability in their proximity to water, surrounding vegetation both at the fine scale of the hibernaculum and the larger ecosystem context, substrate (or lack thereof), and timing of immergence. Our data are also the first to confirm jumping mice underground with the use of temperature-sensitive radio collars and PIT tags. Additional studies investigating hibernacula will promote recovery of New Mexico meadow jumping mice and provide further understanding of the physical characteristics and environmental setting of their hibernacula.

Diet Analysis

The New Mexico meadow jumping mouse demonstrates wide dietary breadth through its polyphagy of plant resources (graminoids and forbs), insectivory (moths, likely larvae), and hypogeous mycophagy. Overall, our dietary taxonomies and patterns in the diet of the jumping mouse agree with previously reported data for *Z. hudsonius* obtained through prey microscopy and field observation (Bailey 1926, Hamilton 1935, Quimby 1951, Whitaker 1963, Wright and Frey 2014). However, previous analysis of diet involved almost 800 individuals to reveal only 25 plant taxonomies (Whitaker 1963a) compared to our genetic analyses. Molecular diet tracing is therefore the most efficient means of investigating the diets of jumping mice, with emphasis on scalability, taxonomy, and dietary diversity.

Herbaceous plants (particularly forbs and graminoids) are the foundation of the New Mexico meadow jumping mouse diet. The variety we revealed lends support to the hypothesis of Whitaker (1963), who suggested that resource use in jumping mice is governed by availability of mature seeds. The ability for New Mexico meadow jumping mice to consume seeds from a variety of species may be advantageous to an individual if seeds suitable to their foraging behavior vary in availability over space and time.

The herbaceous vegetation is also a foundation of the diet because it provides habitat for insect prey. Our study documents the first evidence of insectivory for the subspecies and most insects that are consumed are associated with plants (80%). We recognize that it is impossible to determine whether the arthropod taxa were incidentally consumed. DNA cannot reveal whether a detection was adult-stage, larval, frass, or where the ingestion occurred (e.g., leaf, flower, seed, root). For example, we detected parasitoid wasps, which could have been interacting with other insect herbivores. Yet, the high frequency of lepidoptera (mostly moth) in the current study is consistent with previous studies that document consumption of lepidopteran larvae (Whitaker 1963). Although consumption of adult-stage moths would seem unlikely, this has been empirically documented at relatively low frequency and volume (Whitaker 1963). Based stable isotope data (Chambers and Fofanov, unpublished), plants are the components of diet most assimilated by individuals, which would suggest that plants are more staple to their diet than arthropods. Regardless of how arthropod material is ingested or their assimilation, these represent energy and nutrients (Nielsen et al. 2018). Therefore, further work is needed to understand the contribution of insects to the diet. Our findings and those of other studies suggest that herbaceous plants function as a common denominator for both primary production of diverse foraging resources and habitat for insect prey.

Evidence of hypogeous mycophagy in our study of the New Mexico meadow jumping mouse was infrequent but was consistent with observations of mycophagy in all species of North American jumping mice: *Z. hudsonius*, *Z. princeps*, *Z. trinotatus*, and *Napeozapus insignus* (Dowding 1955; Whitaker 1962; Whitaker 1963a; Hart et al. 2004). Hypogeous fungi are associated with plant roots, do not sporulate to reproduce, are most abundant in the Fall (most detections in our study occurred in

August), and are commonly co-adapted to mycophagy in rodents (Połatyńska 2014). It is uncertain whether these dietary items were sought out by the New Mexico meadow jumping mouse or incidentally consumed (e.g., from digging to construct a hibernaculum).

For the New Mexico meadow jumping mouse, shifts in dietary diversity throughout their short season of activity could suggest that the phenology (e.g., seed maturity) of plants in their habitat governs consumption. Shifts in feeding variability, intensity, and prey type with plant phenology has been observed in other rodents (Sunyer et al. 2014; Goldberg et al. 2020) and heterogeneity in resource availability predicts larger home ranges in herbivores (van Beest et al. 2011; Rizzuto et al. 2021). In general, the size of the home range is inversely related to food availability and quality in rodents of both sexes (Taitt and Krebs 1981; Wauters and Dhondt 1992; Corp et al. 1997) and male rodents tend to have larger home ranges than females (Schroder 1979; Attuquayefio et al. 1986; Erlinge et al. 1990). If seed maturity peaks in the late season, this could explain why in the New Mexico meadow jumping mouse, male diets were correlated with more variability (larger home ranges, low food abundance, and quality) in the early season and female diets were correlated with less variability (smaller home ranges, high food abundance, and quality) in the late season. The net trend of the dietary shift coincides with the general window of hibernation. The New Mexico meadow jumping mouse is not believed to survive on seed caches during hibernation and instead survives off of fat stores (Humphries et al. 2003; Frey 2015). Late-season shifts in diet that we observed could be associated with fat accumulation prior to hibernation.

Plants are the foundation of this subspecies' diet and are a limiting factor in some areas given pressures that reduce plant biomass (e.g., grazing). Graminoids and forbs comprise the core diet and provide habitat for insects that are consumed as well as cover.

Population Genetics

We tested whether separate, adjacent watersheds influenced the population genetic structure of the New Mexico meadow jumping mouse in the White Mountains, AZ. We did not observe strict, stream-delineated differentiation, nor did we observe complete admixture. Rather, our assessment suggests that genetic structure and diversity is discernable among separate watersheds, but gene flow appears to cross overland boundaries at adjacent, terminal headwaters. Drainages appear to influence population structure, and contemporary and pre-historic patterns of gene flow suggest that inter-drainage migrants may play a role in replenishing genetic diversity. Incoming migrants, even at low number, can replenish genetic diversity or substantially lower the rate of drift (Lacy 1987). When multiple lines of evidence are taken together, our results partially support gene flow as a product of hydrological networks, and that overland dispersal should not be excluded as a factor in maintaining genetic diversity among the separate drainages.

The New Mexico meadow jumping mouse may be able to disperse if conditions vary annually or within a season (e.g., monsoon rains). Another factor could include annual variation in population growth rates (i.e., pre-saturation dispersal), which increases dispersal into non-natal localities in fossorial water voles (Berthier et al. 2006). Thus, the movement of individuals and genes among separate drainages is possible; potentially dependent on local moisture conditions and periods of increased population growth rates; and could be mediated by functional linkages or cryptic sub-corridors that allow for short distance dispersal in males. These overland paths likely exhibit elements of habitat that another conspecific could use, which would likely exclude overland paths over dry, rough, open, or coniferous terrain (Quimby 1951).

A fragmented population may exist in the eastern fork of the LCR and into the SF localities. This abrupt discontinuity in the landscape could either result from a discrete barrier (Blair et al. 2012), resistance in the landscape (McRae 2006), or adaptation to the local environment (Wang and Bradburd 2014). The east fork of the LCR and into SF appears most influenced by human development (ranchland,

roads, and private residences) relative to other localities of capture. Contemporary habitat fragmentation may also exist between the West Fork of the LCR and BR. Historic specimens (1963-1991) of the BR in closer spatial proximity to the LCR fall intermediate to the two watersheds. It is unclear if this could indicate a contemporary cline between the two subregions or that change occurred (i.e., BR used to be more similar to LCR). These historic localities were not resampled and would therefore require contemporary survey of those same localities.

Drainages influence population structure and therefore hydrological management units are likely more relevant than Euclidean swaths. However, drainages should not be managed separately given evidence of relatively short overland dispersal. Within the White Mountains, habitat fragmentation is most concerning in eastern fork of LCR as human development could be impeding gene flow along the headwater. The LCR seems to be a historic source of genetic diversity that possibly feeds migrants into both BR and SF. SF, in particular may be a genetic sink.

eDNA

This study presents new evidence of the use of vegetation as a reservoir for detecting eDNA of a terrestrial mammal. We demonstrated that eDNA can be detected on vegetation and can persist for up to 6 months after contact with New Mexico meadow jumping mice. Our study provides a proof of concept that eDNA from vegetation can be effective as a presence/absence survey for terrestrial mammals, particularly when used in conjunction with other ecological survey methods. We successfully designed a high-efficiency, qPCR assay that is specific to the New Mexico meadow jumping mouse and exhibits comparable sensitivity to other eDNA assays for terrestrial and aquatic species (Dysthe et al., 2018; Franklin et al., 2019; Hunter et al., 2017; Spangler et al., 2017). In Arizona and New Mexico, eDNA detections may better indicate sites where New Mexico meadow jumping mice naturally occur, without influence of bait attractants. In addition, capture or observation is difficult with rare or low-density species, and the use of eDNA increases the probability of detection. Reporting detections made using eDNA could thus corroborate site occupancy or aide in population density inferences.

Of the two candidate assays, the short fragment assay provided the highest sensitivity and confidence in detection and is thus our recommended primer set. In the modeling approach (Griffin et al., 2020) used to estimate occupancy and false negative and false positive errors, we found that the probability of detection in the field may be underestimated (probability of true positive field sample θ_{11} was 27.5% whereas the expected value was 90%), as was the probability of true positive qPCR detection (p_{11} was 66.9% whereas the expected value was 90%). The probability of false positives in the field and lab were also underestimated (θ_{10} = 0.8%, p_{10} = 0.3%; expected values were both 10%). That field and lab false positive probabilities were substantially lower than true positive probabilities suggests that most detections are likely true observations. However, sample false-negative probability of 72.5% and qPCR false negative probability of 33.1% indicates room for improvement in both sampling and assay methods. Cantera et al. (2019) has shown that the probability of false negatives can be reduced by increasing biological and technical replicates.

Although the number of uninformed detections was low, this method is promising particularly when comparing the effort between trapping and this study. At one site it required 404 trap nights to capture 12 individuals and only 30 uninformed swabs to detect two jumping mice; at the other site it took 800 trap nights to capture three jumping mice and 30 uninformed swabs to detect one jumping mouse. The expense of supporting a field team for trapping and track plating far exceeded that of collecting and analyzing swabs from vegetation. We aim to improve the rate of uninformed detection via conducting additional field studies and assay optimizations. We will increase sampling effort (more samples per unit area), sample immediately after sunrise, and sample during period when males move more (i.e., within six to eight weeks of emergence from hibernation), and when subadults might be dispersing (i.e., four weeks prior to immergence). We will determine whether particular substrates are

better for eDNA detection. As for improving the amount of eDNA collected on swabs, we will use larger swabs moistened by sterile water prior to swabbing.

This eDNA assay provides means to verify presence of New Mexico meadow jumping mouse and discriminate the endangered subspecies from other sympatric small mammals. Current survey methods may not distinguish between western jumping mice and New Mexico meadow jumping mice; similarly, the eDNA assay has not been extensively tested against all species of jumping mice and thus should not be used to distinguish co-occurring congenerics (*Zapus princeps*). We suggest relying upon geographic range and subsequent use of amplicon sequencing to confirm *Zapus* species identity. Although the assay is inexpensive because it is SYBR-based, adapting the assays with a hydrolysis probe would likely improve sub-specific differentiation between the New Mexico meadow jumping mouse and western jumping mouse in zones of sympatry such as in southern Colorado, unless hybridization occurs. A hydrolysis probe could also improve specificity and sensitivity of the detection (Birdsell et al. 2012, Wilcox et al. 2013).

Additional data reduction and analyses are in progress and manuscripts are being prepared from field studies.

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Outreach

The Life and Times of an Endangered Rodent (November 2020), Seminar for University of Arizona, School of Natural Resources and the Environment, Tucson

Video on New Mexico meadow jumping mouse work: A Space in Time: Movements and Home Ranges of the New Mexico Meadow Jumping Mouse (December 2019) https://youtu.be/V62PNsP7WXk

Presentations

2021

Rozanski, C. A., C. Chambers, S. Tuttle, M. Miller, and W. B. Southerland. *Can fluvial geomorphology predict New Mexico meadow jumping mouse occupancy?* The Wildlife Society 28th Annual Conference (November), Presentation

Rozanski, C. A., C. Chambers, S. Tuttle, M. Miller, and W. B. Southerland. *Can fluvial geomorphology predict New Mexico meadow jumping mouse occupancy?* Arizona/New Mexico Chapters of The Wildlife Society 54th Joint Annual Meeting (February), Presentation

2020

C. L. Chambers, J. L. Zahratka, C. A. Rozanski, and J. G. Martínez-Fonseca. *Life in the Riparian Zone: Home Ranges and Locomotion of an Endangered Jumping Mouse*, The Wildlife Society 27th Annual Conference (October), Presentation

Rozanski, C. A., C. Chambers, S. Tuttle, M. Miller, and W. B. Southerland. *Can fluvial geomorphology predict New Mexico meadow jumping mouse occupancy?* The Wildlife Society 27th Annual Conference (October), Presentation

C. Chambers, J. Zahratka, C. Rozanski, and J. G. Martínez-Fonseca, *The saltatorial, aquatic, and arboreal New Mexico meadow jumping mouse: home ranges and movements*, Arizona/New Mexico Chapters of The Wildlife Society 53rd Joint Annual Meeting (February), Presentation

Hershauer, S., J. Lyman, D. E. Sanchez, C. J. Sobek, C. L. Chambers, and F. M. Walker, *Detecting New Mexico meadow jumping mice using eDNA*, Arizona/New Mexico Chapters of The Wildlife Society 53rd Joint Annual Meeting (February), Poster **(Won Best Student Poster Award)**

2019

Sanchez, D., A. Dikeman, F. Walker, V. Fofanov, and C. Chambers, *Salad within: a genetic survey of diet of the endangered New Mexico jumping mouse (Zapus luteus luteus)*, The 8th International Barcode of Life Conference (June), Presentation

Lyman, J., D. Sanchez, F. Walker, and C. Chambers, *An assay for "Bigfoot": Detecting eDNA of the endangered New Mexico jumping mouse (Zapus luteus luteus)*, Northern Arizona University Undergraduate Symposium (April), Poster

J. G. Martínez-Fonseca, C. Chambers, and J. Zahratka, *Home on the range: How far do New Mexico jumping mice go?*, Arizona/New Mexico Chapters of The Wildlife Society 52nd Joint Annual Meeting (February), Presentation

J. Lyman, D. Sanchez, C. Sobek, C. Chambers, P. Keim, A. E. Springer, G. Caporaso, and F. Walker, *Environmental DNA for ecological monitoring*, Arizona/New Mexico Chapters of The Wildlife Society 52nd Joint Annual Meeting (February), Presentation

Undergraduate Student Research Projects

2019

Austin Dikeman (co-advisor with F. Walker), *Escherichia coli* in the New Mexico meadow jumping mouse (**NAU Gold Axe Award recipient**)

2018

Jacqueline Lyman (co-advisor with F. Walker), Identifying presence of the New Mexico jumping mouse using eDNA

Publications

Completed

Sanchez, D. E. 2021. Hierarchical assessments of gene flow for cryptic and endangered mammals. Ph.D. Dissertation.

Rozanski, C. A. 2021. The importance of fluvial geomorphology and watershed restoration for the New Mexico jumping mouse. M.S. Thesis.

Harrow, R. L., V. J. Horncastle, and C. L. Chambers. 2018. Track plates detect the endangered New Mexico meadow jumping mouse. Wildlife Society Bulletin 42(4):693-700.

Zahratka, J.L., J.G. Martinez-Fonseca, C.C. Chambers. 2020. Thamnophis elegans vagrans. Wandering Gartersnake. Diet. *Zapus hudsonius luteus*. Herpetological Review 51(2):361-362.

In review

Sanchez, D. E., F. M. Walker, and C. L. Chambers. Forbs and graminoids are the foundation: dietary breadth and seasonal variation of the New Mexico meadow jumping mouse (*Zapus hudsonius luteus*), Molecular Ecology. In review.

Lyman, J. A., D. E. Sanchez, S. N. Hershauer, C. J. Sobek, C. L. Chambers, and F. M. Walker. Mammalian eDNA on herbaceous vegetation? Validating a qPCR assay for detection of an endangered rodent. Environmental DNA. In review.

Grants (excludes CEAP funding)

2021

New Mexico meadow jumping mouse ecology, Arizona Game and Fish Department, $82,280 (Pending)

Ecology of the New Mexico Meadow Jumping Mouse, Fish and Wildlife Service, $30,970 (Funded)

2020

New Mexico meadow jumping mouse hibernacula, life transitions, and hormones, Fish and Wildlife Service, $27,500 (Funded)

2019

New Mexico meadow jumping mouse habitat, home range, and diet and a test of an environmental DNA survey method on the Apache-Sitgreaves National Forests, USDA Forest Service, $75,000 (Funded)

2018

Connectivity and barriers to New Mexico jumping mice movements, Arizona Game & Fish Department Heritage IIAPM Program, $139,899 (Funded)

Habitat use and diet of the New Mexico meadow jumping mouse in the Southwest, USDA Forest Service, $187,000 (Funded)

Table 1. Sites surveyed for New Mexico meadow jumping mouse (*Zapus hudsonius luteus*) on the Apache-Sitgreaves National Forest (ASNF, Arizona; 2018-2020), Gila National Forest (GNF, New Mexico; 2018-2019), and Santa Fe National Forest (SFNF, New Mexico; 2018-2019), and Arizona Game and Fish Department lands (AGFD, Arizona; 2018-2019). Jumping mice were detected at 25 of 44 sites (31 unique) (ZAHU Presence = Yes) using live trapping or track plating. Datum for all sites was WGS84.

Year	Forest	Site Number	Site Name	ZAHU Presence	Latitude	Longitude
2018	AGFD	73	EC Bar Ranch	Yes	33.983312	-109.199274
2019	AGFD		Sipe White Mountain Wildlife Area	Yes	34.027935	-109.240104
2019	AGFD	73	EC Bar Ranch	Yes	33.979218	-109.202315
2018	ASNF	26	West Fork Black River Middle	Yes	33.778410	-109.405610
2018	ASNF	22	West Fork Black River Lower	Yes	33.761420	-109.378140
2018	ASNF	99	San Francisco River	Yes	33.865030	-109.180120
2018	ASNF	39	Coyote Creek	No	33.833090	-109.249340
2019	ASNF	68	West Fork LCR Sheep Crossing	Yes	33.959900	-109.507030
2019	ASNF	63	Gabaldon Horse Camp	Yes	33.929460	-109.489930
2019	ASNF	66	East Fork LCR East	No	33.950910	-109.469040
2019	ASNF	49	Turkey Creek	Yes	33.882350	-109.160370
2019	ASNF	60	Nutrioso Creek	Yes	33.916290	-109.176450
2019	ASNF	99	San Francisco River	Yes	33.865030	-109.180120
2020	ASNF	63	Gabaldon Horse Camp	Yes	33.929460	-109.489930
2020	ASNF	68	West Fork LCR Sheep Crossing	Yes	33.959900	-109.507030
2020	ASNF	26	West Fork Black River Middle	Yes	33.778410	-109.405610
2020	ASNF	99	San Francisco River	Yes	33.865030	-109.180120
2020	ASNF	42	Three Forks	Yes	33.850830	-109.312490
2020	ASNF	49	Turkey Creek	No	33.882350	-109.160370
2020	ASNF	22	West Fork Black River Lower	Yes	33.761420	-109.378140
2018	GNF	1	SA Creek	No	33.877567	-108.888250
2018	GNF	2	Centerfire Creek	No	33.884684	-108.858557
2018	GNF	4	Tularosa Gauging Station	No	33.891500	-108.516733
2018	GNF	3	Tularosa Wetlands	No	33.831300	-108.624467
2018	GNF	5	San Francisco River	No	33.746717	-108.767750
2018	GNF	6	Willow Creek	No	33.398650	-108.588417
2018	GNF	7	Upper Dry Blue	No	33.764350	-108.997350
2018	GNF	8	Lower Dry Blue	No	33.735500	-109.032683
2019	GNF	5	San Francisco River	No	33.745947	-108.768746
2019	GNF	6	Willow Creek	No	33.398565	-108.588202
2019	GNF		Dry Blue ZAHU Capture Site	No	33.717699	-109.044219
2019	GNF		Dry Blue	No	33.721087	-109.042383
2019	GNF	8	Lower Dry Blue	No	33.735817	-109.031871
2018	SFNF	0	Fenton Lake	Yes	35.874464	-106.744510
2018	SFNF	5	Rio Cebolla	Yes	35.843700	-106.781690
2018	SFNF	3	Rio Cebolla	Yes	35.852089	-106.766099
2019	SFNF	14	O'Neil Landing	No	35.870581	-106.795129
2019	SFNF	6	Rio Cebolla Vacas Confluence	No	35.819953	-106.788487

2019	SFNF		Lower Cebolla	Yes	35.842048	-106.784288
2019	SFNF	3	Upper Cebolla Culvert	Yes	35.857730	-106.758020
2019	SFNF	1	Lake Fork Corral	No	35.853690	-106.754460
2019	SFNF		Lower Cebolla	Yes	35.842048	-106.784288
2019	SFNF		Seven Springs 1	Yes	35.930099	-106.695720
2019	SFNF		Seven Springs 2	Yes	35.932278	-106.688850

Table 2. Small mammals captured from live trapping on Arizona Game and Fish Department property (AGFD), Apache-Sitgreaves National Forests (ASNF) and Santa Fe National Forest (SFNF) during summer (Jun – Sep 2018 to 2020). One trap night is one Sherman trap open for 1 night.

Scientific name	Common name	2018 AGFD	2018 ASNF	2018 SFNF	2019 AGFD	2019 SFNF	2019 ASNF	2020 ASNF	Total
Peromyscus maniculatus	deer mouse	4	58	12	75	54	194	479	876
Microtus spp.	Unidentified vole	0	12	0	0	1	40	603	656
Microtus montanus arizonensis	montane vole	0	23	3	34	36	118	121	335
Zapus hudsonius luteus	**New Mexico meadow jumping mouse**	**2**	**27**	**18**	**4**	**15**	**26**	**108**	**200**
Sorex spp.	shrew species	0	3	7	0	39	29	47	125
Microtus longicaudus	long-tailed vole	0	2	0	4	10	23	40	79
Microtus mogollonensis navajo	Mogollon vole	1	6	3	13	10	12	5	50
Reithrodontomys megalotis	western harvest mouse	0	0	1	31	0	14	0	46
Neotoma mexicana	Mexican woodrat	0	1	0	0	0	2	6	9
Sorex palustris	American water shrew	0	0	0	0	4	0	0	4
Peromyscus spp.	Unidentified deer mouse	0	3	0	0	0	0	0	3
Callospermophilus lateralis	golden-mantled ground squirrel	0	0	0	0	0	0	1	1
Total captures		7	135	44	161	169	458	1410	2384
Total trap nights		320	1150	503	580	1504	1879	5370	11,306

Table 3. Home range sizes (ha) and movement data (m) for 70 New Mexico jumping mice radio tracked during summers 2017, 2018, 2019, and 2020 on Apache-Sitgreaves National Forests, Santa Fe National Forest, Arizona Game and Fish Department property, and Colorado lands. Summary of home ranges is described using Minimum Convex Polygon (100% MCP) and Kernel Density Probability (95%) in hectares (ha). Mean, standard error (SE), minimum (Min), and maximum (Max) are provided for all animals, females only, and males only. Number of locations for each animal (n) is adequate to calculate home range when n > 30. Mean and maximum distance from last location is the average and maximum distance between consecutive nocturnal locations. Maximum distance from centroid is the furthest distance observed relative to the center of the home range (centroid). Distance from stream is the distance, in meters, to the closest point on the nearest flowline. Maximum distance from stream is the furthest distance observed; mean is the average distance for all locations from flowline and SD is the standard deviation for mean distance from stream.

Metric	Number of days	Mass (g)	n	Mean distance moved from last location (m)	Maximum distance from last location (m)	Maximum distance from centroid (m)	Maximum distance from stream (m)	Mean distance from stream (m)	SD of distance from stream (m)	MCP (ha)	95% KD (ha)
All animals											
Mean	14.8	21.0	51.8	61.9	273.1	273.8	40.7	11.2	10.5	3.02	2.80
SE	0.8	0.6	2.6	4.8	26.7	29.0	4.8	1.7	1.4	0.46	0.64
Min	7	13.9	30	18.4	42.4	36.2	7.4	2.4	1.5	0.15	0.10
Max	40	35.9	150	219.5	1155.0	1151.8	187.1	71.9	57.4	17.96	28.44
Females											
Mean	13.8	21.5	49.8	52.3	204.8	202.3	41.2	11.7	11.3	2.04	1.39
SE	0.7	0.6	2.0	3.0	13.7	16.8	5.0	1.7	1.6	0.28	0.18
Min	9	15.5	32	18.5	42.4	36.2	7.4	2.4	1.5	0.16	0.11
Max	32	35.9	109	132.2	602.6	667.9	155.6	49.6	52.0	11.62	7.55
Males											
Mean	15.6	20.6	53.4	69.1	324.4	327.4	40.3	10.9	9.9	3.76	3.85
SE	0.9	0.5	2.9	5.7	32.2	34.3	4.8	1.7	1.3	0.55	0.81
Min	7	13.9	30	18.4	63.0	47.8	9.9	2.6	2.6	0.15	0.10
Max	40	31.6	150	219.5	1155.0	1151.8	187.1	71.9	57.4	17.96	28.44

Table 4. New Mexico meadow jumping mice (*Zapus hudsonius luteus*) tracked into hibernation during Aug-Oct, 2019 and 2020, Arizona and Colorado. Animal ID and Channel identify individuals. State and site indicate location of the hibernacula. Tracking dates indicated period each animal was tracked (or monitored during hibernaculum), # of days indicate the total days the animal was monitored while active and in hibernation, # telemetry points provide number of individual fixes during the tracking period. Sex is M = male, F = female. Mass indicates weight of the animal at capture and recapture (if recollared to continue tracking prior to hibernation). Animals were tracked until they entered hibernation (hibernation date) and for 10 to 17 days after entering hibernation. We recorded distance to water in the riparian zone and elevation for each hibernaculum.

Animal ID (Transmitter frequencies) [1]	Chan-nel	State	Site [2]	Site#	Year	Tracking dates	# of days	Sex	Mass (g)	End mass (g) [3]	# telem-etry points	Eleva-tion (m)	Hiber-nation date	Hibern-aculum distance to water (m)
150.380; 150.299	13	AZ	SFR	99	2019	15 Sep - 16 Oct	31	F	21.0	25.9	109	2512	1 Oct	5.5
148.787, 150.059	10	AZ	SFR	99	2019	8 Sep - 16 Oct	34	M	20.0	26.0	127	2475	5 Oct	3
151.619	6	AZ	Horse	63	2020	23 Aug - 10 Sep	19	M	31.6	NA	60	2859	27 Aug	100
148.689, 150.306	13	CO	Flo	--	2020	31 Aug - 10 Oct	44	M	18.0	19.7	92	1993	28 Sep	8

[1] Because radio collars had a life of 14 to 21 days, individuals that we tracked for longer periods were recaptured and recollared and received a new transmitter frequency.

[2] Site names: SFR = San Francisco River exclosure, Horse = Gabaldon Horse Camp on East Fork Little Colorado River, Flo = Florida River

[3] Individuals that were not recollared were not reweighed.

Table 5. Feature (OTU or ASV), taxonomy, and sample summaries by marker and target for dietary analysis of New Mexico meadow jumping mice (Sanchez 2021).

	Plants	**Arthropods**	**Hypogeous fungi**
Feature type	LULU OTU	OTU	ASV
	ITS2 | rbcL	**COI**	**ITS2**
Features	444 | 154	374	30 of 598
*Unique taxonomies after curation	69 | 59 106 total	127	4
Phylum	2	1	2
Class	3	4	2
Order	21	17	3
Family	35	79	3
Genus	83	75	3
Species	43	39	2
Num. individuals sequenced and classified (N = 145)	137 94.5%	118 81.4%	131 4.8%

Table 6. List of diet items detected in New Mexico meadow jumping mice with two or more detections among individuals (except for Fungi). In order of frequency of occurrence (FOO) and percent frequency of occurrence (%FOO) (Sanchez 2021).

Target	Order	Family	Genus	Species	Common name	FOO	%FOO
Arthropod	Lepidoptera			*Lepidoptera sp.*	moth or butterfly	43	22.9
Plant	Geraniales	Geraniaceae	Geranium	*Geranium sp.*	geranium	30	21.9
Plant	Asterales	Asteraceae	Rudbeckia	*Rudbeckia laciniata*	cutleaf coneflower	24	17.5
Plant	Rosales	Rosaceae	Geum	*Geum macrophyllum*	large-leaf avens	22	16.1
Plant	Poales	Poaceae	Triticum	*Triticum sp.*	wheat	22	16.1
Plant	Poales	Poaceae	Poa	*Poa sp.*	bluegrass	17	12.4
Plant	Poales	Poaceae	Phalaris	*Phalaris arundinacea*	reed canarygrass	16	11.7
Plant	Ranunculales	Ranunculaceae	Ranunculus	*Ranunculus sp.*	buttercup	16	11.7
Plant	Asterales	Asteraceae	Tragopogon	*Tragopogon dubius*	common salsify	12	8.8
Arthropod	Hymenoptera	Ichneumonidae		*Ichneumonidae sp.*	Ichneumon wasps	12	6.4
Plant	Fagales	Betulaceae	Alnus	*Alnus sp.*	alder	11	8.0
Arthropod	Diptera			*Diptera sp.*	flies	11	5.9
Plant	Rosales	Rosaceae	Geum	*Geum sp.*	avens	10	7.3
Plant	Poales	Juncaceae	Juncus	*Juncus sp.*	rush	10	7.3
Plant	Caryophyllales	Polygonaceae	Persicaria	*Persicaria lapathifolia*	curlytop knotweed	10	7.3
Plant	Pinales	Pinaceae	Pinus	*Pinus sp.*	pine	10	7.3
Plant	Fabales	Fabaceae	Phaseolus	*Phaseolus sp.*	bean	9	6.6
Plant	Malpighiales	Violaceae	Viola	*Viola sp.*	violet	9	6.6
Plant	Poales	Cyperaceae	Eleocharis	*Eleocharis sp.*	spikerush	8	5.8
Plant	Asterales	Asteraceae	Erigeron	*Erigeron formosissimus*	beautiful fleabane	8	5.8
Plant	Fabales	Fabaceae	Medicago	*Medicago lupulina*	black medick	8	5.8
Plant	Asterales	Asteraceae	Taraxacum	*Taraxacum sp.*	dandelion	8	5.8
Arthropod	Hemiptera	Aphididae		*Aphididae sp.*	aphids	8	4.3
Plant	Poales	Poaceae	Avena	*Avena barbata*	slender oat	7	5.1
Plant	Poales	Cyperaceae	Carex	*Carex sp.*	sedge	7	5.1
Plant	Caryophyllales	Caryophyllaceae	Cerastium	*Cerastium sp.*	chickweed	7	5.1
Arthropod	Hymenoptera	Braconidae		*Braconidae sp.*	braconid wasp	7	3.7
Plant	Fabales	Fabaceae		*Fabaceae sp.*	Pea family	6	4.4
Plant	Asterales	Asteraceae	Helianthus	*Helianthus sp.*	sunflower	6	4.4
Arthropod	Hemiptera	Aphididae	Aphis	*Aphis sp.*	aphids	6	3.2
Arthropod	Diptera	Chironomidae		*Chironomidae sp.*	nonbiting midges	6	3.2
Arthropod	Lepidoptera	Erebidae		*Erebidae sp.*	erebid moths	6	3.2
Plant	Rosales	Rosaceae	Agrimonia	*Agrimonia sp.*	agrimony	5	3.6
Plant	Rosales	Cannabaceae	Humulus	*Humulus lupulus*	common hops	5	3.6
Plant	Lamiales	Lamiaceae	Prunella	*Prunella vulgaris*	common selfheal	5	3.6
Plant	Malpighiales	Salicaceae	Salix	*Salix sp.*	willow	5	3.6
Arthropod	Lepidoptera	Crambidae		*Crambidae sp.*	grass moths	5	2.7
Plant	Poales	Poaceae	Bromus	*Bromus sp.*	brome	4	2.9
Plant	Poales	Cyperaceae		*Cyperaceae sp.*	sedge family	4	2.9

Target	Order	Family	Genus	Species	Common name	FOO	%FOO
Plant	Poales	Poaceae	Hordeum	*Hordeum brachyantherum*	meadow barley	4	2.9
Plant	Caryophyllales	Polygonaceae	Rumex	*Rumex sp.*	sorrel	4	2.9
Plant	Asterales	Asteraceae	Tragopogon	*Tragopogon sp.*	salsify	4	2.9
Arthropod	Lepidoptera	Depressariidae	Agonopterix	*Agonopterix sp.*	casebearer moths	4	2.1
Arthropod	Diptera	Cecidomyiidae		*Cecidomyiidae sp.*	gall midges	4	2.1
Arthropod	Lepidoptera	Tortricidae	Cochylis	*Cochylis sp.*	moths	4	2.1
Arthropod	Lepidoptera	Noctuidae	Mythimna	*Mythimna unipuncta*	armyworm moth	4	2.1
Arthropod	Diptera	Tachinidae		*Tachinidae sp.*	tachinid flies	4	2.1
Plant	Brassicales	Brassicaceae	Brassica	*Brassica sp.*	mustard	3	2.2
Plant	Poales	Poaceae	Bromus	*Bromus inermis*	smooth brome	3	2.2
Plant	Asterales	Asteraceae	Carduus	*Carduus sp.*	thistle	3	2.2
Plant	Poales	Poaceae	Dactylis	*Dactylis glomerata*	orchardgrass	3	2.2
Plant	Rosales	Rosaceae	Dasiphora	*Dasiphora fruticosa*	shrubby cinquefoil	3	2.2
Plant	Caryophyllales	Polygonaceae	Fallopia	*Fallopia sp.*	bindweed	3	2.2
Plant	Poales	Juncaceae	Juncus	*Juncus confusus*	Colorado rush	3	2.2
Plant	Lamiales	Phrymaceae		*Phrymaceae sp.*	lopseed family	3	2.2
Plant	Pinales	Pinaceae	Picea	*Picea sp.*	spruce	3	2.2
Plant	Caryophyllales	Polygonaceae	Polygonum	*Polygonum aviculare*	prostrate knotweed	3	2.2
Plant	Rosales	Rosaceae	Potentilla	*Potentilla sp.*	cinquefoil	3	2.2
Plant	Poales	Poaceae	Torreyochloa	*Torreyochloa pallida*	pale false mannagrass	3	2.2
Plant	Lamiales	Verbenaceae	Verbena	*Verbena sp.*	verbena	3	2.2
Arthropod	Coleoptera	Chrysomelidae		*Chrysomelidae sp.*	leaf beetles	3	1.6
Arthropod	Araneae	Clubionidae	Clubiona	*Clubiona sp.*	sac spiders	3	1.6
Arthropod	Coleoptera	Elateridae	Dalopius	*Dalopius sp.*	click beetles	3	1.6
Arthropod	Diptera	Limoniidae	Dicranomyia	*Dicranomyia sp.*	crane flies	3	1.6
Arthropod	Lepidoptera	Momphidae	Mompha	*Mompha unifasciella*	casebearer moths	3	1.6
Arthropod	Lepidoptera	Noctuidae		*Noctuidae sp.*	owlet moths	3	1.6
Arthropod	Hemiptera	Aphididae	Oestlundiella	*Oestlundiella sp.*	aphids	3	1.6
Arthropod	Trombidiformes	Tarsonemidae		*Tarsonemidae sp.*	thread-footed mites	3	1.6
Arthropod	Araneae	Philodromidae	Tibellus	*Tibellus maritimus*	crab spider	3	1.6
Hypogeous fungus	Agaricales	Hymenogastraceae	Hymenogaster	*Hymenogaster rubyensis*	false-truffle	3	2.3
Plant	Caryophyllales	Amaranthaceae	Amaranthus	*Amaranthus sp.*	amaranth	2	1.5
Plant	Caryophyllales	Chenopodiaceae	Bassia	*Bassia sp.*	smotherweed	2	1.5
Plant	Asterales	Asteraceae	Cosmos	*Cosmos parviflorus*	southwestern cosmos	2	1.5
Plant	Myrtales	Onagraceae	Epilobium	*Epilobium sp.*	willowherb	2	1.5
Plant	Geraniales	Geraniaceae	Erodium	*Erodium cicutarium*	redstem stork's bill	2	1.5
Plant	Poales	Poaceae	Festuca	*Festuca rubra*	red fescue	2	1.5
Plant	Poales	Poaceae	Festuca	*Festuca sp.*	fescue	2	1.5
Plant	Poales	Poaceae	Glyceria	*Glyceria striata*	fowl mannagrass	2	1.5

Target	Order	Family	Genus	Species	Common name	FOO	%FOO
Plant	Malpighiales	Linaceae	Linum	*Linum lewisii*	Lewis' flax	2	1.5
Plant	Caryophyllales	Nyctaginaceae	Mirabilis	*Mirabilis sp.*	o'clock	2	1.5
Plant	Myrtales	Onagraceae	Oenothera	*Oenothera sp.*	evening primrose	2	1.5
Plant	Saxifragales	Grossulariaceae	Ribes	*Ribes sp.*	currant	2	1.5
Plant	Poales	Cyperaceae	Schoenoplectus	*Schoenoplectus sp.*	bulrush	2	1.5
Plant	Asterales	Asteraceae	Sonchus	*Sonchus sp.*	sowthistle	2	1.5
Plant	Dipsacales	Caprifoliaceae	Valeriana	*Valeriana edulis*	tobaccoroot	2	1.5
Arthropod	Diptera	Culicidae	Aedes	*Aedes increpitus*	woodland mosquito	2	1.1
Arthropod	Diptera	Anthomyiidae		*Anthomyiidae sp.*	muscoid flies	2	1.1
Arthropod	Lepidoptera	Tortricidae	Cochylis	*Cochylis n. sp.*	tortricid moth	2	1.1
Arthropod	Coleoptera			*Coleoptera sp.*	beetles	2	1.1
Arthropod	Coleoptera	Curculionidae		*Curculionidae sp.*	true weevils	2	1.1
Arthropod	Diptera	Cecidomyiidae	Dasineura	*Dasineura sp.*	plant midges	2	1.1
Arthropod	Lepidoptera	Noctuidae	Helotropha	*Helotropha reniformis*	kidney-spotted Rustic Moth	2	1.1
Arthropod	Hymenoptera			*Hymenoptera sp.*	sawflies, wasps, bees, ants	2	1.1
Arthropod	Hemiptera	Miridae	Lygus	*Lygus robustus*	leaf bug	2	1.1
Arthropod	Lepidoptera	Momphidae	Mompha	*Mompha sp.*	casebearer moths	2	1.1
Arthropod	Diptera	Mycetophilidae		*Mycetophilidae sp.*	fungus gnats	2	1.1
Arthropod	Lepidoptera	Lasiocampidae	Phyllodesma	*Phyllodesma americana*	American lappet moth	2	1.1
Arthropod	Lepidoptera	Gracillariidae	Phyllonorycter	*Phyllonorycter sp.*	moths	2	1.1
Arthropod	Diptera	Syrphidae	Platycheirus	*Platycheirus quadratus*	syrphid fly	2	1.1
Arthropod	Diptera	Tephritidae		*Tephritidae sp.*	fruit flies	2	1.1
Arthropod	Araneae	Theridiidae	Theridion	*Theridion neomexicanum*	cobweb spider	2	1.1
Arthropod	Lepidoptera	Tortricidae	Thyraylia	*Thyraylia sp.*	moths	2	1.1
Arthropod	Diptera	Tipulidae	Tipula	*Tipula sp.*	crane flies	2	1.1
Arthropod	Trombidiformes	Tydeidae		*Tydeidae sp.*	acariform mites	2	1.1
Arthropod	Araneae	Thomisidae	Xysticus	*Xysticus sp.*	ground crab spiders	2	1.1
Hypogeous fungus	Glomerales	Glomeraceae	Glomus	*Glomus macrocarpum*	false-truffle	2	1.5
Hypogeous fungus	Agaricales	Hymenogastraceae		*Hymenogastraceae sp.*	false-truffle	1	0.8
Hypogeous fungus	Boletales	Rhizopogonaceae	Rhizopogon	*Rhizopogon sp.*	false-truffle	1	0.8

Table 7. Existing microsatellite primers used for the New Mexico meadow jumping mouse in the White Mountains, AZ, multiplex cocktails, and genetic parameters (Sanchez 2021).

Locus	Cocktail	5' Label	Primer Sequence (5' to 3')	Allele size range	H_O	H_E	N_a	Source
ZhuC12	A	PET	**F:**GTGCCCATATACACAGACTCAC **R:**AGAGGGAGCATGAGAAGTTAAG	107-119	0.633	0.665	4	King, Eackles, et al., 2006
ZhuC120*	A	6FAM	**F:**AGGGTCATCCTTGGATAAATAG **R:**TCCTTATGTAAATGGTGCAGTGT	141-167	0.433	0.688	6	King, Eackles, et al., 2006
ZhuD07	A	PET	**F:**AGGTCAGAATGAGAGAACAAGAC **R:**GTATTTAGAGGCTTGGTTGACTC	153-186	0.752	0.776	9	King, Eackles, et al., 2006
ZhuD107	A	6FAM	**F:**CACCATGAAAACAAATCTCTTG **R:**ACATCCATAGGAAGAAAACAGAG	237-261	0.723	0.747	7	King, Eackles, et al., 2006
ZhuD109	A	NED	**F:**CCTCCTACCATCACAATACTCTG **R:**ACTCTGTCTGCCTTTTTGTCTC	136-157	0.644	0.715	6	King, Eackles, et al., 2006
ZhuD122	A	NED	**F:**TCCAGTTATCAGTAGCCATCTTC **R:**TTTGGGAACACTTAGCAGATTAG	240-268	0.703	0.851	8	King, Eackles, et al., 2006
ZhuC3	B	6FAM	**F:**AGCCTTCAGTCGGAATGAT **R:**GGGCCTTTAATCCCAGTAC	207-232	0.614	0.711	7	King, Eackles, et al., 2006
ZhuC6	B	VIC	**F:**ACCTGCTCTTTGGAACTTTC **R:**TCGGTCTGTTTGTTCAAGAC	128-140	0.248	0.247	4	King, Eackles, et al., 2006
Ztri2	B	PET	**F:**CCCAGCTTCTGTGGAAAGGC **R:**TGGCATATGAGAGCAGCAGAGTC	127-147	0.697	0.746	6	Vignieri, 2003

* indicates that locus was omitted from final analysis due to null alleles and deviations from HW expectations.

Table 8. Genetic parameters from New Mexico meadow jumping mouse populations in the White Mountains, AZ (assumed by watershed membership) for nuDNA and mtDNA (Sanchez 2021).

	nuDNA (microsatellite - 8 loci)						mtDNA (cyt B - 929 sites)					
Watershed	$N_{mean/locus}$	H_O	H_E	A_R	A_P	F_{IS}	N_{Seq}	*S*	Hap	Hd	π	*k*
BR	50.63	0.69	0.69	5.40	4	-0.027	35	7	4	0.52	0.0022	2.03
LCR	30.00	0.58	0.65	5.25	0	0.115	29	6	5	0.76	0.0024	2.26
SF	19.75	0.53	0.61	3.81	1	0.138	19	6	3	0.43	0.0016	1.49
Total	33.46	0.60	0.65			0.096	83	8	7	0.70	0.0029	2.73

$N_{mean/locus}$ (mean number of individuals per locus; H_O (mean observed heterozygosity); H_E (mean expected heterozygosity); A_R (rarified allelic richness); A_P (number of private alleles);F_{IS} (inbreeding coefficient);N_{Seq} (number of sequences); S (variable sites); Hap (number of haplotypes); Hd (haplotype diversity); π (nucleotide diversity); k (average number of differences).

Table 9. Pairwise F_{ST} (bolded, lower diagonal) and G'_{ST} (upper diagonal) among watershed populations of the New Mexico meadow jumping mouse in the White Mountains, AZ (Sanchez 2021).

	BR	LCR	SF
BR	-	0.165	0.249
LCR	**0.018**	-	0.167
SF	**0.037**	**0.017**	-

P-value < 0.05 for all pairwise comparisons based on 999 permutations.

Table 10. Analysis of molecular variance (AMOVA) results for watershed populations of the New Mexico meadow jumping mouse in the White Mountains, AZ (Sanchez 2021).

Source of variation	Df	SS	MS	Variance σ	Variance (%)	Φ	P-value
Among watersheds	2	64.858	32.429	0.417	6.547	0.065	0.001
Among samples within watersheds	98	629.103	6.419	0.467	7.339	0.079	0.001
Within samples	101	553.941	5.485	4.485	86.114	0.139	0.001
Total	201	1247.903	6.208	6.208	6.369	100.000	

Table 11. BAYESASS output showing mean posterior migration rates with 95% confidence intervals for the New Mexico meadow jumping mouse in the White Mountains, AZ. Bold values indicate non-zero rates of migration (self-migration excluded) and italicized values indicate asymmetry. This analysis only included specimens captured between 2015 and 2020 (Sanchez 2021).

Recipient population	BR	LCR	SF
BR	0.95 (0.9 to 1.0)	0.03 (-0.02 to 0.07)	0.03 (-0.01 to 0.06)
LCR	0.03 (-0.02 to 0.08)	0.78 (0.67 to 0.89)	***0.19 (0.06 to 0.31)***
SF	0.05 (-0.01 to 0.11)	0.02 (-0.01 to 0.05)	0.93 (0.87 to 1.0)

Table 12. Observed haplotypes for the New Mexico meadow jumping mouse in the White Mountains, AZ with aggregated specimen counts per haplotype and watershed (Sanchez 2021).

	Haplotypes (No. individuals)						
Watershed	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
BR	23	8	1	3	0	0	0
LCR	6	11	0	0	7	4	1
SF	1	14	0	0	0	4	0

Table 13. CytB assays for eDNA detection of the New Mexico meadow jumping mouse.

Assay	Primer pair	Amplicon length (bp)	Amplicon Melt Temperature (°C)
Short fragment	97F: CTCTTTTAGGAGCATGCCTAGCT 166R: TCGGCAAATATGGGTGACTGAAG	110	74.47
Long fragment	97F: CTCTTTTAGGAGCATGCCTAGCT 219R: GGAAGCTCCATTAGCGTGGAG	161	77.81

Table 14. Observed and expected values of true and false positive probabilities, from the model of Griffin et al. (2020).

Parameter	2.5 credible interval	Mean	97.5 credible interval	Expected value
Occurrence probability (ψ)	0.103	0.32	0.629	0.5
Sample true-positive probability (θ_{11})	0.085	0.275	0.505	0.9
Sample false-negative probability (1-θ_{11})		0.725		
Sample false-positive probability (θ_{10})	6.97E-05	0.008	0.044	0.1
qPCR replicate true-positive probability (p_{11})	0.407	0.669	0.897	0.9
qPCR false-negative probability (1- p_{11})		0.331		
qPCR replicate false-positive probability (p_{10})	3.42E-05	0.003	0.013	0.1

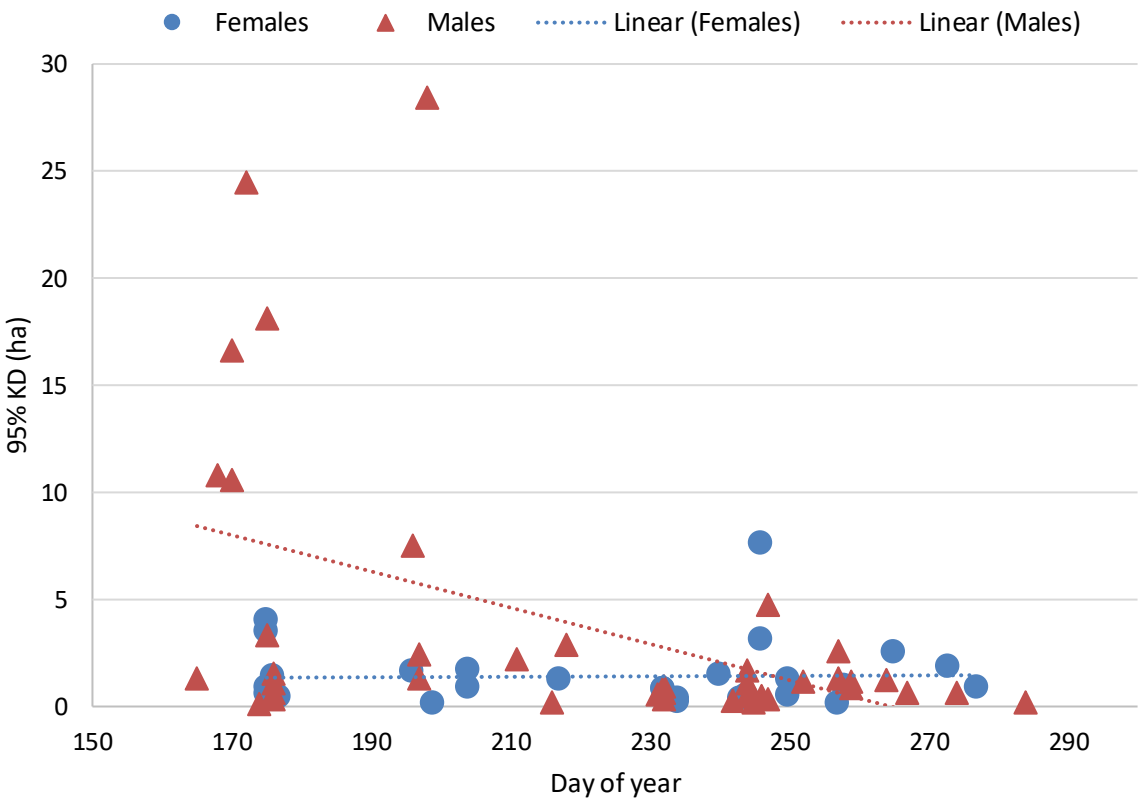

Figure 1. Kernel Density (95% Probability) estimate of home range sizes for 70 New Mexico jumping mice radio tracked during summers 2017, 2018, 2019, and 2020 on Apache-Sitgreaves National Forests, Santa Fe National Forest, Arizona Game and Fish Department property, and Colorado lands. Linear trend (dashed) lines indicate a decrease in size of home ranges from June to October for males but not for females. For Day of year, 1 June = 152; 1 October = 274.

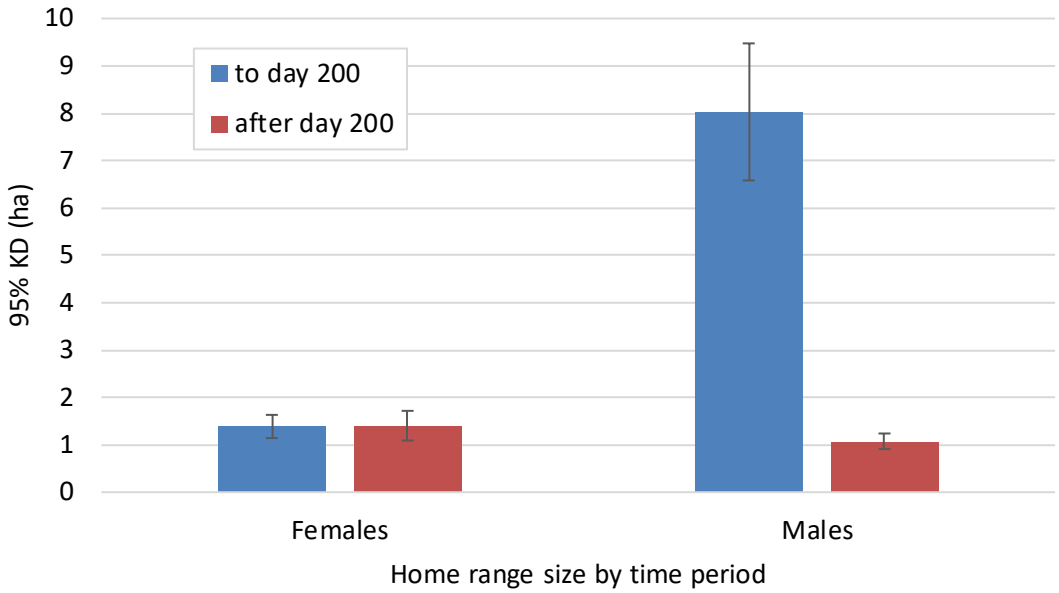

Figure 2. Kernel Density (95% Probability) estimate ± SE of home range sizes for 70 New Mexico jumping mice radio tracked during summers 2017, 2018, 2019, and 2020 on Apache-Sitgreaves National Forests, Santa Fe National Forest, Arizona Game and Fish Department property, and Colorado lands. For males, home range sizes between emergence (May / early Jun) and 19 July (Day 200) were 8 times larger than home ranges from 20 July to immergence (late Aug to Oct). Home ranges for females showed little variation throughout the active period.

A.

B.

Figure 3. Hibernaculum location for female New Mexico meadow jumping mouse (frequency 150.380 and 150.299) on 1 Oct on the San Francisco River (12S 667777, 3748330), 5.5 m from the stream, Apache-Sitgreaves National Forests, 2019. A. Technician Jordyn Gladden points to loudest signal from the radio collar. B. Strongest signal was at a grass patch indicated by blue arrow.

A.

B.

Figure 4. Hibernaculum location for female New Mexico meadow jumping mouse (frequency 150.380 and 150.299) on 1 Oct on the San Francisco River (WGS84 12S 667777, 3748330) excavated 30 Jun 2020, Apache-Sitgreaves National Forests. A. Blue arrow points to the cavity where we found the nest; the cavity was created partially adjacent to and below a tree root. The cavity was 10.5 cm in diameter and 15 cm below ground. Depth of soil to top of the nest material was 6.5 cm. B. The animal had constructed a nest from vegetation (grasses, Gambel oak [*Quercus gambelii*] leaves).

A.

B.

C.

Figure 5. Hibernaculum location (under orange arrow) for male New Mexico meadow jumping mouse (frequency 151.619) on 27 Aug on the East Fork Little Colorado River (12S 639604, 3755227), 100 m from the stream. We detected the animal both by the telemetry signal from his collar and from his PIT tag. A. The white tube contains soil temperature sensors and is ~2 m from suspected hibernaculum. B. The hibernaculum was outside the exclosure, in grasses, 100 m from the stream. C. The animal was not in a day nest on the surface level but below ground. Evidence of arousal from torpor (on 29 Aug and 7 Sep) the animal arousing and pushing soil to seal the entrance to the burrow) indicated by fresh soil. The animal was detected from its PIT tag in its hibernaculum on 25 May 2021.

Figure 6. Hibernaculum location (under blue arrow in shrubs) for male New Mexico meadow jumping mouse (frequency 148.689, 150.306) on 28 Sep on the Florida River, Colorado, 8 m from the stream. We detected the animal both by the telemetry signal from his collar and from his PIT tag. The animal was not in a day nest on the surface level but below ground.

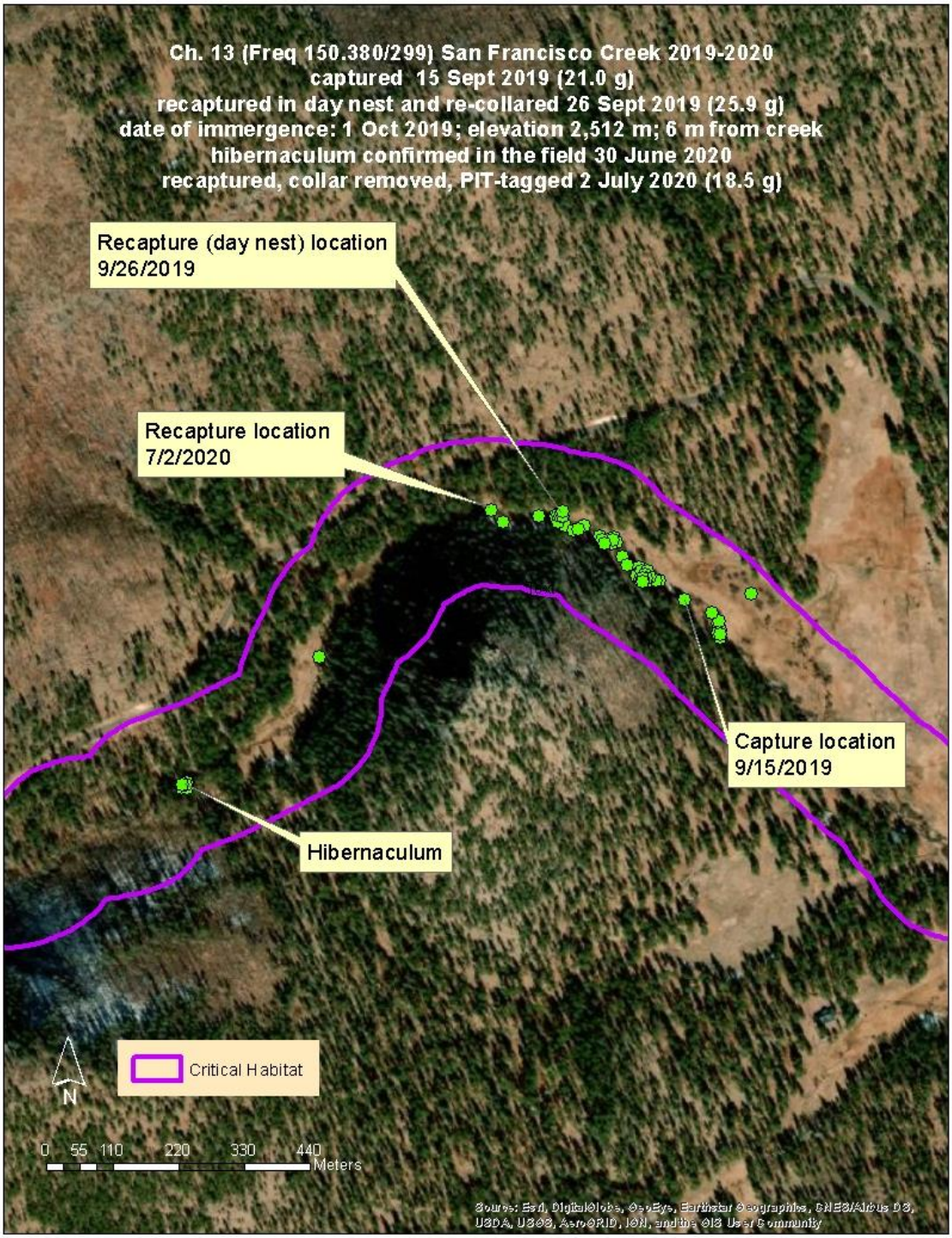

Figure 7. Green circles represent 2019 capture location, fixes taken for home range calculation (2019), hibernaculum location (2019-2020), and 2020 recapture location for female New Mexico meadow jumping mouse (frequency 150.380 and 150.299) on the San Francisco River, Apache-Sitgreaves National Forests. This hibernaculum was within designated critical habitat (purple lines).

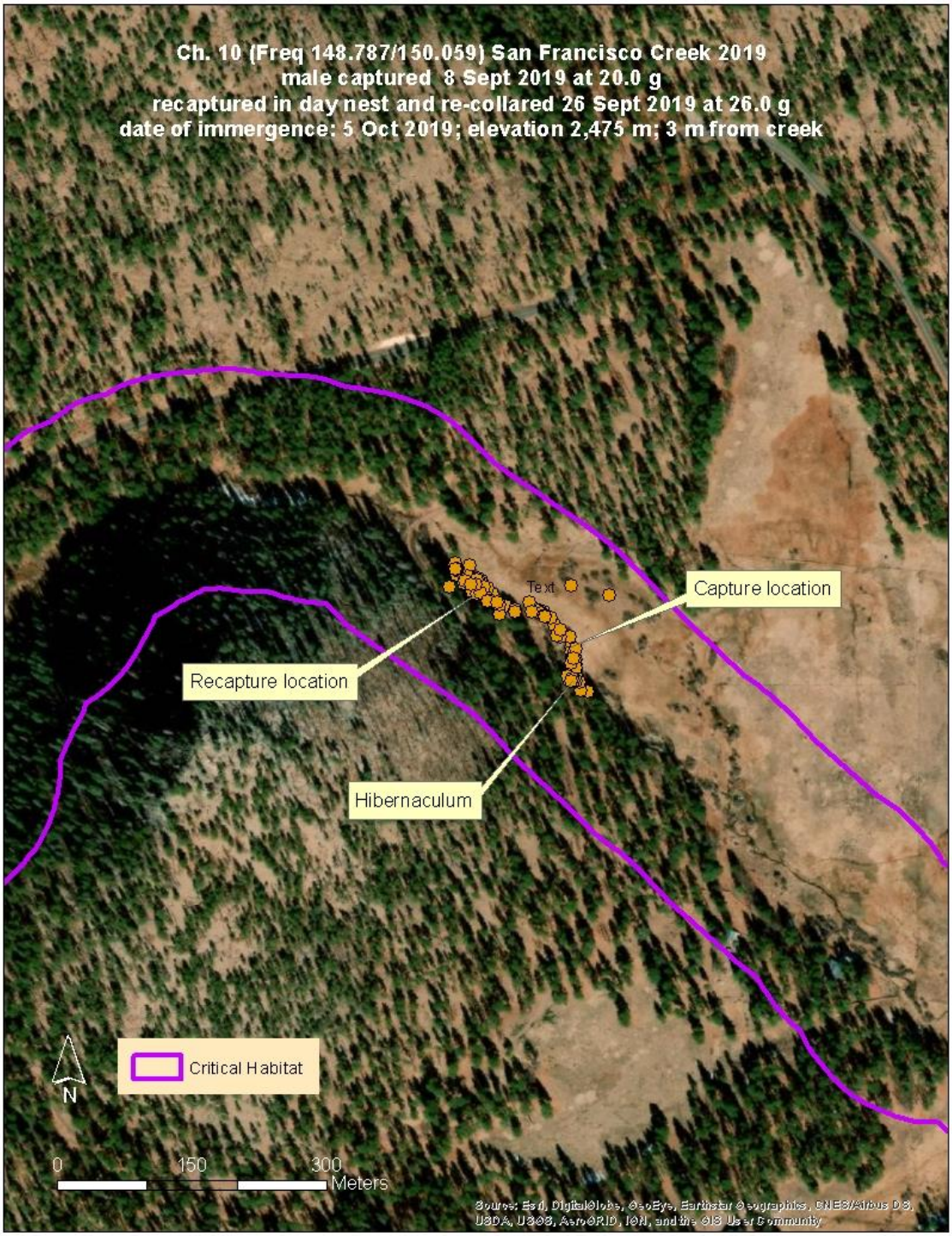

Figure 8. Orange circles represent capture location, fixes taken for home range calculation, presumed hibernaculum location for male New Mexico meadow jumping mouse (frequency 148.787 and 150.059) on the San Francisco River, Apache-Sitgreaves National Forests, 2019. This hibernaculum was within designated critical habitat (purple lines). We were unable to confirm the exact location underground of the hibernaculum.

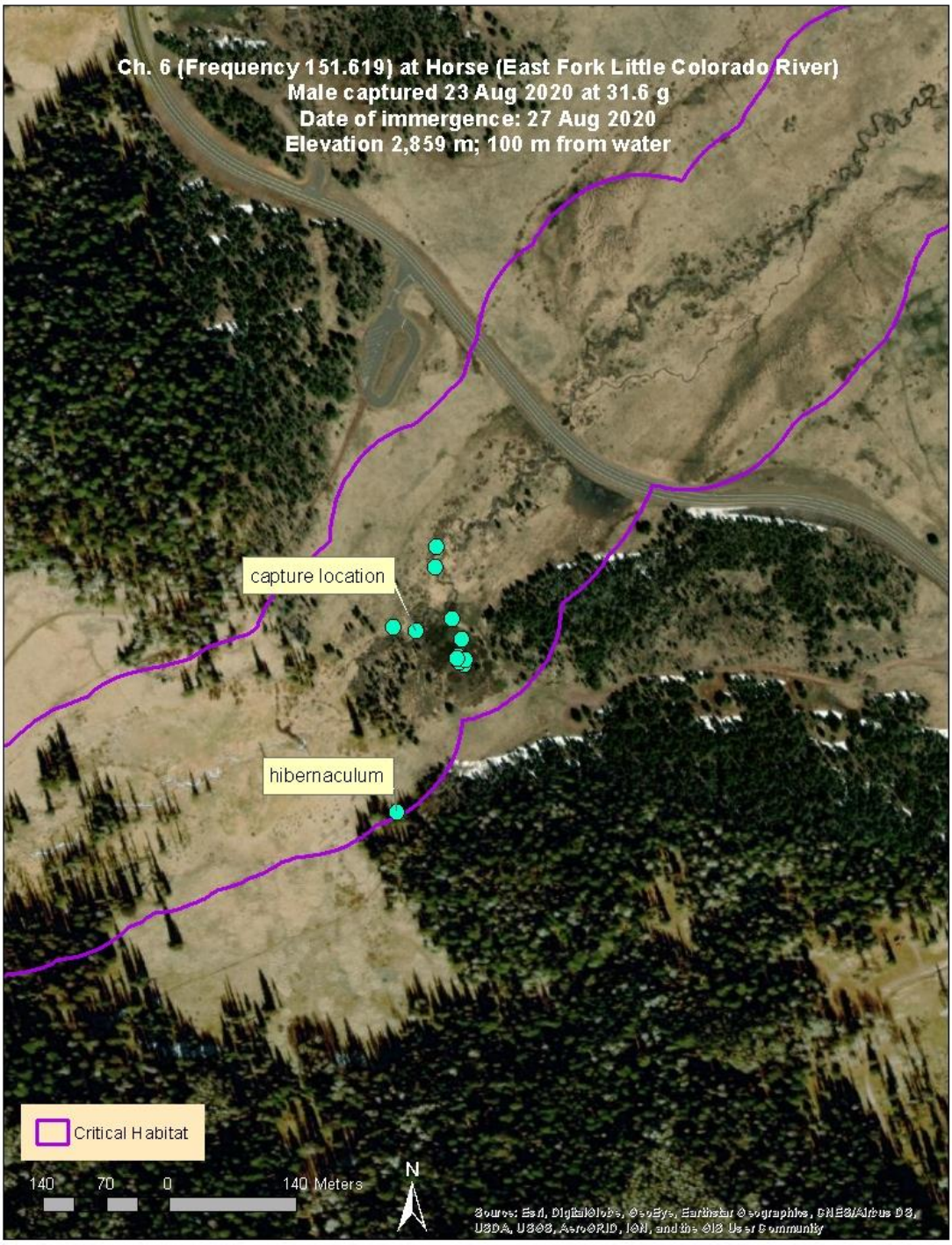

Figure 9. Blue circles represent capture location, fixes taken for home range calculation, and hibernaculum location for male New Mexico meadow jumping mouse (frequency 151.619) on the East Fork Little Colorado River, Apache-Sitgreaves National Forests, 2020. This hibernaculum, at ~100 m from the stream, was just within designated critical habitat (purple lines).

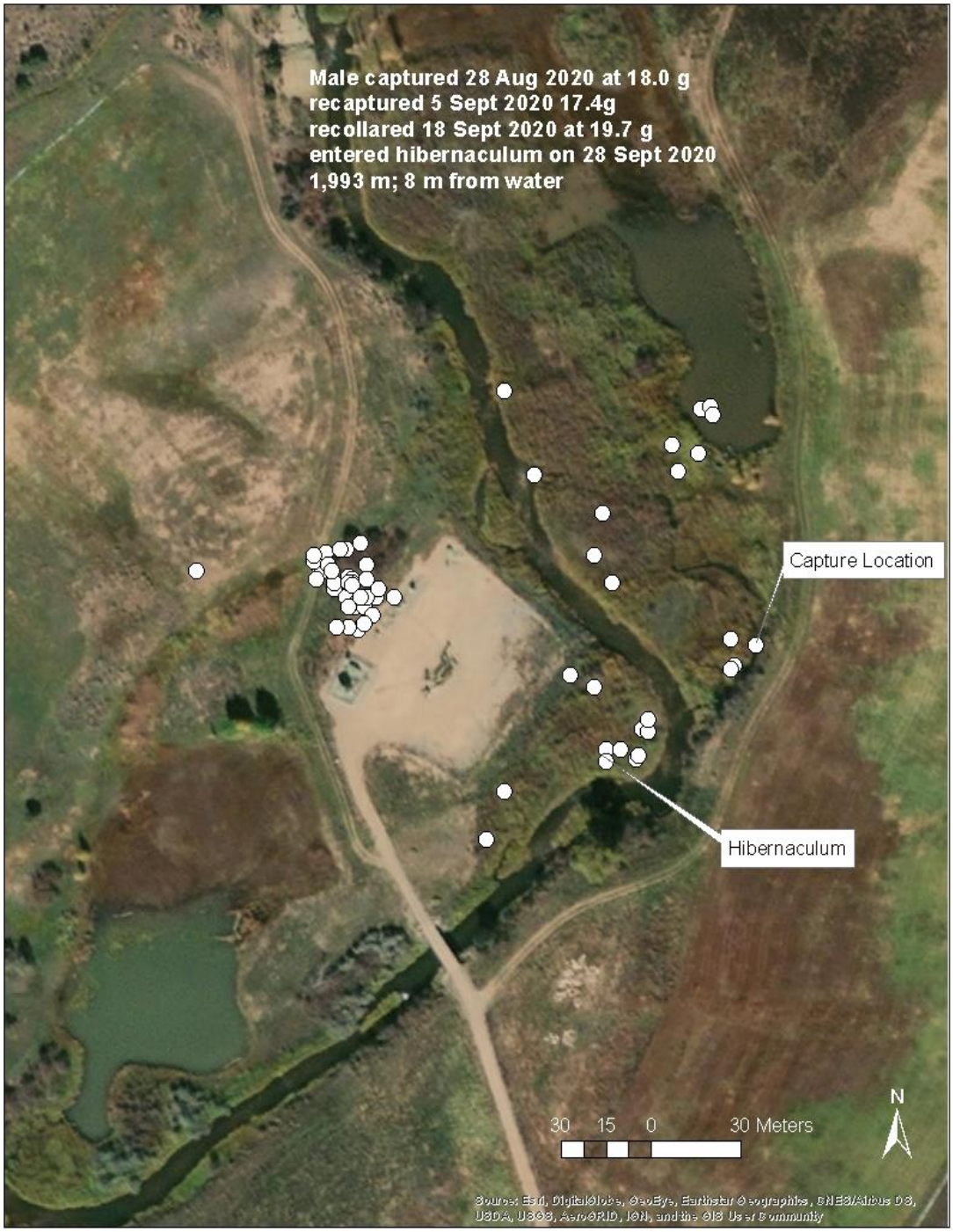

Figure 10. White circles represent capture location, fixes taken for home range calculation, and hibernaculum location for male New Mexico meadow jumping mouse (frequency 148.689, 150.306) on the Florida River, Southern Ute Indian Reservation, 2020. Critical habitat is not designated on tribal lands, but the hibernaculum was 8 m from water.

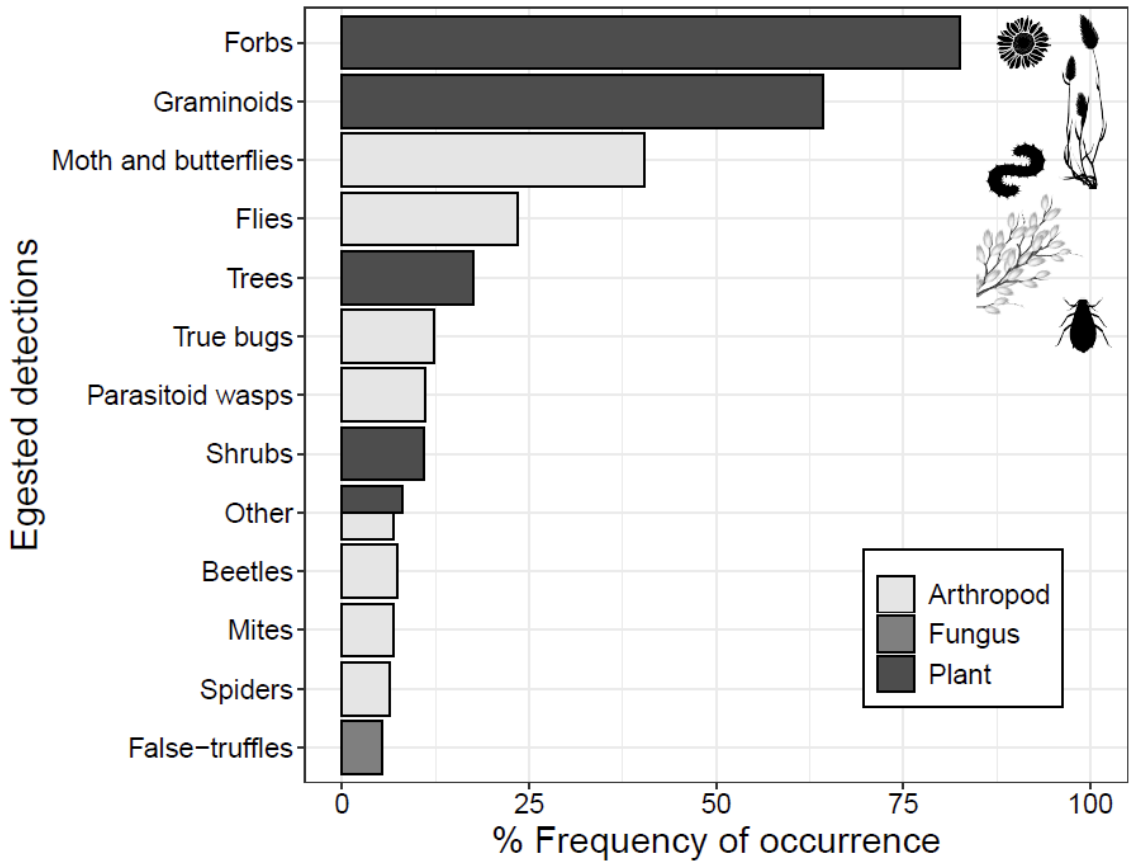

Figure 11. Frequency of occurrence of broader dietary groupings (among all metabarcoding markers) that were detected in *Z. h. luteus* (n=145 individuals across the subspecies range) (Sanchez 2021).

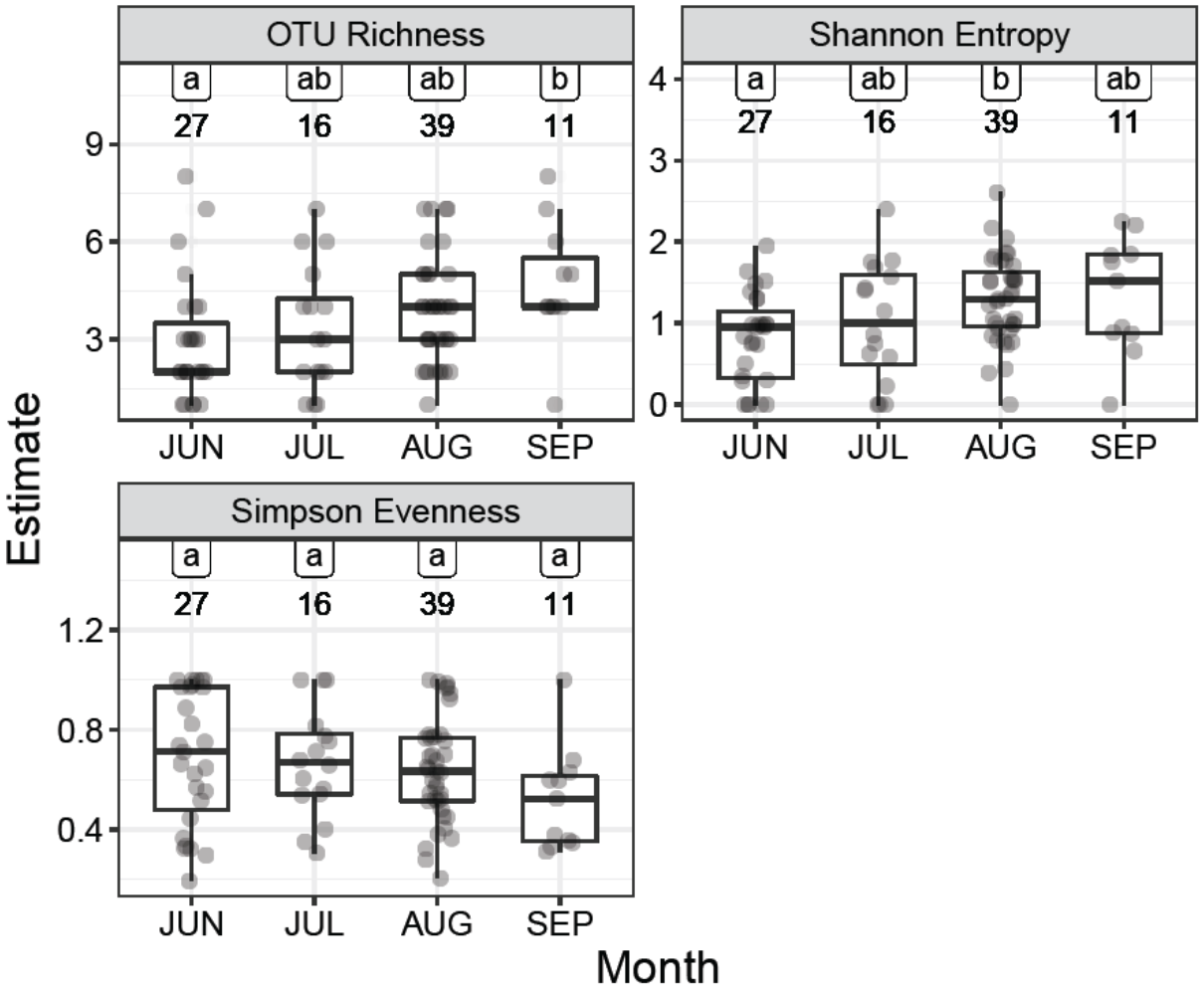

Figure 12. Alpha diversity comparisons for *Z. h. luteus* among month groupings. Differing letter codes within a metric indicate significant groupings ($p < 0.05$) following post-hoc comparison from a Kruskal-Wallis analysis of variance (Sanchez 2021).

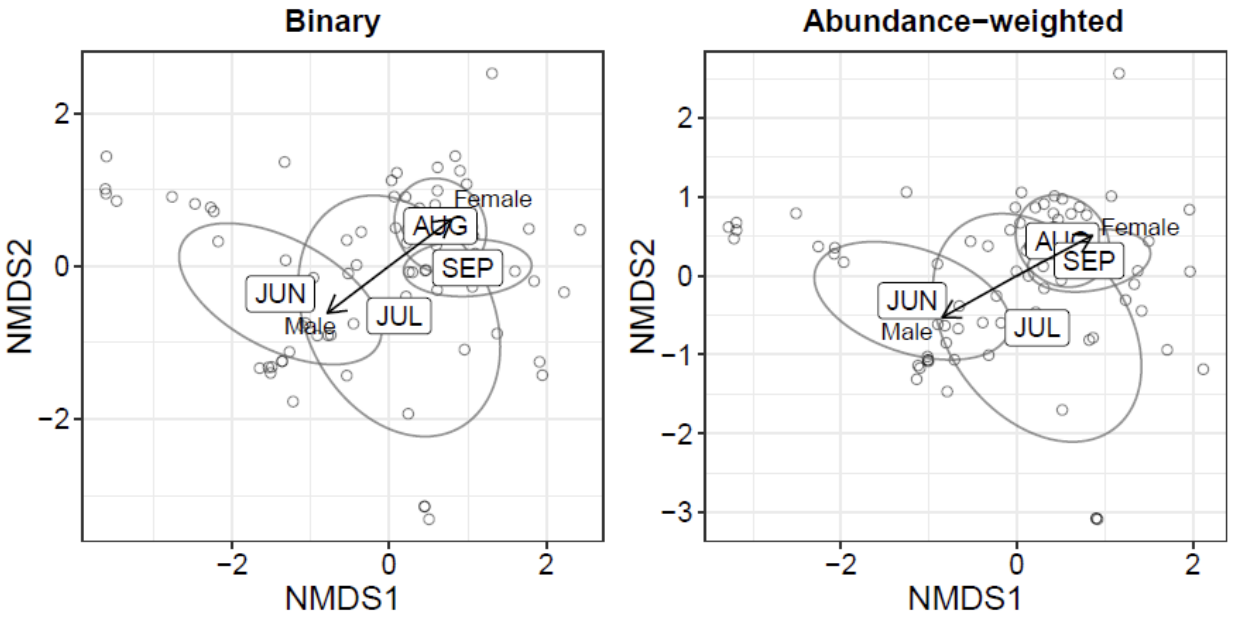

Figure 13. NMDS ordinations for *Z. h. luteus* with standard deviation ellipses around month groupings and correlation vectors for sex. The binary ordination is based on Jaccard dissimilarity, whereas the abundance-weighted ordination is based on Bray-Curtis dissimilarity (Sanchez 2021).

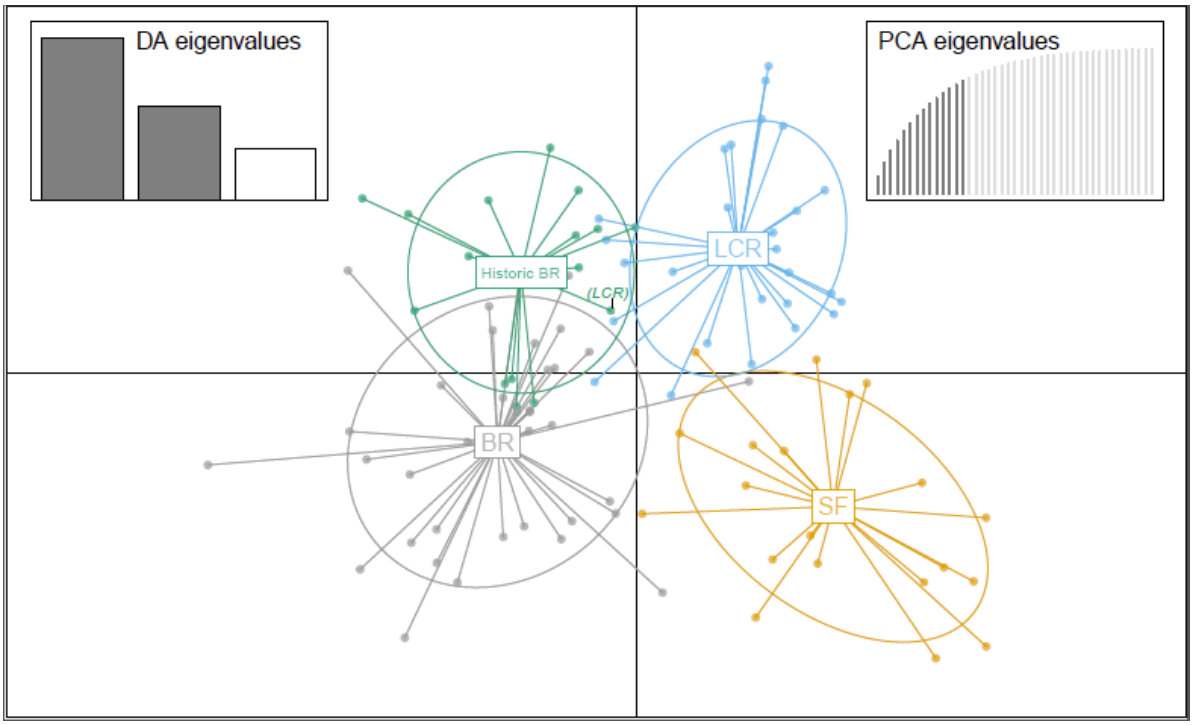

Figure 14. Time and space variation of a scatter plot for DAPC analysis for all New Mexico meadow jumping mice sampled between 1963 and 2020 (White Mountains, AZ). This plot shows relationship of groupings based on watershed membership (indicated by color, centroid, and inertia ellipses). Individuals are represented as points. The "Historic BR" grouping contains all historical individuals (1963-1991), largely captured in the BR watershed but at different localities than contemporary individuals (note that one individual in this grouping is from the LCR) (Sanchez 2021).

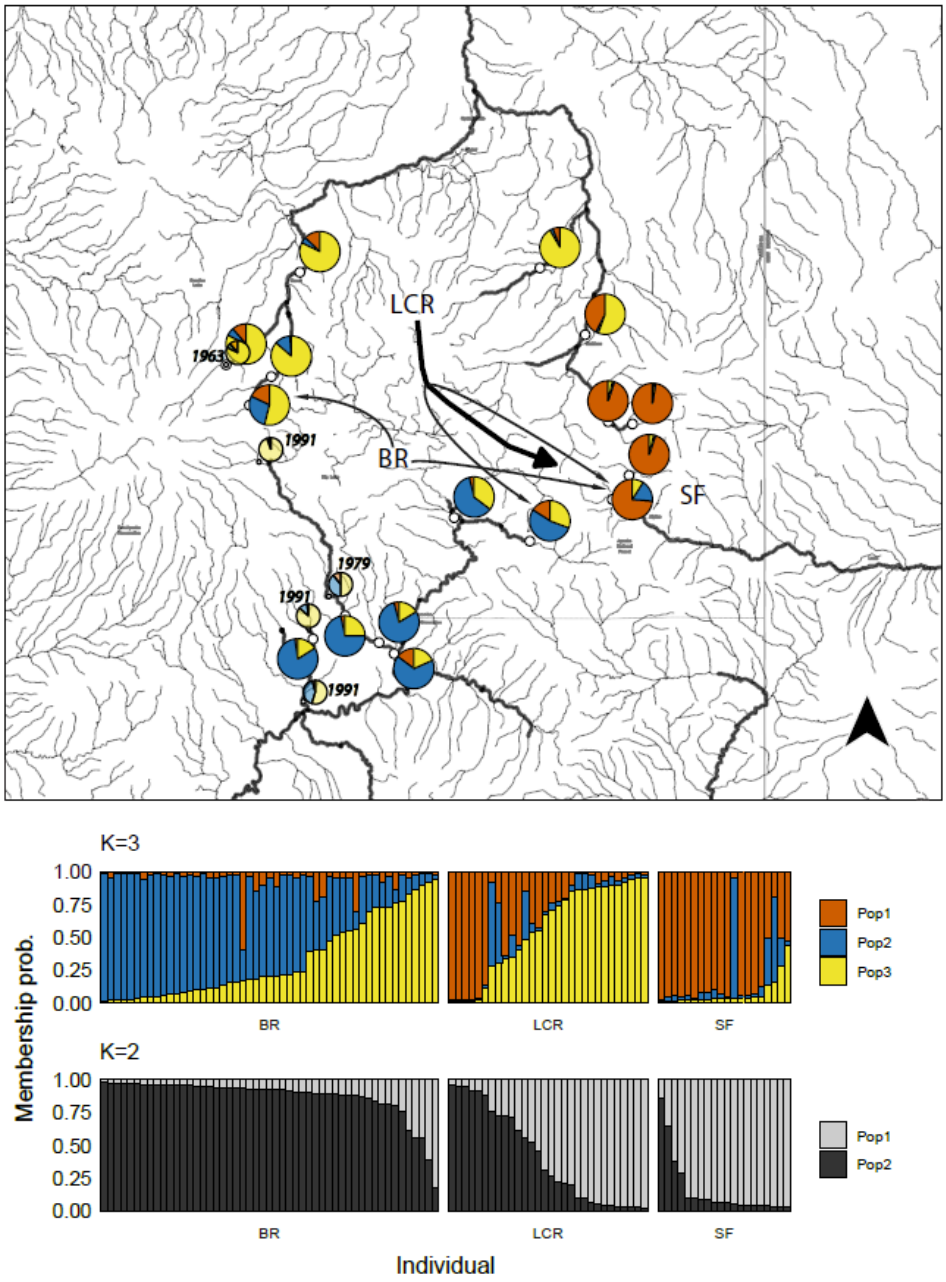

Figure 15. Results from STRUCTURE (K=3 and K=2), BAYESASS, and assignment tests (GeneClass2) for New Mexico meadow jumping mice in White Mountains, AZ. The map shows mean Q score per individuals in a locality (STRUCTURE). Each pie is a site and each slice is the proportion of membership to an inferred cluster (K=3 shown). Smaller transparent pies are historical samples (1963-1991) and are superimposed if the locality is the same as contemporary. The bold arrow represents a significant migration rate from BAYESASS (95% confidence intervals did not overlap 0), whereas the thin arrows represent a potential dispersal event to a particular locality among watersheds. The bottom diagrams are individual membership probabilities (Q score) to an inferred cluster in STRUCTURE where each bar is an individual (Sanchez 2021).

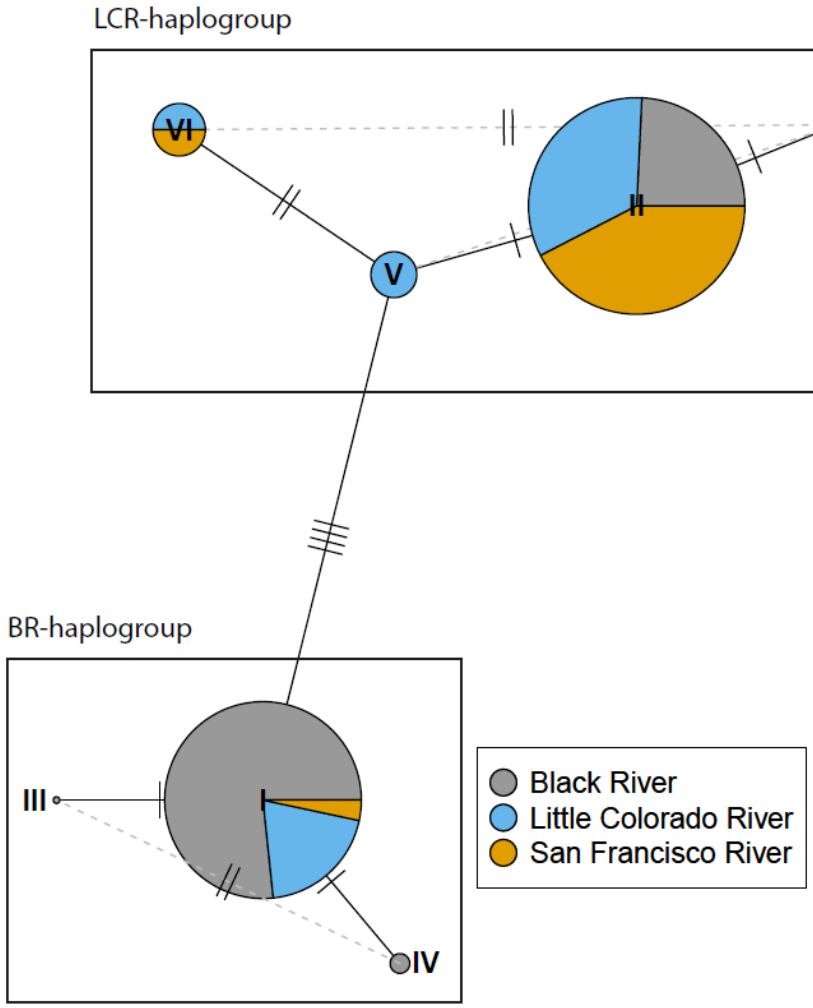

Figure 16. Haplotype network for individual New Mexico meadow jumping mice, sampled among different watersheds in the White Mountains, AZ. Boxes around a group of haplotypes bound hypothesized haplogroups. The size of a pie represents the frequency of individuals within haplotype, color-coded by the watershed that an individual was captured from. Haplotypes are represented by Roman numerals, the number of perpendicular dashes along linkages indicate number of nucleotide differences between two haplotypes, and the lighter dashed branches indicate alternative linkages (Sanchez 2021).