Diet, disease, and hibernation behavior of northern Idaho ground squirrels

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Authorization to submit dissertation

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Abstract

Anthropogenic changes have caused wildlife extinctions across the world. Climate change, invasive species, habitat destruction, and human introductions of non-native diseases often have interactive effects and pose challenges to endemic wildlife populations. Understanding the processes that constrain population abundance is essential for effective management of rare species. The northern Idaho ground squirrel (*Urocitellus brunneus*) is a threatened species that persists in a relatively small number of isolated populations within 0.05% of their former range. The primary hypothesis for their decline is due to years of fire suppression that enabled conifer trees to encroach on the open spaces these squirrels occupy. However, this habitat loss hypothesis may not be the only cause for the species decline. We tested 3 alternative hypotheses that may also be suppressing population growth of this threatened ground squirrel: (1) sylvatic plague hypothesis, (2) food limitation hypothesis, and (3) climate sensitivity hypothesis. The sylvatic plague hypothesis suggests that plague may be directly decreasing abundance of northern Idaho ground squirrels. To test this hypothesis, we combed and removed fleas from northern Idaho ground squirrels and 3 coexisting small mammal species (*Urocitellus brunneus, Tamias amoenus*, and *Peromyscus maniculatus*). We identified 7 species of fleas; all 7 species are known to be capable of transmitting plague and 3 of the 7 species were collected from all 4 small mammal species (i.e., 3 species of fleas can readily transmit plague across these 4 sympatric small mammal species). We also designed an experiment where we removed fleas from paired and un-paired sites to test an explicit prediction of the plague hypothesis: that animals without fleas will have less opportunity to get plague and therefore have higher survival than control animals. We found that survival was between 2% and 50% greater (depending on species and study design) for flea-removal animals compared to un-treated (control) animals for all 4 species of small mammals in both the paired and un-paired flea-removal studies. We found the same pattern (higher survival for animals in flea-removal treatments) for all 4 species in both experimental designs, suggesting that plague is present and negatively impacting survival of northern Idaho ground squirrels and coexisting small mammals. The food limitation hypothesis is a more explicit, mechanistic version of the fire suppression hypothesis and suggests that shifts in plant community composition (from fire suppression) may be limiting certain key nutrients required by northern Idaho ground squirrels and the limited availability of these key nutrients has caused the past population declines in squirrels. Northern Idaho ground squirrels appear to be selective feeders (they consume some plants more often than they are found in their environment). In addition, survival was higher for squirrels consuming *Perideridia* sp. in the summer and lower for those consuming *Frasera* sp. The climate sensitivity hypothesis suggests that thermal conditions are no longer ideal for northern Idaho ground squirrel overwinter survival due to reductions in snow pack. We documented the habitat features that influence winter season (hibernacula) and summer season (active season) habitat selection in northern Idaho ground squirrels. We found that northern Idaho ground squirrels preferred hibernacula locations which had steeper slopes, higher canopy cover, and more trees than the areas that they used during the summer active season. Survival was greater for squirrels that hibernated near logs, and squirrels that hibernated in locations with more snow (duration and depth) emerged later in the spring (a behavior that likely improves survival). Overall, results from all 3 of these alternative hypotheses point to areas of concern for populations of northern Idaho ground squirrels. However, management solutions are possible to address these concerns and hopefully lead to population increases and eventual recovery of the species.

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Dedication

I want to dedicate my dissertation to my mother, Phyllis Emsig. She was incredibly supportive of me and my career choices along the way (even though I know she was always nervous about me being out in the "middle of nowhere"). She was always available by phone or in person when I needed her (even when times were difficult) and I could not have done this without her.

In addition, I want to thank my father (Mark Goldberg) and sister (Brianna Goldberg) for their time and support over the past few years. Thank you for making it possible for me to live so far away and for always being ready for a great meal or movie night when we were in the same place at the same time. I want to thank all my family, friends, and colleagues who have helped me grow both personally and professionally during my time here at the University of Idaho.

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Chapter 1: Are fleas shared among coexisting small mammal hosts? Implications for plague transmission

Abstract

For vector-borne diseases, the abundance and competency of the vector will impact the ability of the vector to transfer the disease among hosts. Sylvatic plague is a non-native vector-borne disease caused by the bacterium *Yersinia pestis*, whose main vector is the flea. Sylvatic plague is present in much of the western United States and impacts several small mammal species. Plague may be suppressing northern Idaho ground squirrel (*Urocitellus brunneus*), a threatened species, population growth, if a competent vector community is allowing plague to be maintained within the remaining several dozen sites that support the rare ground squirrel. We collected fleas from northern Idaho ground squirrels and 3 coexisting small mammals to evaluate which flea species were present at occupied sites and whether fleas were shared among the small mammal community. We documented 7 species of fleas from 3,356 fleas collected from the 4 species of small mammals. All 7 species of fleas are known vectors of plague. We used generalized linear mixed models to evaluate which abiotic and biotic factors influence flea abundance on northern Idaho ground squirrels, Columbian ground squirrels (*Urocitellus columbianus*), and yellow-pine chipmunks (*Tamias amoenus*). Factors that impacted flea abundance varied by host species, but flea abundance: 1) changed over the summer, 2) was greater on males, and 3) increased if the previous summer had above average precipitation. Our results suggest the flea community has the capacity to transfer plague among the 4 small mammal host populations if *Yersinia pestis* is present. Furthermore, some sites may be more susceptible to the disease than others because they have higher flea abundances and such high-risk sites can be identified based on their abiotic and biotic factors.

Keywords: abundance, flea community, northern Idaho ground squirrel, vector-borne disease, *Yersinia pestis*.

Introduction

Wildlife diseases are recognized as one of the leading causes of species declines (Wilcove et al. 1998) but their effects may be underrepresented in the literature (Scott 1988, Smith et al. 2009). Parasites are vectors for many vector-borne diseases impacting both human and wildlife health. Fleas are parasites and known vectors for a number of harmful bacteria such as *Yersinia pestis, Rickettsia felis,* and *Bartonella spp*. (Bitam et al. 2010) which are all associated with both human and wildlife diseases. The abundance of the vector, the host, and the bacteria all affect the incidence of disease (Davis et al. 2004, Keesing et al. 2006, Tripp et al. 2009). Furthermore, the competency of the vector will impact its ability to transfer the bacterium between itself and the host (Ostfeld and Keesing 2000, Eisen et al. 2009). Hence, the risks of vector-borne diseases are influenced not only by the pathogen itself (i.e., the bacteria), but also by traits of the host and the vector. For example, some pathogens are transmitted by a single vector whereas other pathogens have several species that serve as vectors. The number and identity of the vectors that play a role in the transmission process is important, particularly if those vectors are parasitic on several hosts (e.g., if a vector-borne bacterium frequently moves between sympatric hosts in an ecosystem).

The relationship between the host and the flea may play an important role in understanding the risk of transmission of vector-borne diseases. Both abiotic and biotic factors influence the richness and abundance of the flea community. Numerous intrinsic and extrinsic factors can impact species richness, diversity and/or abundance of fleas, such as: 1) host community diversity (Maher and Timm 2014, Young et al. 2015), 2) host body condition (Eads et al. 2016*a*), 3) host density (Krasnov et al. 2002*b*, Stanko et al. 2002, Young et al. 2015), 4) precipitation (Young et al. 2015, Eads and Hoogland 2016, 2017), 5) air temperature (Stenseth et al. 2006), and 6) elevation (Maher and Timm 2014). Understanding the factors that affect the flea community may help us better comprehend where and when both humans and wildlife are most at risk of infection of vector-borne diseases.

Fleas are considered the main vector of sylvatic plague (Gage and Kosoy 2005). Moreover, numerous species of fleas can serve as vectors, and the efficiency of plague transmission differs among flea species (Wheeler and Douglas 1945, Burroughs 1947). These characteristics create challenges for fully understanding the scope of the disease transmission web. Plague is an extremely virulent disease (Lorange et al. 2005) and abundance and species composition of fleas (among other factors) likely affects the persistence and spread of plague in an area. Despite this, we know surprisingly little about the vectors that transmit plague. Approximately 230 species of fleas have been identified in the western U.S., but only a handful have been tested to evaluate whether they serve as vectors for plague (Eisen and Gage 2012) and such studies have primarily focused on prairie dogs (*Cynomys* sp.). However, many other host species are adversely affected by plague and we know very little about their flea communities and whether fleas are shared among sympatric rodents.

Northern Idaho ground squirrels (*Urocitellus brunneus*) were listed as threatened under the federal Endangered Species Act in 2000 (U.S. Fish and Wildlife Service 2000). Northern Idaho ground squirrels persist in isolated populations within a small fraction of their former range. The primary cause(s) of the population decline is not known, but one proposed hypothesis is the increased mortality caused by the introduction of plague. Furthermore, the leading hypothesis for the ground squirrels decline is habitat loss (U.S. Fish and Wildlife Service 2000). Pathogens can interact with other factors (such as habitat loss and climate change) further reducing populations (Smith et al. 2009). Heard et al. (2013) found that species at risk due to factors other than disease, were also those most likely to be simultaneously threatened by disease. Any reduction in population size is especially concerning for a species of conservation concern and the need to understand and manage to minimize a threat is of great importance.

Plague affects at least 203 rodent species (Gage and Kosoy 2005), but not all of these species are effective hosts that help maintain the persistence of plague in an ecosystem. Hence, it is important to not only understand the flea community of northern Idaho ground squirrels, but also that of the coexisting small mammals (i.e., species that may act as the main host for the disease within the ground squirrel's restricted distribution). Plague may persist in a more common species (e.g. one that is the primary host that maintains the disease) and periodically spill over into northern Idaho ground squirrel populations. Northern Idaho ground squirrels are a threatened species and exist in small isolated populations. A sympatric (and more abundant) species may be the primary host for the disease (if it is indeed present in the system). It is possible that northern Idaho ground squirrel populations exist in such small, isolated populations, that plague may only occasionally be introduced into many of these isolated populations at the same time, resulting in large declines of the population as a whole. In addition or alternatively, if density is low, transmission between host species may be lower because these squirrels would be less likely to come into contact with infected hosts (Eisen and Gage 2009). Hence, we sought to document flea abundance and the differences in the flea communities between and among northern Idaho ground squirrels and 3 sympatric rodents: Columbian ground squirrels (*Urocitellus Columbians*), yellow-pine chipmunks (*Tamias amoenus*), and deer mice (*Peromyscus maniculatus*). All 4 of these species occupy burrows and sometimes visit burrows of the other 3 species (personal observation) which provides a possible mechanism for transfer of fleas among species.

Our primary objective was to test whether the suite of flea species on northern Idaho ground squirrels overlapped with those from any of 3 coexisting (and more abundant) small mammals: deer mice, yellow-pine chipmunks, and Columbian ground squirrels. We also examined the effect of 10 biotic and abiotic factors on the abundance of fleas on small mammals in our system. Previous research in other systems have found varying effects of biotic and abiotic factors on flea abundance and it may be that flea abundance is system specific (Stanko et al. 2002, Amatre et al. 2009, Young et al. 2015, Eads and Hoogland 2017).

Materials and Methods

Study area

We sampled fleas on northern Idaho ground squirrels and 3 coexisting small mammals at 19 study sites between April and August during 2013-2017 (not all sites were sampled all 5 years). All 19 sites were in the Payette National Forest, Adams County, Idaho between 1,200 and 1,700-m elevation. Distance to the next-closest study site (i.e., degree of isolation) varied among the 19 sites from 147-m to 6,449-m to the nearest site (mean 1,868-m). The sites were in remote areas with low human densities: ~4000 people live in Adams County and our 17 of the 19 study sites are 9-35 km as the crow flies from the nearest town on gravel roads except for 2 sites; one which is ~6 km from the town of New Meadows, Idaho close to a state highway and the other is less than 1 km from a wood mill. Vegetation at the sites varied from mesic and xeric open meadows to forested areas dominated by ponderosa pine (*P. ponderosa*) or Douglas fir (*P. menziesii).*

Capture methods

We used Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, WI, USA) to trap northern Idaho ground squirrels, Columbian ground squirrels, and yellow-pine chipmunks. The size of the trapping grids at the 19 sites varied between 1.5-ha and 9.2-ha. We baited the traps with an oat, peanut butter, and vanilla mixture. We checked traps every ~15 minutes at the 16 sites that supported northern Idaho ground squirrels and every 2 hours at the 3 sites that did not support northern Idaho ground squirrels (only coexisting small mammal species). The effort (the length of time that traps were open) varied depending on logistics and weather conditions. At each site, we placed traps in a grid and the density of traps with the grid varied among sites (3.75/ha -18.00/ha). Trap density varied among the field sites because: (1) sites containing northern Idaho ground squirrels had to have fewer traps so technicians could check them more often, (2) some sites were simultaneously being used for another study so trap density had to remain at a previously established quantity, and (3) we received more traps part-way into the study which enabled us to increase trap densities at our non-northern Idaho ground squirrel sites. We also used a focal trap method to catch northern Idaho ground squirrels (at the 16 sites where they occurred): following squirrels to a burrow, plugging all nearby surrounding burrows, and placing a specially designed focal trap over the only remaining opening. We watched focal traps closely and the trap was removed if a northern Idaho ground squirrel did not enter the trap within ~1 hour. We ceased trapping if the ambient temperature rose above 27°C. We also set 49-56 Sherman traps (HB Sherman Inc., Tallahassee, FL, USA) at 5 sites and placed a small ball of polyester fill for bedding in each trap every night. Traps were evenly spaced in an established grid 20m apart. We opened Sherman traps in the evenings (after sunset at sites with northern Idaho ground squirrels present and after 5:00pm at sites without northern Idaho ground squirrels present).

We trapped sites during two seasons (spring: April-early June and summer: June-August). We trapped squirrels for 3-4 days within each season at each site for both the Tomahawk and Sherman traps.

We anesthetized a subset of the captured animals to collect fleas by placing them in a chamber (size of chamber varied by species based on body size) with a cotton ball dosed with 0.4 – 1.3mL of isoflurane. Dose of isoflurane depended on animal sex, size, and species. We removed anesthetized animals from chambers and combed for 30 seconds such that dislodged fleas fell into a white dish. We anesthetized and collected fleas from an individual only once per season (some individual animals were trapped and anesthetized multiple times throughout the study but never twice within the same season). We anesthetized 10-20 adult animals (and both juvenile and adult deer mice) of each species within each site and season (spring and summer). We did not anesthetize any female northern Idaho ground squirrels in the spring trapping sessions. We removed anesthetized animals from chambers and combed for 30 seconds such that dislodged fleas fell into a white dish. We used either metal ear tags (National Band and Tag Co., Newport, KY, U.S.A) or Passive Integrated Transponder (PIT) tags (Biomark Inc., Boise, ID, U.S.A) to individually mark new captures and to identify recaptured individuals. We recorded the sex, reproductive condition (scrotal, pregnant, lactating, or none), and 2 body measurements (foot length and total body length) for each individual captured. We placed animals back into traps until they fully recovered from anesthesia. We released all captured individuals back to the initial trapping location. We used forceps to collect and count fleas. We placed fleas in vials filled with 70% ethyl alcohol for later identification. All trapping and processing was conducted in accordance with protocols approved by the University of Idaho Institutional Animal Care and Use Committee (#2015-53).

Flea identification and diversity

We followed Hubbard (1968) to identify fleas to species. We identified all the fleas within a vial that we could (occasionally a flea was in too poor condition to be identified either due to dehydration or missing parts from handling). We assigned the species to currently accepted names according to Lewis (1988). We were unable to process and identify all of the 7,616 fleas that we collected due to lack of funding/personnel and had to selectively choose which subset of fleas to identify in the lab. We identified all the fleas we collected from 7 of the 19 study sites that we sampled. We prioritized those 7 study sites based on 3 criteria: 1) sites that contained northern Idaho ground squirrels, 2) sites that varied regarding which of the 3 other small mammals were present, and 3) sites that were spread out spatially. We identified 3,556 fleas (46.7% of the total fleas collected) from 676 individual animals (39 deer mice, 271 yellow-pine chipmunks, 216 northern Idaho ground squirrels, and 150 Columbian ground squirrels). We used these data to summarize the number of different flea species on each host species and the number of flea species shared among the 4 host species.

We used the vegan package (Oksanen et al. 2017) in program R (R Core Team 2017) to compare the extent of dissimilarity in flea species between northern Idaho ground squirrels and each of 3 coexisting small mammals: 1) northern Idaho ground squirrels versus Columbian ground squirrels, 2) northern Idaho ground squirrels versus yellow-pine chipmunks, and 3) northern Idaho ground squirrels versus deer mice. We used an abundance-based Morisita-Horn dissimilarity index (Chao et al. 2006) to compare all samples combined for each of the 3 pairs of species above. We calculated 95% confidence intervals by using non-parametric bootstrapping to resample randomly from our individual data samples 1000 times.

Mixed-effects modelling of flea abundance

We used data collected between 2013-2017 at all 19 sites to assess which of 10 factors best explained variation in flea abundance where we anesthetized and counted fleas on squirrels. We focused our efforts on the 3 species in the *Sciuridae* family because: 1) sciurids have higher average flea loads than mice, and 2) we have larger sample sizes for the 3 sciurids (we only trapped mice at 3 sites). We counted the number of fleas collected from 1,865 squirrels (463 northern Idaho ground squirrels, 493 Columbian ground squirrels, and 909 yellow-pine chipmunks; some individuals were sampled more than one time but never in the same trapping session). We only used samples from sites with at least 5 samples within each species (e.g. if we trapped 3 chipmunks and 6 Columbian ground squirrels at site A we only included data from site A within the Columbian ground squirrel models and no samples from site A in the chipmunk models). We used a negative-binomial model to address overdispersion caused by a large number of zero counts (we also considered Poisson and quasi-Poisson distributions). We examined the following fixed effects: 1) density of Columbian ground squirrels, 2) density of yellow-pine chipmunks, 3), density of northern Idaho ground squirrels, 4) previous summer precipitation, 5) current summer precipitation, 6) previous winter precipitation, 7) elevation, 8) season (spring or summer), 9) date, and 10) canopy cover.

We tested the effects of precipitation (snow and rain) and density on flea abundance. We obtained data from the SNOTEL station closest to our study sites (West Branch, Idaho USA) for precipitation measurements. We calculated the density of each small mammal species by dividing the minimum number of adults known alive (within a site each year) by the area of use at that site. We used the adehabitatHR package in program R version 3.4.3 (Calenge 2006) to calculate the 95% minimum convex polygon (MCP = area of use) for each species and site separately. We used the 'Minimum Bounding Geometry' tool to calculate a minimum bounding polygon in ArcMap 10.4.4 for any sites with fewer than 5 locations because we were unable to calculate a 95% MCP with such sparse data. We added the 75-m buffers to all polygons (both minimum bounding polygons and 95% MCPs) to increase the likelihood we covered the area used by the animals. We used ArcMap and National Land Cover Database 2011 layer (Homer et al. 2015) to calculate canopy cover for each MCP. We used the National Elevation Dataset (Gesch et al. 2002) to calculate the average elevation at each site. Male ground squirrels have larger body surface areas (and typically have higher flea loads) so we included sex as a fixed effect in the model. We included Julian date or season as a parameter in the model (but never both because they are highly correlated). We included site as a random effect. We only included a random intercept model as models had difficulty converging when we tried including random slopes.

We used the glmmTMB package in program R (Brooks et al. 2017) to run mixed-effects models. We only included additive effects and we examined candidate models with all possible combinations of explanatory variables with the exception of time (Julian date and season). We initially ran 2 global models: one with Julian date and one with season. We used Akaike's Information Criterion (AICc; Akaike 1974, Burnham et al. 2011) to determine which predictor should be used in all the models. We standardized all continuous predictor variables. We conducted separate analyses for each of the 3 species because factors that affect flea abundance may vary among species. We assumed the best model was the one with the lowest AICc. We considered all models with a ΔAICc less than 2.0 as well supported. We averaged all fixed parameters over all models using the MuMIn package (Barton 2018). Hence, when a variable is not in a model the parameter is assigned the value of 0, and is thus a conservative approach leading to smaller effect sizes (Burnham and Anderson 2002). We did not include any interactions, ran every combination possible, and removed any correlated variables to meet asumptions necessary for model averaging (Cade 2015).

Results

We collected an average of (1) 8.36 (± 0.51 SE, range between 0 and 82) fleas on Columbian ground squirrels, (2) 2.38 (± 0.23 SE, range between 0 and 49) fleas on northern Idaho ground squirrels, (3) 2.12 (± 0.09 SE, range between 0 and 21) fleas on yellow-pine chipmunks, and (4) 0.73 (± 0.09 SE, range between 0 and 18) fleas on deer mice. We collected a total of 4,140 fleas from 495 anesthetized Columbian ground squirrels (320 individuals), 1,112 fleas from 467 anesthetized northern Idaho ground squirrels (355 individuals), 2,091 fleas from 983 anesthetized yellow-pine chipmunks (644 individuals), and 273 fleas from 374 anesthetized deer mice (275 individuals).

Flea Community

We identified 7 species of fleas: *Eumolpianus eumolpi, Thrassis pandorae, Oropsylla idoensis, Oropsylla tuberculata tuberculata, Neopsylla inopina, Aetheca wagneri,* and *Malaraeus* *telchinus*. All 7 species of fleas we collected and identified are known plague vectors (Brown 1944, Marchette et al. 1962, Gratz 1999, Eisen et al. 2009, Abbott and Rocke 2012). Three of the 7 species of fleas were detected on all 4 of the small mammal species we caught (northern Idaho ground squirrels, Columbian ground squirrels, yellow-pine chipmunks, and deer mice). Deer mice had fewer species of fleas (4) but species richness did not differ among the 3 squirrel hosts (6 flea species were identified on all 3 squirrel host species; Fig. 1.1). Flea community diversity differed more between northern Idaho ground squirrels and yellow-pine chipmunks (0.95) and northern Idaho ground squirrels and deer mice (0.92) than between northern Idaho ground squirrels and Columbian ground squirrels (0.03; Fig. 1.2).

Flea Abundance

Previous summer precipitation was positively correlated with flea abundance for 3 hosts (northern Idaho ground squirrels, Columbian ground squirrels and yellow-pine chipmunks) and was in every top model (Table 1.1). Season or Julian date was in every top model for all 3 hosts but the direction of the effect differed among hosts (Tables 1.1, 1.2). Flea abundance was higher in the summer for yellow-pine chipmunks but lower in summer for Columbian ground squirrels and northern Idaho ground squirrels. Density of northern Idaho ground squirrels was included in 9 of the 10 top models for northern Idaho ground squirrels, all the top models for Columbian ground squirrels, and 2 out of the 9 top models for chipmunks. Elevation was in every top model for northern Idaho ground squirrels,3 of 6 top models for Columbian ground squirrels, and only 1 top model for chipmunks. Northern Idaho ground squirrels' flea abundance decreased at higher elevations while Columbian ground squirrels' flea abundance increased at higher elevations. Canopy cover was in 9 of the 11 top models for yellow-pine chipmunk and all of the top models for Columbian ground squirrel (Table 1.1) and was negatively correlated with flea abundance in both species. Sex was in all top models for all 3 squirrel species; males had higher flea abundance than females for all 3 species (Table 1.2).

The proportion of parasitized individuals differed among the 3 small mammal species (F_2 = 154.8, $P < 0.001$): 247 of 457 (54.1%) northern Idaho ground squirrels, 426 of 493 (86.4%) of Columbian ground squirrels, and 584 of 909 (64.2%) of yellow-pine chipmunks had at least 1 flea. The parasite loads on parasitized individuals (those with at least 1 fleas) differed among the 3 small mammal species ($\chi^2 = 123.24$, d.f. = 2, $P < 0.001$). Post hoc comparisons using the Tukey HSD test indicated that mean flea load of parasitized individual Columbian northern Idaho ground squirrels ($\overline{x} = 9.67 \pm 0.56$ SE) was significantly different than northern Idaho ground squirrels ($\overline{x} = 4.41 \pm 0.37$ SE) and yellow-pine chipmunks ($\overline{x} = 3.35 \pm 0.12$ SE) but did not significantly differ between northern Idaho ground squirrels and yellow-pine chipmunks.

Discussion

Flea community

We identified 7 species of fleas on northern Idaho ground squirrels and coexisting small mammals and all 7 fleas are known vectors of plague (Brown 1944, Marchette et al. 1962, Gratz 1999, Eisen et al. 2009, Abbott and Rocke 2012). Furthermore, the 4 coexisting mammal hosts share flea species. Our results corroborate a previous study that also reported that the fleas on northern Idaho ground squirrels are shared by other rodents (Yensen et al. 1996). An adult flea relies on blood from a living host to survive and typically exhibits host specificity (Medvedev and Krasnov 2006). However, a flea will switch to a less ideal host if necessary (i.e., if its host dies; Krasnov et al. 2004). The fact that infected fleas can move among multiple sympatric hosts is a concern for plague management and conservation of the northern Idaho ground squirrel because plague could easily be transferred to northern Idaho ground squirrels if it is present in any of the host species. All three of the coexisting species are known hosts of plague in the western United States (Twigg 1978, Zipser Adjemian et al. 2008, Smith et al. 2010).

Our results corroborate those of the only other study that has provided information regarding the fleas present on northern Idaho ground squirrels; that study reported 4 of the 6 species of fleas we identified on northern Idaho ground squirrels but did not report *E. eumolpi* or *A. wagneri* (Yensen et al. 1996). These 2 flea species may have been missed in the Yensen et al. (1996) study because only 43 animals were examined (29 freshly dead and 12 live) and they did not anesthetize the 12 live animals to collect fleas. We used anesthesia because fleas can be difficult to see and collect when they dig deep in the fur of animals. The anesthesia likely increased our ability to remove a larger percentage of the flea community than we would have by simply combing, searching and using tweezers to remove fleas. It is also unlikely that *E. eumolpi* or *A. wagneri* are new to the region because both have been recorded as present in Idaho as early as the 1904 (*A. wagneri*; (Lewis et al. 1988)) and the 1960s (*A. wagneri* and *E. eumolpi*, Allred 1968). Furthermore, *E. eumolpi* is the most commonly reported flea of western U.S. chipmunks (Lewis et al. 1988) and *A. wagneri* is a common flea of *P. maniculatus* across a broad range of locations (Lewis et al. 1988). Northern Idaho ground squirrel flea prevalence was greater in our study than in Yensen et al. (1996). Approximately 2x more northern Idaho ground squirrels had at least 1 flea (54.1%) than Yensen et al. (1996; 28%). The difference in flea prevalence may be due to differences in samples sizes, sampling methods, or actual differences in flea prevalence (perhaps as a result of differences in precipitation, season, or other environmental factors).

Flea abundance

Multiple biotic and abiotic factors influenced flea abundance on all 3 squirrel species (Columbian ground squirrels, northern Idaho ground squirrels, and yellow-pine chipmunks). Flea loads increased when the previous summer had higher precipitation. Previous summer precipitation was in all top models and showed similar relationships with abundance for all 3 host species. Our results contradict those from numerous previous studies conducted in other locations with other species who found that flea abundance was driven more by the current year weather conditions than the previous year's weather (Linardi and Krasnov 2013, Young et al. 2015, Eads and Hoogland 2016). Studies that did find a previous year influence of precipitation on abundance found that flea abundance increased after a previous dry year (Eads and Hoogland 2016, 2017, Eads et al. 2016*b*, Eads and Biggins 2017). Our results may diverge from previous studies because we did not experience an intense enough drought during our study period to influence our results. Furthermore, it may be that precipitation interacts with host density resulting in higher populations (higher survival and reproduction) during wet years from increased vegetation, which in turn results in greater flea abundances.

Date or season was in all our top models but the direction of the effect differed among host species. Flea abundance on both northern Idaho ground squirrels and Columbian ground squirrels' decreased as the summer progressed whereas yellow-pine chipmunks showed the opposite temporal pattern. Differences in the flea community may explain the varying relationships between flea abundance and time, as different flea species may become dominant at different times of the year depending on their life history (Krasnov et al. 2002*a*, Tripp et al. 2009). For example, *E. eumolpi* (a chipmunk specialist flea) may peak later in the year than fleas more commonly associated with northern and Columbian ground squirrels. Other studies have reported contradicting results as well: 3 of the 4 flea species on Richardson's ground squirrels (*Urocitellus richardsonii*) increased throughout the season while the 4th species slightly decreased in abundance during the season (Lindsey and Galloway 1997). Seasonality of maximum species abundances may differ by flea species due to differences in climate tolerances (heat and humidity) and may allow more species to colonize a host by temporally separating when species need the maximum amount of host surface for feeding.

Flea abundance in northern Idaho ground squirrels decreased at higher elevations. The relationship between elevation and flea abundance is not well understood and the few past studies that have done so reported positive (Amatre et al. 2009), negative (Amatre et al. 2009), or no correlation (Eisen et al. 2012) between elevation and flea abundance. While elevation was included in 3 of 6 top models for Columbian ground squirrels it was not a significant parameter (Table 1.2). Thermal conditions impact flea reproduction and thus flea abundance (Krasnov et al. 2001*a*, *b*) and thermal gradients may differ among different elevation gradients.

Conspecific density was in 14 of 16 top models for northern Idaho ground squirrels: flea abundance was positively correlated with northern Idaho ground squirrel density. Northern Idaho ground squirrel density was also included in all of the top models for Columbian ground squirrel and in 3 of 11 top models for yellow-pine chipmunk. Increased density in hosts may lead to: (1) increased contact rates among individuals and, thus, increased potential sharing of fleas or (2) increased probability of coming into contact with fleas searching for a host, and thus increased potential for flea transfer from a deceased host to a new one (Arneberg et al. 1998, Patterson and Ruckstuhl 2013). However, behavioral factors such as grooming may mitigate density and lead to decreased flea loads (Eads et al. 2016*a*, 2017). Past studies have found conflicting relationships between parasite abundance and host density (Krasnov et al. 2002*a*, Stanko et al. 2002, Sanchez et al. 2014, Young et al. 2015). Differences may be a result of spatial scale, how density is calculated, or flea-host specificity (are the flea species in the study specialists or generalists). Future work to evaluate the relationship of host density and flea abundances across multiple study systems should aim to standardize methods of density measurements, work on the small mammal community and not just one species, and document flea specificity.

We identified numerous abiotic and biotic factors that influence flea abundance on 3 different host species (Columbian ground squirrels, northern Idaho ground squirrels, and yellow-pine chipmunks). And we have identified 7 flea species capable of transmitting plague on 4 host species (Columbian ground squirrels, northern Idaho ground squirrels, yellow-pine chipmunks, and deer mice). However, we still do not know transmission rates of plague for most of these species (Eisen et al. 2009). Not every site where northern Idaho ground squirrels exist have the same small mammal community, same flea abundances, or same flea community. Thus, every northern Idaho ground squirrel site may not require the same management strategies. Some locations may be better targets for restoration efforts than others because they are less likely to be at risk for plague. An area may be at lower risk because their flea communities may not be as good at transmitting plague as others. We designed our modeling framework (approach) to help identify the factors that influence flea abundance across our study sites. Our results may help managers decide which sites are most likely to be impacted by plague (and hence which sites to target first for control actions). Site-specific precipitation data (rather than annual averages) and soil data may further elucidate site-specific differences in flea abundance.

Additional work is needed to identify which fleas are the most efficient at transmitting plague. Not every site where northern Idaho ground squirrels exist have the same small mammal community, same flea abundances, or same flea community. Thus, every northern Idaho ground squirrel site may not require the same management strategies. Some locations may be better targets for restoration efforts than others because they are less likely to be at risk for plague. An area may be at lower risk because their flea communities may not be as good at transmitting plague as others. The better we understand the relationship among fleas, hosts, and *Y. pestis*, the better we will be able to manage the disease impacts on wildlife. One of the most widely used methods to control plague in the United States is vector control using insecticides (e.g. Delta Dust ®), where the insecticides is manually applied to burrows throughout a specified area (Biggins et al. 2010, Tripp et al. 2016). The better we understand which year(s) or spatial locations with the highest risk, the better we can target our flea control efforts.

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Table 1.1. Best fixed-effects models (those within 2 ΔAICc) for factors that explain variation in flea abundance on northern Idaho ground squirrels (NIDGS), Columbian ground squirrels (COGS), and yellow-pine chipmunks (Chipmunk). Site was included as a random effect with a random intercept in all models. We used a negative binomial distribution.

Model	ΔAIC_c	df	w_i
Northern Idaho ground squirrel			
Date + Sex + Previous summer precip + Elevation + NIDGS density	0.00	8	0.050
Date + Sex + Previous summer precip + Elevation + NIDGS density + Current summer precip	0.11	9	0.047
Date + Sex + Previous summer precip + Elevation + NIDGS density + Winter precip	0.18	9	0.045
Date + Sex + Previous summer precip + Elevation + NIDGS density + COGS density	0.43	9	0.040
Date + Sex + Previous summer precip + Elevation + NIDGS density + Winter precip + COGS density	0.70	10	0.035
Date + Sex + Previous summer precip + Elevation + NIDGS density + Current summer precip + COGS density	0.74	10	0.034
Date + Sex + Previous summer precip + Elevation	0.81	7	0.033
Date + Sex + Previous summer precip + Elevation + COGS density	1.20	8	0.027
Date + Sex + Previous summer precip + Elevation + NIDGS density COGS density + Chipmunk density	1.47	10	0.024
Date + Sex + Previous summer precip + Elevation + NIDGS density + Chipmunk density	1.59	9	0.022
Global	6.12	13	0.002
Null	50.10	3	0.000
Columbian ground squirrel			
Season + Sex + Previous summer precip + Canopy cover + NIDGS density + Chipmunk density + Elevation	0.00	10	0.111
Season + Sex + Previous summer precip + Canopy cover + NIDGS density + Chipmunk density	0.31	9	0.095
Season + Sex + Previous summer precip + Canopy cover + NIDGS density + Chipmunk density + Elevation + Current summer precip	0.55	11	0.084
Season + Sex + Previous summer precip + Canopy cover + NIDGS density + Chipmunk density + Current summer precip	0.75	10	0.076
Season + Sex + Previous summer precip + Canopy cover + NIDGS density + Chipmunk density + Elevation + Winter precip	0.80	11	0.074
Season + Sex + Previous summer precip + Canopy cover + NIDGS density + Chipmunk density + Winter precip	1.03	10	0.066
Global	4.74	13	0.010
Null	75.29	3	0.000
Yellow-pine chipmunk			
Season + Sex + Previous summer precip + Canopy cover	0.00	7	0.044
Season + Sex + Previous summer precip + Canopy cover + NIDGS density	0.91	8	0.028
Season + Sex + Previous summer precip	1.57	6	0.020
Season + Sex + Previous summer precip + Canopy cover + Winter precip	1.58	8	0.020
Season + Sex + Previous summer precip + NIDGS density	1.63	7	0.020
Season + Sex + Previous summer precip + Canopy cover + Current summer precip	1.64	8	0.020
Season + Sex + Previous summer precip + Canopy cover + COGS density	1.84	8	0.018
Season + Sex + Previous summer precip + Canopy cover + Elevation	1.88	8	0.017
Season + Sex + Previous summer precip + Canopy cover + Chipmunk density	1.95	8	0.017
Global	9.01	13	0.000
Null	23.91	3	0.000

Table 1.2. Comparison of parameter estimates for the best fitting flea count models for northern Idaho ground squirrels (NIDGS), Columbian ground squirrels, and yellow-pine chipmunks using generalized linear mixed models (GLMM). All models included site as a random effect and a random intercept. All continuous variables were centered about their mean. Only the top model for each species is included. The parameter estimate for sex is in relation to males.

	N. Idaho ground squirrel			Columbian ground squirrel			Yellow-pine chipmunk		
Term	**Parameter estimate**	SE	*P*	**Parameter estimate**	SE	*P*	**Parameter estimate**	SE	*P*
Intercept	**0.179**	0.229	0.218	**1.880**	0.081	< 0.001	**0.434**	0.102	< 0.001
Julian Date	**-0.298**	0.097	0.001						
Season				**-0.516**	0.104	< 0.001	**0.205**	0.107	0.028
Sex	**0.559**	0.214	0.005	**0.526**	0.098	< 0.001	**0.381**	0.087	< 0.001
Previous summer precipitation	**0.229**	0.108	0.017	**0.094**	0.067	0.080	**0.067**	0.057	0.122
Current summer precipitation	**-0.032**	0.109	0.387	**-0.029**	0.074	0.347	**-0.005**	0.048	0.459
Winter precipitation	**-0.033**	0.097	0.367	**-0.014**	0.059	0.407	**-0.011**	0.046	0.402
Canopy cover	**-0.015**	0.147	0.460	**0.242**	0.054	< 0.001	**-0.082**	0.081	0.156
NIDGS density	**0.193**	0.188	0.152	**0.241**	0.061	< 0.001	**0.040**	0.059	0.249
COGS density	**0.078**	0.122	0.262	**-0.004**	0.047	0.464	**0.021**	0.051	0.340
Chipmunk density	**-0.027**	0.104	0.399	**-0.219**	0.085	0.006	**-0.003**	0.037	0.472
Elevation	**-0.430**	0.207	0.020	**0.042**	0.055	0.223	**0.009**	0.042	0.416

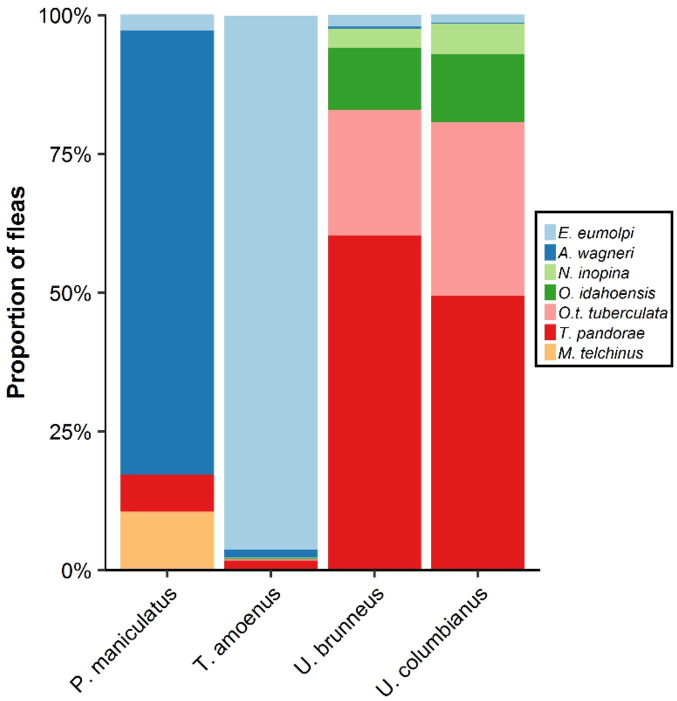

Figure 1.1. Proportion of fleas collected from northern Idaho ground squirrels and 3 coexisting small mammal hosts. The same 6 flea species were found on all 3 squirrel species (*T. amoenus, U. brunneus,* and *U. columbianus*) and 3 of the 6 same flea species were found on all 4 small mammal hosts. *M. telchinus* was only found on deer mice (*P. maniculatus*).

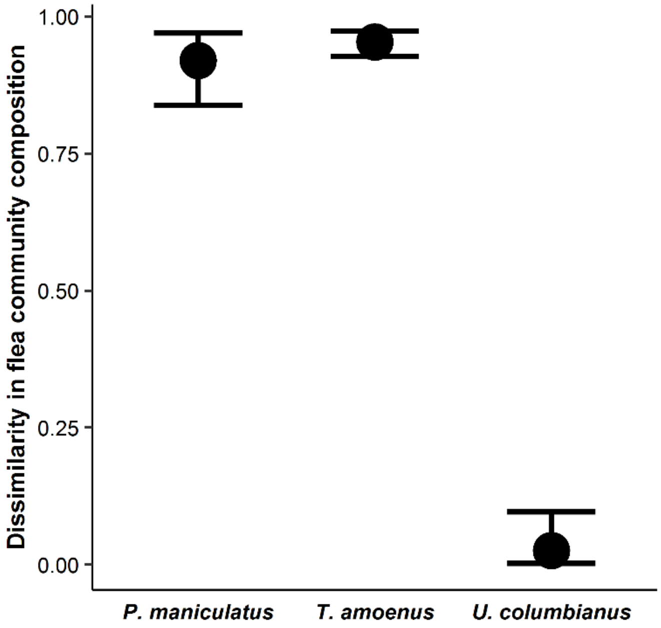

Figure 1.2. The dissimilarity in flea species composition between northern Idaho ground squirrels and 3 co-existing small mammals. The error bars are the 95% confidence intervals. Dissimilarity index is on a scale between 0 and 1 where 0 represents communities that are identical while a 1 represents communities that are completely different.

Chapter 2: Effects of flea removal and experiment vaccine on survival of northern Idaho ground squirrel and coexisting rodents: Sylvatic plague as a potential cause of population declines

Abstract

Plague is a non-native disease that reduces survival of many mammals in the United States. Previous studies have focused on epizootic plague which causes high mortality and dramatic declines in abundance. We know much less about enzootic plague which causes less dramatic reductions in survival and thus abundance of infected populations. As a result, enzootic plague is much more difficult to detect because changes in population dynamics are more subtle and *Yersinia pestis* prevalence is likely lower relative to epizootic plague outbreaks. The northern Idaho ground squirrel (*Urocitellus brunneus*) is a threatened species which coexists with Columbian ground squirrels (*Urocitellus columbianus*), yellow-pine chipmunks (*Tamias amoenus*), and deer mice (*Peromyscus maniculatus*). All 3 of these coexisting species are known hosts for plague. Furthermore, plague epizootics have been documented less than 125-km away from the last remaining northern Idaho ground squirrel populations. We conducted 3 experiments to test for the presence of plague in northern Idaho ground squirrels and the 3 coexisting species: (1) a paired flea-removal experiment, (2) an non-paired flea-removal experiment, and (3) a plague vaccine experiment. If enzootic plague is present and causing reductions in survival, animals with fleas (the main vector for plague) should have lower survival than those in areas where fleas are experimentally removed or reduced. Survival was higher (2%-50% higher among 4 species and 2 experimental designs) for flea-removal animals compared to non-treated animals. Our results suggest that plague is present and negatively impacting survival of northern Idaho ground squirrels and coexisting species.

Keywords: *Urocitellus brunneus*, *Yersinia pestis*, deltamethrin, enzootic, conservation, threatened, flea

Introduction

Wildlife disease is often a primary driver of wildlife population dynamics, species interactions, and life history evolution (Deem et al. 2001, Smith et al. 2006, Pedersen et al. 2007, Johnson et al. 2012). However, wildlife diseases are probably underestimated in the literature as a driver of population declines (Smith et al. 2009). Indeed, wildlife diseases are a concern worldwide for many species of conservation concern, and infectious diseases are emerging more and more frequently (Jones et al. 2008). The increase in discovery of new infectious diseases in wildlife populations may reflect new pathogens or parasites, mutations, or new discoveries of extant diseases that were merely not previously known to exist in a region (Smith et al. 2006, 2009, Keesing et al. 2010). Increased incidence of diseases can lead to changes in population densities and community dynamics. Wildlife disease is believed to be underrepresented in the literature as the cause for at-risk species decline (Smith et al. 2006, 2009, Pedersen et al. 2007, Keesing et al. 2010). Furthermore, pathogens are moving into new areas via human movement of wildlife and plants carrying pathogens, or via climate change (Roy et al. 2017). Diseases and their adverse effects on wildlife populations are often overlooked until the population is extinct or numbers are perilously low and the suite of possible management actions are limited (Dobson and Foufopoulos 2001, Heard et al. 2013). To prevent this problem, we need to evaluate whether wildlife diseases are impacting the survival of rare species before they risk extinction.

Sylvatic plague is an example of a disease that has the potential to dramatically affect wildlife populations but for which we know very little. Sylvatic plague is a highly virulent, infectious, vector-borne disease that is caused by the bacterium *Yersinia pestis*. Sylvatic plague was introduced from Asia to North America in the early 1900s (Barnes 1993) and is a disease to which many mammals endemic to North America show little natural resistance. Sylvatic plague is a host generalist that has infected >200 species of mammals worldwide, and the disease is most common in small rodents (Gage and Kosoy 2005). Plague is often considered to have two phases: epizootic and enzootic. Epizootic sylvatic plague outbreaks have caused widespread die-offs in small mammal populations such as prairie dogs (*Cynomys* sp.; Gage and Kosoy 2005). These epizootic events are characterized as affecting a large portion of the population (Lincoln 1998), but we know very little about how sylvatic plague persists in the intervening years between the large epizootic outbreaks or the potential demographic impacts of enzootic sylvatic plague (Gage and Kosoy 2005, Matchett et al. 2010). Enzootic sylvatic plague refers to periods of time when plague persists and may be associated with low flea abundances and/or host disease persistence (Wimsatt and Biggins 2009). Understanding the potential impacts of enzootic sylvatic plague is important for rare species because even a small decrease in survival for a species of conservation concern can have long-term effects on the ability of the species to increase in abundance or recover from declines caused by other threats.

Effects of enzootic sylvatic plague on small mammal populations might be substantial but are much more likely to go unnoticed. For example, experimental removal of enzootic sylvatic plague (via reducing flea vectors with deltamethrin dust) improved annual survival of prairie dogs by 31-45% (Biggins et al. 2010). And recent experimental studies in Colorado and New Mexico suggest that enzootic sylvatic plague reduces survival in several other species of small mammals in the western United States (D.E. Biggins, unpubl. data). Additional experimental studies are needed to quantify the threat that sylvatic plague (both enzootic and epizootic) poses for population viability in free-ranging mammal populations, especially rare endemic species that face other threats to their persistence. Furthermore, direct tests for the presence of *Y. pesti*s may be inaccurate (possibility of false-negatives from testing fleas; (Matchett et al. 2010) or because it is difficult to find carcasses from small mammals that died from plague when only a small percentage of the population dies.

The northern Idaho ground squirrel (*Urocitellus brunneus*) is a threatened species with a restricted distribution (i.e. it is found in only 2 counties in Idaho; U.S. Fish and Wildlife Service 2003) and, hence, may be particularly susceptible to reductions in survival caused by sylvatic plague. The leading hypothesis for the squirrel's rarity and past population declines is habitat loss: years of fire suppression is thought to have caused conifer trees to encroach into the forest openings where these squirrels live (U.S. Fish and Wildlife Service 2003). However, this fire suppression hypothesis has not been tested empirically, nor have alternative hypotheses been tested. If sylvatic plague is present in northern Idaho ground squirrels, it may be responsible (or partially responsible) for local declines or extirpations of some populations. Indeed, the Recovery Plan for northern Idaho ground squirrels identifies the effects of sylvatic plague as a research need (U.S. Fish and Wildlife Service 2003). However, no previous studies have examined whether sylvatic plague is present in northern Idaho ground squirrels.

Sylvatic plague has not been documented in northern Idaho ground squirrels; however, it has been documented in a number of sympatric and parapatric species, as well as in geographic regions that are in close proximity to northern Idaho ground squirrel populations. Specifically:

1) Sylvatic plague has been documented in 27 counties in Idaho (Fig. 2.1).

2) A sylvatic plague epizootic was identified in voles and a sister-species of ground squirrel in southern Idaho in 2015 and 2016 (~125 km away; Ridler and Phillips 2015) and in badgers (*Taxidea taxus*) (~50 km away; Messick et al. 1983) not far from the current range of northern Idaho ground squirrels.

3) Sylvatic plague has been documented in carnivores numerous times in the past decade from both Adams and Valley counties (Abbott and Rocke 2012): the two counties in Idaho where all remaining northern Idaho ground squirrel colonies occur (Fig 2.1).

4) Most recently, one bobcat (*Lynx rufus*) in 2012 in Adams County and one gray wolf (*Canus lupus*) in 2014 in Valley County tested positive for plague from sero-surveys conducted in the 2 counties that support northern Idaho ground squirrels.

Northern Idaho ground squirrels often co-occur or live in close proximity with Columbian ground squirrels (*Urocitellus columbianus*), yellow-pine chipmunks (*Urocitellus amoenus*), and deer mice (*Peromyscus maniculatus*). All 3 of these co-existing small mammal species are known hosts of sylvatic plague in the United states (Nelson 1980, Barnes 1993, Biggins and Kosoy 2001, Smith et al. 2010) and are often found at higher densities than northern Idaho ground squirrels. If sylvatic plague is present, one or more of these coexisting species is likely to be the primary host for the disease which then may spill over into the less-abundant and more patchily distributed northern Idaho ground squirrel population. Hence, efforts to test for the presence of sylvatic plague and, if present, efforts to manage the disease would be more effective if those efforts included these coexisting species.

We designed a study to test the hypothesis that enzootic sylvatic plague is reducing survival of northern Idaho ground squirrels. To test this hypothesis, we conducted 3 field experiments: 1) a spatially paired flea-removal experiment where we removed fleas from a subset of occupied ground squirrel sites to investigate whether sylvatic plague is present in northern Idaho ground squirrels and 3 coexisting small mammals; 2) a non-paired flea-removal experiment (similar to #1 above but the 2 treatments were not spatially paired), and 3) a vaccine experiment to test for the presence of sylvatic plague in 2 coexisting species: yellow-pine chipmunk and deer mice. These 3 controlled field experiments allowed us to address two questions: 1) Is sylvatic plague present in northern Idaho ground squirrels or any of 3 coexisting small mammal species? and 2) Does sylvatic plague reduce survival of these 4 species of small mammals?

Methods and Analysis

We conducted this study on northern Idaho ground squirrels, Columbian ground squirrels, yellow-pine chipmunks, and deer mice at 16 study sites in Adams County, Idaho from 2014-2018 (Fig. 2.1). We designed and implemented 3 field experiments to examine whether sylvatic plague was reducing survival of these 4 rodents: 1) spatially paired sites where adjacent pairs of plots were randomly assigned to one of two treatments: flea-removal or not-treated; 2) non-paired sites that were randomly assigned to one of two treatments: flea-removal or not-treated; and 3) vaccine plots where we compared survival of vaccinated animals versus animals given a control placebo. These 3 experiments were independent of each other and allowed us to more rigorously test our hypothesis and provide strong inferences regarding the potential impact of sylvatic plague.

Study designs

Experiment 1: We used a spatially paired study design with 6 flea-removal and 6 non-treated plots similar to Biggins et al. (2010) to examine whether *Y. pestis* was reducing survival in northern Idaho ground squirrels, Columbian ground squirrels, yellow-pine chipmunks, and deer mice from 2014-2017. The paired study design with treated and non-treated plots adjacent to each other allowed us to control for site-specific variation in survival of small mammal populations. We applied a 0.05% deltamethrin formulation (DeltaDust®, Bayer Environmental Science, Research Triangle Park, NC) to all burrows and deployed bait stations treated with 0.06% deltamethrin (Suspend SC, Bayer Environmental Science, Research Triangle Park, NC) within the 6 treated plots. We applied DeltaDust® with handheld bulb dusters into every burrow located within each treatment plot and within a 30-m buffer surrounding each treatment plot. We applied 2 – 4g of DeltaDust® per burrow (2g for small northern Idaho ground squirrel burrows and up to 4g for large Columbian ground squirrel burrows). We applied DeltaDust® on average at least 2 weeks prior to the first trapping session of the year at each of the 6 treated plots (4 April – 30 April). We also deployed bait stations (but without deltamethrin) consisting of an upside-down gutter with 5 millileters of peanut butter smeared to the top of each gutter to encourage animals to enter the bait stations. The bait stations in the 6 treatment plots included a piece of carpet under the gutter that was dipped in 0.06% deltamethrin solution and dried. Bait stations in the 6 non-treated plots and we placed an equal number of bait stations on the treatment and non-treatment plots (31 bait stations per ha). The bait stations in the 6 non-treatment plots included gutters with bait but not carpet pieces. Our bait station design was modified from previous studies that used bait stations to apply insecticide (Bronson and Smith 2002; Biggins unpubl. data). We chose to use these two different methods (dusting burrows and treated bait stations) to remove fleas in our 6 treatment plots to increase the probability of treating all the small mammals present within our treatment plots (because it is difficult to locate and treat every burrow, especially mouse burrows). We conducted these paired experiments at 6 study sites: 3 with northern Idaho ground squirrels (1 site also contained Columbian ground squirrels and chipmunks) and 3 with Columbian ground squirrels and yellow-pine chipmunks but no northern Idaho ground squirrels (because northern Idaho ground squirrels are rare and potential sites with northern Idaho ground squirrels for which we had access were limited). We trapped sites during three seasons (spring: April-end of June, summer: June-early August, and late summer (Sherman traps only): July-early September; Fig. 2.2). We trapped squirrels for 3-4 days within each season at each site for both the Tomahawk and Sherman traps.

Experiment 2: We also tested the effects of flea removal on 9 other non-paired sites (4 treatment plots and 5 non-treated plots) from 2015-2017. We applied the 2 deltamethrin treatments described above (under Experiment 1) at the 4 (unpaired) treatment plots where we were concurrently trapping rodents as part of a separate study (a 10-year forest restoration study). And we compared results from those 4 unpaired treated plots to 5 non-paired, non-treated plots (that were also part of the 10-year forest restoration study). Northern Idaho ground squirrels, Columbian ground squirrels, and yellow-pine chipmunks occupied all 9 of the non-paired study sites that were part of Experiment

#2. Hence, we had 6 paired study sites (where we had a treatment plot and a non-treatment plot at each of these 6 sites), and 9 unpaired study sites (4 were treatment plots and 5 were non-treatment plots). We trapped each site 2 times each year (spring: April-early June and summer: June-August; Fig. 2.2). We analyzed data from the 2 experiments separately (given that one included spatially paired treatments and the other did not).

Experiment 3: We conducted a third experiment (2016-2018) where we compared survival between vaccinated and control animals to help evaluate the effects of sylvatic plague on deer mice and yellow-pine chipmunks. We used the F1 vaccine (Anderson Jr et al. 1998) to vaccinate animals against plague. The F1 vaccine has been used in similar studies to test for the presence of sylvatic plague in woodrats (*Neotoma*) and *Peromsycus* (D. E. Biggins, pers. comm.) by evaluating differences in survival between treated and un-treated individuals. Furthermore, the F1 vaccine has proven to be effective at protecting mice in the lab from *Y. pestis* infection (Anderson et al. 1997). We vaccinated half of all mice and half of all chipmunks caught within each of the 10 plots. We administered a placebo injection to the other individuals caught (the control group) to control for any potential impact of the stress and discomfort from the injection itself. We administered a 0.1 ml solution which contained 0.5 ug of the F1 protein subcutaneously to chipmunks and mice in the treatment group. The placebo contained a mixture of dulbecco and alhydrogel which are also in the F1 vaccine. In 2016, we compared survival between vaccinated and control animals on 8 vaccine plots (Table 2.1). We trapped individuals over 3 trap sessions in 2016: spring (29 April – 5 June), early summer (10 June – 12 July), and late summer (15 July – 9 August; Fig. 2.2). We only vaccinated individuals during the spring and early summer trap session. We only vaccinated individuals during the first 2 trap sessions because we designed the study to be a 1 year study and we needed to estimate survival during intervals post-injection. In 2017, we added 2 additional vaccine plots to improve our ability to test the effectiveness of the vaccine (providing a total of 10 plots for Approach #3; Table 2.1). We also added a booster injection to chipmunks in 2017 because some studies have shown that a booster injection increases the effectiveness of the vaccine (Mencher et al. 2004, Rocke et al. 2008, 2014).

We trapped during 4 trap sessions in 2017: spring (7 May – 5 June), early summer (6 June – 10 July), mid summer (9 July – 30 July), and late summer (3 August – 19 August; Fig. 2.2). We vaccinated/boosted individuals during the spring, early summer, and mid summer. (not late summer). In 2018, we returned to the 6 vaccine plots where we trapped chipmunks to assess overwinter survival for 1 early summer trap session at each site (3 June – 29 June). We did not vaccinate any individuals in 2018.

Trapping Methods

Experiment 1: We used Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, WI, USA) to trap northern Idaho ground squirrels, Columbian ground squirrels, and yellow-pine chipmunks and Sherman traps (HB Sherman Inc., Tallahassee, FL, USA) to trap yellow-pine chipmunks and deer mice (Table 2.1). We baited all traps with an oat, peanut butter, and vanilla mixture. We placed traps in a non-fixed grid (grid varied every day on northern Idaho ground squirrel plots and traps were occasionally moved on non-northern Idaho ground squirrel plots to try and trap new individuals). The density of tomahawk traps (8.89/ha – 15.6/ha) and Sherman traps (21.78/ha – 24.89/ha) varied among sites but was the same for each pair within a site. We checked traps every ~15 minutes at the 3 paired sites with northern Idaho ground squirrels. In addition, used a focal trap method to catch northern Idaho ground squirrels (at the 3 sites where they occurred): following squirrels to a burrow, plugging all nearby surrounding burrows, and placing a specially designed focal trap over the only remaining opening. We watched focal traps closely and the trap was removed if a northern Idaho ground squirrel did not enter the trap within ~1 hour. We checked traps ~ every 2 hours at the 3 sites that did not contain northern Idaho ground squirrels. We ceased trapping after 1:00pm or when the ambient temperature rose above 27°C. We set 49-56 Sherman traps on 8 plots (4 sites). Sherman traps were evenly spaced in an established grid 20m apart. We trapped and handled all northern Idaho ground squirrels following protocols developed by Idaho Department of Fish & Game (D. Evans Mack, unpubl. data). We opened Sherman traps later (after dusk) on sites that had northern Idaho ground squirrels. Northern Idaho ground squirrels are small enough to fit into a Sherman trap and we did not want them to be confined in a trap throughout the night. Sherman traps were checked at dawn before northern Idaho ground squirrels emerged from their burrows for the day. We opened Sherman traps in the evenings after 5:00pm at sites without northern Idaho ground squirrels present).

Experiment 2: We set 30 Tomahawk live traps at each site (3.75/ha) and followed the same trapping protocols as described in Experiment 1 for northern Idaho ground squirrel sites. However, we trapped squirrels until the early evening (when northern Idaho ground squirrel activity stopped) in the spring and until 1:00pm or the ambient temperature was above 27°C in the summer.

Experiment 3: We set 24 – 30 Tomahawk live traps (10.67/ha – 16.85/ha) and 49-70 Sherman traps (21.78/ha – 39.33/ha) within each plot. We only set Tomahawk traps at sites without northern Idaho ground squirrels (at sites where we were targeting both chipmunks and deer mice). We followed the same trapping methods as described in Experiment 1. Each of the 4 trap sessions were designed to coincide with phenology of the squirrels' hibernation behavior: 1) before juvenile ground squirrels' emergence (spring), 2) after juvenile ground squirrels have emerged (early summer), 3) after adult ground squirrels entered hibernation (mid summer), and 4) after juvenile ground squirrels entered into hibernation (late summer). Hence, only yellow-pine chipmunks and deer mice were caught during the mid summer (#3) and late summer (#4) trapping sessions and we only set Sherman traps during those sessions (see below). We added the 4th trap session in 2017 because we received additional funding that year and were able to hire additional technicians. Our initial results from 2016 indicated that chipmunk flea abundance increased as the summer progressed suggesting that if plague was present it was likely to be more active later in the season and an additional trap session would increase our ability to detect plague later in the summer.

Animal handling

We marked all animals other than yellow-pine chipmunks with a metal ear tag in each ear (National Band and Tag Co., Newport, KY, U.S.A.). If a northern Idaho ground squirrel or Columbian ground squirrel did not have enough of an ear to tag properly, we used a PIT tag instead (occasionally squirrels ears were ripped either from previous tag loss or other cause). In addition, we added PIT tags to individuals who had only 1 tag because we were concerned about full tag loss). We marked all yellow-pine chipmunks with PIT tags (Biomark Inc., Boise, ID, U.S.A.) to minimize tag loss (many of the chipmunks we initially tagged with ear tags lost them by the following trap session in 2013).

We anesthetized animals with Isoflurane to comb and collect fleas and to document the effectiveness of the flea-removal treatments (insecticide at burrows and bait stations). Anesthesia not only anesthetizes the hosts but also the fleas which makes it easier to comb and collect them. We anesthetized and combed all individual deer mice the first time each was trapped every session. We anesthetized $\leq$10 adult northern Idaho ground squirrels per study site (non-paired study design) and plot (paired study design; for females, we only anesthetized them during the summer trapping session to limit any potential adverse effects on northern Idaho ground squirrel populations). We anesthetized $\leq$10 adult (equal number of males and females) Columbian ground squirrels and yellow-pine chipmunks at each site (non-paired study design) or plot (paired study design). We counted the number of fleas that fell from all anesthetized animals while combing them and compared the number of fleas per captured animal between treated and non-treated plots (if our treatments are effective we should have few to no fleas on the treatment plots and some fleas on the non-treated plots). We also preserved all the fleas in 70% ethyl alcohol for later identification and analyses (see Chapter 1).

Analytical methods

We used a Cormack-Jolly-Seber model (CJS) implemented in RMark (Laake 2013, R Core Team 2017) to estimate seasonal and annual survival for each species in Program Mark (White and Burnham 1999). We used Akaike's Information Criterion corrected for small sample size (AIC_c) to compare a suite of candidate models in program MARK and used the model with the lowest AIC_c to simultaneously estimate survival and detection probability of each species in both treatment and non-treatment plots. We included treatment in all models (except for a null model) apriori because this is the predictor variable we are most interested in predicting. Furthermore, we expected effect sizes to be relatively small based on our samples sizes and the fact that we are evaluating enzootic plague (which by definition results in only a small percentage of the population dying from plague). We believed the effect of the treatment, if present, may be lost by more parsimonious models, even if it is biologically relevant, due to the small effect it is having on survival (but an important effect nonetheless). In addition to the explanatory variable of primary interest (flea-removal versus non-treatment), we evaluated candidate models with different combinations of 3 other explanatory variables that might explain variation in survival: sex, season, and time.

We calculated apparent survival estimates (hereafter, survival) for flea-removal (treatment) and non-treatment plots for 2014-2017 on the 6 paired study sites (approach #1), and for treatment and non-treatment sites for 2015-2017 on the 9 non-paired study sites (approach #2). We compared survival estimates between the 2 treatments separately for the 6 paired study sites and the 9 non-paired study sites because the top model in Program MARK included a 2-way interaction between study type (paired versus non-paired) and treatment when data from both experiments were combined (suggesting that the results differed between the 2 experiments). We did not include data for yellow-pine chipmunks from 2014 because we only trapped 15 individuals in both trapping sessions combined in 2014. We only used spring captures for Columbian ground squirrels at the 6 paired study sites to estimate annual survival because we trapped too few individuals during the summer in 2014 and 2015, resulting in difficulty obtaining model convergence when we included the summer season. We also calculated survival for Columbian ground squirrels based only on captures from the spring and summer data at the 6 paired study sites in 2016 and 2017, and we found similar patterns to the spring-only data estimates (for which we could include all 4 years). We only included data from adults for the 3 squirrel species (northern Idaho ground squirrels, Columbian ground squirrels, and chipmunks) because CJS estimates of survival are confounded with dispersal, those estimates (i.e., apparent survival) may actually decrease in response to a treatment that increases true survival. We combined all age classes for mice because age was difficult to determine consistently. For both experiments, we also used Mann-Whitney U-tests to compare overall flea abundance between flea-removal and non-treatment plots and we conducted a separate U-test for each of the 4 species of small mammals.

For the vaccine experiment, we used logistic regression to estimate survival of vaccinated versus placebo animals for both yellow-pine chipmunks and deer mice in program R (R Core Team 2017). We assume that probability of capture is the same for both treatments because there are not spatial differences between the 2 treatments (individuals within a plot were injected with either the vaccine or the placebo). Any difference in probability of capture due to environment, density, or trauma of injection will be the same for both treatments. We modeled age separately for the yellow-pine chipmunks due to differences in time of year they were trapped and potential impacts of dispersal on survival. We included sex as a covariate in mouse models but not age due to the difficulty in ageing mice consistently and all ages were trapped throughout the study period. We did not include year in the model due to small sample sizes in 2016. We modeled treatment state of each individual as a single covariate with 4 levels: 1) interval following first injection, 2) interval following booster injection, 3) all other intervals following first injection, and 4) all other intervals following booster. These 4 levels allowed us to test for and account for the possibility that the effects of the vaccine may not be immediate and that a booster might increase the effectiveness of the vaccine. We also included a season covariate: 1) all time intervals (spring, early summer, late summer, and winter); and 2) early summer and late summer combined (spring, summer, and winter). We administered an initial injection in the spring, early summer, or late summer to all species. We administered booster injections to chipmunks only in the early summer or late summer. Animals were put in the after injection category following the first interval after they were given an injection or booster injections which could occur over multiple intervals during the early summer, late summer, and winter (Fig. S1). All candidate models included a treatment effect (vaccine versus placebo) because that was the variable of primary interest and the focus of the experiment. We only used individuals in the chipmunk model that were initially treated in the spring or early summer due to the difficulty we had accurately aging individuals (body size of adults and juveniles become too similar to accurately distinguish age classes). We used AIC_c to rank all candidate models and we used the top model for each species, age (for chipmunks), and vaccination stage combination. We removed any groups with fewer than 5 individuals because we assumed that the sample size of such groups was too small to make strong inferences from the data.

Results

Flea-removal treatment

Our flea-removal treatments were very effective (Fig. 2.3). Northern Idaho ground squirrels had on average 97.5% fewer fleas on flea-removal plots compared to those on non-treatment plots at the 6 paired study sites ($P < 0.001$) and 99.3% fewer fleas on flea-removal sites compared to non-treatment sites at the 9 non-paired study sites ($P < 0.001$). Deer mice had on average 57.2% fewer fleas on flea-removal sites than non-treatment sites ($P < 0.001$). Yellow-pine chipmunks had on average 24.1% fewer fleas on flea-removal plots compared to those on non-treatment plots on the 6 paired study sites ($P < 0.001$) and 89.2% fewer fleas on flea-removal sites compared to non-treatment sites ($P < 0.001$) at the 9 non-paired study sites. Columbian ground squirrels had on average 99.1% fewer fleas on flea-removal plots compared to those on non-treatment plots on the 6 paired study sites ($P < 0.001$) and 97.8% fewer fleas on the flea-removal sites compared to the non-treatment sites ($P < 0.001$) on the 9 non-paired study sites. Average flea abundance on non-treated sites was positively correlated with body size across species. Columbian ground squirrels had the highest flea abundance per individual animal ($\overline{x}$ = 7.81), followed by northern Idaho ground squirrels $\overline{x}$ = 2.04), yellow-pine chipmunks ($\overline{x}$= 1.87), and deer mice ($\overline{x}$ = 0.72).

Survival: flea-removal experiment

Annual survival point estimates were higher on flea-removal plots than on non-treatment plots for northern Idaho ground squirrels, Columbian ground squirrels, and yellow-pine chipmunks on both types of study sites (experiment #1 and #2): 1) Female northern Idaho ground squirrels on flea-removal plots had 12.6% and 1.7% higher survival (for paired and non-paired study sites, respectively) and male northern Idaho ground squirrels on flea-removal plots had 22.1% and 2.8% higher survival (for paired and non-paired study sites, respectively) than those on non-treatment plots; 2) Female Columbian ground squirrels on flea-removal plots had 5.6% and 18.6% higher survival (for paired and non-paired study sites, respectively) and male Columbian ground squirrels on flea-removal plots had 15.2% and 30.5% higher survival (for paired and non-paired study sites, respectively) than those on non-treatment plots; and 3) Female yellow-pine chipmunks on flea-removal plots had 11.4% and 6.0% higher survival (for paired and non-paired study sites, respectively) and male yellow-pine chipmunks on flea-removal plots had 16.7% and 9.8% higher survival (for paired and non-paired study sites, respectively) than those on non-treatment plots; (Figs. 2.4, 2.5). Results for deer mice were more nuanced: female deer mice and male deer mice had 6.8% and 5.8% lower survival, respectively, on flea-removal sites compared to non-treatment sites during the spring-early summer trapping interval, but they had 50.0% and 41.2% higher survival, respectively, on flea-removal sites compared to non-treatment sites during the early summer-late summer trapping interval (Fig. 2.6).

Survival differed between sexes and across time intervals (either just by season or every interval): for all species in the 9 non-paired study sites and for all species except Columbian ground squirrels in the 6 paired study sites (Tables 2.2, 2.3).

Survival: sylvatic plague vaccine experiment

We used data from both 2016 and 2017 to calculate survival for vaccinated and placebo animals at our 10 vaccine plots. For all groups, except late summer juveniles (see below), survival immediately following initial vaccine was lower for vaccinated animals than placebo animals (Figs. 2.7, 2.8). Vaccinated animal survival following the initial injection was 22.8% lower compared to placebo for mice, 12.4% lower for adult chipmunks in the spring, and 12.0% lower for juvenile male chipmunks. The only groups that did not show a negative effect of the initial vaccine injection were summer adult chipmunks (survival was 8.3% higher for vaccinated versus placebo adults) and juvenile female chipmunks (survival was the same for vaccinated and placebo juveniles).

Boosted chipmunks (those that received a second dose of vaccine in a different trap session) showed opposite trends for adults and juveniles. Vaccinated booster adults had 17.6% higher survival rates compared to placebo boosted animals in the summer, and vaccinated animals that were re-trapped during the first interval following the booster injection had 12.6% higher survival rates compared to placebo chipmunks. In contrast, vaccinated booster female juveniles (we only re-trapped 2 vaccinated males and administered a booster vaccine) had 34.7% lower survival rates compared to placebo booster animals in the late summer and those that did not receive a booster but survived the initial injection had 44.5% lower survival if they were vaccinated than given the placebo. Survival of mice was 7.0% higher for vaccinated animals during intervals after the initial injection. Survival differed among sexes only for juvenile chipmunks (Table 2.4). Overall it appears that the initial vaccine injection reduces survival of mice and chipmunks compared to placebo in the spring, but vaccinated mice and adult chipmunks have higher survival compared to placebo individuals after a lag period.

Discussion

Our experimental treatments greatly reduced flea abundance on our flea-removal plots and survival was higher on those plots without fleas in all years, for all 4 species, and for each of 2 independent study designs. The results of our vaccine experiment were more ambiguous.

The following are some explanations for why the vaccine experiment did not conclusively corroborate the flea-removal results (especially for juveniles): 1) plague is not present and the results of the flea experiments reflect random effects, 2) plague is not present and the results of the flea-removal experiments reflect other survival benefits of removing fleas, 3) short-term negative effects of vaccine make it difficult to show subsequent positive effects with the sample sizes and study duration we used, 4) herd immunity protected placebo individuals because vaccinated and control animals were in the same plots for the vaccine experiment, 5) juvenile dispersal effects apparent survival, 6) low statistical power due to small sample sizes, and 7) plague activity may have been low during the only year of the vaccine study. We think explanation #1 is unlikely given how many scenarios showed the same effect (higher survival in flea-removal plots for 4 species and 2 study designs), but more research is warranted to verify whether or not plague is indeed the mechanism behind the survival benefits we saw in the flea-removal experiments. Our sample sizes were small; we injected (vaccine or placebo) 346 individual animals (155 mice, 107 adult chipmunks, and 134 juvenile chipmunks). Our sample size was 539.0% smaller than the sample size used in the Biggins et al. (2010) study which evaluated enzootic plague in prairie dogs. More research is needed regarding the effectiveness of the vaccine, with a larger sample size and perhaps over multiple years to minimize Type II errors.

Flea loads in our study sites differed by year, season, and species (Chapter 1; Fig. 2.9). The average spring flea loads of Columbian ground squirrels were more than sufficient to sustain enzootic sylvatic plague in a population if the flea species in our system are equal or better vectors for plague than those in previous studies (Lorange et al. 2005, Eisen et al. 2007). The number of fleas required for maintenance of enzootic sylvatic plague may differ by flea species because their vector efficiency may differ, and additional research is needed to better quantify the flea loads necessary to sustain plague in a system and if/how that threshold varies among flea species and host species. However, the estimates of flea loads on our study sites are minimum counts because they do not include fleas that remain in squirrel nests but still regularly feed from squirrels (Eads et al. 2013). Flea counts for northern Idaho ground squirrels (Chapter 1) are undoubtedly lower than what was actually present because we did not anesthetize them as completely as the other species: we took extra caution given the conservation status of the species. Furthermore, all 4 small mammal species had some individuals with flea loads in the double digits. While we were able to substantially reduce flea loads on all species, we were more successful at removing fleas from northern Idaho ground squirrels and Columbian ground squirrels compared to deer mice and yellow-pine chipmunks. Chipmunks often move larger distances during their daily activities than the two ground squirrel species (pers. observ.). Hence, our plot size may not have been large enough to prevent chipmunks within our plots from regularly coming into contact with non-treated burrows (and getting re-infected with fleas). We more effectively reduced fleas on flea-removal sites in the non-paired experiment compared to the plots in the paired experiment, and the non-paired sites were larger (4-ha compared to the 2.25-ha paired plots). However, our results suggest that while our flea-removal was not perfect for chipmunks on the paired-study sites, we may have reduced flea loads enough to reduce plague activity. We are also unlikely to be as proficient at removing fleas from mouse burrows because they are difficult to find. We hoped to combat this problem by deploying the bait stations. We observed sign of mouse activity in the bait stations (e.g., fecal pellets left on the carpet). However, for this study, we were unable to test the effectiveness of the bait stations on flea-removal of the mice compared to dusting.

All 4 species of small mammals had higher survival on flea-removal sites compared to non-treatment sites. We analyzed data for each species and experimental approach separately and, in all instances, had similar results. The effect of the flea-removal treatment on survival did differ by year and/or season for some of the species. We would expect to see temporal variation in the effects of sylvatic plague on survival (and hence temporal variation in the effects of flea-removal treatments) in wild populations and adverse effects of plague should peak when environmental conditions are such that they elevate flea loads and bacteria levels (Tripp et al. 2009). Despite annual variation in the magnitude of the relationships, the general pattern remained the same: higher survival on flea-removal plots. The increase in survival varied between 2% and 50% for animals in the flea-removal treatments compared to the non-treatment sites. Even a small decrease in survival due to plague can impact the future viability of a species of conservation concern such as the northern Idaho ground squirrel. The standard error bars around the survival estimates overlapped between the two treatments, but the consistency in the pattern among the 4 species, across 3 years, and for the 2 separate experiments suggests that the differences are likely biologically relevant. Other studies that have evaluated enzootic sylvatic plague in areas where sylvatic plague is known to be present have reported similar results: some species responded with modest but biologically meaningful differences between treated and non-treated animals and overlapping confidence intervals (Biggins et al. 2010) while other had more statistically clear results (Biggins et al. 2010, Ramakrishnan 2017). Multiple studies also found a combination of flea-removal and vaccine resulted in the greatest improvement in survival compared to non-treatment groups (Matchett et al. 2010)

For the flea-removal experiment, we chose to focus on adults and not juveniles (except for deer mice). We know little about juvenile dispersal of northern Idaho ground squirrels, but many ground squirrels have high juvenile dispersal rates (Greenwood 1980, Dobson 1982, Holekamp 1984) and juvenile dispersal is often density-dependent (Matthysen 2005, Bonte and de la Pena 2009). Hence, if survival of adults and juveniles increases on flea-removal sites, a higher proportion of juveniles likely disperse (if juvenile dispersal is density-dependent). And because our CJS estimates of survival are confounded with dispersal, those estimates (i.e., apparent survival) may actually decrease in response to a treatment that increases true survival. Furthermore, if survival is lower on non-treatment sites, more juveniles are likely to remain and thus further confounding our results

(Biggins et al. 2010). Hence, it may be difficult to detect the impact of flea-removal treatments on juveniles (Biggins et al. 2010). We did evaluate juveniles for the vaccine experiment because we initially assumed that the vaccine experiment would occur during just a single summer (i.e., only examine within-season survival).

We added the vaccine experiment in 2016 because the differences observed from our flea-removal experiment may reflect some other benefit (aside from eradication of enzootic plague) that flea-removal has on survival. The F1 vaccine specifically targets sylvatic plague and, hence, the vaccine experiment is complimentary to the flea-removal experiments. However, the point estimates from the vaccine experiment are ambiguous. Similar to the flea-removal experiments, the estimates of survival were not significantly different between vaccinated and placebo animals. Survival point estimates were greater for vaccinated animals compared to placebo animals for both mice and adult chipmunks for all intervals except the interval following the initial administration of the vaccine/placebo. We do not know why the vaccine appears to have a negative impact on survival during the first interval, but vaccinated individuals have higher survival than placebo individuals for all subsequent intervals. While our results are statistically unclear, we believe the point estimates for the vaccine experiment are biologically relevant. For adult chipmunks and mice, we independently observed the same relationship in survival between vaccinated and placebo groups; higher survival for those vaccinated. This is the same relationship we observed over multiple years, experimental designs, and species in our flea-removal study. For a species of conservation concern, a reduction in survival by 10-30% annually can have a negatively impact our ability to recover a species. The vaccine experiment was only conducted as a full-scale study for 1 year. We still had relatively low samples sizes which increases the risk of a type II error.

Juvenile chipmunks do not follow the same patterns as adults. As mentioned above, if juvenile dispersal is density-dependent, we would not expect to detect higher survival of vaccinated juveniles even if the vaccine increased true survival. Hence, the overall effect of the vaccine on survival of juveniles may be impossible to document without also measuring survival of dispersing juveniles (e.g., via telemetry). Male juvenile chipmunks had low re-capture rates and thus we were unable to evaluate whether survival differed between vaccine and placebo for intervals after the initial injection. The vaccine effectiveness may also differ by age (Rocke et al. 2008, 2015, Stacy et al. 2008). Future studies should collect blood samples at the various stages of inoculation for all age classes to determine whether the vaccine effectiveness differs by age. In addition, it would be even more useful to conduct laboratory challenge trials on some of these species to test both the effectiveness of the vaccine and the best vaccine dosage. However, results in the lab are not guaranteed to mimic the response of these animals in a free ranging wild population who may be exposed to other external pressures (e.g. other pathogens) and stress.

If sylvatic plague is more active later in the summer (when flea loads on chipmunks are highest), perhaps the negative short-term impact of the vaccine on survival is overshadowed by the positive longer-term effect of the vaccine on survival. We only tested the vaccine during one year and prevalence of sylvatic plague likely varies among years. When the vaccine is used during high sylvatic plague activity periods, the overall vaccine effect may be better able to overcome the initial short-term negative effects of the vaccine on survival. We suggest future plague vaccine studies be conducted over multiple years to: 1) increase sample sizes, 2) account for differences in plague activity among years (increase likelihood of detecting plague), and 3) have more intervals post initial injection to better evaluate differences in long-term survival (and account for an initial period of short-term negative effects).

The effect size of the difference in survival may be biased low for several reasons: 1) small sample sizes, 2) we are unable to differentiate between dispersal and death, 3) vaccine may initially decrease survival, and 4) herd immunity. We define herd immunity is when a population is protected against a pathogen (e.g. vaccinated animals) but is short of 100% coverage, the circulation of the pathogen may be reduced or ceased because there are not enough susceptible individuals in a population to maintain the pathogen (John and Samuel 2000). Because we vaccinated over half the animals we trapped (Table 2.5) and both vaccinated and placebo animals were in the same population, we may have inadvertently also reduced the incidence of infection in the non-vaccinated (placebo) animals on our sites due to herd immunity (Fine 1993, John and Samuel 2000, Keeling et al. 2003).

Sylvatic plague intensity (the abundance of both the bacteria and the vectors) typically varies among years and seasons (Ben Ari et al. 2011). The number of diseased animals most likely fluctuates with the abundance of both *Y. pestis* and fleas (Stapp et al. 2004). Sylvatic plague is more difficult to detect during years of low activity because during these enzootic periods sylvatic plague may not reduce abundance of populations on a noticeable level without directly testing for it (Matchett et al. 2010). Epizootic sylvatic plague in some species (especially colonial species such as black-tailed prairie dogs, *Cynomis ludovicianus*) is not difficult to detect both visually (dead carcasses are often visible on the ground and a dramatic drop in abundance of live animals can be apparent) and through PCR detection of the bacterium in carcasses and fleas. Carcasses are easy to locate because the sheer number of dead animals overwhelms the predator community. However, when only a small percentage of the population dies from sylvatic plague, we expect a predator will find the carcass before a human researcher which makes detection of dead animals more difficult. Furthermore, when bacteria levels are low, our ability to directly test for *Y. pestis* within fleas may be difficult with current methods (Matchett et al. 2010). Previous studies have shown plague treatment effects increase survival of animals but little to no positive samples from PCR detection on large samples of fleas collected at the same site (Biggins et al. 2010, Matchett et al. 2010). As a result, we currently believe the best tools we have available to detect sylvatic plague at low levels (enzootic) is through more experimental flea-removal and vaccine experiments.

Sylvatic plague may be impacting many more species than previously believed in the western United States. Sylvatic plague is likely not the only cause for the decline of the northern Idaho ground squirrel (U.S. Fish and Wildlife Service 2003, Burak 2011). However, without accounting for sylvatic plague or controlling for it, studies may have a harder time assessing the impacts of other threats. Furthermore, we need to better understand the impact of restoration efforts on sylvatic plague maintenance. Increases in population density or dispersal can directly impact sylvatic plague transmission rates but may also increase recovery after a population has declined. Changes in vegetation and the small mammal community can impact flea species richness and abundance which can impact transmission rates and the ability of sylvatic plague to be maintained in a system. We expect that as new information and tools to test for the presence and effect of sylvatic plague on small mammals grows, we will better understand the impacts that this introduced disease has on small mammal communities in the western United States.

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Table 2.1. Number of study sites for each of 3 experiments where we trapped and marked northern Idaho ground squirrels and coexisting small mammals to examine the effects of plague on survival. The 9 non-paired study sites consisted of a 4-ha plot that is currently occupied by northern Idaho ground squirrels and an adjacent forested 4-ha plot. We applied DeltaDust and treated bait stations to the 4 non-paired flea-removal plots in 2015 to remove fleas. In contrast, each of the 6 paired study sites consisted of both a treatment plot where we removed fleas and a paired control plot adjacent to the treatment plot (within the same northern Idaho ground squirrel or Columbian ground squirrel site). The 5 vaccine study sites included 2 plots each where we vaccinated half of the animals caught and injected the other half with a placebo.

Type of Study Site	# Sites	# Flea-Removal Plots	# Non-Flea Removal Plots	# Vaccine Plots
Non-paired flea removal	9	4	5	0
Paired flea-removal	6	6	6	0
Vaccine	5	0	0	10

Table 2.2. Top models based on data from 9 unpaired study sites to evaluate the impacts of flea-removal treatments on survival of Columbian ground squirrels, northern Idaho ground squirrels, and yellow-pine chipmunks. Models include data from 2015-2017. Models that include season as a covariate are those where survival does not differ annually but survival differs between the active versus the hibernation seasons. All models included the trt effect (flea-removal versus no treatment). We evaluated a total of 316 candidate models for each species but only displayed those with a ΔAIC_c less than 2.00 plus the next model with the lowest AIC_c.

Model	AIC_c	ΔAIC_c	w_i	K
Columbian ground squirrels				
φ(sex + trt + season) p(sex + trt * time)	929.19	0.00	0.10	15
φ(sex + trt + season) p(sex + time)	930.65	1.46	0.05	10
φ(sex + trt + time) p(time)	930.80	1.60	0.04	12
φ(sex + trt + season) p(trt * time)	930.84	1.65	0.04	14
φ(sex + trt + time) p(trt * time)	931.16	1.97	0.04	17
φ(sex * trt + season) p(sex + trt * time)	931.24	2.04	0.04	16
Northern Idaho ground squirrels				
φ(sex + trt + season) p(sex + time)	2765.34	0.00	0.08	10
φ(sex + trt + season) p(time)	2765.63	0.29	0.07	9
φ(sex + trt + season) p(sex * trt + time)	2766.27	0.94	0.05	12
φ(sex + trt + time) p(trt + time)	2766.29	0.95	0.05	12
φ(sex * trt + time) p(time)	2766.55	1.21	0.04	12
φ(sex + trt + time) p(time)	2766.67	1.33	0.04	12
φ(sex + trt + season) p(sex + trt * time)	2767.04	1.71	0.03	15
φ(sex + trt + season) p(sex + trt + time)	2767.08	1.75	0.03	11
φ(sex * trt + season) p(sex + time)	2767.14	1.80	0.03	11
φ(sex + trt * season) p(sex + time)	2767.23	1.90	0.03	11
φ(sex + trt + season) p(trt * time)	2767.33	1.99	0.03	14
φ(sex * trt + season) p(time)	2767.36	2.03	0.03	10
Yellow-pine chipmunks				
φ(sex + trt + season) p(trt * time)	1703.03	0.00	0.08	14
φ(sex + trt + season) p(sex + trt * time)	1703.57	0.54	0.06	15
φ(sex * trt + season) p(trt * time)	1703.60	0.57	0.06	15
φ(sex * trt + season) p(sex + trt * time)	1704.15	1.12	0.05	16
φ(sex + trt * season) p(trt * time)	1704.27	1.23	0.05	15
φ(sex + trt * time) p(sex + time)	1704.64	1.61	0.04	16
φ(sex + trt * season) p(sex + trt * time)	1704.80	1.76	0.03	16
φ(sex + trt * time) p(sex + season)	1705.00	1.97	0.03	14
φ(sex + trt + season) p(time)	1705.07	2.04	0.03	9

Table 2.3. Top models based on data from 6 paired study sites to evaluate the impacts of flea-removal treatments on survival of Columbian ground squirrels, northern Idaho ground squirrels, yellow-pine chipmunks, and deer mice. Models include data from 2014-2017 (except for chipmunks which we included data only from 2015-2017). Models that include season as a covariate are those where survival does not differ annually but survival differs between the active versus the hibernation seasons. All models included the trt effect (flea-removal versus no treatment). Season was not included in any Columbian ground squirrel models because we only used data from spring trapping sessions due to low capture rates in the summer. We evaluated a total of 117 (Columbian ground squirrel), 315 (northern Idaho ground squirrel and deer mice), and 280 (yellow-pine chipmunk) candidate models for each species but only displayed those with a ΔAIC_c less than 2.00 plus the next model with the lowest AIC_c.

Model	AIC_c	ΔAIC_c	w_i	K
Columbian ground squirrels				
φ(sex + trt) p(time)	325.95	0.00	0.19	6
φ(sex * trt) p(time)	327.65	1.70	0.08	7
φ(sex + trt) p(trt + time)	327.94	2.00	0.07	7
φ(sex + trt) p(sex + time)	328.08	2.14	0.07	7
Northern Idaho ground squirrels				
φ(sex + trt + time) p(sex + time)	2001.17	0.00	0.27	17
φ(sex + trt + time) p(sex + trt + time)	2002.96	1.78	0.11	18
φ(sex + trt + time) p(time)	2002.99	1.82	0.11	16
φ(sex * trt + time) p(sex + time)	2003.24	2.07	0.10	18
Yellow-pine chipmunks				
φ(sex + trt + time) p(time)	2902.26	0.00	0.22	12
φ(sex + trt + time) p(trt + time)	2903.42	1.16	0.12	13
φ(sex + trt + time) p(sex + time)	2904.14	1.88	0.09	13
φ(sex * trt + time) p(time)	2904.23	1.97	0.08	13
φ(sex + trt * time) p(sex * trt + time)	2904.91	2.65	0.06	16
Deer mice				
φ(sex + trt * season) p(trt)	966.47	0.00	0.05	9
φ(sex + trt + time) p(sex)	966.94	0.47	0.04	16
φ(sex * trt + time) p(sex)	966.94	0.47	0.04	17
φ(sex + trt + time) p(trt)	967.50	1.03	0.03	16
φ(sex + trt + season) p(sex)	967.50	1.03	0.03	7
φ(sex * trt + time) p(trt)	967.54	1.07	0.03	17
φ(sex + trt + season) p(trt)	967.70	1.23	0.02	7
φ(sex * trt + season) p(sex)	967.79	1.32	0.02	8
φ(sex + trt * season) p(sex + trt)	967.91	1.44	0.02	10
φ(sex * trt + season) p(trt)	968.14	1.67	0.02	8
φ(sex + trt * season) p(sex)	968.21	1.74	0.02	9
φ(sex + trt + season) p(trt + season)	968.24	1.77	0.02	9
φ(sex * trt + time) p(sex + trt)	968.41	1.94	0.02	18
φ(sex + trt + time) p(sex + trt)	968.58	2.11	0.02	17

Table 2.4. Top models based on data from 10 vaccine plots designed to examine the effectiveness of a plague vaccine on apparent survival of yellow-pine chipmunks and deer mice. All data were collected from 2016-2018 (only 2016-2017 for deer mice). Trt(boostandafterboost) includes all intervals: initial shot, all intervals after the first interval after shot, booster shot, and all intervals after the first interval after booster. Trt(after) includes: initial shot and combines all other intervals into one (after shot, booster, and after booster). Mice were not given a booster shot so trt(after shot) includes only initial shot and intervals after the first interval after the shot. Season_summer refers to the last two trap session (mid and late summer did not differ). Only models with a ΔAIC_c less than 10.00 and the null model are included.

Model	AIC_c	ΔAIC_c	w_i	df
Adult yellow-pine chipmunks				
Trt(boostandafterboost) * season_summer	283.728	0.000	0.60	16
Trt(boostandafterboost) * season_summer + sex	285.923	2.195	0.20	17
Trt(after) * season_summer	287.462	3.733	0.09	10
Trt(after) * season	289.425	5.696	0.03	12
Trt(after) * season_summer + sex	289.495	5.766	0.03	11
Trt(boostandafterboost) * season	290.512	6.783	0.02	19
Trt(after) * season + sex	291.453	7.725	0.01	13
Trt(boostandafterboost) * season + sex	292.804	9.075	0.01	20
Null	310.550	26.821	0.00	1
Juvenile yellow-pine chipmunks				
Trt(boostandafterboost) * sex	223.39	0.00	0.43	16
Trt(boostandafterboost)	225.66	2.27	0.14	8
Trt(after) * season_su	226.81	3.42	0.08	6
Trt(after) * season	226.81	3.42	0.08	6
Trt(boostandafterboost) + sex	227.00	3.61	0.07	9
Trt(boostandafterboost) * season_su	228.24	4.85	0.04	10
Trt(boostandafterboost) * season	228.24	4.85	0.04	10
Trt(after) * season_su + sex	228.36	4.97	0.04	7
Trt(after) * season + sex	228.36	4.97	0.04	7
Trt(after) * sex	229.68	6.29	0.02	8
Trt(boostandafterboost) * season_su + sex	229.69	6.30	0.02	11
Trt(boostandafterboost) * season + sex	229.69	6.30	0.02	11
Null	236.27	12.88	0.00	1
Deer mice				
Trt(after shot)	316.11	0	0.34	4
Trt(after shot) * season_summer	316.55	0.44	0.27	6
Null	317.77	1.66	0.15	1
Trt(after shot) + sex	318.15	2.04	0.12	5
Trt(after shot) * season_summer + sex	318.58	2.47	0.1	7
Trt(after shot) * sex	322.75	6.64	0.01	8
Trt(after shot) * season	324.44	8.33	0.01	10

Table 2.5. Number of individuals administered either a vaccine or placebo at 10 vaccine plots. We vaccinated every other individual trapped within an age/sex class within each session.

Species	Sex	Age	Year	# Vaccinated	# Placebo
T. amoenus	Male	Adult	2016	2	1
T. amoenus	Female	Adult	2016	5	3
T. amoenus	Male	Juvenile	2016	0	0
T. amoenus	Female	Juvenile	2016	0	0
T. amoenus	Male	Adult	2017	24	14
T. amoenus	Female	Adult	2017	35	25
T. amoenus	Male	Juvenile	2017	39	31
T. amoenus	Female	Juvenile	2017	32	32
P. maniculatus	Male		2016	9	3
P. maniculatus	Female		2016	8	6
P. maniculatus	Male		2017	39	26
P. maniculatus	Female		2017	39	25

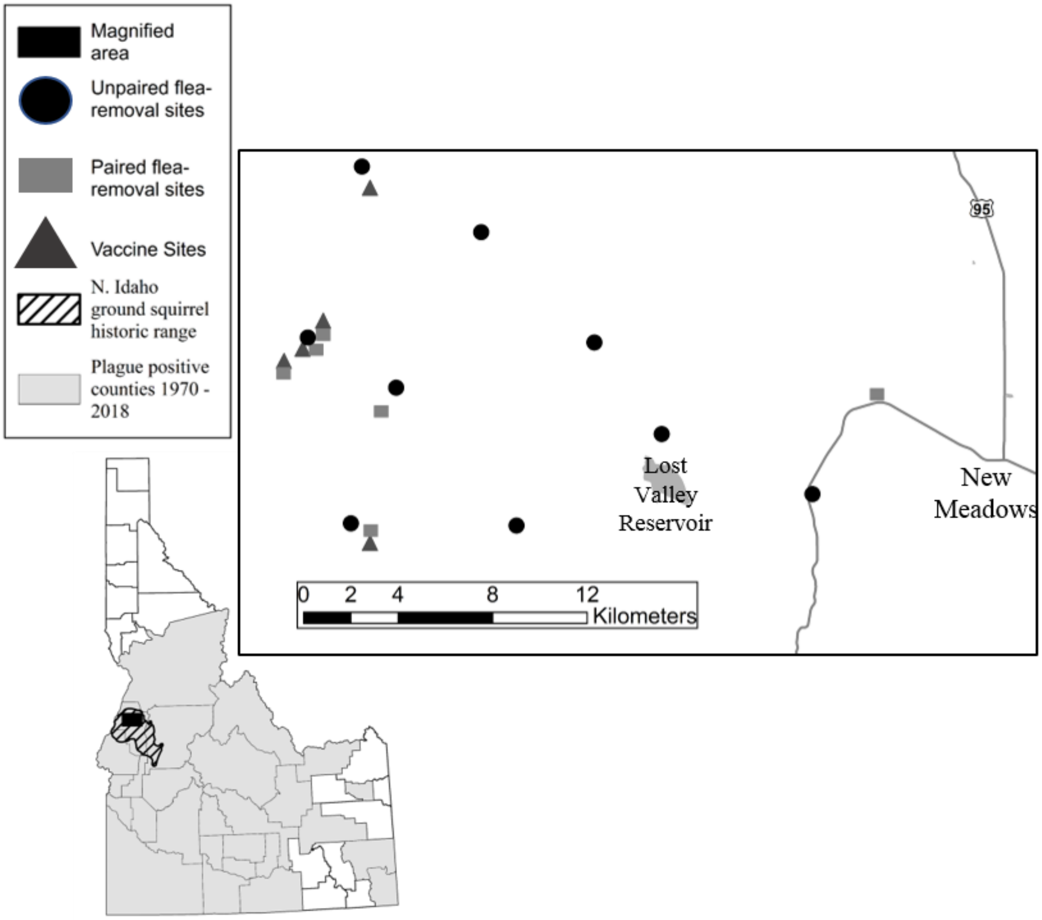

Figure 2.1. Maps of the plague study sites including all 3 experimental designs: non-paired flea-removal study sites, paired flea-removal study sites, and vaccine study sites. Map of Idaho includes all counties that have reported at least 1 plague-positive animal or flea sample between 1970 – 2018 (data from 1970-2009 from Abbott and Rocke 2012, data from 2010 – 2018 from S.R. Stopack and Idaho Department of Fish and Game). All study sites were located within Adams County, Idaho.

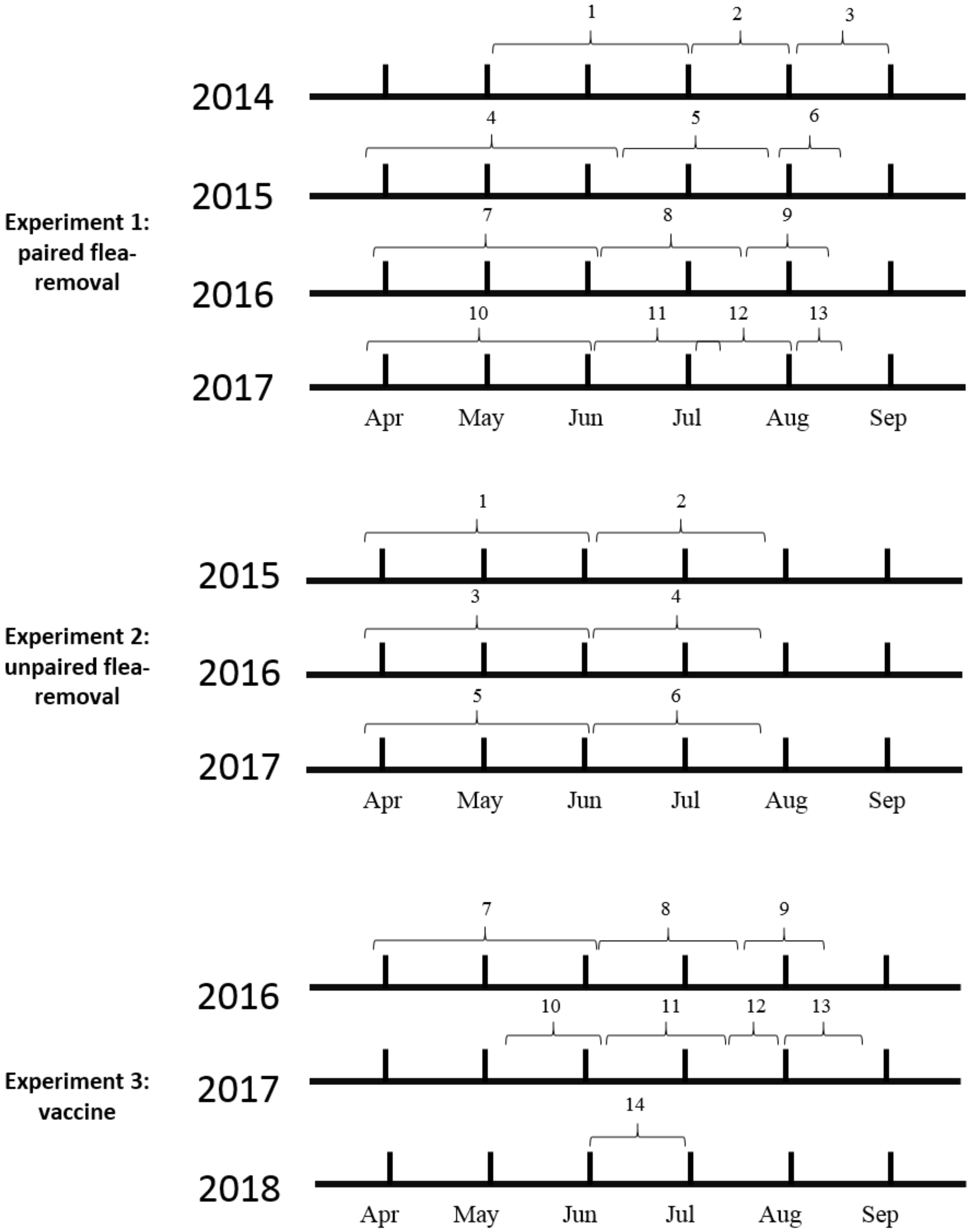

Figure 2.2. Trapping timeline for all 3 experimental designs. Each number represents the sequential trapping session. We defined seasons (covariate included in many of the models) as: spring (first session each year), summer (second session each year) and late summer (third session each year). Timing of trapping varied between experiments and years because of weather or notification of funding.

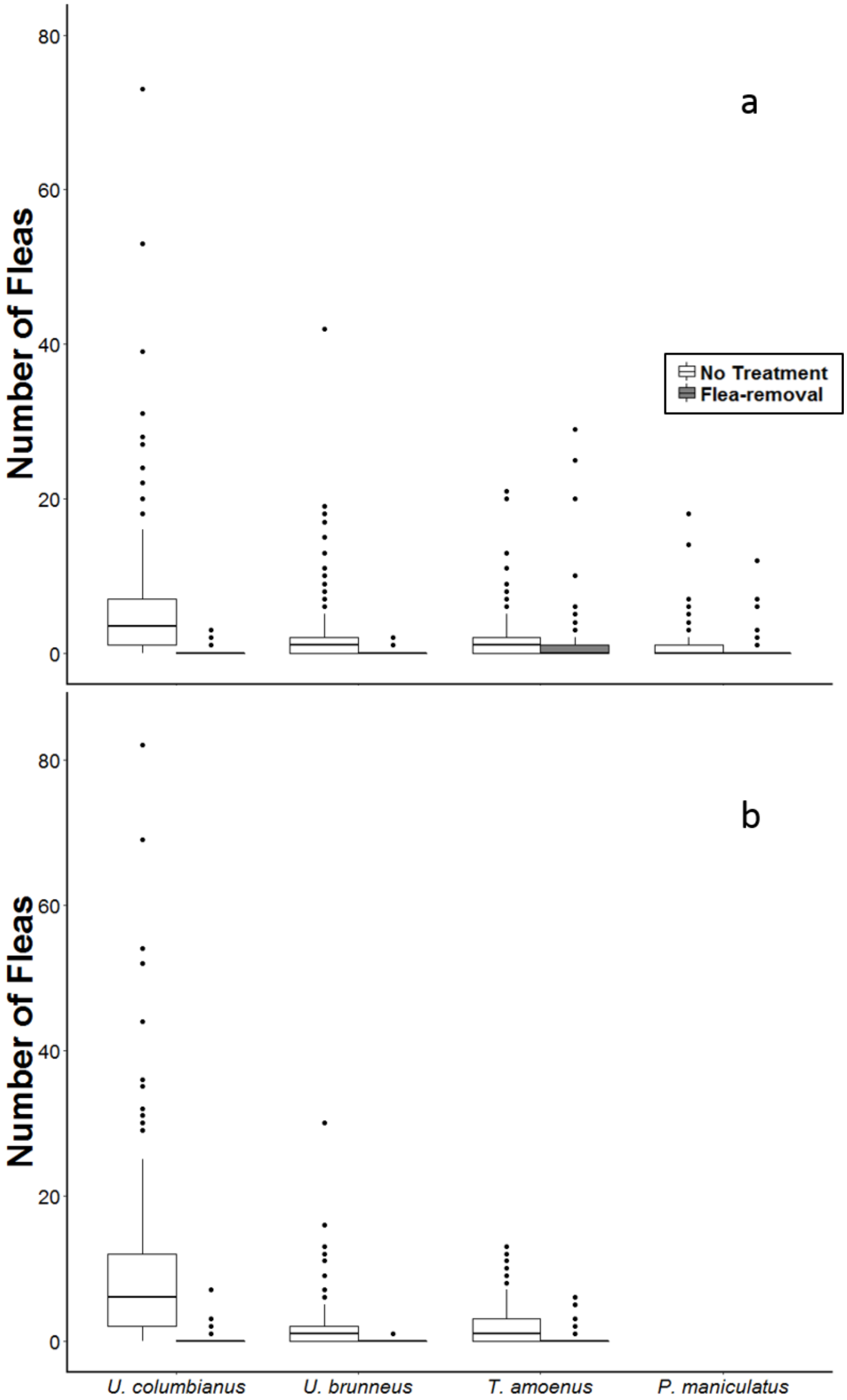

Figure 2.3. Flea abundance on flea-removal plots and non-treatment plots for 4 species of rodents at a) 6 paired plots and b) 9 non-paired plots. The bottom and top limits of each box are the 1st and 4th quartile (25th and 75th percentiles). Outliers are more than 1.5 times the interquartile range.

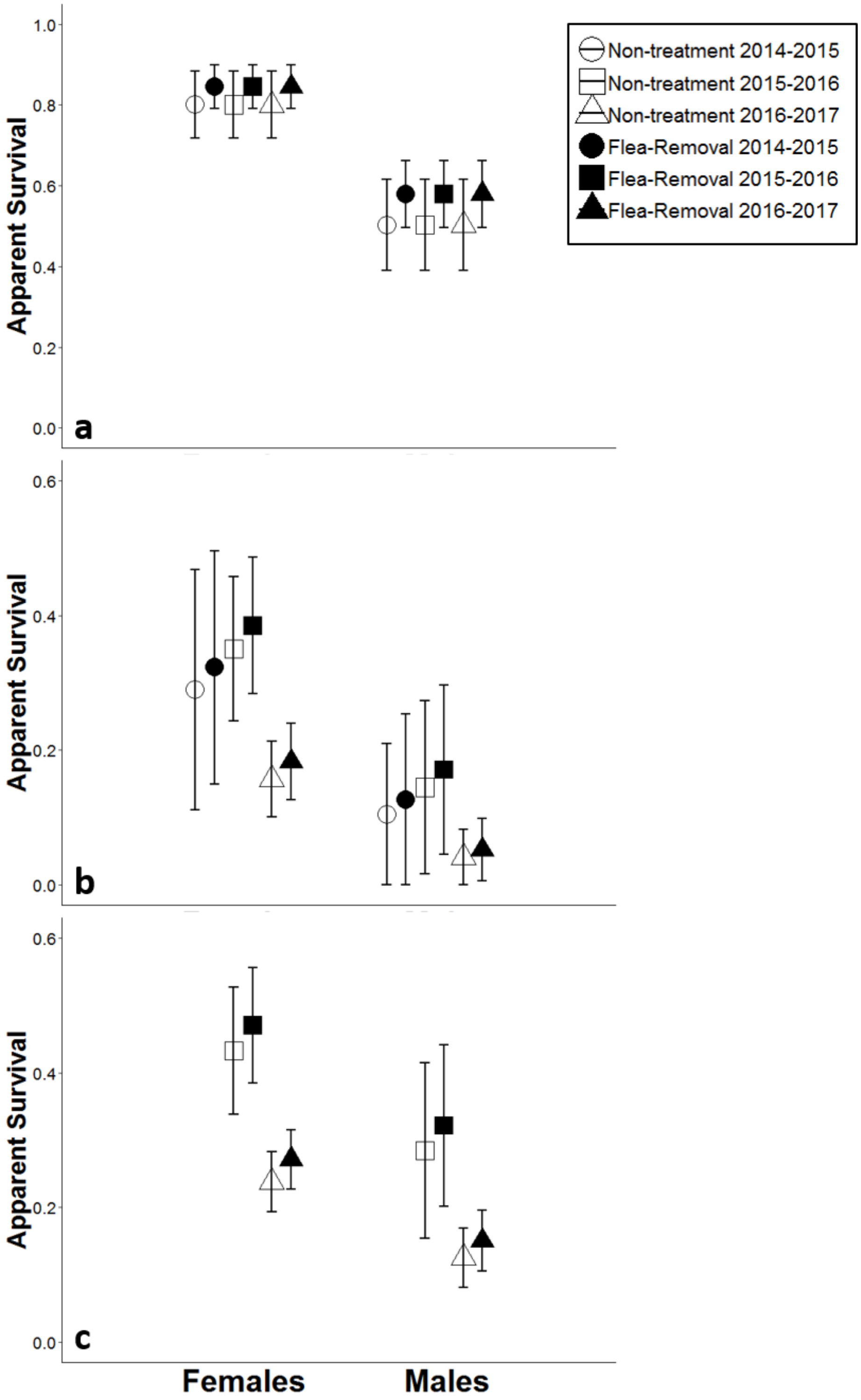

Figure 2.4. Apparent annual survival based on data from 6 paired plots from Program MARK for (a) Columbian ground squirrels, b) northern Idaho ground squirrels, and (c) yellow-pine chipmunks from 2014-2015, 2015- 2016, and 2016-2017. Error bars indicate SE. We did not estimate survival for chipmunks from 2014-2015 because we trapped too few individuals. We also trapped very few Columbian ground squirrels during the summer in 2014 so we estimated annual survival using only spring captures.

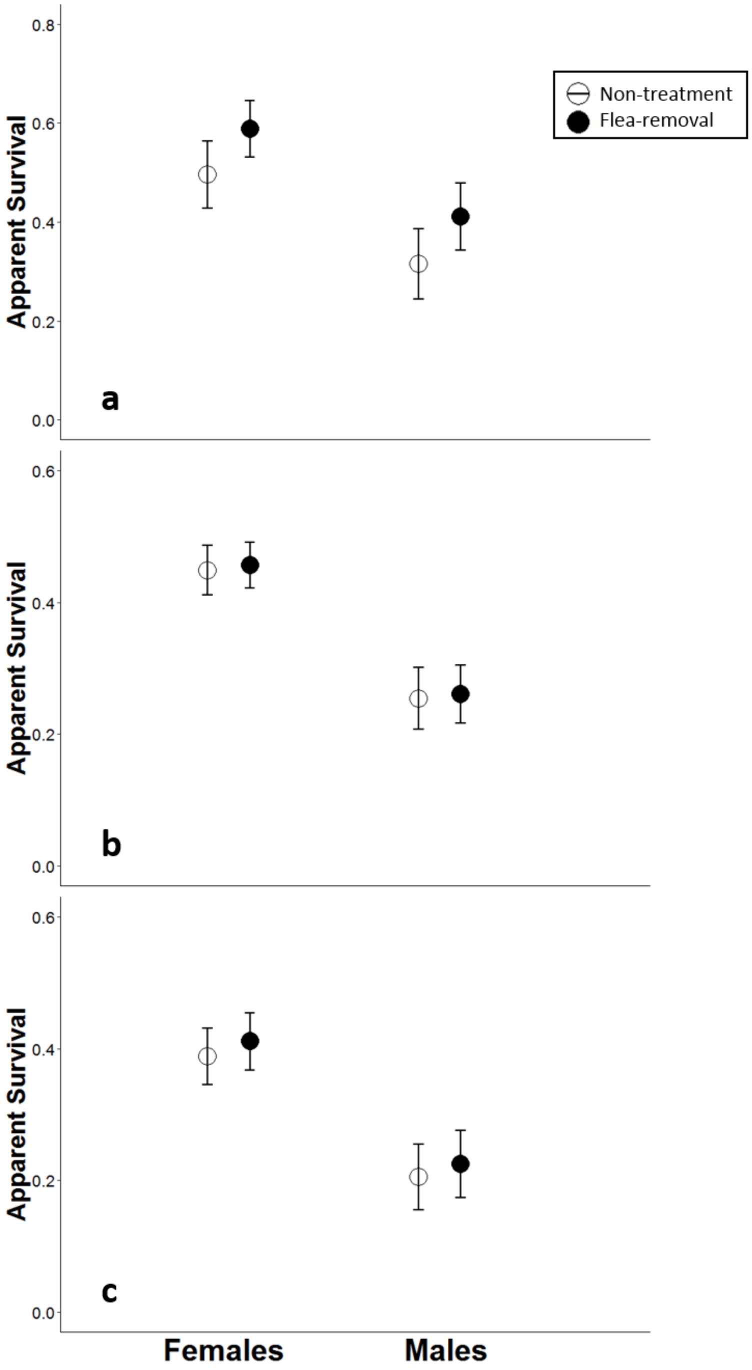

Figure 2.5. Apparent annual survival based on data from 9 non-paired study sites. Estimates were calculated from Program MARK for (a) Columbian ground squirrels, b) northern Idaho ground squirrels, and (c) yellow-pine chipmunks from 2014-2015, 2015-2016, and 2016-2017. Error bars indicate SE. The top model for all 3 species did not include a covariate for year so point estimates are for all 3 years that the plague study was implemented on the non-paired sites.

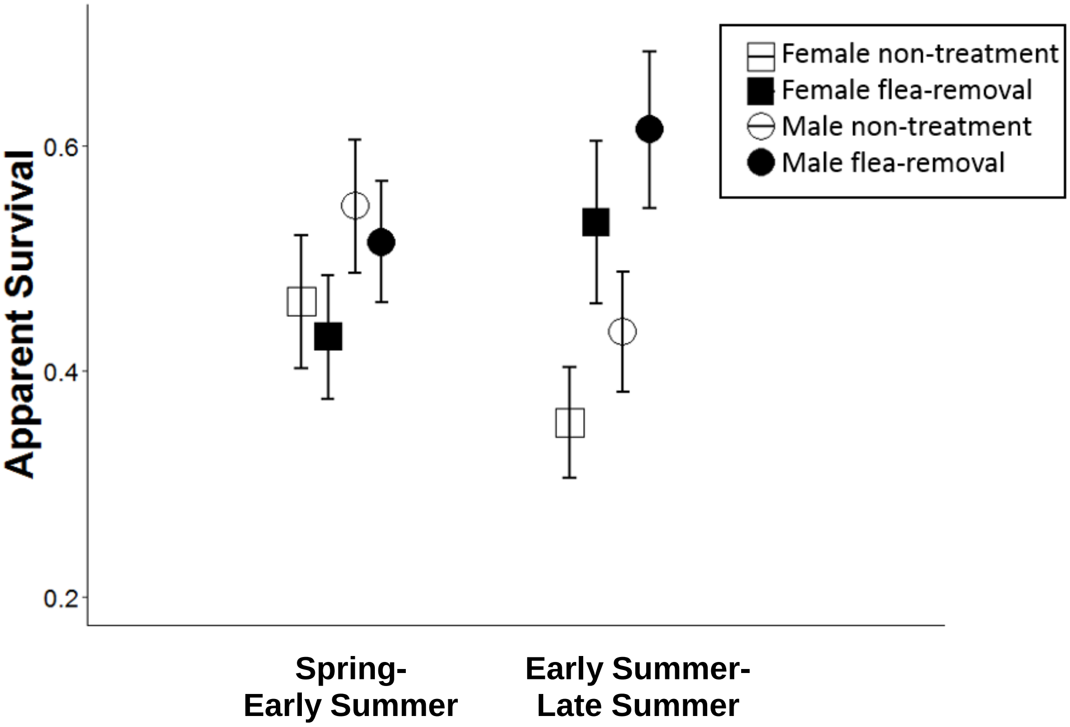

Figure 2.6. Apparent survival estimates for deer mice based on data from 6 paired study sites (6 flea-removal plots and 6 control plots) from Program MARK for each of 4 years (2014-2017). Trapping intervals represent apparent survival rates during the trapping season (spring through late summer). Error bars indicate SE. The top model did not include the effect of year so estimates are for all 4 years combined.

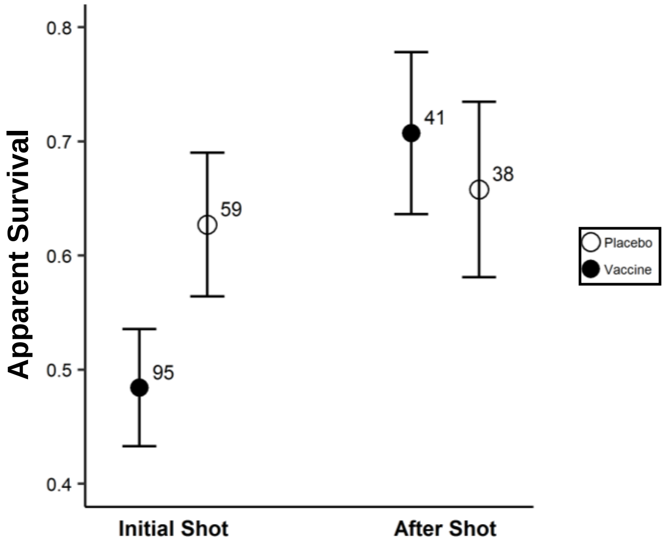

Figure 2.7. Apparent survival estimates for vaccinated and placebo deer mice based on data from 10 vaccine plots for 2 years combined (2016-2017). Initial shot included all intervals immediately following the initial shot (vaccine or placebo). All intervals after the first (initial shot) are considered the after shot interval. Sample sizes are included next to each point. Error bars indicate SE.

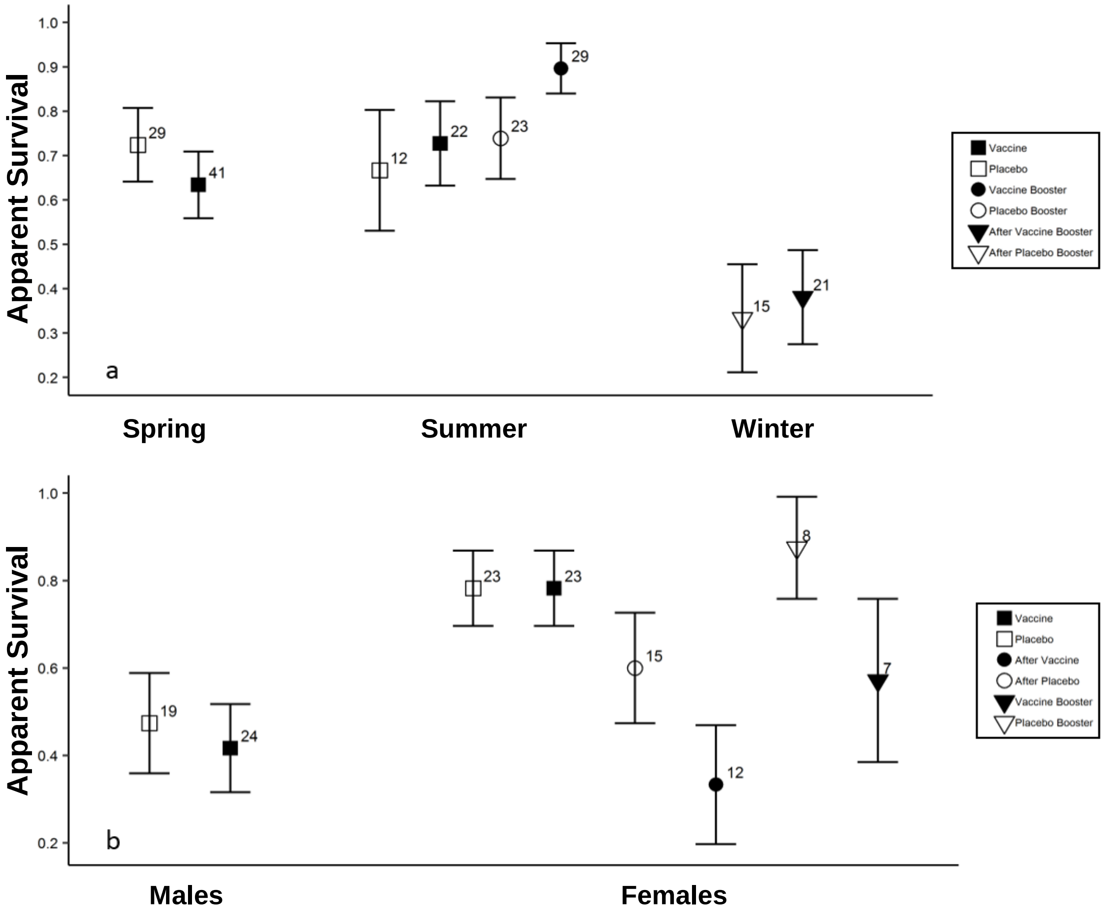

Figure 2.8. Apparent survival estimates of vaccinated and placebo animals for a) adult yellow-pine chipmunks and b) juvenile yellow-pine chipmunks based on data from 10 vaccine plots for all years combined (2016-2018). Vaccine and placebo represent the first interval following the initial shot, vaccine and placebo booster represent the first interval following the booster shot, after vaccine and after placebo represent interval(s) following the 1st interval following the initial shot, and after vaccine or placebo booster represents the interval(s) following the 1st interval following the booster shot. All intervals after the first (initial shot) are considered the after shot interval. Sample sizes are included next to the point estimates. Error bars indicate SE.

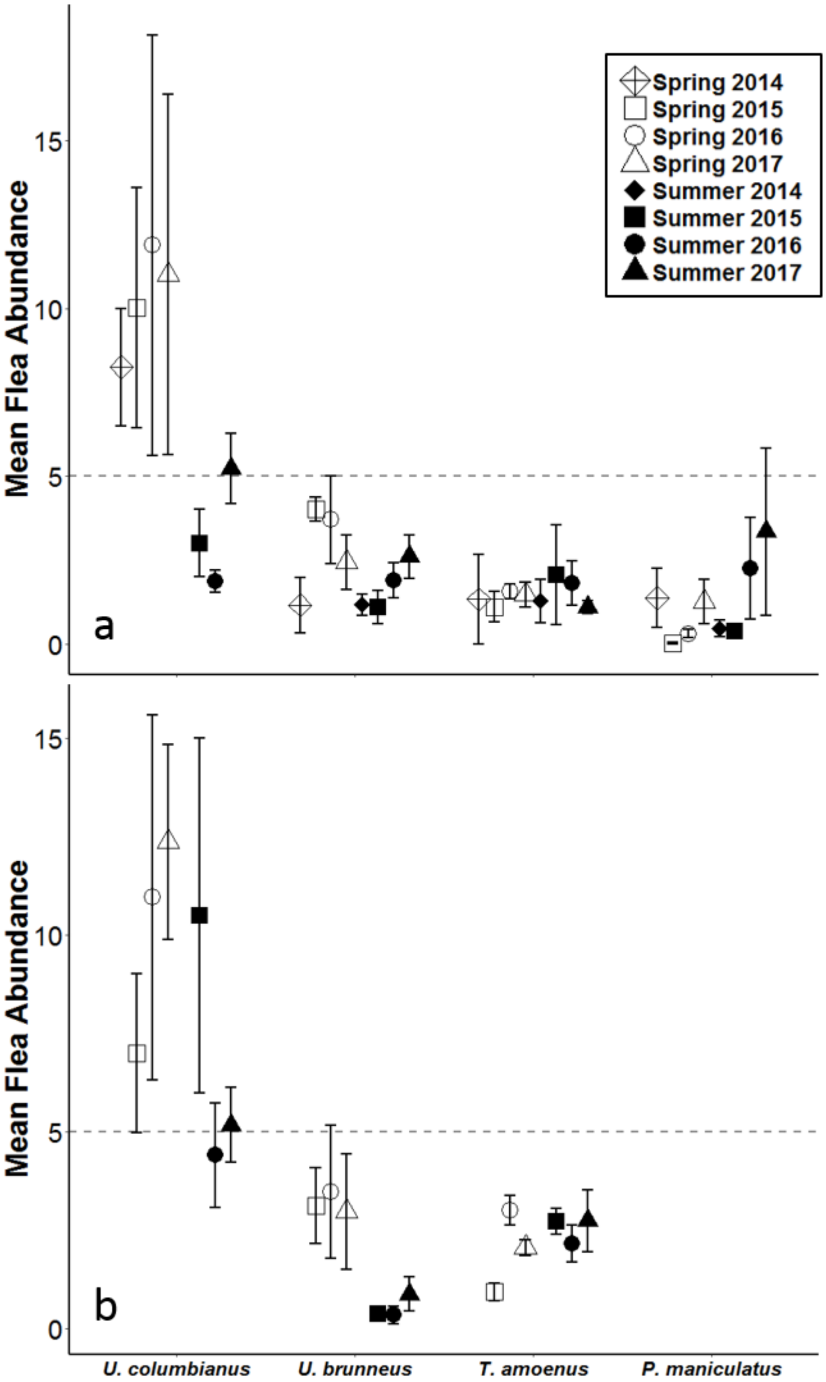

Figure 2.9. Average flea abundance on Columbian ground squirrels (*U. columbianus*), northern Idaho ground squirrels (*U. brunneus*), yellow-pine chipmunks (*T. amoenus*), and deer mice (*P. maniculatus*) on non-treatment sites at a) 6 paired study sites and b) 9 un-paired study sites. All points include standard error bars. The grey horizontal dashed line represents the estimated minimum number of fleas needed to sustain enzootic plague (Lorange et al. 2005). Flea abundances differed among species, seasons, and years. All flea abundance estimates are minimum counts; we undoubtedly missed some unknown number of fleas due to the sampling design (see text).

Chapter 3: Diet of the northern Idaho ground squirrel based on fecal DNA: selection, seasonality, and survival correlates

Abstract

Herbivore survival and reproduction are thought to be influenced by food availability. Animals that hibernate may be particularly sensitive to changes in food quantity or quality because they often have a short period of time to consume energy to reproduce and store enough fat to survive the hibernation season. The northern Idaho ground squirrel (*Urocitellus brunneus*) is listed as federally Threatened under the Endangered Species Act and is only found in 2 counties in Idaho. The northern Idaho ground squirrel hibernates for approximately 8 months each year. The leading hypothesis for the decline is due to habitat loss from years of fire suppression which has enabled conifer trees to encroach on the open canopy areas the squirrels prefer. Fire suppression and other anthropogenic threats may reduce both abundance and quality of necessary forage for these squirrels. We used genetic metabarcoding techniques to identify the diet of 188 squirrels at 11 study sites, and we compared frequency of plants in the diet to their frequency within vegetation plots (i.e., availability). We identified 42 families, 126 genera, and 120 species of plants in the squirrel's diet (29 genera could not be classified to the species level). Northern Idaho ground squirrel diets differed between spring and summer, and frequency of many plants in the diet differed from their frequency in plots within foraging areas. *Perideridia* and *Allium* were positively associated with survival while *Frasera* was negatively associated with survival. Our results suggest that northern Idaho ground squirrels are selective feeders that may be limited by food quality and availability.

Keywords: *Urocitellus brunneus*, conservation, double barcode, non-invasive sampling, food selection, survival, season

Introduction

You are what you eat. There is a lot of truth in this school-yard phrase because animals are often defined by their diet and the search for food typically affects most other aspects of an animal's behavior and ecology. For example, herbivores are often thought to be food limited (Bobek 1977, Sinclair et al. 1985, Skogland 1985, Belovsky 1986, Fryxell 1987, White 2008) and reductions in high-quality forage may reduce population size via impacts to demographic parameters. Optimal foraging theory assumes that the main goal of a generalist herbivore is to maximize quantity or quality of food while foraging (MacArthur and Pianka 1966, Charnov 1976, Belovsky 1986) or to obtain food efficiently while avoiding predation. Both survival and reproduction of herbivores have been associated with increased energy intake (White 1983, Ritchie 1990). Hence, obtaining a sufficient amount of high-quality forage quickly and safely are behaviors assumed to be under strong selection for many herbivores. Herbivores face a number of anthropogenic threats that may reduce food availability or food quality such as: (1) changing plant community composition due to invasive species (D'Antonio and Vitousek 1992), (2) reduced plant biomass due to grazing (Hayes and Holl 2003), and (3) reductions in optimal forage plants due to changes in precipitation and temperature as as a result of climate change (Thuiller et al. 2005, Bertrand et al. 2011).

We need to understand the diet of animals to be able to assess if and/or how land use changes (and management actions) impact populations. Moreover, the response of plants to management actions may be taxa-specific. Hence, documenting dietary preferences of specific taxa rather than broad categories, and documenting the influence specific forage plants on survival, will help determine whether anthropogenic-induced habitat changes have a negative impact on forage quality and, ultimately, survival of herbivores. This information is particularly important for species of management and conservation concern.

Animals that hibernate may be particularly sensitive to changes in food quantity or quality because they may have different nutritional needs than non-hibernators and these nutrients must be obtained during a short period of time each year. Many hibernating ground squirrels alter their diets seasonally and often switch to eating plants high in particular polyunsaturated fatty acids prior to hibernation (Frank 1994, Lehmer et al. 2006). Therefore, documenting seasonal changes in preferred forage is particularly important for hibernating herbivores and diet studies need to have sufficient taxonomic resolution to detect subtle shifts in preferred forage items.

The northern Idaho ground squirrel (*Urocitellus brunneus*) is a threatened species that hibernates for approximately 8 months each year (U.S. Fish and Wildlife Service 2003). Habitat loss is thought to be the cause of their past population declines and range contraction. Years of fire suppression have enabled conifer trees to encroach into meadows and forest openings where these ground squirrels live. Changes in canopy cover and loss of fire may have led to changes in food quantity or quality. We need more detailed information on diet of the squirrel to better assess the effects of fire suppression on preferred food items and to determine how management treatments affect preferred forage plants. Northern Idaho ground squirrels have a diverse diet (Dyni and Yensen 1996), but we lack information regarding the relative importance of different forage plants or whether diet differs among sex or age classes. Food preference and nutritional needs may vary or change seasonally among age or sex classes within the same species (juveniles vs adults and/or females vs males). Diet may differ among age classes because smaller individuals have smaller guts and, hence, they may be more selective in their diet choices to optimize their energy intake (Demment and Van Soest 1985). Diets of ungulates often differ between sexes (Beier 1987, Oakes et al. 1992) due, in part, to increased nutritional needs of females during pregnancy and lactation (Barboza and Bowyer 2000, Rothman et al. 2008). In contrast, microhistological studies on ground squirrels have found no difference in diet between males and females (Van Horne et al. 1998, Yensen et al. 2013). However, subtle differences may be difficult to detect with microhistology. Understanding if and how diet varies seasonally and among demographic classes would help determine which plants influence persistence of northern Idaho ground squirrel populations.

We used DNA metabarcoding of fecal pellets to identify both the composition and frequency of plants in the diet of northern Idaho ground squirrels. All previous noninvasive studies that assessed the diet of hibernating herbivores have relied on microhistological techniques. However, new DNA metabarcoding techniques have been shown to have higher resolution compared to microhistological methods (Valentini et al. 2009, Bybee et al. 2011). Furthermore, microhistological analysis requires substantial training in identification of plant taxa from tiny fragments left in fecal samples and fragment sizes must be large enough to distinguish (resulting in potential bias towards more easily identified and less easily digested species; Soininen et al. 2009). In contrast, DNA metabarcoding is faster and potentially more accurate at identifying food items (Soininen et al. 2009). DNA metabarcoding should enable us to evaluate diets at lower taxonomic levels and with less bias, and thereby allow us to better assess dietary preferences and identify forage items that impact survival. We had 3 objectives:

(1) determine whether the diet of northern Idaho ground squirrels differs among age classes (adult vs juvenile), sexes, seasons (spring vs summer), or plant-community composition (i.e., relative to available food items among study sites),

(2) determine whether particular plant genera are associated with northern Idaho ground squirrel survival, and

(3) create an updated and more comprehensive list of plants consumed by northern Idaho ground squirrels to inform future management practices.

Materials and Methods

Study area and species

We collected northern Idaho ground squirrel fecal pellets in 2015 and 2016 from 13 study sites in Adams County, Idaho. The study sites were mostly in remote areas on lands managed by the U.S. Forest Service, Idaho Department of Lands, and privately-owned lands. Study sites varied in elevation (1,280 – 1,700 m) and distance to nearest incorporated town (8 – 35 km straight line distance). The average maximum snow depth for the two years of our study was 1.21 m and average annual precipitation was 0.92 m at the closest SNOTEL weather station to our sites (ns (www.wcc.nrcs.usda.gov). The study sites were 13 of the 124 extant sites known to support northern Idaho ground squirrels and were part of a long-term restoration project employing 2 study designs: (1) 8-ha plots that straddled the ecotone between forest and non-forest (meadow/clearing) (Fig. 3.1a), and (2) 4 to 8-ha non-forest plots (Fig. 3.1b).

Northern Idaho ground squirrels mate in the spring soon after females emerge from hibernacula in late March – April, reproduce only once per year, and immerge back into hibernacula in July – August (Yensen and Sherman 1997). Hence, they have a short active season above ground (~4 months) when they must reproduce, raise offspring, and increase their body mass before reentering into hibernation. Hence, we assumed that these squirrels should forage quickly and efficiently on the most preferred food items available to them.

Sampling

We trapped and handled all northern Idaho ground squirrels following protocols developed by Idaho Department of Fish & Game (D. Evans Mack, unpubl. doc). We trapped squirrels twice per year at each site: in the spring (end of April – early June) and in the summer (June – end of July). We collected fecal samples within a site over approximately a 2-week period of time. We recorded the age (adult or juvenile) and sex of each squirrel trapped. We rarely captured juveniles in the spring as they do not emerge from their natal burrows until the end of the spring trapping season (late May or early June).

We carried each captured squirrel in the trap to a nearby processing station. We placed the trap on top of a paper towel while the northern Idaho ground squirrel was waiting to be processed or after it was processed but waiting to be released. We placed all fecal pellets that fell onto the paper towel into a paper coin envelope. We recorded the individual squirrel ID, date, and location for each fecal pellet collected. We placed the envelopes in a zip-top bag with silicone gel beads to dry the samples out. All samples were placed in a 0°C freezer at the end of the field season to minimize degradation of the samples (within 1-4 months).

DNA extraction

We used QIAamp DNA Stool Mini Kits (Qiagen, Inc., Hilden, Germany) to extract genomic DNA from the pellet samples. The number of pellets per sample extraction varied from one to three. We combined the pellets from each sample together (when there was >1) for DNA extraction and used all fecal pellet material combined. We used a Qubit 2.0 fluorometer to quantify the DNA in the resulting extractions. Initial tests to quantify the amount of DNA from samples with varying number of pellets suggested a positive relationship between pellet number and DNA amount so we only used samples with $\geq$2 pellets. Thereafter we combined all fecal pellets ($\geq$2) for each individual northern Idaho ground squirrel samples for DNA extraction. We analyzed 188 fecal samples from our 13 study sites, including those from 27 juvenile males (all captured during the summer), 45 juvenile females (1 captured in the spring and 44 in the summer), 43 adult males (22 in the spring and 21 in the summer), and 73 adult females (41 in the spring and 32 in the summer).

PCR and Amplicon Sequencing

We used 3 metabarcoding primer sets to amplify 2 gene regions, the nuclear ribosomal (nr) DNA internal transcribed spacer (ITS) region and a portion of the chloroplast (cp) DNA *trn*L intron (trnL). The ITS region was amplified in two fragments (ITS1 and ITS2) using the "universal" plant primers pairs its5/its2 (ITS1) and its3/its4 (ITS2) of (Baldwin 1992). To amplify a portion of the cpDNA *trn*L intron, a region of the plastome commonly sequenced for species-level plant systematic studies, we designed universal primers for seed plants to amplify an ~200bp portion of the 5' end of the *trn*L intron (trnLi_SP_9F: TGGATTGAGCCTTGGTATGGAA, trnLi_SP_189R: AGCTTCCATTGAGTCTCTGCA), based on prior knowledge of the plant composition data that we collected as part of a companion study (Andrews et al. 2017). To test the performance of these primers, we conducted PCRs for each primer set on genomic DNA extracted from herbarium specimens of two plant species that occur in northern Idaho ground squirrel habitat: *Balsamorhiza sagittata* and *Salix scouleriana.*

PCR amplification followed a two-round PCR strategy. Following Uribe-Convers et al. (2016) each target-specific primer sequence contained a conserved sequence tag that was added to the 5' end at the time of oligonucleotide synthesis (CS1 for forward primers and CS2 for reverse primers). The purpose of the added CS1 and CS2 tails is to provide an annealing site for the second pair of primers. After an initial round of PCR using the CS-tagged, target-specific primers (PCR1), a second round of PCR was used to add 8 bp sample-specific barcodes and high-throughput sequencing adapters to both the 5' and 3' ends of each PCR amplicon (PCR2). From 5' to 3', the PCR2 primers included the reverse complement of the conserved sequence tags, sample-specific 8 bp barcodes, and either Illumina P5 (CS1-tagged forward primers) or P7 (CS2-tagged reverse primers) sequencing adapters. Sequences for the CS1 and CS2 conserved sequence tags, barcodes, and sequencing adapters were taken from Uribe-Convers et al. (2016). PCR conditions were as follows: PCR1 – 25 ul reactions included 2.5 ul of 10x PCR buffer, 3 ul of 25 mM $MgCl_2$, 0.30 ul of 20 mg/ml BSA, 1 ul of 10 mM dNTP mix, 0.125 ul of 10 uM CS1-tagged target specific forward primer, 0.125 ul of 10 uM CS2-tagged target specific reverse primer, 0.125 ul of 5000 U/ml Taq DNA polymerase, 1 ul template of DNA, and PCR-grade H_2O to volume; PCR1 cycling conditions - 95°C for 2 min.

followed by 20 cycles of 95°C for 2 min., 50-60°C for 1 min. (depending on T_m of target specific primers), 68°C for 1 min., followed by a final extension of 68°C for 10 min.; PCR2 – 20 ul reactions included 2 ul of 10x PCR buffer, 3.6 ul of 25 mM $MgCl_2$, 0.60 ul of 20 mg/ml BSA, 0.40 ul of 10 mM dNTP mix, 0.75 ul of 2 uM barcoded primer mix, 0.125 ul of 5000 U/ml Taq DNA polymerase, 1 ul of PCR1 product as template, and PCR-grade H_2O to volume; PCR2 cycling conditions - 95°C for 1 min. followed by 15 cycles of 95°C for 30 sec., 60°C for 30 sec., 68°C for 1 min., followed by a final extension of 68°C for 5 min. Following PCR2, the resulting amplicons were pooled together and sequenced on an Illumina MiSeq platform with 150bp paired end reads using the 300 cycle MiSeq Sequencing v2 Nano kit.

Sequence processing

Pooled reads from the Illumina MiSeq run were demultiplexed using the dbcAmplicons pipeline (https://github.com/msettles/dbcAmplicons) following the workflow detailed in Uribe-Covers et al. (2016). For each sample, read-pairs were identified, sample-specific dual-barcodes and target specific primers were identified and removed (allowing the default matching error of 4 bases), and each read was annotated to include the sample name and read number for the gene region. To eliminate fungal contamination that may have been amplified with ITS, and non-specific amplification of poor PCR products for both gene regions, each read was screened against a user-defined reference file of annotated sequences retrieved from GenBank (using the "-screen" option in dbcAmplicons). Reads that mapped with default sensitivity settings were kept, and unique sequences were identified using the clustering approaches implemented in PURC v.1.02 (Rothfels et al. 2017). The fluidigm2purc pipeline (Blischak et al. 2018) was used to convert demultiplexed data from dbcAmplicons to inputs for PURC. The fluidigm2purc pipeline takes the paired-end FASTQ files, filters them using Sickle (Joshi and Fass 2011; minimum length = 100 bp, PHRED threshold = 20), merges the filtered reads using FLASH2 (Magoč and Salzberg 2011), and then converts the resulting FASTQ files into FASTA files for each gene region with sequence header information that is compatible with the *purc_recluster.py* script (Rothfels et al., 2017) using the iterative clustering thresholds of 0.975, 0.950, 0.925, and 0.975, and defaults for other settings. The *purc_recluster.py* script is used to iteratively run chimera detection and sequence clustering (performed with USEARCH; Edgar 2010, Edgar et al. 2011) on each gene region individually to produce a reduced set of putative haplotypes that includes size information about the number of original reads forming each cluster (for more details on fluidigm2purc and PURC see https://github.com/pblischak/fluidigm2purc and https://bitbucket.org/crothfels/purc, respectively).

Sequence identification

Clustered sequences recovered from PURC were identified using a combination of GenBank blast hits, and an annotated list of plants known to occur at the 13 study sites based on species documented within 16-32 1-m^2 quadrats that we sampled at each of the 13 study sites (see below) and information from previous studies at these sites (Dyni and Yensen 1996, Suronen and Newingham 2013, Yensen et al. 2013). To identify the closest sequence in GenBank (accessed January 18, 2018), blastn v.2.60 from the command line ncbi tools was used, and the top hit was recorded based on comparison to the annotated list of plants known to occur at all sites combined.

Vegetation sampling

We used 1-m^2 quadrats to sample vegetation composition at all 13 study sites in June-July of 2015 and 2016. We evenly distributed the quadrats throughout the study sites (4 quadrats per 1-ha; Fig. 3.1). We recorded all plants present within the quadrats below waist level and identified all plants to the lowest taxonomic level possible.

We examined two subsets of the vegetation data: (1) data from all of the 383 quadrats at all of the 13 study sites (16-32 quadrats per site; 4 quadrats/ha) hereafter referred to as all vegetation quadrats, and (2) only data from those 191 quadrats that fell within the minimum convex polygons (MCPs) based on all trapped northern Idaho ground squirrels at each site (hereafter referred to as MCP quadrats; Fig. 3.1). MCPs were calculated around all trap locations where we captured a squirrel within a site. We used the minimum bounding geometry – convex hull tool in ArcGis 10.4.1 (ESRI Inc., Redlands, CA) to calculate the MCPs.

Fecal sample diet identification

We created a master list of all known plants that we detected at $\geq$1 of our 13 study sites. In addition, we added 2 families, 20 genera and 83 species that we didn’t record on our 383 quadrats but they had been identified in past studies at $\geq$1 of our 13 study sites (Dyni and Yensen 1996, Suronen and Newingham 2013, Yensen et al. 2013). Combined, we had a list of 276 species, 188 genera, and 44 families on the master list that we believe represented a nearly complete list of the possible food plants that were available to northern Idaho ground squirrels at our 13 study sites.

We matched the top hit from all samples in GenBank to our list of 276 known plants that occur at the 13 study sites. We matched the lowest taxonomic level from the top hits to our list of 276 plants. We also were able to deduce a species or genus if only 1 known plant occurred in that category under the next higher taxonomic level (e.g., only one species within the genus *Microseris* is on our master list of 276 species so any top hit of *Microseris sp.* was assumed to be *Microseris nutans*). We used the genus level for all analyses (besides our list of species identified in the diet).

Genus was the lowest taxonomic resolution possible for many plants because field technicians that conducted our vegetation quadrats were unable to identify some plants beyond genus.

Data analysis

Differences among groups - We used Pianka's niche overlap index implemented in the spaa package in R (Zhang 2016) to estimate the extent of dietary overlap among ground squirrel demographic classes (sex and age) and seasons (Pianka 1973). We used the iNEXT R package to compile sample-based rarefaction curves for our fecal data (Chao et al. 2014, Hsieh et al. 2018). We used the randomForest package in Program R (Liaw and Wiener 2002, R Core Team 2017) to determine which plant genera best discriminated whether a sample came from: (1) a squirrel fecal sample versus a vegetation quadrat (based on all vegetation sampling quadrats), (2) a squirrel fecal sample versus a vegetation quadrat (based on only the MCP vegetation sampling quadrats), (3) an adult fecal sample collected in the summer versus a juvenile fecal sample collected in the summer, and (4) an adult female fecal sample versus and adult male fecal sample. We did not include trees (deciduous or coniferous) in the comparison between fecal samples and vegetation quadrats (comparisons #1 and #2 above) because we did not sample the upper canopy in the 1-m^2 vegetation quadrats (we only sampled vegetation below waist level). We used random forest models because they do not overfit the data and have high predictive accuracy (Breiman 2001). We tuned the random forest models to determine the number of variables (n) to try at each node of the tree and the number of trees (m) to grow. We conducted all statistical analyses in R version 3.43 (R Core Team 2017). Furthermore, we examined partial plots to identify whether taxa used to discriminate were more common in one group or the other.

Survival - We used logistic regression to assess whether the presence of individual plant genera in the diet impacted overwinter survival of northern Idaho ground squirrels. We ran a separate model for each of the 13 plant genera that were detected in $\geq$25% of the summer northern Idaho ground squirrel pellet samples because we were interested in addressing how each of the 13 most common plant genera in the diet affected northern Idaho ground squirrel survival. All models included squirrel age because overwinter survival of juveniles is lower than adults (Sherman and Runge 2002). An interaction between age and presence-absence of the plant did not significantly improve the models ($P > 0.05$ for all 13 models) so we only included the additive effect of age.

Results

We identified 42 families, 126 genera, and 120 species of plants (Table S1) in the northern Idaho ground squirrel fecal samples. Each of the 3 different gene primers from 2 regions identified a similar number of total genera within the northern Idaho ground squirrel diet (Table S2), but a genus was only picked up by one gene region 35.2% of the time. Thus, we would have identified only ~85 genera if we had used only 1 gene region. Every fecal sample contained >1 species of forbs and 85% of the fecal samples contained >1 species of grass (Table 3.1). Rushes and sedges were rarely detected in fecal samples (4.3%). Adult spring fecal samples contained 80.2% more shrubs and 53.5% more trees than summer fecal samples. In contrast, adult summer fecal samples contained 13.8% more grasses and 18.9% more rushes and sedges than spring fecal samples. The frequency of fecal samples containing the vegetation types (forb, grass, shrub, tree, and rush/sedge) significantly differed between spring and summer ($P = 0.044$, two-tailed Fisher's exact test). Our sample size of fecal samples was effective at documenting the diverse diet of squirrels (all seasons combined) and rarefaction curves based on the 124 summer samples came close to approaching the asymptote (i.e., we likely would have detected additional plants had we collected more samples, but our sample sizes were approaching adequate for documenting the wide diet breath of the squirrel). However, more samples in the spring season were likely needed to more completely document the wide diversity of plants eaten (Fig. 3.2).

On average, 11.36 (± 0.42 SE) plant genera were identified in each fecal sample (range 1-31 genera per fecal sample). Diets of adult ground squirrels on average contained more genera in the spring (13.19 ± 0.79 SE genera) than summer (9.47 ± 0.75 SE genera. Dietary overlap was lowest (i.e., diet was most different) between adult males in the spring versus the summer (Table 3.2). Adult northern Idaho ground squirrel fecal samples contained fewer genera (9.47 ± 0.75) than did juvenile fecal samples (11.12 ± 0.62 SE) in the summer. However, diet overlap was high between adults and juveniles (Table 2). Hence, diets of adults differed seasonally (between the spring versus the summer) more so than juveniles versus adults (Table 3.3).

Lomatium, *Poa*, and *Allium* were the 3 most common genera found in the squirrel's diet (each was found in greater than 50% of the 188 fecal samples). *Lomatium* was the most frequent genus in fecal samples (detected in 71.3% of all samples and 63.7% of summer samples). Despite its frequency in the diet, *Lomatium* was found in only 17.8% of the MCP vegetation quadrats and only 13.1% of all vegetation quadrats. *Allium* (detected in 51.6% of fecal samples) was found in 31.9% of all MCP vegetation quadrats and 24.3% of all vegetation quadrats. *Poa* (detected in 55.3% of fecal samples) was found in 63.9% of the MCP vegetation quadrats and in 55.6% of all the vegetation quadrats. *Achillea* was the most frequently occurring genus in all vegetation quadrats (61.1%) and *Poa* was the most frequently occurring genus in the MCP vegetation quadrats (63.9%; Fig 3). Twenty five of the 30 most-frequent genera identified in the northern Idaho ground squirrel diet (ignoring *Pinus* because it was not sampled in the vegetation quadrats) were more frequently found in northern Idaho ground squirrel fecal pellets than in all the plant quadrats (Fig. 3.4). Only 5 genera were more often found in the vegetation quadrats than in the northern Idaho ground squirrel fecal samples (Fig. 3.4). The portion of the study sites occupied by northern Idaho ground squirrels (the area with the MCPs at each study site) were more likely to have the foods preferred by squirrels (i.e., those more common in the pellets than expected). Only 7 out of the 30 most common genera in the squirrel's diet (with the exception of *Pinus*) were found more often in all plant quadrats than in the MCP quadrats (Fig. 3.5).

Fecal samples versus plant availability

Phlox, *Lomatium*, and *Carex* were the most important genera for predicting if the sample was from a fecal sample versus from a vegetation quadrat (Fig. 3.6a). *Perideridia*, *Carex,* and *Phlox* were also the most important genera for predicting if the sample was from a fecal sample versus a vegetation plot within the MCPs (Fig. 3.6b).

We compared northern Idaho ground squirrel summer diet composition to (1) all vegetation quadrats, and (2) MCP vegetation quadrats. We compared summer (not spring) fecal samples with the data from our vegetation quadrats because we conducted our vegetation sampling during the same time period that summer fecal samples were collected. Hence, any differences in frequency of genera between summer fecal samples and vegetation quadrats can provide inferences regarding preference or avoidance of those genera. *Carex, Agoseris,* and *Perideridia* were the most important genera for discriminating between fecal samples and vegetation quadrat samples (Fig. 3.7a) and for discriminating between fecal samples and MCP vegetation quadrat samples (Fig. 3.7b). *Agoseris* and *Perideridia* were more often found in the summer fecal samples than in the vegetation quadrats and *Carex* was more often found in the vegetation quadrats than fecal samples (Fig. 3.7). Our analyses suggested that some genera are more often found in fecal samples than in the vegetation quadrats because the 4 random forest models all had low out-of-bag (OOB) error rates (ranged from 3.15% - 5.80%; Table 3.4).

Fecal sample group comparisons

Lithophragma, Microsteris and *Phlox* were the most important genera for predicting if a fecal sample was from the spring versus the summer (Fig. 3.8). *Sidalcea, Calochortus,* and *Pinus* were the most important genera for predicting if a sample was from an adult versus a juvenile (Fig. 3.9). However, differences between seasons (spring versus summer) were more pronounced than differences between age classes (adults versus juveniles). The OOB error rate is relatively high (37.10%; Table 3.4).

Association between Diet and Overwinter Survival

Apparent over-winter survival was greater for individuals that consumed *Perideridia or Allium* versus those that did not (Fig. 3.10). In contrast, apparent over-winter survival was lower for individuals that consumed *Frasera* versus those that did not (Fig. 3.10). Apparent survival was 1.39 and 1.70 times higher for individuals that consumed *Perideridia* compared to those that did not for adults and juveniles, respectively. Apparent survival was 1.47 and 1.79 times lower for those that consumed *Frasera* compared to those that did not for adults and juveniles, respectively.

Discussion

Our results suggest that northern Idaho ground squirrels have a diverse diet and they corroborate results from 2 prior microhistological studies which suggested that the northern Idaho ground squirrel is a generalist herbivore that eats a wide variety of plants (Dyni and Yensen 1996, Yensen et al. 2013). The diet of northern Idaho ground squirrels is dominated by forbs and grasses, with shrubs, trees, rushes, and sedges accounting for a much small component. We identified ~70 more plant species (140% increase) and 88 more genera (232% increase) in the squirrels' diet than previous microhistological studies (Dyni and Yensen 1996, Yensen et al. 2013). Furthermore, 4 of the most frequently occurring genera in the fecal samples that we analyzed (*Navarretia, Phlox, Allium, Epilobium*) were not mentioned in one or both of those two previous studies (Dyni and Yensen 1996, Yensen et al. 2013). However, we did not detect 1 genera (*Descurainia*) reported by Dyni and Yensen (1996) and 4 genera (*Phleum*, *Equisetum*, *Pedicularis*, and *Saxifraga*) reported by Yensen et al. (2013). Furthermore, we did not identify *Descurainia* , *Equisetum*, or *Pedicularis* in any of our 383 quadrats. We may not have identified these plants our fecal samples, let alone our vegetation samples because they may rare in the environment (it is possible they were rare also during other studies or have since become more rare) or flower and desiccate before we sample in the summer so we are unable to identify them. Two recent studies on other animals have reported improved resolution with DNA methods for plants compared microhistology for documenting diet (Soininen et al. 2009, Khanam et al. 2016)

Northern Idaho ground squirrels consumed some plant genera (e.g., *Lomatium*) more than expected based on their frequency within the squirrels' foraging areas and consumed other plants (e.g., *Poa, Achillea*) less than expected based on their frequency (Fig. 3.4). Our results suggest that northern Idaho ground squirrels are preferentially eating several uncommon plants (e.g. *Phlox* and *Periderida*) - a pattern that corroborates a previous diet study (Yensen et al. 2013). However, northern Idaho ground squirrels also eat common plants (e.g., *Poa*). The protein content of most grasses decreases as the growing season progresses (Frase and Armitage 1989), but squirrels frequently eat grass seeds later in the summer when the leaves and stems of most herbaceous vegetation has dried out. Grasses are widely considered to rarely employ chemical defenses so they may be easier to digest but they may not be as nutritious as forbs; some herbivores select a diet containing both forbs and grasses to maximize energy intake and digestibility (Belovsky 1984, 1986). Optimal foraging theory predicts that when food is abundant, individuals are more likely to be choosy and should select higher-quality foods (Pyke et al. 1977). Perhaps squirrels are able to select forbs they prefer in the spring, but then must incorporate less nutritious but more common grasses as the summer progresses to ensure they obtain enough sufficient energy. Or perhaps squirrels switch to eating grass seeds in the summer because they provide essential fatty acids valuable for hibernation and because seeds are not available in the spring. Further studies are needed to evaluate how the quantity of high-quality versus low-quality foods in northern Idaho ground squirrel diets differs in relationship to availability.

Our results imply that habitat use of northern Idaho ground squirrels may reflect diet constraints; the 30 most commonly eaten plants were more common in squirrel use areas (MCPs) compared to the areas immediately surrounding the MCPs (Fig. 3.5). Future studies should collect plant abundance data in a larger buffer surrounding MCP use areas (particularly in open canopy locations) to further evaluate whether northern Idaho ground squirrels forage and live in areas with higher frequencies of preferred vegetation compared to available.

Our study is one of only a few studies that have documented diet at the genus level; most other studies have done so at the family level (Iwanowicz et al. 2016). Furthermore, only 2 other metabarcoding studies of animal diets have used 2 different gene regions (Kartzinel et al. 2015, Lopes et al. 2015) and ours is the only study to use both gene regions (3 primer pairs) to more thoroughly categorize an animal's diet. Most previous studies focused on trnL alone or used a second region (1 or 2 primers) to gain resolution within one or two families. Our results demonstrate that the use of multiple gene regions is imperative for studies that use metabarcoding methods to document diet of a generalist herbivore – reliance on only one gene region would have caused us to miss important taxa in the diet. Hence, our results can help inform future studies that hope to use this new method.

Northern Idaho ground squirrels spend the majority of their lifetime in hibernation. High overwinter survival (followed by high spring reproductive success) are likely critical for the species recovery. We found some intriguing relationships between squirrel overwinter survival and diet that warrant further study. Squirrels that consumed *Perideridia and Allium* tended to have higher survival whereas squirrels that consumed *Frasera* tended to have lower survival. *Perideridia* produces large tuberous roots underground (Clarke 1977) and *Allium* produces an edible bulb (Brewster 1994). All parts of *Perideridia* are edible and it is relatively high in protein and energy content, and low in fiber compared to many other plants (Eshelman and Jenkins 1989). Furthermore, *Perideridia* is high in starch, vitamin A, vitamin C, potassium, and protein (Kaldy et al. 1980). *Allium* is high in water, calcium, potassium, zinc, and magnesium (Morris et al. 1981). In late June and July, after the plants have dried, squirrels are most likely consuming old stems, roots, tubers, or seeds as few flowers or green leaves remain. As above-ground vegetation dries during the summer, fiber typically increases, reducing the digestibility of a plant (Elliott and Flinders 1984). Future studies should compare nutritional values of *Perideridia*, *Allium*, and *Frasera* to understand why they appear to affect squirrel survival and what parts of the plants squirrels are consuming during both spring and summer. *Perideridia* was recorded in 5.7% (48th most common plant) of the MCP vegetation quadrats that we sampled, Allium was recorded in 34.0% (8th most common plant) of the MCP vegetation quadrats that we sampled, and *Frasera* was recorded in 9.9% (the 33rd most common plant) of those quadrats. Squirrels may primarily consume below-ground parts (roots) or dropped seeds of some plants and, hence, the frequency of plants in our 1-m^2 vegetation quadrats may underestimate their availability to the squirrels. Despite this caveat, an important next step would be to evaluate whether availability of *Perideridia* and *Allium* is a limiting factor for northern Idaho ground squirrels. Future studies might also examine whether squirrels are consuming *Frasera* only because more-nutritious forage is unavailable.

In ground squirrels, survival during the hibernation season is often associated with the ability to consume enough energy during the summer (Murie and Boag 1984). Juvenile northern Idaho ground squirrels have lower overwinter survival than adults (Sherman and Runge 2002) and juvenile’s ability to gain enough weight to survive the winter is a potential cause of that difference. Juvenile and adult northern Idaho ground squirrel diets were fairly similar, but juveniles more often consumed *Sidalcea, Calochortus* and *Pinus* compared to adults. Further studies should evaluate why these 3 plant genera are consumed more often by juveniles than adults. Juveniles enter into hibernation later than adults (Michener 1992, Buck and Barnes 1999) and ground squirrels and other hibernating herbivores often increase their consumption of polyunsaturated fatty acids prior to entering into hibernation (Frank 1994, Ruf and Arnold 2008). Perhaps ground squirrels (both adults and juveniles) switch over to seeds and other plants that are high in polyunsaturated fatty acids in preparation for the oncoming hibernation season and the differences we recorded merely reflect slight differences between age classes regarding when that switch occurs. Future studies should focus on more fine-scaled seasonal changes in diet as individuals approach hibernation.

Our analyses indicated that northern Idaho ground squirrel diet differed between spring and summer and these results corroborate previous studies that have reported seasonal differences in diet in northern Idaho ground squirrels and other rodents (Schitoskey Jr and Woodmansee 1978, Fagerstone et al. 1981, Frase and Armitage 1989, Lehmer et al. 2006, Yensen et al. 2013). Different plants are available at different times throughout the growing season and squirrels may be forced to alter their diet based on plant phenology. Adult northern Idaho ground squirrels consumed a greater number of plant genera in the spring than in the summer and this pattern could reflect either seasonal changes in plant availability (functional response) or seasonal changes in the squirrels' nutritional requirements or both. Future studies should sample vegetation during both spring and summer to help evaluate the 2 potential causes of the seasonal changes we recorded. One limitation of DNA metabarcoding methods is that they do not allow investigators to determine which part of a plant (seeds vs roots vs leaves) the animal eats and whether the use of different plant parts varies seasonally. Animals might not only select different plants as the season progresses, but they also might select different parts of the same plants. Numerous species of ground squirrels increase their intake of seeds just prior to hibernation to increase their polyunsaturated fatty-acid intake (Frank 1992, 1994, Florant 1998, Munro and Thomas 2004). Squirrels have also been shown to shift to other plant parts (e.g., roots and seeds) in relationship to availability (Fagerstone et al. 1981, Karasov 1982).

The seasonal shifts in diet that we observed can potentially be interpreted based on phenological data from herbarium specimens (Consortium of Pacific Northwest Herbaria). For example, northern Idaho ground squirrels consumed *Lithophragma* more often in the spring than in the summer (Figs. 36). *Lithophragma* goes to seed on the 9th of June (on average) within Adams County at the elevational range in which northern Idaho ground squirrels are found. This suggests that squirrels are targeting the leaves, and flowers of *Lithophragma* during the spring (when seeds are not yet available). In contrast, *Lomatium* is commonly consumed both in the spring and summer (Fig. 3.3) and it begins to grow early in the spring (Ogle and Brazee 2009) and typically begins fruiting ~22 June. Hence, northern Idaho ground squirrels are most likely eating all parts (leaves, roots, flowers, and seeds) of *Lomatium*. We have often observed signs of ground squirrel digging in the summer months and these diggings are presumably squirrels eating plant roots (such as *Lomatium* roots). Polyunsaturated fatty acids, particularly linoleic and linolenic acid, are considered critical nutrients for some hibernating mammals (Florant 1998, Frank et al. 1998, Munro and Thomas 2004, Ruf and Arnold 2008). Seeds in particular contain high levels of polyunsaturated fatty acids (Frank et al. 1998, Lehmer and Horne 2001). Piute ground squirrels (*Urocitellus mollis*) and arctic ground squirrels (*Urocitellus parryii*) consume more shrubs in the summer than spring (McLean 1985, Van Horne et al. 1998) and shrubs are higher in linoleic acid which is an important fat for hibernating small mammals (Van Horne et al. 1998). We found that northern Idaho ground squirrels rarely eat shrubs but may shift to seeds in late summer for the same reason.

Northern Idaho ground squirrels are a threatened species. A popular hypothesis for their decline is habitat loss and declines in habitat quality due to years of fire suppression (U.S. Fish and Wildlife Service 2000), but the explicit mechanism(s) by which fire suppression reduces habitat quality is not clear. Several potential mechanisms by which fire suppression may reduce habitat quality involve changes in the quality or quantity of important plant foods: 1) periodic ground fires may lead to increases in plant reproductive growth, number of inflorescences, and size of many grasses and forbs (Wrobleski and Kauffman 2003); 2) fires can increase availability of protein and other nutrients in plants which may be important to the diet of northern Idaho ground squirrels (Hobbs and Spowart 1984, Yensen 2004); and 3) fires may increase vegetation diversity (or increase abundance of certain plant species) by increasing available sun and nutrients to understory plants (Jameson 1967, Thysell and Carey 2001, Barbier et al. 2008).

Food quality and availability may be limiting the recovery of northern Idaho ground squirrels. Anthropogenic changes to the landscape occupied by these squirrels include altered grazing regimes, fire suppression, and climate change. Anthropogenic changes may alter the vegetation composition and phenology of the plants these squirrels depend on. Northern Idaho ground squirrels are generalists which may help them adjust to changes in the plant community. However, studies are needed to evaluate the impacts that changes in plant phenology, nutrition, and abundance have on: (1) diet selection, (2) body condition, and (3) timing of hibernation. Northern Idaho ground squirrels may be able to utilize a wide range of plants, but squirrels may not be able to meet their energetic and nutritional needs if only suboptimal forage plants are available. For example, if specific plants begin to seed at earlier dates when squirrels are hyperphagic, how will that alter the amount and type of fatty acids (linolenic vs linoleic) they consume prior to hibernation? Information regarding nutritional value of the plants will inform future habitat restoration efforts to improve the quantity of preferred plants at the time of the growing season when they are most important to squirrels. Additional information on diet of northern Idaho ground squirrels, building upon the results presented in this paper, will help managers design management actions that provide optimal habitat conditions for recovery and de-listing.

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Table 3.1. Number and percent of 188 northern Idaho ground squirrel fecal samples that contained at least one species from each of 5 general vegetation types.

Vegetation Type	Number of Samples	Percent of Samples
Forb	188	100.0%
Grass	160	85.1%
Tree	61	32.4%
Shrub	23	12.2%
Rush/Sedge	8	4.3%

Table 3.2. Dietary overlap in northern Idaho ground squirrels in Adams County, Idaho based on Pianka's niche overlap indices. We compared diets by season, and specific sex and age categories.

	Spring		Summer		
	Adult Males	Adult Females	Adult Males	Juvenile Males	Adult Females
Spring Adult Females	0.888				
Summer Adult Males	0.718	0.766			
Summer Juvenile Males	0.788	0.810	0.895		
Summer Adult Females	0.718	0.764	0.890	0.861	
Summer Juvenile Females	0.732	0.768	0.911	0.908	0.933

Table 3.3. Dietary overlap in northern Idaho ground squirrels in Adams County, Idaho based on Pianka's niche overlap indices. We compared diets by season, sex, and age.

	Spring			Summer			
	Females	Adults	All	Males	Females	Adults	Juveniles
Spring Adults	0.985						
Spring All	0.985	1.000					
Summer Males	0.817	0.821	0.824				
Summer Females	0.785	0.785	0.788	0.934			
Summer Adults	0.792	0.789	0.792	0.952	0.977		
Summer Juveniles	0.807	0.812	0.815	0.974	0.978	0.951	
Summer All	0.811	0.812	0.815	0.976	0.989	0.983	0.992

Table 3.4. Optimal values for the random forest models used to classify 6 comparisons of fecal and/or vegetation samples.

Model Comparison	Number of Variables at Each Split	Number of Trees	OOB Error Rate
All fecal samples vs all vegetation quadrats	7	800	5.80%
All fecal samples vs vegetation quadrats within MCP	6	1000	5.30%
Summer fecal samples vs all vegetation quadrats	12	600	3.15%
Summer fecal samples vs vegetation quadrats within MCP	7	1500	5.06%
Spring fecal samples vs summer fecal samples (adults only)	12	1000	9.48%
Adult fecal samples vs juvenile fecal samples (summer only)	8	800	37.10%

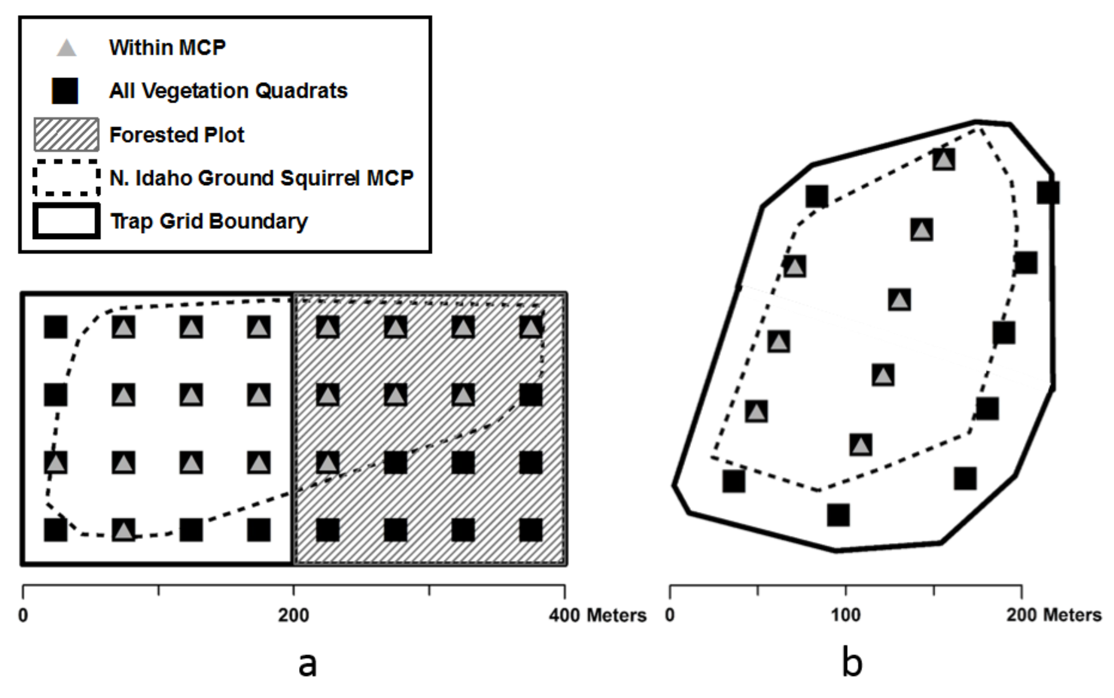

Figure 3.1. The 2 northern Idaho ground squirrel study site designs: (1) 9 of the 13 study sites included two 4-ha plots (one in non-forest and one in the adjacent forest) and (2) 4 of the 13 study sites included a 4-8ha plot in a non-forested area. Small black squares are the evenly spaced vegetation quadrats. We placed 16 quadrats in every 4-ha plot. The dotted line represents the area occupied (minimum convex polygon) of northern Idaho ground squirrels at the site (based on trapping data). Grey triangles indicate the vegetation quadrats that are within the squirrel MCP.

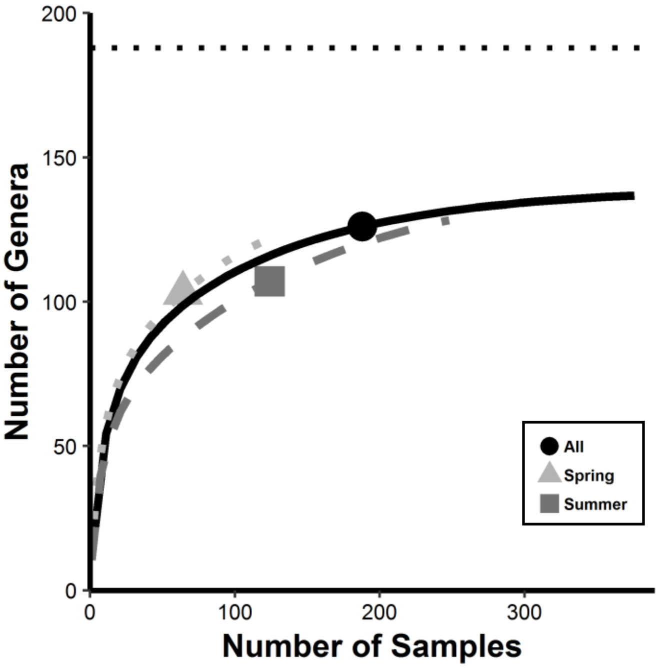

Figure 3.2. Sample-based rarefaction curves for spring only (64), summer only (124), and all fecal samples combined (188). We extrapolated the curves past the points (number of samples) to double the number of samples in each category to better illustrate the projection of the accumulation curve. The 3 symbols (circle, triangle, and square) show the number of genera actually detected for each category.

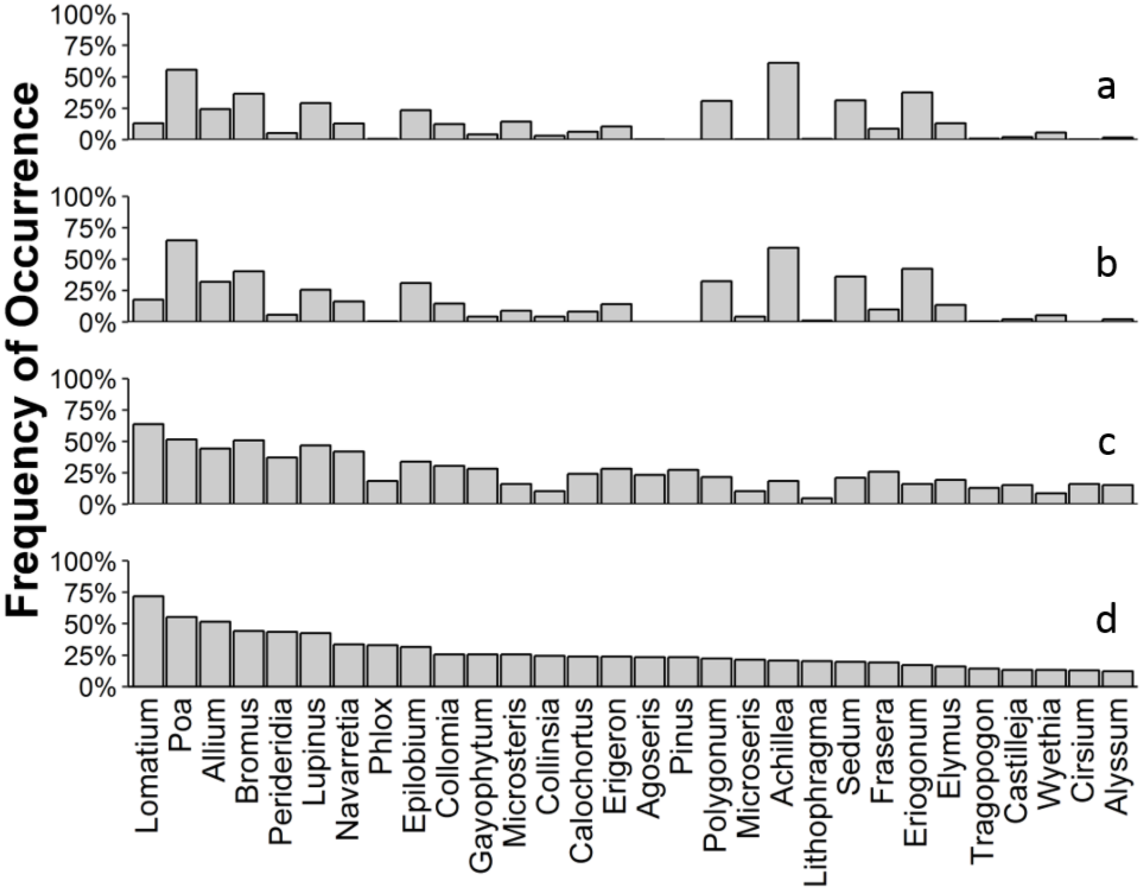

Figure 3.3. Frequency of occurrence of the 30 most common plant genera in a) all vegetation quadrats, b) MCP vegetation quadrats, c) summer fecal samples from northern Idaho ground squirrels, and d) all fecal samples (spring and summer samples) from northern Idaho ground squirrels. We included the summer fecal samples separately (panel c) because we only sampled the vegetation in the summer. We did not include *Pinus* in the vegetation quadrats (a, b) because we only sampled below 1-m (we did not sample the upper canopy).

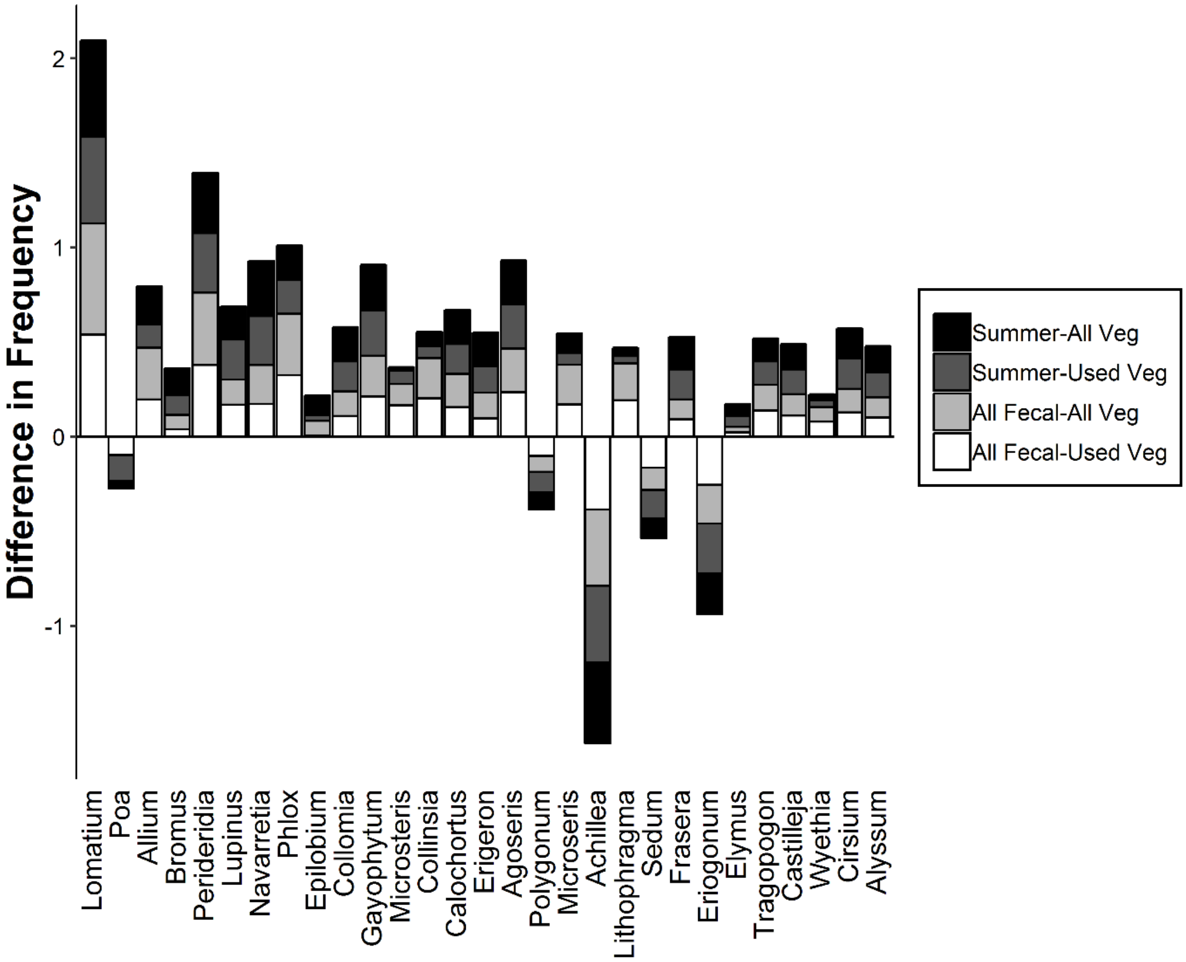

Figure 3.4. Difference in frequency between genera in: (1) all northern Idaho ground squirrel fecal samples compared to all vegetation quadrats, (2) all fecal samples compared to MCP vegetation quadrats, (3) summer fecal samples compared to all vegetation quadrats, and (4) summer fecal samples compared to MCP vegetation quadrats. Bars less than zero represent genera that were found more frequently in the vegetation quadrats (environment) compared to the fecal samples (diet) (i.e., those that squirrels may have avoided). We did not include *Pinus* because they were not assessed in the vegetation quadrats

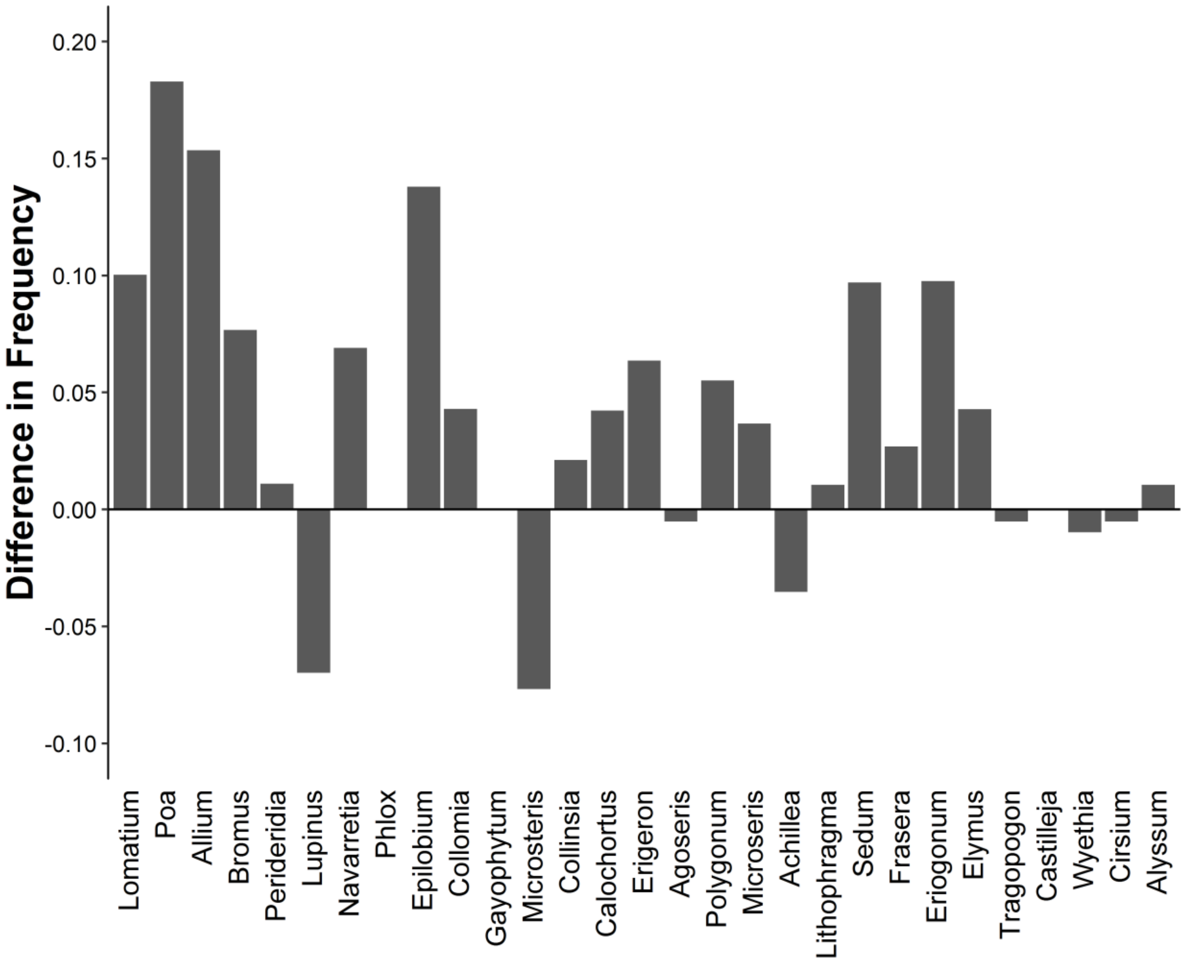

Figure 3.5. Difference in frequency between genera in all northern Idaho ground squirrel MCP vegetation quadrats and all other quadrats (those outside of the MCPs). Bars less than zero represents genera that were more frequent outside the MCPs compared to within the MCPs.

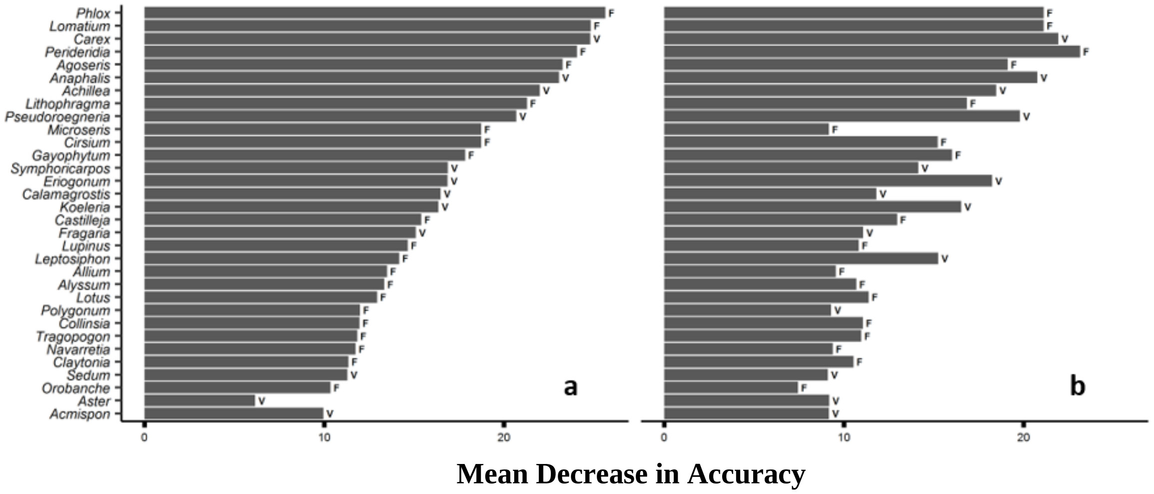

Figure 3.6. Variable importance contribution of northern Idaho ground squirrel (*Urocitellus brunneus*) for: a) fecal samples versus 383 vegetation quadrats (size of trapping area differs among sites), and b) fecal samples versus a subset of 191 vegetation quadrats (those within northern Idaho ground squirrel MCPs at each study site). The mean decrease in accuracy is a measure of the impact of each plant genera on the accuracy of the model (e.g., if *Phlox* is removed from the model in Panel a, the accuracy would decline by 26%). Only the top 30 genera for each category of quadrats are included in each panel (last 4 genera are in the top 30 for the MCP vegetation quadrats but not all quadrats), representing the genera that are most important to the model's ability to distinguish between a sample from a fecal pellet or a sample from a vegetation quadrat. A genus was more often found in a fecal sample if the letter "F" is next to the bar. A genus was more often found in a vegetation quadrat if a 'V' is next to the bar.

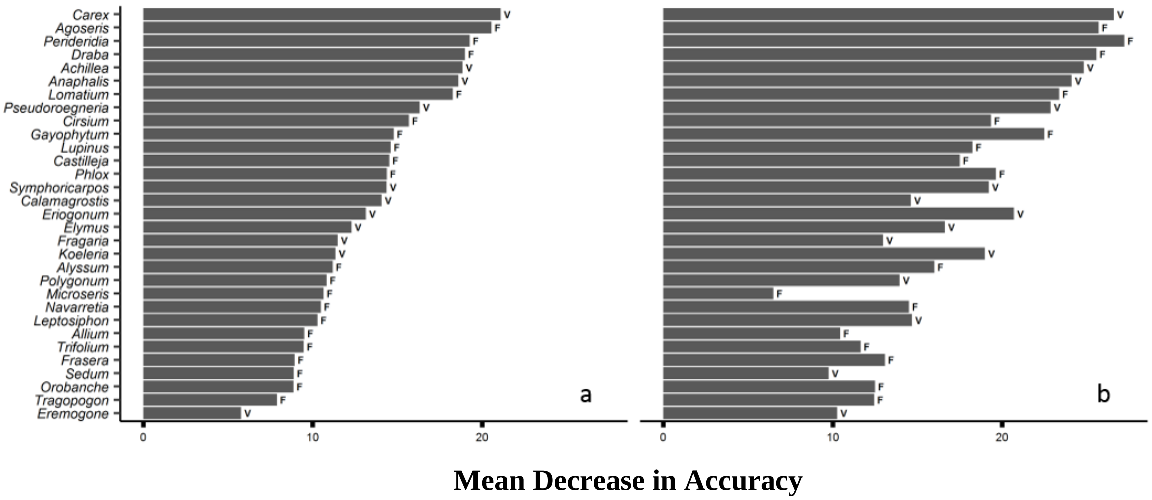

Figure 3.7. Variable importance contribution of: a) northern Idaho ground squirrel summer fecal samples versus 383 vegetation quadrats, and b) northern Idaho ground squirrel summer fecal samples versus a subset of 191 vegetation quadrats (those within northern Idaho ground squirrel MCPs at each study site). The mean decrease in accuracy is a measure of the impact of each plant genera on the accuracy of the model (e.g., if *Carex* were removed from the model in Panel a, the accuracy would be reduced by 21%). Only the top 30 genera are included in each panel (last 3 genera are in the top 30 for the MCP vegetation quadrats but not for all quadrats), representing the plant genera that are most important to the model's ability to distinguish between a sample from a fecal pellet and sample from a vegetation quadrat. A genus is more often found in a fecal sample if the letter "F" is next to the bar and is more often found in a vegetation quadrat if a 'V' is next to the bar. No tree genera were included in this analysis because we did not sample trees in the vegetation quadrats.

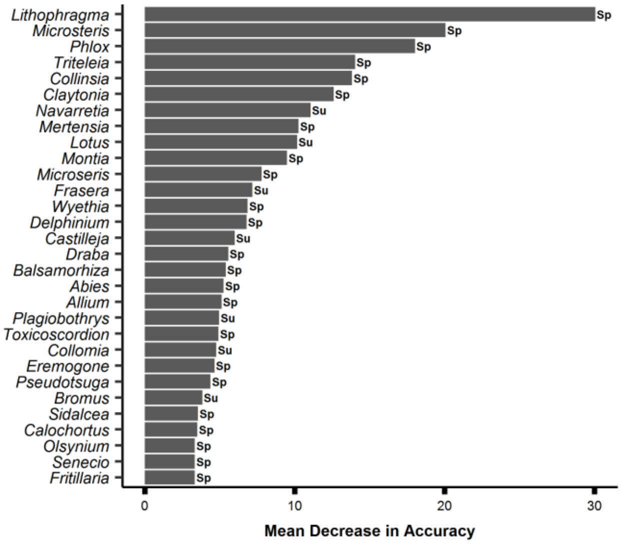

Figure 3.8. Variable importance contribution that shows which plant genera best discriminate between adult spring and summer fecal samples from northern Idaho ground squirrels (*Urocitellus brunneus*). A genus is more often found in spring diets if the letters 'sp' are next to a bar and more often found in summer diets if the letters 'su' are next to the bar. Only the top 30 genera are included, representing the genera that are most important to the model's ability to distinguish between seasons.

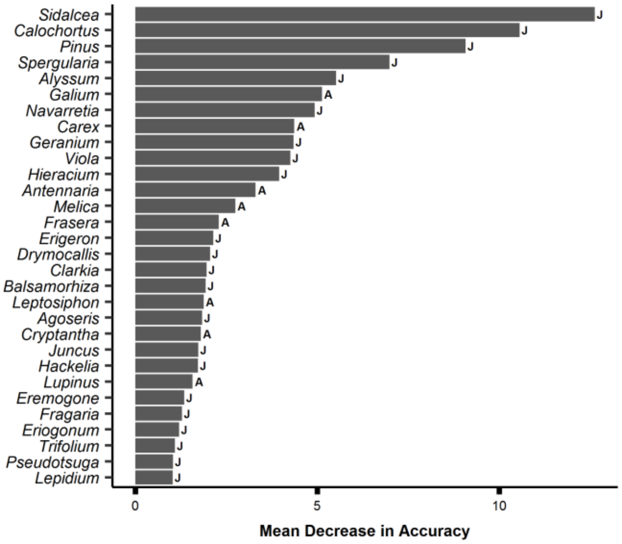

Figure 3.9. Variable importance contribution that shows which plant genera best discriminate between adult and juvenile fecal samples from northern Idaho ground squirrels (*Urocitellus brunneus*) collected during the summer trapping session. A genus is more often found in adult diets if the letter 'A' is next to a bar and more often found in juvenile diets if the letter 'J' is next to the bar. Only the top 30 genera are included, representing the genera that are most important to the model's ability to distinguish between age classes.

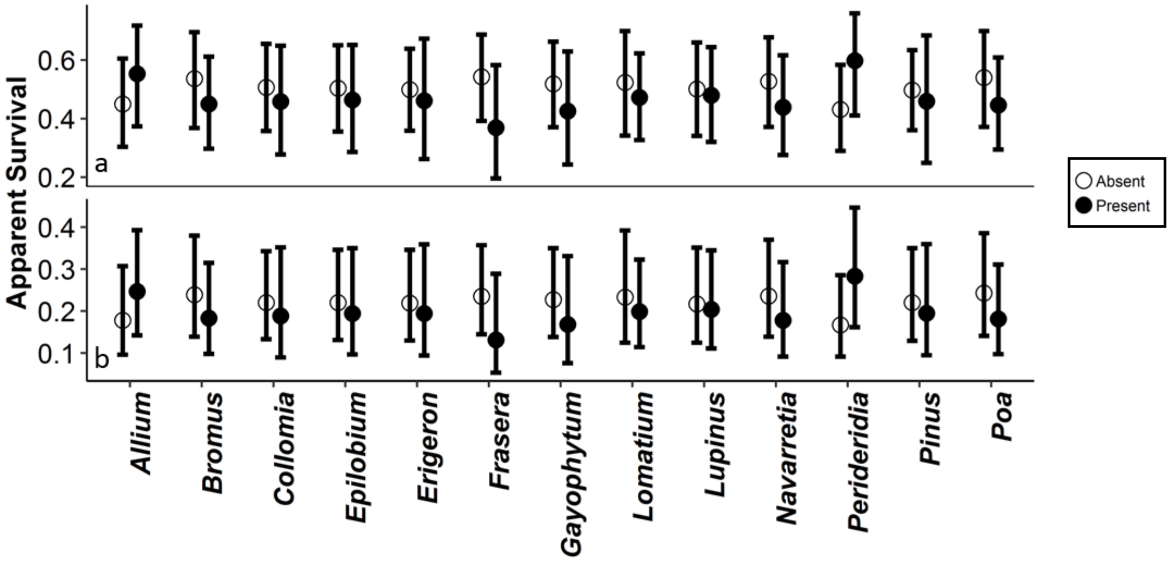

Figure 3.10. Difference in apparent overwinter survival for adult (a) and juvenile (b) northern Idaho ground squirrels (*Urocitellus brunneus*) based on whether a plant genus was present or absent in the squirrel's fecal sample. The 13 plant genera shown are those that were found in at least 25% of the 124 summer fecal samples (71 juveniles and 53 adults) analyzed. Standard error bars are included.

Chapter 4: Consequences of habitat selection in a threatened ground squirrel: effects of habitat metrics and weather on hibernation behavior and overwinter survival

Abstract

Hibernation is an adaptation to survive periods of stress (e.g., food limitation or harsh thermal conditions). A key question is whether rare range-restricted species can adapt by changing their behavior in response to climate change (i.e., through behavioral plasticity). The northern Idaho ground squirrel (*Urocitellus brunneus*) is a threatened species in Idaho that hibernates for approximately 8 months per year. Changes in temperature, snow accumulation, and summer precipitation as a result of climate change may reduce survival or fecundity of northern Idaho ground squirrels if they cannot adequately adapt to these climatic changes. Hibernating species can respond to climate change in 2 ways: they can change their hibernation behavior (i.e., emergence date, number of torpor bouts, etc.) or they can alter their environment (i.e., change hibernacula depth or location). We found that northern Idaho ground squirrels often select hibernacula outside of the areas they use during the summer active season and that habitat features of hibernacula locations differed from habitat features of summer use areas. Hibernacula had 427.1% higher canopy cover compared to active season locations (36.9% and 7.0%, respectively). Furthermore, we explored a suite of intrinsic and extrinsic factors to document if they influenced hibernation behavior and survival of northern Idaho ground squirrels. Squirrels that hibernated near a fallen log had higher survival than those that did not, and snow pack was positively associated with emergence date and hibernation duration. These results suggest that changes in snowfall and forest management actions will likely impact survival of this rare ground squirrel.

Keywords: ground squirrel, *Urocitellus brunneus*, climate change, hibernation, body temperature, hibernacula, emergence

Introduction

Climate change is likely to impact plants and animals at many scales: individuals, populations, communities, and ecosystems. Some species respond to changes in climate either through micro-evolution or behavioral plasticity (Parmesan 2006, Hoffmann and Sgrò 2011, Bellard et al. 2012). Species not capable of responding appropriately to changes in climate to meet their bioenergetic or thermal constraints may ultimately face extinction (McCain and King 2014). Plants and animals can potentially mitigate the impacts of climate change via one of two responses: 1) spatial (i.e., move to follow appropriate conditions) or 2) temporal or phenological (i.e., change timing of migration, daily activity, or reproduction to better match peak in resources or thermal tolerance zones (Humphries et al. 2004, Bellard et al. 2012).

The frequency and magnitude of harsh climatic conditions are projected to change due to anthropogenic climate change (Mote et al. 2003, Klos et al. 2014, Lute et al. 2015, Gergel et al. 2017). Many mammals use one of four strategies to survive harsh climate conditions: 1) migrate to areas with more favorable conditions, 2) hoard food caches and live within the subnivean zone, 3) morphological adaptations (e.g., grow thick fur to stay warm during cold winters), or 4) hibernate and/or estivate. Western North America is expected to have warmer summers, earlier spring snowmelt, and less snow accumulations (i.e., shallower snow depth) which could impact the relative effectiveness of these 4 strategies if mammals do not adapt accordingly (Stewart 2009).

Animals that hibernate typically rely on hibernacula with relatively constant temperatures and must select or create a hibernaculum with the desired temperatures. Some hibernators enter into hibernation when ambient air temperature is warm (i.e., estivate) and some of those species become torpid during the summer and remain so throughout the winter (Wang 1978, Wilz and Heldmaier 2000, Staples 2016). Animals may enter into estivation before hibernation as a mechanism to avoid periods of heat, water or food stress (Geiser and Körtner 2010). These animals that select a hibernaculum where they both estivate and hibernate may be particularly sensitive to climate change. We know relatively little about the plasticity of behaviors related to hibernation and the capacity of hibernators to respond to climate change. Moreover, choice of the hibernaculum may have enormous consequences given that a hibernator's fitness is influenced by the conditions of that hibernaculum for a large portion of its annual cycle. And the optimal hibernaculum under one set of climatic conditions may not be the optimal hibernaculum under a different set of conditions. For example, northern Idaho ground squirrels (*Urocitellus brunneus)* hibernate approximately 8 months per year from July through March (Yensen and Sherman 1997, Sherman and Runge 2002) and thus, begin torpor bouts during the warm summer months (estivation) and continue throughout the winter (hibernation). Survival probability of northern Idaho ground squirrels during the 8-month heterothermal period (both estivation and hibernation) is thought to be low relative to other hibernators (citation) and may be influenced by the: (1) availability of suitable hibernacula locations, (2) squirrels' ability to dig a burrow with an optimal microclimate that allows them to meet their physiological needs (during both estivation and hibernation), and (3) squirrels' ability to alter their behavior or metabolism during suboptimal environmental conditions. Climate-induced changes in environmental conditions over the past few decades may have changed the likelihood of one or more of these 3 requirements for survival.

The landscape that supports northern Idaho ground squirrels has changed over the past few decades. Fire suppression has allowed coniferous trees to encroach into some of the forest openings that support northern Idaho ground squirrels. Canopy cover of overstory trees reduces snow depth but increases the seasonal duration of snow pack (Davis et al. 1997, D'Eon 2004, Varhola et al. 2010). Snow cover has an insulative effect and likely helps maintain a constant burrow temperature within hibernacula (Young 1990*a*, Buck and Barnes 1999*a*, Geiser and Turbill 2009) and the insulative effect of snow cover likely is related to hibernacula depth. As snow depth increases, the depth of frost in the soil column decreases, and squirrels are better able to maintain constant body temperatures above freezing for longer periods (Hardy et al. 2001, Decker et al. 2003). Hence, temperature of the hibernaculum, mediated by snow pack and hibernaculum depth, may influence overwinter survival of northern Idaho ground squirrels. Furthermore, canopy cover provides shade, reducing direct effects of the sun on soil temperatures, resulting in cooler soil temperatures with less temperature fluctuation prior to snow cover (Breshears et al. 1998, Royer et al. 2012). We sought to examine these relationships between hibernation behavior, weather, habitat selection of hibernacula, and overwinter survival. Climate change is gradual and so examining its consequences is difficult in field studies. Hence, we used space-for-time substitution (Blois et al. 2013) in snow conditions (brought on by variation in elevation) to evaluate the potential influence of climate change on northern Idaho ground squirrel hibernation behavior and survival. To examine the potential influence of climate change on northern Idaho ground squirrels, we addressed three questions: (1) how do winter (hibernacula) and summer (active season) use areas differ?; (2) what habitat features are northern Idaho ground squirrels selecting (use versus availability) for both hibernacula and summer use areas?; (3) what habitat features effect overwinter survival?; and (4) are hibernation behaviors (torpor/arousal bouts, duration of hibernation, and immergence/emergence dates) affected by weather, body condition, or habitat features?

Materials and Methods

Study Location and Design

We conducted field work in Adams County, Idaho on both public and private land. We trapped northern Idaho ground squirrels, attached VHF radio-collars to a subset of squirrels, and used telemetry receivers and hand-held antennas to locate hibernacula. We collected habitat metrics at hibernacula locations and active season locations at 11 study sites from 2013-2017.

Locating hibernacula

We deployed VHF collars on 122 adult northern Idaho ground squirrels from 2013-2017 at 11 study sites. We also attached light loggers (geolocators; Migrate Technology Ltd., Cambridge, U.K.) to 71 of the 122 squirrels (10 in 2015, 28 in 2016, and 33 in 2017). We deployed 2 models of light loggers: 5 Intigeo C65 in 2015 (older model) and 66 Intigeo F100. The Intigeo C65 models had ±3.0°C accuracy and the newer F100 models had ±0.5°C accuracy. We attached VHF collars to adult squirrels that weighed >120g from 2013-2015 and those >140g in 2016-2017. We deployed collars only at locations that supported $\geq$10 adult northern Idaho ground squirrels. We collared <33% of the adults at any one site (restrictions established by a species working group to minimize potential negative effects that collars may have on squirrels). Light loggers recorded both the light level (which allowed us to document whether a northern Idaho ground squirrel was above or below ground) and the skin temperature at 15-min intervals (4-hr intervals on 2 older model collars deployed in 2015). We refer to these measurements as body temperature because light loggers produced temperature readings during hibernation similar to those from implanted thermochrons in arctic ground squirrels (*Urocitellus parryii*) (William et al. 2014). We used both Holohil (Holohil Systems Ltd., Ontario, Canada) and Biotrack (Biotrak Ltd., Wareham, U.K.) VHF collars to track squirrels to their hibernacula. The Biotrack collars were designed to emit a signal on a pre-selected schedule (we programmed the collars to emit a signal 2 or 3 days per week to maximize the battery life). We also set the Biotrack collars to turn off during the winter so they had enough power to turn back on in the spring and still emit signals for 2 – 3 months (thereby, allowing us to locate and catch squirrels that retained their collar over winter or to retrieve just the collar for those that had slipped from squirrels' necks in the spring). We located the hibernacula for 106 of the 122 radio-collared squirrels between 2013-2017. We assumed that a location was a hibernaculum if a northern Idaho ground squirrel was detected in the same location on 3 sequential daytime visits during late summer. Typically ground squirrels move throughout the day within their home range and an active (non-hibernating) squirrel would be very unlikely to be located within the exact location (within 30cm) as the two previous days it was located. Furthermore, we often noticed that signals of active squirrels moved underground in response to our presence above ground walking on the soil.

Many of the collared northern Idaho ground squirrels that we re-captured in the spring had dropped their collars. If a squirrel had slipped its collar and we could hear the collar's signal emitting in the spring (Biotrack collars only) (*n* = 19 collars), we were able to: (1) dig those collars up (or recover on the ground if it was slipped or predated in the spring after emergence), (2) measure the depth at which the collar was located in the hibernaculum (if the collar was belowground), and (3) recover the light logger. For squirrels that retained their collars until spring (both Holohil and Biotrack), we made concerted efforts to recapture them (*n* = 37 collars). The 20 recovered light loggers and temperature sensors (we retrieved 4 additional collars the spring after they were attached: 1 failed, 1 was not programmed correctly, and 2 were slipped prior to hibernation) enabled us to evaluate: (1) the exact dates of immergence and emergence into/out of hibernation, (2) overwinter body temperature, (3) duration and frequency of winter euthermic bouts during hibernation, and (4) depth of the hibernaculum. We were able to measure the true depth of the hibernaculum for the 9 collars that we recovered within the hibernacula (5 with light loggers attached). Typically, collars were left within nest material in the hibernaculum, so we believe the depth of the collar closely corresponded to the squirrel's hibernaculum depth.

Hibernacula habitat characteristics

We collected a suite of habitat measurements at each hibernaculum location in 2016 and 2017 to document the habitat conditions used by hibernating northern Idaho ground squirrels. In 2018, we returned to every documented hibernaculum location between 2013-2017 to collect the same measurements so we could use all years of data. At each hibernaculum, we measured: 1) slope, 2) aspect, 3) distance between capture location and hibernaculum location, 4) percent canopy cover, 5) number of trees within 5-m (up to 4), 6) DBH of all trees within 5m, 7) distance to nearest tree within 30m for 3 size classes (1-8cm, 8-12cm, and >12cm), 8) distance to nearest log within 5m, and 9) maximum width of nearest log.

Snow depth and duration of winter snowpack may be important for northern Idaho ground squirrel survival via several mechanisms: (1) its insulating quality during hibernation, (2) deep snow may afford protection from terrestrial predators, and (3) its effect on the date of vegetation green-up. The latter may affect reproduction and survival during the subsequent year. Therefore, we measured 1) daily snow depth 2), ambient air temperature, and 3) light intensity at each hibernacula location in 2016 and 2017. We placed a graduated staff gauge and a trail camera at or within 1-km of 6 of our study sites each fall from 2014 to 2017 to document winter snowpack (Table 4.1). Snow poles were not always placed within a site either due to ownership requests or limited number of available poles. We pre-programmed cameras to photograph the staff gauge 2 times per day throughout the winter. We estimated the snow depth and duration of snow cover throughout the winter at those 6 study sites based on the photographs of the staff gauges. Additionally, in 2016 and 2017 we placed a staff gauge at each of 22 hibernacula locations. By placing staff gauges at hibernacula, we were able to document snow depth and duration of snowpack in the immediate area around northern Idaho ground squirrel hibernacula and compare those to snow depth within the summer use areas. Cameras occasionally died or failed due to disturbance (human or wildlife) or malfunction (25.7% failure rate).

We placed 2 soil temperature probes in the ground at 7 of our 11 study sites: (1) within an open canopy area (meadow) where squirrels are active during the summer, near a previously used summer burrow location, and (2) in the adjacent forest (near a hibernaculum location from a previous year). Each soil probe consisted of one 1.5-m long PVC pipe with 3-11 thermochron temperature loggers (Maxim Integrated Products, CA, U.S.A.) spaced at 15-cm and separated by foam. Temperature probes were inserted into the ground by first digging a 1-inch wide hole with a hand-auger. Due to the high density of rocks and tree roots, we were unable to dig a 1.5-m deep hole at most locations. We cut the PVC pipe to fit the depth to which we were able to dig. All soil probes ended up between 0.3-m and 1.5-m in length. We deployed the soil temperature probes in June 2016 and downloaded the temperature data from the soil probes after we retrieved the thermochrons in spring 2017.

We also deployed Hobo temperature loggers (Onset Computer Corporation, Bourne, MA, U.S.A.) to measure light intensity and air temperature during the active season: one in the open canopy area and one in the forest (under tree canopy). Over the winter, we deployed the Hobo temperature loggers at: the hibernacula locations after the squirrel had entered into hibernation and at the annual snow poles in the open canopy areas. Temperature and light were recorded every 30min to 2hrs (depending on logger’s storage capacity). We used these temperature and light intensity measurements to provide a baseline of ambient conditions at each study site and at each hibernaculum to help interpret the data generated from the measurements from collars on squirrels.

Animal mass

We weighed all squirrels that we captured. Body mass increases across the summer active season and so we sought to standardize our body mass measurements given that we caught animals throughout the summer active season (May – July). Hence, we regressed adult body mass against capture date for each sex and site (given that body mass differs between sexes and likely among study sites) by using data from 2013-2017, and we recorded the residual body mass from those regressions for each adult squirrel. We combined data from all years for these regressions because we had too few data for any one year at most study sites.

Hibernation behavior analysis

We used univariate general linear models to evaluate the relationships between our 35 explanatory variables and each of 5 hibernation behaviors: (1) immergence date, (2) emergence date, (3) total length of the heterothermal period, (4) number of euthermic bouts, and (5) hibernaculum depth. We also examined relationships among the 5 hibernation behaviors. We did not evaluate all combinations of variables but rather selected a priori those that we believed were most likely to impact each hibernation behavior (Table S3). We conducted 166 different univariate GLM models. Given the large number of univariate models and the exploratory nature of this project, we used the Benjamini-Hochberg method (Benjamini and Hochberg 1995) with a threshold of 0.1 to correct for experiment-wise error and thereby control the false discovery rate.

We assumed that a squirrel had entered its hibernaculum when all above-ground activity ceased for more than 1 day (based on data from the light loggers; Fig. 4.1). We assumed that a squirrel had entered the heterothermal period when the first torpor bout was greater than 24 hours. We assumed a torpor bout had begun when a squirrel's body temperature (T_b) began to decrease and we assumed a euthermic bout had begun when a squirrel's T_b began to increase steadily (Fig. 4.2). We assumed that a heterothermal period had ended when torpor bouts were less than 24 hours long. Total torpor length and euthermic bout length were only those that occurred during the heterothermal period.

Resource selection analysis

We measured the following abiotic and biotic factors to document winter and summer habitat selection of northern Idaho ground squirrels: (1) canopy cover, (2) slope, (3) elevation, and (4) aspect. We compared data from available locations within squirrels' use areas at each site to both summer active season locations (above ground activity) and winter hibernacula locations. At each study site, we used capture locations and hibernacula locations of all squirrels to create an MCP of the year-round use area of squirrels (hereafter referred to as year-round use area) and we sampled random locations within these year-round use areas to represent habitat features that were readily 'available' to the squirrels (e.g., third-order habitat selection ala(Johnson 1980)). To better illustrate the seasonal differences in habitat selection behaviors of squirrels, we split the random available locations at each site into two categories: those in open canopy areas within the year-round use areas (northern Idaho ground squirrels are associated with open areas during the summer active season (U.S. Fish and Wildlife Service 2003) and those within forested areas within the year-round use areas. We assumed that the year-round use areas available to squirrels included everything within a 150-m radius buffer around every active season trap location and hibernacula location at each study site. The diameter of the largest home range documented for an adult northern Idaho ground squirrel (we only collared adults) based on telemetry data for 16 adult squirrels in a prior study was 138-m (D. Evans Mack, unpublished data). Hence, we used a 150-m radius buffer to ensure that we included all of the area readily available to a squirrel at each study site into the year-round use areas that we designated. We then merged all of the 150-m buffer polygons from both the hibernacula and trap locations (active season) to create an overall year-round use area that we assumed was readily available to squirrels at each study site. We excluded a small area within this availability polygon at 1 site because it extended into unusable land uses (open water and a human use area). The size of the polygons for year-round use areas varied among the 8 study sites from 10.5 to 45.0 ha. We excluded data from 3 of the 11 study sites for these analyses because we had fewer than 5 hibernacula locations at those sites. We used 2,688 trap locations during the summer active season from 660 squirrels and 99 hibernacula locations from 96 squirrels at 8 study sites. To document the habitat metrics within the year-round available use areas at each study site, we first determined the number of random points to generate within each site by generating a sample mean and confidence interval for each covariate at various numbers of random points (100-1000). We determined that the number of random points necessary to reach a stable mean (minimized variance; Kershaw 1964) was 550-700 across our 8 study sites.

We further split the year-round available use areas into forested areas or open canopy (meadow) areas based on 2015 National Geospatial Data Asset NAIP Imagery. Random points were then assigned as either forest or non-forest (within a meadow or open canopy area). We used canopy cover measurements published by the National Land Cover Database (Homer et al. 2015) and we obtained elevation, slope and aspect estimates in ArcGis 10.4.1 from the National Elevation Dataset (Gesch et al. 2002). We then assigned each location (summer active season squirrel location, winter hibernacula location, random forest location, random open canopy location) a value for canopy cover, slope, aspect, and elevation. We generated exploratory box plots for each of the 4 habitat metrics. We used MANOVA to compare 3 of the 4 habitat metrics (canopy cover, slope, and elevation) and aov.anova in the circular package (Lund et al. 2017) to compare aspect among the 4 location types (summer active season, winter hibernacula, random open canopy, and random forest). We employed a linear discriminant function analysis to determine which factors influence habitat selection by using the flipMultivariates package to visualize how the 4 location types differed from one another. We set priors equal for all 4 location types.

Active season versus hibernacula selection analysis

We used a generalized logistic mixed-effects model implemented in the lme4 package (Bates et al. 2015) to evaluate differences in habitat selection between summer active season locations and winter hibernacula locations. We also measured the following variables directly (on-the-ground measurements) at each summer active season location (only individuals for whom we had hibernacula locations) and winter hibernacula location: (1) canopy cover, (2) aspect, (3) slope, (4) number of trees within 5m that were larger than 1cm dbh (we counted up to 4 trees), distance to nearest tree within 5m, (5) dbh of nearest tree within 5m, (6) dbh of largest tree within 5m, and (7) diameter of nearest log within 5m. For this analysis, we only included the 103 hibernacula locations from the 9 study sites that had at least 4 hibernacula locations. Candidate models included the 7 habitat variables listed above and sex, plus squirrel number and study site as nested random effects. We used AICc to compare candidate models (Burnham and Anderson 2002) and we removed any models that did not converge from model selection. We used a generalized linear regression model to evaluate differences in soil temperature across elevations and between open canopy areas versus forest areas. We used soil temperatures at 30-cm depth for these comparisons because that was the deepest depth that we had thermochrons within our soil temperature probes at all 7 study sites (see above). We also used generalized linear regression models to evaluate: 1) differences in canopy cover across elevations, and 2) the relationship between elevation and squirrel emergence date relative to snow melt.

Survival analysis

We examined whether any habitat features at hibernacula influenced overwinter survival based on 94 hibernation events (90 squirrels, 4 of which we had two hibernation-year events). We trapped squirrels within a site during 2 sessions (spring and summer) each year. We used logistic regression to evaluate the effects of the following 8 predictor variables on overwinter survival (all individuals used were believed alive when they entered into hibernation): (1) sex, (2) canopy, (3) slope, (4) aspect, (5) number of trees within 5m, (6) log width within 5m, (7) distance to log within 5m, (8) elevation, and (9) year. We considered downed wood to be a log if it was greater than 2cm in diameter. We only included radio-collared squirrels at study sites where we attempted to re-capture squirrels the year after they were collared (i.e., those that we could document whether they had survived until the following summer). For the 51 hibernacula that did not have a log within 5m, we assigned them a distance to log of 5.1m and a log width of zero so that we could include them in the analysis. We used AICc to compare a suite of candidate models for both logistic regression analyses (Burnham and Anderson 2002). All analyses were conducted in program R v 3.4.3. (R Core Team 2017) unless otherwise stated.

Results

Hibernation behavior

We retrieved data from 20 light loggers that provided information on hibernating northern Idaho ground squirrels (10 females and 10 males) from 7 of our 11 study sites (Supplementary A). We determined the emergence date for 14 of the 20 squirrels (73.7%) and hibernacula depth for 7 of the 20 squirrels (35.0%). The lowest recorded body temperature (0.20°C) was at the second lowest elevation study site but was from the older model with less temperature precision (Table 4.1). The minimum body temperature during hibernation did not differ between sexes ($P = 0.903$; Table 4.1). The lowest and average body temperatures of hibernating squirrels were only slightly higher than the lowest (0.5°C) and average minimum (1.43°C ± 0.29 SE) soil temperature at the sensor closest to the depth of the hibernacula (at the 7 hibernacula with known depths; Table 4.2; 5 of the 7 sensors were located at shallower depths than squirrels hibernated). The average soil temperature at hibernacula between 1 September and 1 March was 5.58°C (±0.51 SE at 7 known hibernacula; Table 4.2). Torpor bouts were typically longest when soil temperatures were coolest (Fig. 4.3). The relationship between elevation and both average ($P < 0.001$) and minimum ($P = 0.018$) soil temperature differed between meadows (open canopy areas) and forests (closed canopy areas) (Figs. 4.4 & 4.5). Soil temperature was positively correlated with elevation in the open canopy areas, but soil temperature did not vary with elevation in the adjacent forested areas (Fig. 4.5)

Hibernation behavior was associated with elevation, snow, body weight, slope, and fallen logs (Supplemental A). The number of days with snow on the ground, maximum snow depth, date of snow melt, and slope were all positively correlated with hibernation emergence date (Fig. 4.6). Squirrels emerged later (relative to snowmelt date) at lower elevations compared to those at higher elevations ($P = 0.003$; Fig. 4.7). The presence of a fallen log and the width of the closest fallen log were both positively correlated with hibernacula depth (Fig. 4.8). Elevation was positively correlated, and immergence date was negatively correlated, with duration of hibernation (Fig. 4.9). Squirrel body mass was negatively correlated with immergence date (Fig. 4.10) and positively correlated with number of euthermic bouts. The number of euthermic bouts was negatively correlated with the average length of torpor bouts and positively correlated with the total time spent euthermic (Fig. 4.11).

Resource Selection

We used 2,922 summer active season locations, 99 hibernacula locations, and 4,807 random locations within year-round use areas (1,659 in open canopy areas and 3,148 in forested areas) to explore habitat selection of northern Idaho ground squirrels. We detected differences between winter and summer use areas (winter hibernacula locations and summer active season locations) and the two types of available locations within squirrels' year-round use areas (open canopy locations and forest locations). These 4 types of points differed in canopy cover ($F = 3768.9$, df = 3, $P < 0.001$), slope ($F = 259.9$ df = 3, $P < 0.001$), elevation ($F = 184$, df = 3, $P < 0.001$), and aspect ($F = 40.58$, df = 3, $P < 0.001$); Figs. 4.12 & 4.13). We used a linear discriminant function analysis to better illustrate how the 4 types of points differed based on canopy cover, slope, and elevation (Fig. 4.14). We did not include aspect in the discriminant function analysis because average aspect of all 4 types of points was always south-facing (Fig. 4.13) and inclusion of a circular variable like aspect is difficult in a DFA. The 1^{st} dimension of the discriminant function contained 97.6% of the variance. Winter hibernacula locations had less canopy cover than available forest points but more canopy cover than summer active season locations. Summer active season locations had lower canopy cover than available open canopy points within year-round use areas. Both types of available points (open canopy and forest locations) within year-round use areas had steeper slopes and higher elevations than both summer active season locations and winter hibernacula locations. However, canopy cover was the dominant difference among the 4 types of points. Summer active season locations were most often mis-classified as available open canopy locations (27.6%) and winter hibernacula locations were most often mis-classified as available forest locations (33.3%).

Summer active season locations versus hibernacula locations

Winter hibernacula locations and summer active season locations differed with regards to slope, canopy cover, and number of trees (Table 4.3). Distance to the nearest tree was the only other variable that had some support as a discriminating variable (i.e., included in a model with a ΔAICc under 2.0). The probability of a location being a winter hibernacula location compared to a summer active season location increased with canopy cover, slope and number of trees within 5m (Fig. 4.15). In addition, northern Idaho ground squirrel hibernacula were more strongly associated with higher canopy cover at high-elevation sites compared to low-elevation sites ($P < 0.001$; Fig. 4.16).

Survival analysis

Ground squirrel overwinter survival was negatively correlated with distance to the nearest fallen log. Female apparent survival rates were greater than males (Fig. 4.17). Distance to closest tree, size of closest tree, year, and distance to forest-meadow ecotone were also included in the top competing models (AICc $\leq$ 2.0; Table 4.4).

Discussion

Northern Idaho ground squirrels selected different habitat components during the summer active season versus the winter hibernation season. For both male and female squirrels, winter hibernacula locations had higher forest canopy cover, steeper slopes, and more trees within 5m than summer active locations. We can think of 3 plausible reasons why northern Idaho ground squirrels may avoid canopy cover during the summer active season: (1) heightened predation risk (Thorson et al. 1998), (2) preferred forage, and (3) competition for resources with Columbian ground squirrels (Dyni and Yensen 1996). Future studies should test these (and perhaps other) hypotheses to provide a better understanding of the mechanisms underlying the habitat selection patterns we report here. In contrast to their habitat affinities during the summer, northern Idaho ground squirrels select areas with higher canopy cover for their winter hibernacula locations. The reason for the seasonal change in habitat selection is not clear. The seasonal shift in habitat use of the northern Idaho ground squirrels is relatively unique among hibernating squirrels. While many hibernating squirrels use different burrows between the active versus the hibernation season, hibernacula are typically located within the footprint of space used by the squirrels during the active season (Svendsen 1976, Michener 2002, Borgo 2003). Hence, why do northern Idaho ground squirrels often travel away from active season habitat for hibernation?

Squirrels may prefer to hibernate in areas with higher forest canopy cover because such areas may: (1) retain snow cover longer on the ground (Davis et al. 1997) and thereby provide better insulation, (2) have reduced maximum snow depth (Davis et al. 1997, D'Eon 2004) but still retain enough snow to insulate the soil (at least 30-40cm; Edwards et al. 2007) and hence benefit from longer snow pack (see above) at least in areas receiving enough snow (high-elevation locations), (3) have lower soil temperature when snow does not cover the ground (Decker et al. 2003) and thereby allow the squirrel to save energy during estivation (the start of hibernation prior to snowfall), (4) have less daily temperature fluctuation during estivation, (5) have deeper and/or less rocky soil making it easier to dig deeper burrows and hibernate lower in the soil column, (6) have more tree roots that make it difficult for predators to dig up hibernating squirrels, or (7) have mycorrhizal fungi that squirrels may eat before, during, or after torpor bouts. Future studies should test these (and other) alternative hypotheses to explain why northern Idaho ground squirrels often, but not always, move into forested areas to hibernate so that we better understand the important components of optimal winter hibernacula habitat for northern Idaho ground squirrels. Such information would allow forest managers to ensure that both summer and winter habitat needs are being met in forest management plans. Steeper slopes may be important for minimizing flood risk especially in the late winter when the snow melts but the squirrels have not immerged from their burrows (Shaw 1926, Svendsen 1976). Hibernacula at higher elevations tended to have higher canopy cover than those at lower elevations suggesting that perhaps northern Idaho ground squirrels select hibernacula locations based on snowfall or summer soil temperature. The current management paradigm for recovery of northern Idaho ground squirrels includes reductions in forest canopy cover to improve summer habitat, but such actions may inadvertently reduce winter hibernacula habitat needs. Future management plans should consider the need for both open space summer habitat and canopy cover for winter habitat, and future research efforts should seek to determine which of the two seasonal habitat conditions are most limiting. Further studies should evaluate the importance of a forest mosaic (open spaces patchily distributed throughout the forest) and the optimal size of open spaces to forest areas and how these requirements may differ by elevation.

Northern Idaho ground squirrels that built their hibernacula near a fallen log (relative to available areas within their home range) had higher overwinter survival. Logs may impact soil temperature, winter snow pack, and predation risk. Juvenile ground squirrels have lower survival than adults (Sherman and Runge 2002) and selecting an optimal hibernacula location may be especially important for juvenile squirrels because they enter into hibernation at a smaller body size than adults (Buck and Barnes 1999*a*). Juvenile squirrels have a much shorter active season despite entering into hibernation later than adults (Knopf and Balph 1977, Young 1990b, Michener 1992) because they do not emerge from natal burrows until May-June (Yensen and Sherman 1997). Late summer food resources are typically less digestible and less nutritious than early-season food resources (Elliott and Flinders 1984, Frase and Armitage 1989). Furthermore, juveniles in late summer (when adults have already entered hibernacula) are probably more at risk of predation because there are fewer squirrels to attack at any given time (Delm 1990). Juveniles most likely enter into hibernation with less body mass than adults (a pattern observed in other ground squirrel species (Young 1990*b*, Millesi et al. 1999). The need to maximize energy savings may be even more important for a squirrel with less fat to begin the hibernation season. An optimal hibernacula location may be one that: 1) maintains optimal temperatures for the maximum amount of time, 2) minimizes temperature fluctuations in the summer, 3) maintains winter temperatures just above freezing with little variation. We did not attach collars to any juvenile ground squirrels for this study but putting collars on both adult and juvenile squirrels would document whether juveniles select different hibernacula locations and determine whether those choices impact energy loss and overwinter survival. Specifically, such an effort would help elucidate why juveniles have lower winter survival that adults. For example, do they make poor choices regarding the location of their hibernacula, are they more nutritionally limited than adults (Murie and Boag 1984, Millesi et al. 1999), or both?

The climate where northern Idaho ground squirrels live is changing. Snowfall totals and duration of snowpack are predicted to decline (Mote et al. 2003, Klos et al. 2014, Lute et al. 2015, Gergel et al. 2017) and winter temperatures are predicted to rise, resulting in more rain and less snow (Mote et al. 2003, Gergel et al. 2017). Furthermore, summers are expected to be warmer with less rainfall (Mote and Salathe 2010), resulting in a likely change in plant phenology and diversity (Bertin 2008, Khanduri et al. 2008). Our results suggest that changes in climate will likely alter the emergence date of hibernating ground squirrels and such changes may affect survival. Other species of hibernating mammals have altered both immergence and emergence dates in response to climate change (Lane et al. 2012, Williams et al. 2014), but the effects of these behavioral changes on demographic traits have varied among species (Inouye et al. 2000, Lane et al. 2012, Tafani et al. 2013, Rézouki et al. 2016). Climate-induced changes in hibernation behavior may result in phenological mismatches with preferred food items or changes in competitive interactions with other herbivores. This is the first study to quantify hibernation behaviors, and the factors that affect those behaviors, in northern Idaho ground squirrels; future studies should build upon these results to better evaluate the likely consequences of predicted climatic changes on this rare species.

While emergence date was linked to snow, immergence date did not differ between elevations (snow tends to be deeper and melt later at higher elevation sites). Hence, if immergence date does not shift along with emergence date, earlier snow melt dates may lead to a prolonged active season. A prolonged active season may be less desirable if the ratio of energy consumed versus used is negative. If summers become warmer and the availability of green vegetation is reduced or shifts to earlier dates, squirrels immerging on the same date as previous years may have trouble meeting active season energetic demands and/or be water stressed. Furthermore, squirrels may be more at risk of predation in the active season compared to during hibernation (Turbill et al. 2011). As such, animals should enter into hibernation as soon as they have enough fat to survive the hibernation period and stay in hibernation as long as possible but still emerge healthy in the spring for reproduction when forage and water is available (Bieber et al. 2014). Future studies should explicitly examine the potential survival and fitness consequences of climate-induced changes in hibernation behavior in northern Idaho ground squirrels.

The timing of emergence is important in terms of maximizing energy gain for both fitness (see above) and overwinter survival the following winter. An individual should strive to time their emergence date to take advantage of forage as soon as it is available. If an individual emerges too early, they risk having no food to consume. In contrast, emerging late reduces the time an individual has to consume food while it is most nutritious and easily digested compared to later in the summer when plants dry out and senesce. Individuals that gain more weight during the active season (heavier individuals) immerge sooner into hibernation and have more euthermic bouts. Euthermic bouts are energetically expensive and have been shown to account for about 70% of the energy spent during hibernation (Wang 1978). But all individuals need to arouse periodically from torpor (euthermic bouts). However the reasons for these euthermic bouts are still widely debated (Barnes and Buck 2000, Körtner and Geiser 2000, Dark 2005). Our data suggest that if a squirrel has extra energy, they select to stay in torpor for shorter periods (have more euthermic bouts) most likely to reduce risks associated with prolonged torpor. Risks include: (1) suppressed immune system (Prendergast et al. 2002), (2) sleep deprivation (Daan et al. 1991), and (3) renal function (Humphries et al. 2003) among others. Similarly, heavier woodchucks (*Marmota monax*) had warmer body temperatures and spent less time in torpor (Zervanos et al. 2013). However, we cannot rule out that differences in torpor bout length are due to differences in polyunsaturated fatty-acid stores, and not mass along, which have also been found to correlate to torpor bout length in other studies (Munro and Thomas 2004, Ruf and Arnold 2008). Future studies should explicitly examine the potential risks of lower overwinter survival if northern Idaho ground squirrel body mass is lower than expected (perhaps as a result of a mismatch in emergence date). Furthermore, a better understanding of differences in diet (specifically fatty acid consumption) between individuals with long torpor bouts and those with shorter bouts.

Northern Idaho ground squirrel body temperature (while in torpor) closely mimics the soil temperature and likely does so as long as the soil temperature does not fall below a fatal threshold (Geiser et al. 1990, Michener 1992). Arctic ground squirrels use relatively little thermogenesis when the soil temperature is above 0 degrees (Barnes and Buck 2000). Body temperature for all 19 northern Idaho ground squirrels reached a minimum between 0 and 3°C during hibernation, which is within the range of minimum soil temperatures 30cm below ground at our study sites. Body temperature during hibernation correlates with torpor duration (Geiser and Kenagy 1988, Barnes and Buck 2000). Squirrels should select hibernacula depths where soil temperature stays greater than 0°C but cool enough to stay in torpor without raising metabolic processes (Barnes and Buck 2000). Shallower hibernacula likely experience more fluctuation in soil temperature before snowfall (Kay and Whitford 1978, Baker and Baker 2002) and cooler soil temperature when snow has fallen (Hardy et al. 2001, Zhang 2005). Soil temperatures in our study were warmer during the hibernation season (1 Sept – 1 March) in the forest compared to adjacent areas with open canopy (where squirrels spend the summer active season). Shrub covered soil has been shown to have higher average temperatures than adjacent open areas in southern Idaho (Flerchinger and Pierson 1991) and the difference was more pronounced at higher elevations. Northern Idaho ground squirrels may select hibernacula in forests rather than in open areas, especially at higher elevations, because these areas have warmer average soil temperatures in the winter and cooler soil temperatures in the summer (reducing metabolic needs in the summer during estivation and in the winter when they need to avoid freezing). Furthermore, hibernacula do not necessarily need to be as deep at higher elevations to remain above 0°C. Shallower burrows require less energy to create and unplug for emergence in the spring (but may be more susceptible to predation). Further study is needed to understand how snow depth and duration directly impact soil temperature and how that interacts with canopy cover to provide optimal thermal conditions for hibernation of this rare ground squirrel. Furthermore, more data are needed to understand why hibernacula depth varies. Is the depth of hibernacula regulated by: (1) predator avoidance, (2) ideal thermal conditions (Young 1990*b*, Michener 1992, Buck and Barnes 1999*b*);

taking into account site specific factors such as snow depth/duration and canopy cover), or (3) energetics (the amount of energy necessary to dig a deeper burrow).

Concluding remarks

This study is the first to document hibernation behavior of northern Idaho ground squirrels, to document detailed habitat features of their hibernacula, and to look for links between these intrinsic and extrinsic traits. A better understanding of the habitat features of hibernacula is the first step in understanding how these choices impact ground squirrel survival and fecundity. Most northern Idaho ground squirrel research has focused on the summer active season. The leading hypothesis for the decline of the species is that fire suppression has reduced available summer habitat (openings within forests) for these squirrels. However, this study demonstrates that the surrounding forest also plays an important role for the year-round life cycle of these squirrels. We need to better understand what resources these squirrels need during the active season as well as what they need for the approximately 8 months when they are below ground in hibernation. Furthermore, as the climate continues to change, it may be vitally important that the squirrels respond and can cope with these changes. To avoid extinction, northern Idaho ground squirrels may need to adjust their hibernacula location, depth, and immergence/emergence timing in response to a changing climate. They must avoid a mismatch in emergence from hibernation and vegetation availability. Moreover, they must have enough time during the growing season (particularly, for juveniles) to gain enough energy to survive the winter.

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Table 4.1. Summary of the 20 collared northern Idaho ground squirrels (*Urocitellus brunneus*) from which we also retrieved light loggers. Age of most individuals was unknown. Any ages with a '+' sign indicate the minimum age known based on previous years trapping. We only included hibernacula depths for individuals that we recovered collars from within their hibernacula. Emergence date was unknown for any individual that did not resurface with their collar attached. One light logger failed in January so only data on immergence date is included.

Sex	Elevation (m)	Age (yrs)	Year	Immerge date	Time between immergence and hibernation (hours)	Number of euthermic bouts	Average euthermic bout length (days)	Total euthermic time (days)	Total hetero-thermal length (days)	Longest torpor length (days)	Min T_b (°C)	Depth (m)	Days between final arousal and emergence	Emerge date
F	1288	1+	2016	17-Jul	3.40	21	0.48	10.15	270.72	25.03	0.88		1.98	14-Apr
F	1288	1+	2017	5-Jul	25.32	26	0.46	12.08	279.21	24.38	1.75		1.74	13-Apr
F	1359	1+	2015	2-Jul	64.91	23	0.55	12.59	277.72	30.73	2.13			
F	1376	2	2017	11-Jul	28.41	18	0.41	7.47	263.40	25.79	1.38	0.76		
F	1376	1	2016	16-Jul	17.57	19	0.44	8.40	260.32	26.40	1.63		1.83	4-Apr
F	1585	2+	2016	20-Jul	5.42									
F	1591	2+	2017	15-Jul	20.28	18	0.58	10.46	275.80	29.60	1.50	0.72	2.12	19-Apr
F	1591	2+	2017	15-Jul	48.40	33	0.38	12.47	282.36	27.52	0.25	0.26		
F	1696	2	2015	4-Jul	7.66	27	0.56	15.11	282.45	19.91	1.38		1.25	14-Apr
F	1696	2+	2015	7-Jul	22.57	23	0.44	10.23	283.08	29.58	2.63		3.97	21-Apr
M	1288	1	2017	30-Jul	4.98	15	0.63	9.38	243.82	33.74	1.00		4.00	4-Apr
M	1288	1+	2017	31-Jul	5.24	15	0.54	8.11	226.54	30.77	0.88	0.52		
M	1359	1+	2015	6-Jul	8.84	17	0.70	11.83	252.17	24.00	0.20		10.53	26-Mar
M	1359	2+	2017	5-Jul	2.49	22	0.78	17.24	261.99	29.44	2.63		5.95	30-Mar
M	1376	1+	2015	10-Jul	69.47	30	0.68	20.33	250.50	26.17	0.50		0.59	21-Mar
M	1376	3+	2017	2-Jul	76.24	22	0.69	15.15	264.55	23.00	1.75	0.36		
M	1591	1+	2017	19-Jul	18.83	16	0.67	10.79	267.33	34.27	1.63		6.75	19-Apr
M	1603	1+	2016	15-Jul	5.58	18	0.59	10.65	257.64	25.51	2.25	0.43	4.16	2-Apr
M	1603	2+	2017	7-Jul	4.66	19	0.89	16.95	274.49	33.44	2.25		3.40	12-Apr
M	1696	3+	2017	6-Jul	42.49	29	0.59	17.23	283.20	30.03	1.50	0.78	1.25	18-Apr
Female avg	**1485**			**11-Jul**	**24.39**	**23.11**	**0.48**	**11.00**	**275.01**	**26.55**	**1.50**	**0.58**	**2.15**	**14-Apr**
Male avg	**1454**			**13-Jul**	**23.88**	**20.30**	**0.68**	**13.77**	**258.22**	**29.04**	**1.46**	**0.52**	**4.58**	**5-Apr**
Avg for all	**1469**			**12-Jul**	**24.14**	**21.63**	**0.58**	**12.45**	**266.17**	**27.86**	**1.48**	**0.55**	**3.54**	**9-Apr**

Table 4.2. Body temperature (T_b), associated soil temperature (T_s) and depth of the 20 northern Idaho ground squirrels we retrieve light loggers from. All temperature measurements were collected between 1 Sept and 1 March. We did not measure T_s in 2015. We retrieved 7 slipped collars from hibernacula so we can confirm the depth the squirrel was actually hibernating (known depth). We estimated the depth for all hibernacula in 2016 and 2017 based on the T_s. Depth of the T_s logger used for T_s measurements are included because we were unable to insert probes as deep as some hibernacula at many of the sites. Mean minimum T_b refers to the average lowest body temperature within each torpor bout.

Year	Elevation (m)	Sex	Mean minimum T_b (°C)	Mean T_s (°C)	Minimum T_b (°C)	Minimum T_s (°C)	Known hibernaculum depth (m)	Estimated depth based on T_s (m)	Depth of T_s logger (m)
2015	1696	F	9.3		1.4				
2015	1696	F	8.7		2.6				
2015	1376	M	10.9		0.5				
2017	1376	M	9.6	5.9	1.8	1.6	0.36	0.35	0.30
2015	1359	M	8.1		0.2				
2016	1585	F		5.4	2.52	1.1		0.30	0.30
2017	1376	F	9.2	7.0	1.4	2.6	0.76	0.55	0.45
2016	1376	F	10.2	6.3	1.6	2.6		0.25	0.30
2017	1696	M	9.6	4.5	1.5	1.1	0.78	0.80	0.30
2017	1359	M	13.9	6.9	2.6	0.6		0.45	0.45
2016	1603	M	9.2	7.7	2.3	1.6	0.43	0.40	0.30
2017	1591	F	6.1	4.3	1.5	2.0	0.72	0.65	0.60
2017	1591	F	8.9	4.4	0.3	0.5	0.26	0.30	0.30
2017	1288	M	8.0	3.7	1.0	0.0		0.65	0.30
2016	1288	F	7.6	4.7	0.9	0.5		0.40	0.30
2015	1359	F	5.6		2.1				
2017	1603	M	8.2	5.9	2.3	0.6		0.60	0.30
2017	1288	F	10.1	5.4	1.8	0.6		> 0.30	0.30
2017	1591	M	7.9	5.1	1.6	1.1		0.40	0.45
2017	1288	M	10.0	5.4	0.9	0.6	0.52	> 0.30	0.30

Table 4.3. Top mixed-effects logistic models to assess which factors best classify northern Idaho ground squirrel seasonal use areas (summer active season locations versus winter hibernacula locations). All tree measurements were within 5m of the locations. Only models with a weight > 0.01 are included. Null model is also included. Global model is not provided because it failed to converge.

Models	AICc	ΔAICc	df	w_i
Slope + Canopy + # of trees	475.9	0.0	6	0.25
Slope + Canopy + Distance to closest tree + # of trees	477.7	1.8	7	0.11
Slope + Canopy + Distance to closest tree + DBH closest tree + # of trees	477.7	1.8	8	0.10
Slope + Canopy + # of trees + Aspect + Sex	478.4	2.4	9	0.08
Slope + Canopy + # of trees + Aspect	479.4	3.4	8	0.05
Slope + Canopy + Distance to closest tree + DBH of largest tree	479.4	3.4	7	0.05
Slope + Canopy + Distance to closest tree + DBH closest tree + DBH of largest tree	479.9	4.0	8	0.04
Slope + Canopy + Distance to closest tree + # of trees + Aspect + Sex	480.1	4.2	10	0.03
Slope + Canopy + Distance to closest tree + # of trees + DBH of closest tree + Aspect + Sex	480.1	4.2	11	0.03
Slope + Canopy + DBH of largest tree	480.4	4.5	6	0.03
Slope + Canopy + DBH of closest tree	480.7	4.8	6	0.02
Slope + Canopy + Distance to closest tree + # of trees + DBH of closest tree + Aspect	480.9	5.0	10	0.02
Slope + Canopy + Distance to closest tree + # of trees + Aspect	481.1	5.1	9	0.02
Slope + Canopy + Distance to closest tree + DBH of closest tree + Log width	481.1	5.2	8	0.02
Slope + Canopy + Distance to closest tree + DBH of largest tree + Aspect + Sex	481.3	5.3	10	0.02
Slope + Canopy + Distance to closest tree + DBH of closest tree + Aspect + Sex	481.8	5.9	10	0.01
Slope + Canopy + Distance to closest tree + DBH of closest tree + DBH of largest tree + Aspect + Sex	481.8	5.9	11	0.01
Slope + Canopy + DBH of largest tree + Aspect + Sex	482.0	6.1	9	0.01
Slope + Canopy + DBH of closest tree + Aspect + Sex	482.4	6.4	9	0.01
Canopy + # of trees	482.4	6.4	5	0.01
Slope + Canopy + Distance to closest tree + DBH of largest tree + Aspect	482.8	6.9	9	0.01
Slope + Canopy + Distance to closest tree + DBH of closest tree + Aspect + Sex + Log (y/n)	483.0	7.0	11	0.01
Slope + Canopy + Aspect + Sex	483.1	7.1	8	0.01
Slope + Canopy + Distance to closest tree + DBH of closest tree + DBH of largest tree + Aspect	483.3	7.3	10	0.01
Slope + Canopy + Log width	483.3	7.3	6	0.01
Slope + Canopy + Distance to closest tree + DBH of closest tree + Aspect + Sex + Log width	483.3	7.4	11	0.01
Slope + Canopy + Distance to closest tree Aspect + Sex	483.7	7.7	9	0.01
Slope + Canopy + DBH of closest tree + Aspect	483.8	7.8	8	0.01
Slope + Canopy + DBH of largest tree + Aspect	483.7	7.8	8	0.01
Null model	622.6	146.7	3	<0.001

Table 4.4. Top logistic regression models evaluating northern Idaho ground squirrel overwinter survival. Data include 94 radio-collared ground squirrels at 9 study sites from 2013-2017. Only models with a weight greater than 0.01 are included as well as the null model and global model. "# of trees" is the number of trees (a maximum of 4 were counted) within 5m of each squirrel's hibernaculum.

Model	AICc	ΔAICc	df	w_i
Sex + Distance to log	123.01	0.00	3	0.02
Sex + Distance to log + DBH closest tree	124.78	1.78	4	0.01
Sex + Year + Distance to log	124.83	1.82	4	0.01
Sex + Distance to log + Distance to closest tree	124.88	1.87	4	0.01
Sex + # of trees + Distance to log	124.91	1.91	4	0.01
Sex	124.95	1.94	2	0.01
Sex + Distance to log + Distance	125.00	1.99	4	0.01
Sex + Distance to log + DBH largest tree	125.02	2.01	4	0.01
Distance to log	125.03	2.02	2	0.01
Sex + Slope + Distance to log	125.05	2.04	4	0.01
Sex + Elevation + Distance to log	125.09	2.08	4	0.01
Sex + Canopy + Distance to log	125.15	2.15	4	0.01
Sex + Log width + Distance to log	125.17	2.17	4	0.01
Null	129.62	6.61	1	0.00
Global Model	149.22	26.21	15	0.00

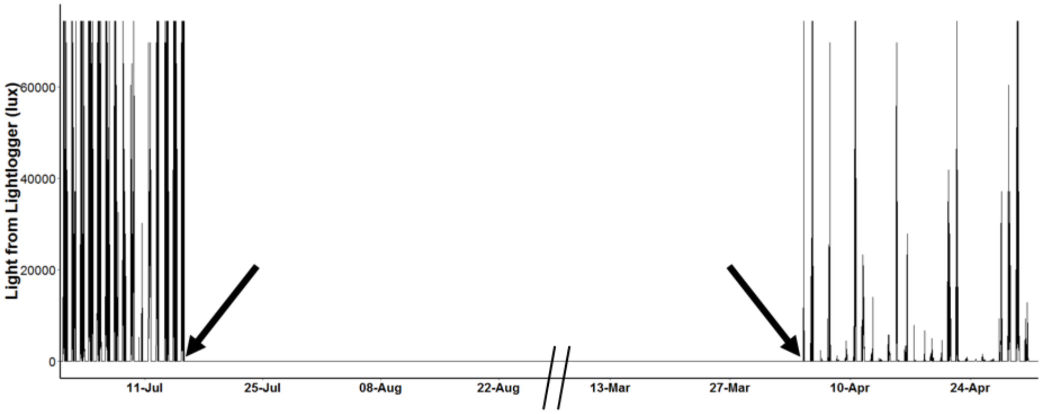


Figure 4.1. Example of the data from a light logger retrieved from a northern Idaho ground squirrel. Vertical lines represent light readings. Large black arrows represent when the squirrel immerged in July and emerged in April.

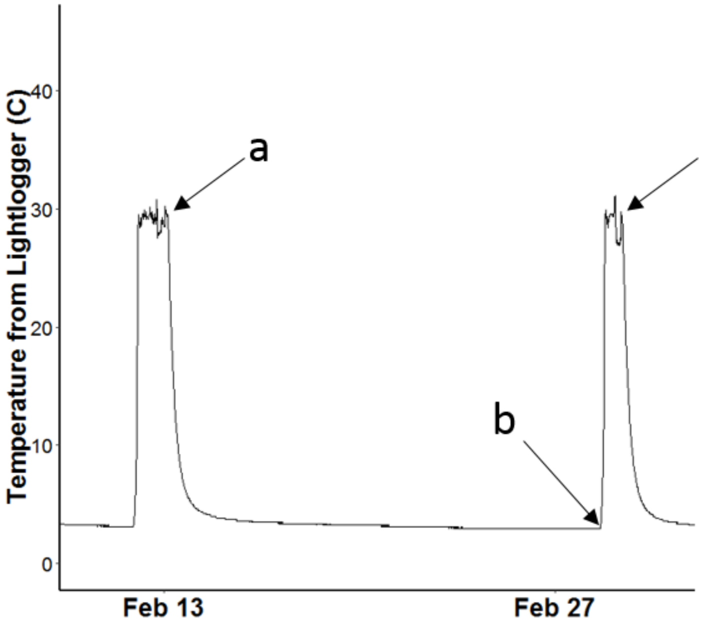


Figure 4.2. Example of the skin temperature data we collected from light loggers on northern Idaho ground squirrels. The line represents the squirrel's skin temperature over 2.5 weeks. Torpor bout begins at point 'a' and ends at point 'b'. A euthermic bout begins at point 'b' and ends at point 'c'. We examined data from all 20 collars that we retrieved and identified the date and time when torpor ended and euthermic bouts began for the entire period each squirrel was below ground.

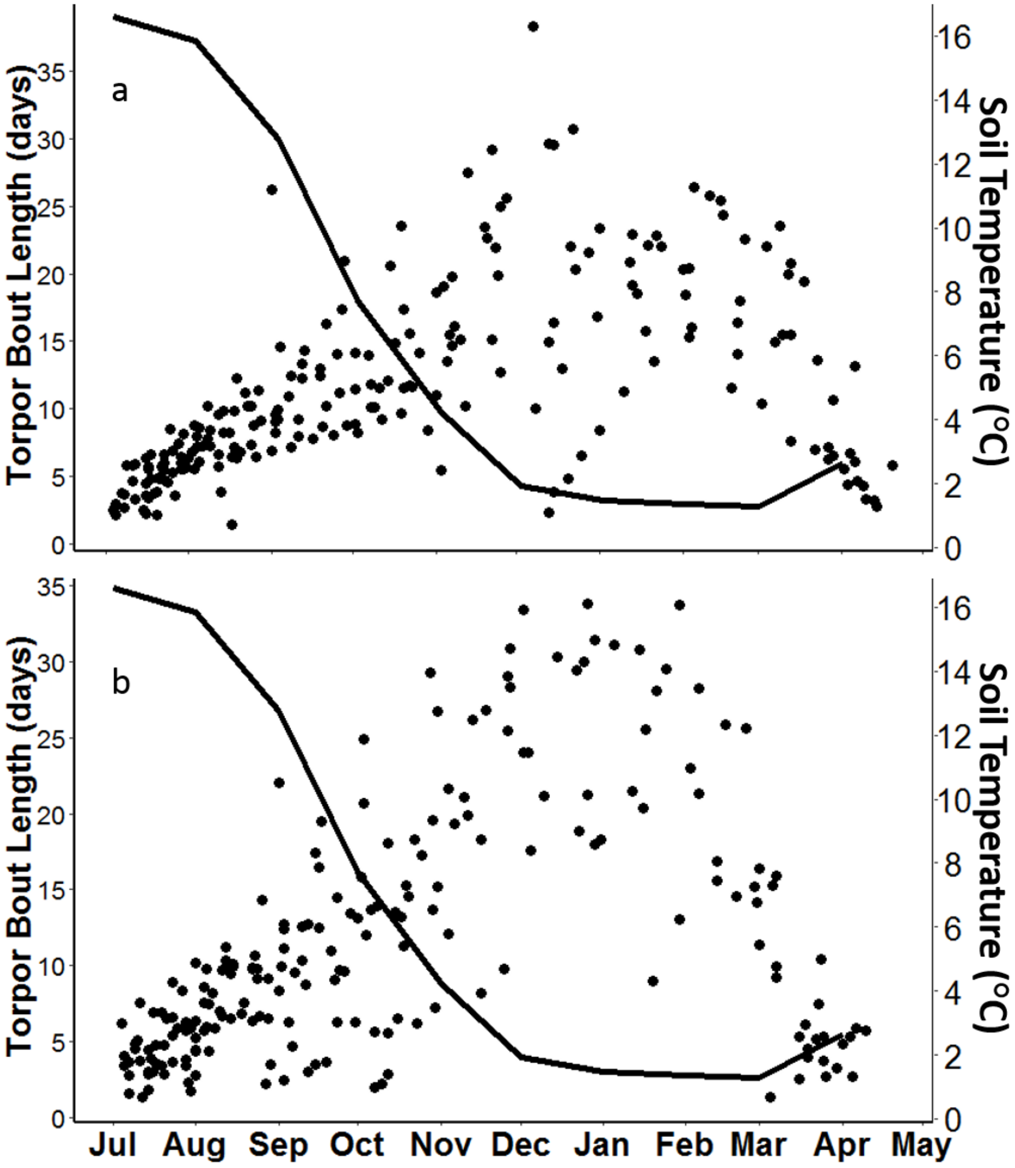


Figure 4.3. Duration of torpor bouts for a) 10 adult female and b) 10 adult male northern Idaho ground squirrels at 6 different field sites. Elevation was 1,288 – 1,696m for all squirrels. Line represents the average monthly soil temperature at 30m depth in the forest for all 6 sites combined.

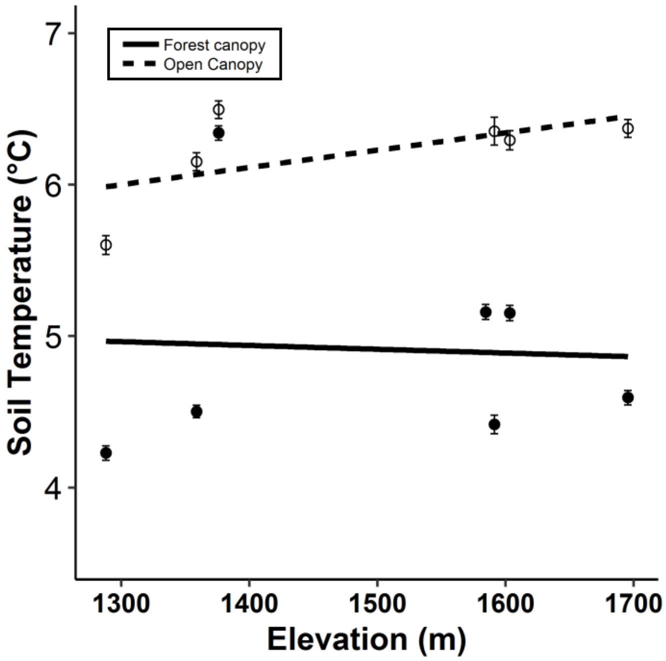


Figure 4.4. Average soil temperature between 1 September and 1 March for 2 years (2016-2017 and 2017-2018) at 30-cm depths. Open circles (and dotted line) are from soil probes placed in areas of open canopy and black circles (and solid line) are from soil probes placed under tree canopy (forest).

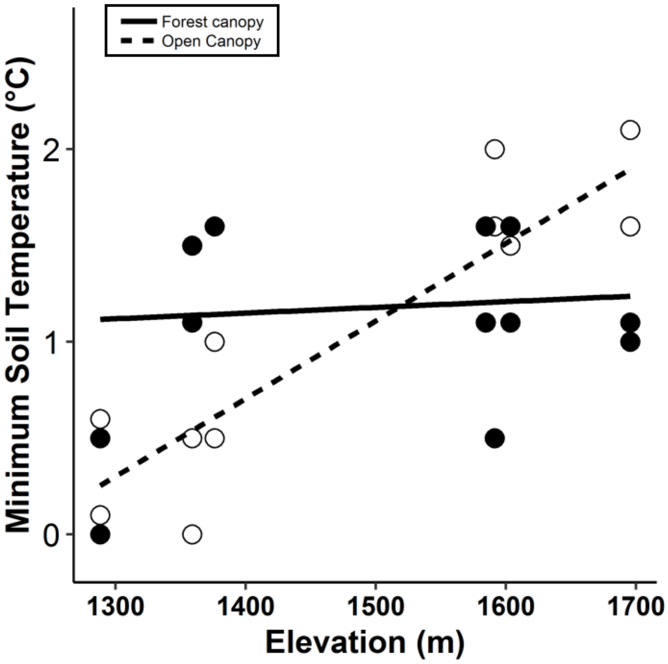


Figure 4.5. Minimum soil temperature between 1 September and 1 March for 2 years (2016-2017 and 2017-2018) at 30-cm depths. Points are the minimum soil temperature for 7 sites, both years combined. Open circles (and dotted line) are from soil probes placed in areas of open canopy and black circles (and solid line) are from soil probes placed under tree canopy (forest).

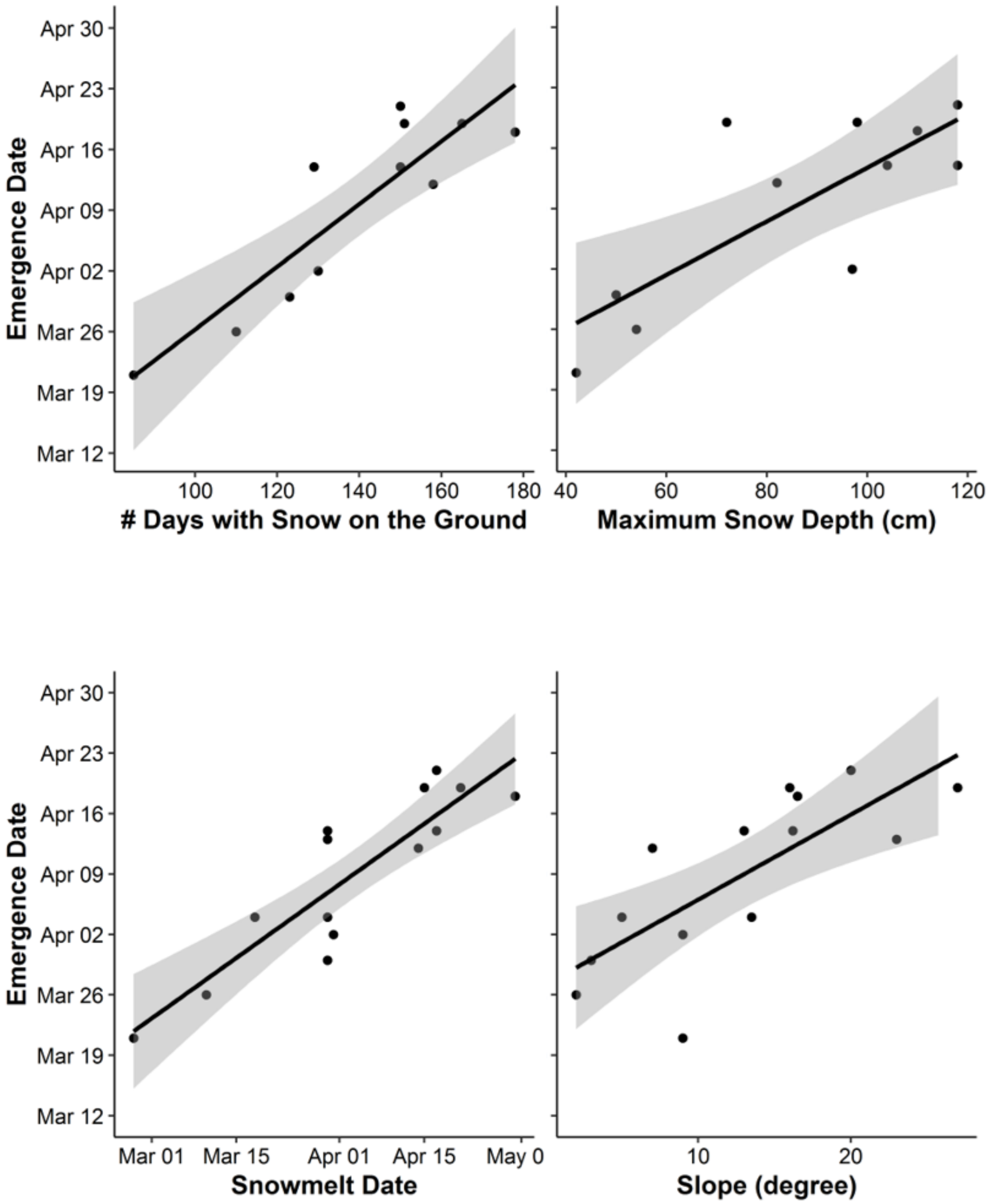


Figure 4.6. Relationship between date of emergence from hibernation and: (a) # days with snow on the ground, (b) maximum snow depth, (c) snowmelt date, and (d) slope at hibernacula site for northern Idaho ground squirrels.

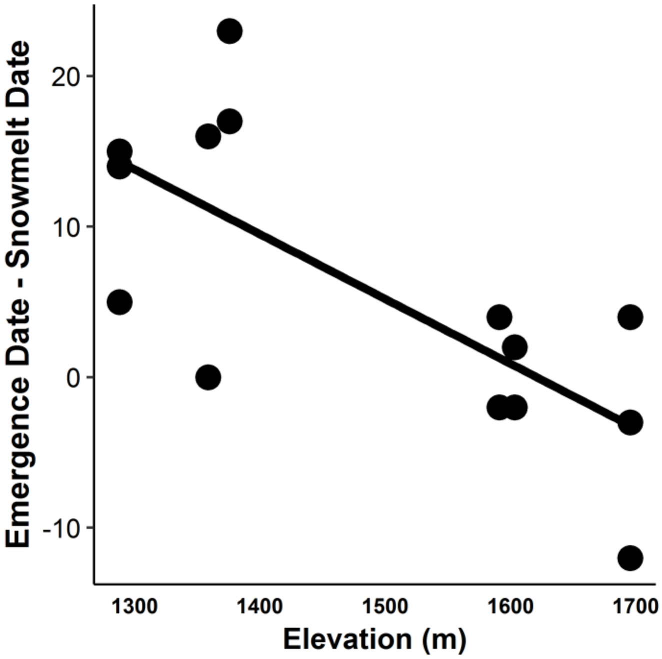


Figure 4.7. Relationship between elevation and the number of days northern Idaho ground squirrels emerged after the snow melted at their hibernacula locations. We included data from 14 squirrels (6 females and 8 males) who we knew the exact date they emerged from hibernation in the spring and the date the snow melted.

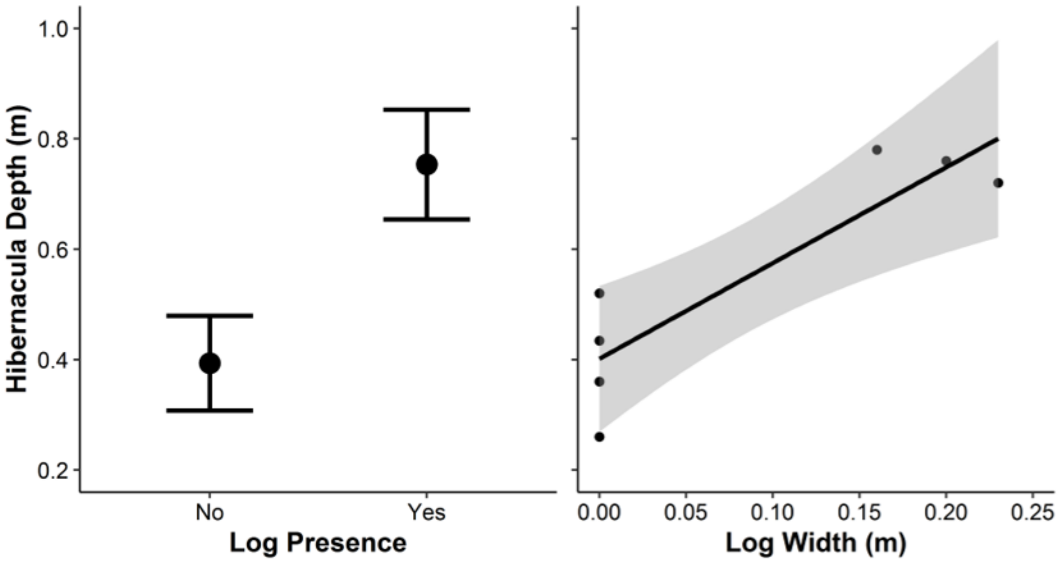


Figure 4.8. Relationship between hibernacula depth and both presence of logs (a) and width of logs (b) at hibernacula site for northern Idaho ground squirrels.

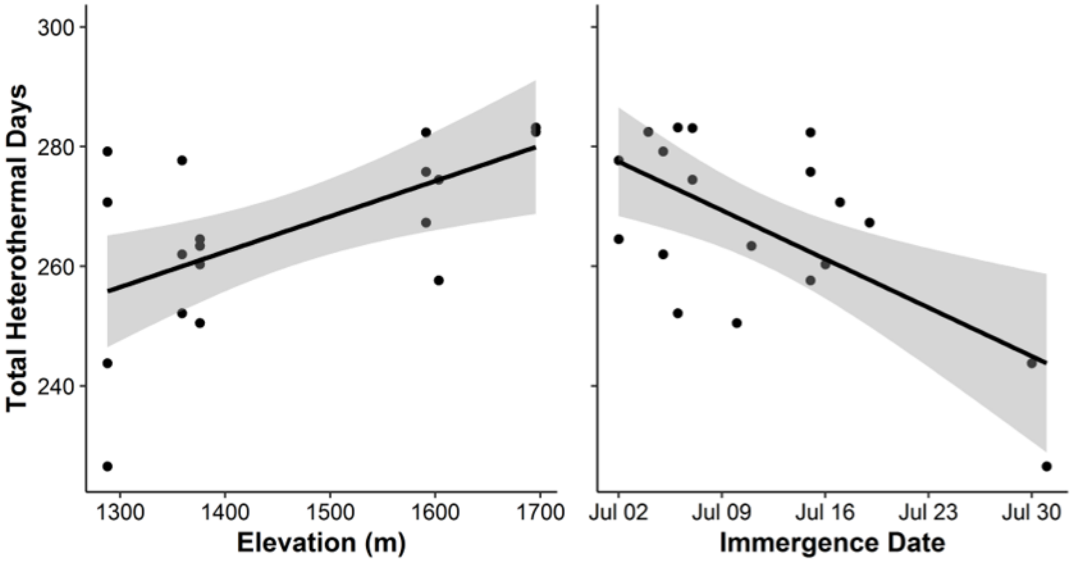


Figure 4.9. Relationship between the number of heterothermal days and both elevation (a) and immergence date (b) for northern Idaho ground squirrels. Heterothermal period begins at the start of the 1st torpor period that is over 24 hours long and ends at the end of the last 24-hour torpor period.

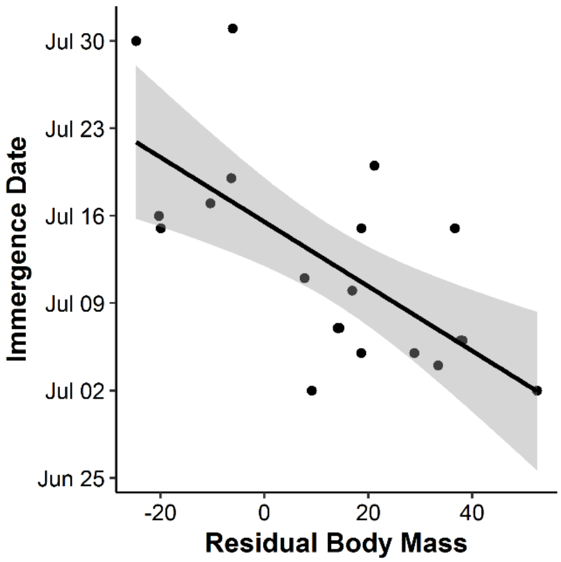


Figure 4.10. Relationship between residual body mass and date of immergence into hibernation for northern Idaho ground squirrels. Residual body mass represents the squirrel's body mass (corrected for capture date) in the summer prior to hibernation. Larger squirrels enter hibernation earlier in the summer.

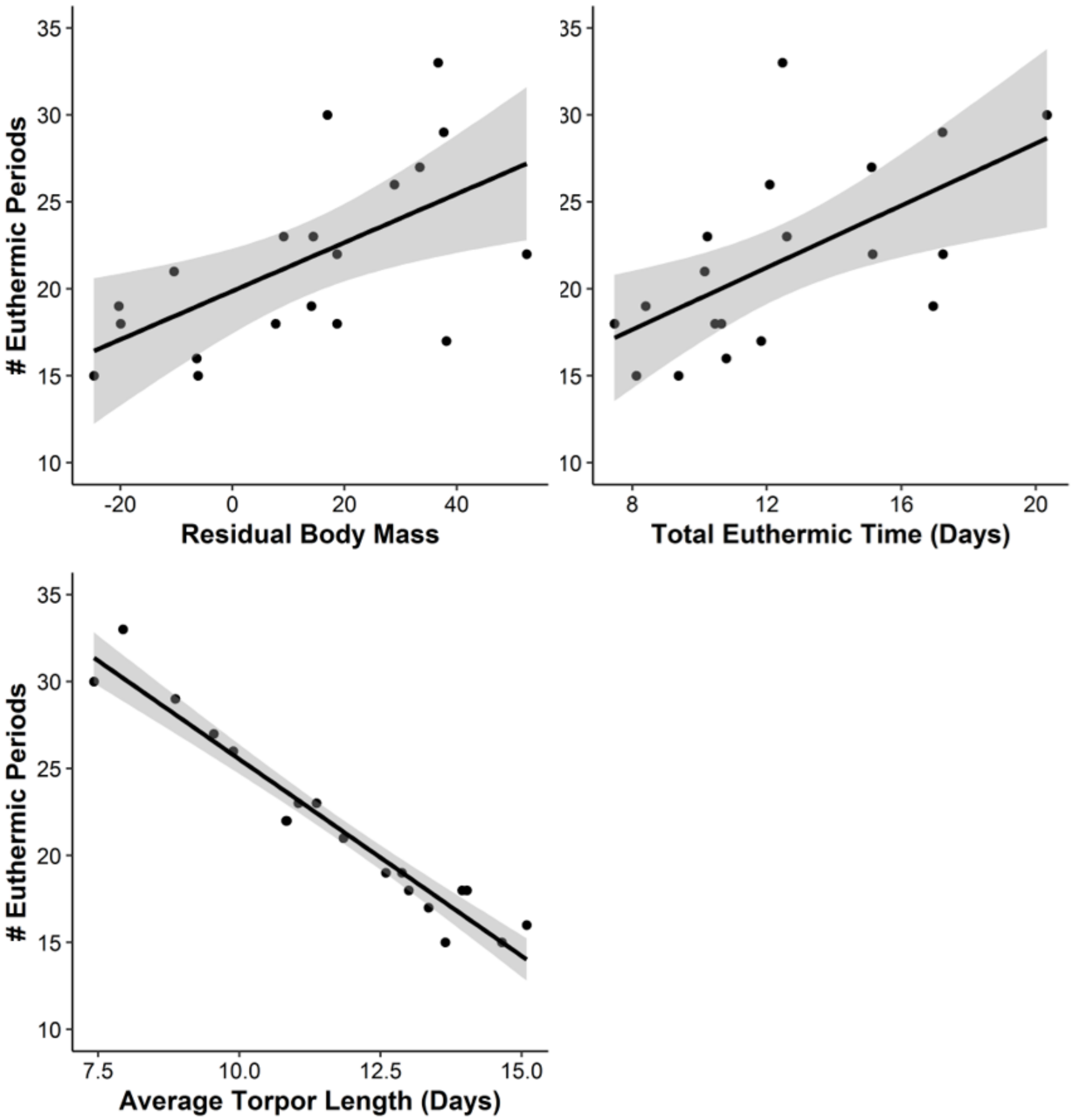


Figure 4.11. Relationship between the number of euthermic periods and: (a) residual body mass, (b) total euthermic time, and (c) average torpor length for northern Idaho ground squirrels. Residual body mass represents the squirrel's body mass (corrected for capture date) in the summer prior to hibernation. Total euthermic time is all euthermic periods following the first 24-hour torpor bout until the last 24-hour torpor period. Average torpor length is the average length of torpor periods that were over 24 hours (heterothermal period).

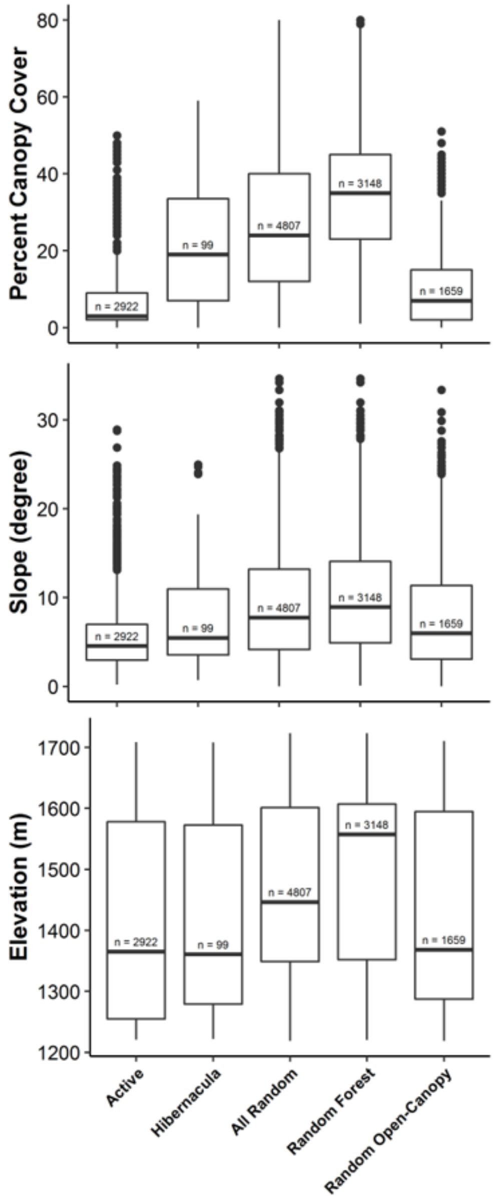
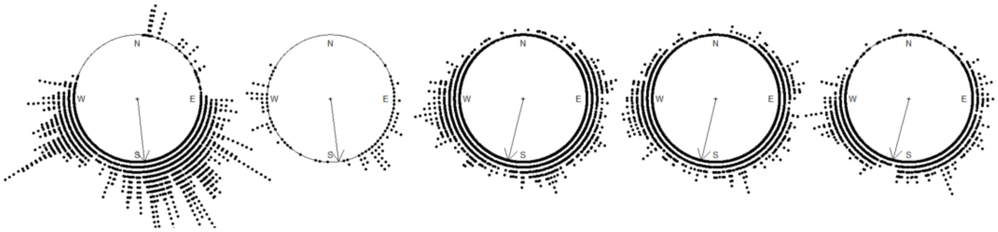


Figure 4.12. Percent canopy cover (a), slope (b), and elevation (c) for each of 5 location types for northern Idaho ground squirrels: summer active-season locations, winter hibernacula locations, random locations within the year-round use areas, random locations within the forested areas, and random locations within the non-forested open-canopy areas.



Active **Hibernacula** **All Random** **Random Forest** **Random Open-Canopy**

Figure 4.13. Aspect (orientation) for each of 5 location types for northern Idaho ground squirrels: summer active-season locations, winter hibernacula locations, random locations within the year-round use areas, random locations within the forest areas, and random locations within the non-forested open-canopy areas. Arrows point to the mean aspect.

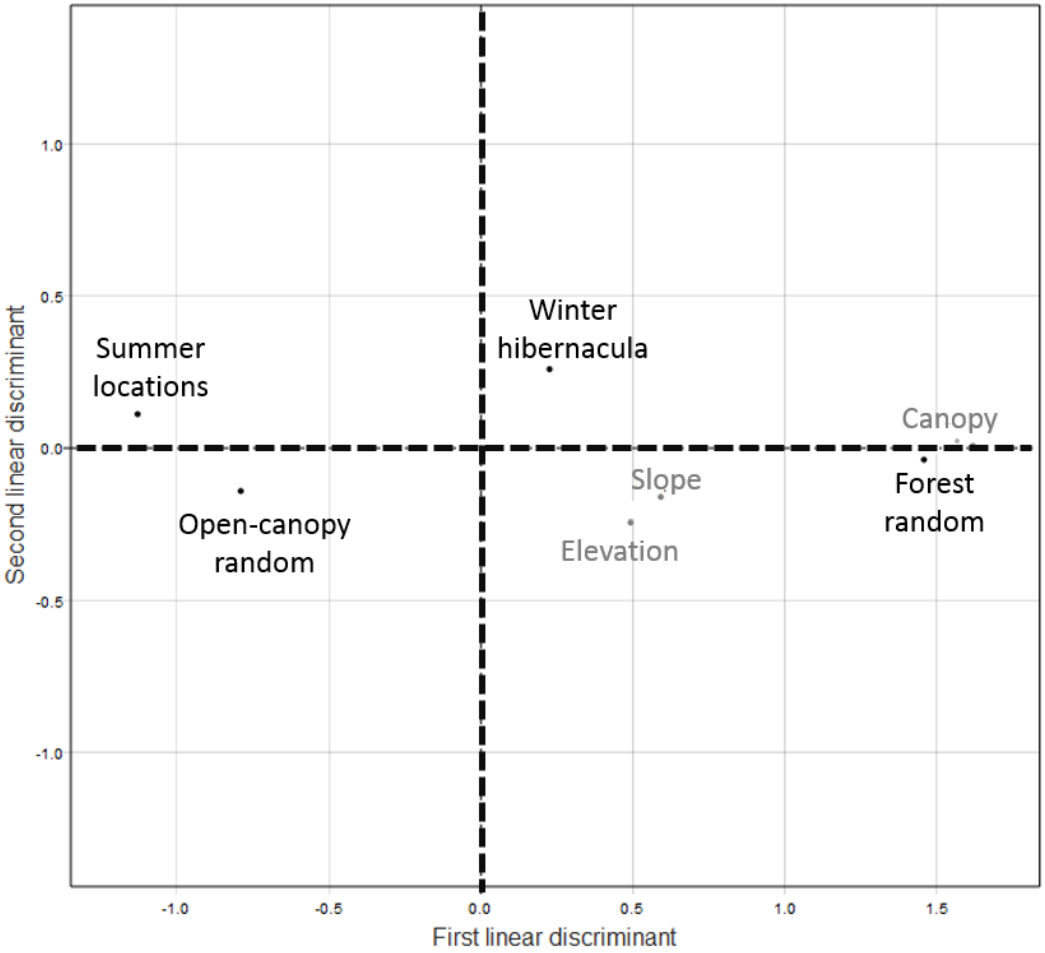


Figure 4.14. Linear discriminant function illustrating differences among 5 location types for northern Idaho ground squirrels: summer active-season locations, winter hibernacula locations, random locations within the forested areas, and random locations within the non-forested open-canopy areas (i.e., available habitat within the species' home range). Slope, elevation, and canopy cover were the variables used in the discriminant function analysis. Summer active season locations were in very open-canopy areas whereas hibernacula were intermediate between open canopy and forested locations available to squirrels.

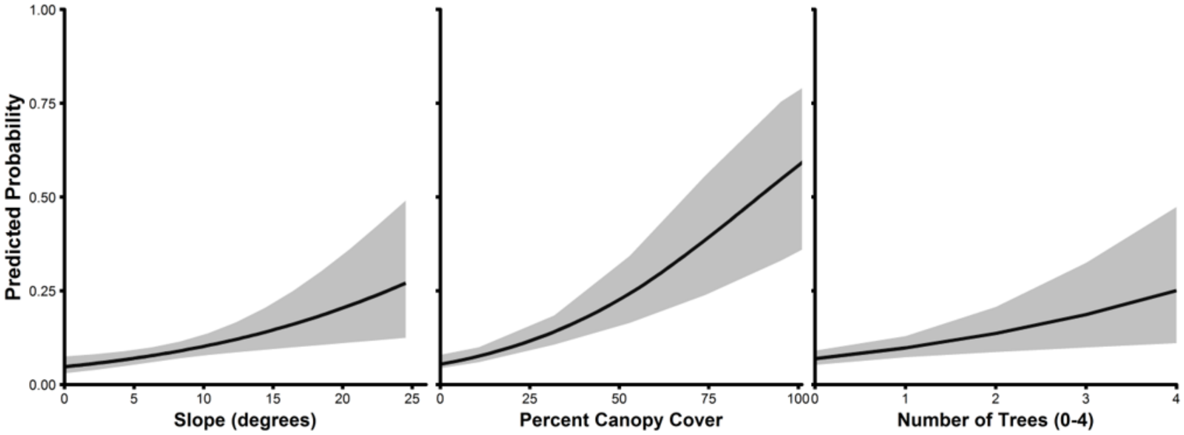


Figure 4.15. The relationship between 3 habitat metrics (slope, percent canopy cover, and number of trees) and whether a point was a summer active season location or a winter hibernacula location for northern Idaho ground squirrels. Data were collected at 9 study sites in Adams County, Idaho between 2013 and 2017. Only adult ground squirrel's locations were included in the model.

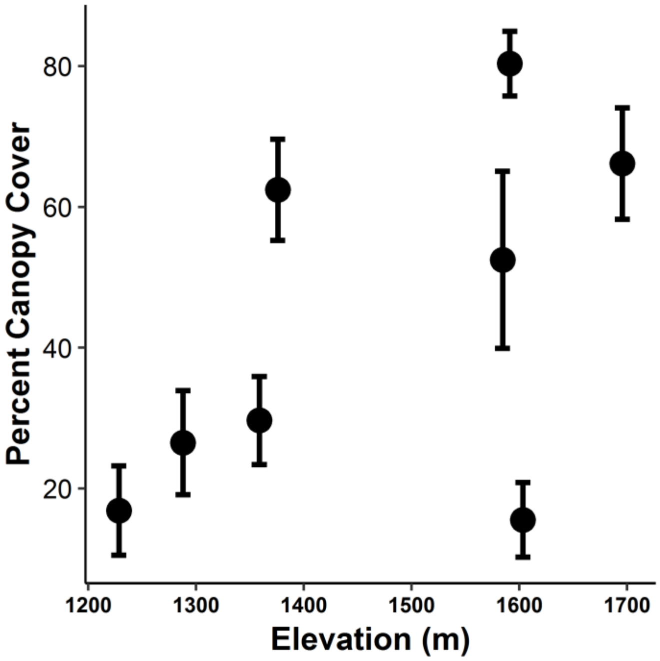


Figure 4.16. The relationship between elevation and percent canopy cover at adult northern Idaho ground squirrel hibernacula locations. Data were collected at 8 study sites in Adams County, Idaho between 2013 and 2017. Standard error bars are included.

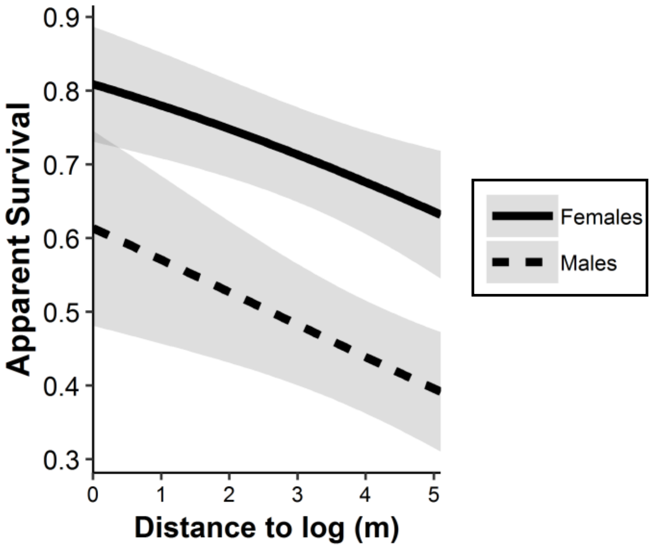


Figure 4.17. Apparent overwinter survival of radio-collared northern Idaho ground squirrels that we believed to be alive when they entered into hibernacula. Survival is based on whether a squirrel was trapped any interval following hibernation. Apparent survival was estimated using a logistic regression model. Data were collected at 9 study sites in Adams County, Idaho between 2013 and 2017. Only adult ground squirrel locations were included in the model. Hibernacula were located using VHF radio-collars.

Appendix

Table S1. Complete list of plant species: 1) used in our master reference list (plants we identified in our vegetation quadrats at our 13 study sites or were identified as present at our sites from past studies), or 2) identified as present in at least 1 northern Idaho ground squirrel fecal sample.

Family	Species: Reference List	Species: Fecal Samples
Amaryllidaceae	*Allium*	*Allium*
	Allium acuminatum	*Allium acuminatum*
	Allium brandegeei	
	Allium douglasii	
	Allium fibrillum	
	Allium tolmiei	*Allium tolmiei*
Apiaceae	*Ligusticum canbyi*	
	Lomatium	*Lomatium*
	Lomatium grayi	*Lomatium grayi*
	Lomatium leptocarpum	
	Lomatium nudicaule	*Lomatium nudicaule*
	Lomatium triternatum	*Lomatium triternatum*
	Orogenia	*Orogenia*
	Perideridia bolanderi	*Perideridia bolanderi*
	Perideridia gairdneri	*Perideridia gairdneri*
Apocynaceae	*Apocynum androsaemifolium*	
Asparagaceae	*Camassia quamash*	*Camassia quamash*
	Dichelostemma congestum	
	Maianthemum racemosum	
	Maianthemum stellatum	
	Triteleia grandiflora	*Triteleia grandiflora*
Asteraceae	*Achillea*	
	Achillea millefolium	*Achillea millefolium*
	Adenocaulon bicolor	
	Agoseris	*Agoseris*
	Agoseris glauca	
	Agoseris heterophylla	*Agoseris heterophylla*
	Anaphalis margaritacea	
	Antennaria	*Antennaria*
	Antennaria luzuloides	
	Antennaria microphylla	*Antennaria microphylla*
	Antennaria rosea	
	Arnica	*Arnica*
	Arnica chamissonis	
	Arnica cordifolia	*Arnica cordifolia*
	Arnica sororia	*Arnica sororia*
	Artemisia	*Artemisia*

Artemisia arbuscula	
Artemisia rigida	
Artemisia tridentata	
Artemisia tripartita	
Aster	
Balsamorhiza	*Balsamorhiza*
Balsamorhiza hookeri	
Balsamorhiza sagittata	*Balsamorhiza sagittata*
Chrysothamnus viscidiflorus	
Cirsium	*Cirsium*
Cirsium vulgare	
Crepis	*Crepis*
Crepis acuminata	
Crepis occidentalis	
Ericameria nauseosus	
Erigeron	*Erigeron*
Erigeron filifolius	*Erigeron filifolius*
Erigeron pumilus	*Erigeron pumilus*
Erigeron speciosus	
Eriophyllum lanatum	
Eurybia integrifolia	
Gnaphalium	*Gnaphalium*
Grindelia hirsutula	
Grindelia nana	*Grindelia nana*
Helianthella uniflora	*Helianthella uniflora*
Hieracium	*Hieracium*
Hieracium albiflorum	
Hieracium scouleri	
Hieracium triste	
Madia	*Madia*
Madia glomerata	
Madia gracilis	*Madia gracilis*
Microseris	
Microseris nutans	*Microseris nutans*
Nothocalais nigrescens	*Nothocalais nigrescens*
Oreostemma alpigenum	
Pyrrocoma	
Pyrrocoma carthamoides	
Senecio	*Senecio*
Solidago	*Solidago*
Solidago lepida	
Solidago missouriensis	*Solidago missouriensis*
Taraxacum	

	Taraxacum ceratophorum	
	Taraxacum officinale	*Taraxacum officinale*
	Tragopogon	*Tragopogon*
	Tragopogon dubius	*Tragopogon dubius*
	Wyethia	*Wyethia*
	Wyethia amplexicaulis	
	Wyethia helianthoides	*Wyethia helianthoides*
Berberidaceae	*Mahonia repens*	
Boraginaceae	*Cryptantha*	*Cryptantha*
	Cryptantha affinis	*Cryptantha affinis*
	Cynoglossum officinale	*Cynoglossum officinale*
	Hackelia	*Hackelia*
	Hackelia deflexa	*Hackelia deflexa*
	Lappula redowskii	
	Lithospermum ruderale	
	Mertensia longiflora	*Mertensia longiflora*
	Myosotis	
	Myosotis micrantha	
	Plagiobothrys	*Plagiobothrys*
	Plagiobothrys leptocladus	
Brassicaceae	*Alyssum*	*Alyssum*
	Alyssum alyssoides	*Alyssum alyssoides*
	Arabis	
	Boechera holboellii	
	Capsella bursa-pastoris	
	Descurainia	
	Draba verna	*Draba verna*
	Lepidium	*Lepidium*
	Lepidium campestre	
	Lepidium graminifolium	
	Lepidium latifolium	
	Lepidium virginicum	*Lepidium virginicum*
Caprifoliaceae	*Linnaea borealis*	
	Lonicera utahensis	*Lonicera utahensis*
	Symphoricarpos	*Symphoricarpos*
	Symphoricarpos albus	
	Symphoricarpos oreophilus	
	Moehringia lateriflora	
Caryophyllaceae	*Arenaria*	*Arenaria*
	Dianthus armeria	*Dianthus armeria*
	Eremogone	*Eremogone*
	Eremogone aculeata	
	Eremogone congesta	*Eremogone congesta*

	Holosteum umbellatum	
	Moehringia lateriflora	*Moehringia lateriflora*
	Moehringia macrophylla	*Moehringia macrophylla*
	Silene menziesii	*Silene menziesii*
	Spergularia rubra	*Spergularia rubra*
Celastraceae	*Paxistima myrsinites*	*Paxistima myrsinites*
	Sedum stenopetalum	*Sedum stenopetalum*
Cyperaceae	*Carex*	*Carex*
	Carex geyeri	*Carex geyeri*
	Carex hoodii	
	Carex pachystachya	
	Carex petasata	
Equisetaceae	*Equisetum*	
Ericaceae	*Chimaphila umbellata*	
	Hypopitys monotropa	
	Vaccinium	*Vaccinium*
	Vaccinium membranaceum	
Fabaceae	*Acmispon americanus*	
	Arachis	*Arachis*
	Astragalus	*Astragalus*
	Lathyrus	*Lathyrus*
	Lathyrus lanszwertii	
	Lathyrus nevadensis	
	Lotus purshianus	
	Lotus unifoliolatus	*Lotus unifoliolatus*
	Lupinus	*Lupinus*
	Lupinus argenteus	
	Lupinus laxiflorus	
	Lupinus leucophyllus	
	Lupinus polyphyllus	*Lupinus polyphyllus*
	Lupinus sericeus	
	Medicago	*Medicago*
	Thermopsis montana	
	Trifolium	*Trifolium*
	Trifolium eriocephalum	
	Trifolium macrocephalum	*Trifolium macrocephalum*
	Trifolium pratense	
	Vicia	
	Vicia americana	*Vicia americana*
Gentianaceae	*Frasera albicaulis*	*Frasera albicaulis*
	Frasera speciosa	*Frasera speciosa*
	Erodium cicutarium	*Erodium cicutarium*
	Geranium viscosissimum	*Geranium viscosissimum*

Grossulariaceae	*Ribes cereum*	*Ribes cereum*
Hydrophyllaceae	*Hydrophyllum capitatum*	*Hydrophyllum capitatum*
	Nemophila breviflora	*Nemophila breviflora*
	Nemophila pedunculata	
	Phacelia	*Phacelia*
	Phacelia franklinii	
	Phacelia hastata	
Hypericaceae	*Hypericum perforatum*	
	Hypericum scouleri	
Iridaceae	*Olsynium douglasii*	*Olsynium douglasii*
Juncaceae	*Juncus*	*Juncus*
	Juncus parryi	
Lamiaceae		
	Prunella vulgaris	
	Scutellaria angustifolia	
Liliaceae		
	Calochortus	*Calochortus*
	Calochortus elegans	*Calochortus elegans*
	Calochortus eurycarpus	*Calochortus eurycarpus*
	Erythronium grandiflorum	
	Fritillaria pudica	*Fritillaria pudica*
Malvaceae	*Sidalcea oregana*	*Sidalcea oregana*
	Sidelacea	
Melanthiaceae	*Toxicoscordion*	*Toxicoscordion*
	Toxicoscordion paniculatum	
	Toxicoscordion venenosum	*Toxicoscordion venenosum*
	Trillium ovatum	
	Veratrum	*Veratrum*
	Veratrum californicum	
	Veratrum viride	
Montiaceae	*Claytonia*	*Claytonia*
	Claytonia cordifolia	
	Claytonia perfoliata	
	Claytonia rubra	*Claytonia rubra*
	Montia linearis	*Montia linearis*
Onagraceae	*Camissonia subacaulis*	
	Chamerion angustifolia	
	Chamerion angustifolium	
	Clarkia pulchella	
	Clarkia rhomboidea	*Clarkia rhomboidea*
	Epilobium	*Epilobium*
	Epilobium angustifolium	
	Epilobium brachycarpum	*Epilobium brachycarpum*

	Epilobium ciliatum	
	Gayophytum	*Gayophytum*
	Gayophytum diffusum	*Gayophytum diffusum*
	Calypso bulbosa	
	Goodyera oblongifolia	
Orobanchaceae	*Castilleja*	*Castilleja*
	Castilleja cusickii	
	Castilleja pallescens	
	Castilleja tenuis	
	Cordylanthus capitatus	*Cordylanthus capitatus*
	Orobanche uniflora	*Orobanche uniflora*
	Orthocarpus tenuifolius	*Orthocarpus tenuifolius*
	Pedicularis	
Paeoniaceae	*Paeonia brownii*	
Phrymaceae	*Mimulus breweri*	
	Mimulus cusickii	
Pinaceae	*Abies*	*Abies*
	Abies concolor	
	Abies grandis	
	Pinus	*Pinus*
	Pinus contorta	
	Pinus ponderosa	*Pinus ponderosa*
	Pseudotsuga menziesii	*Pseudotsuga menziesii*
Plantaginaceae	*Besseya rubra*	
	Collinsia	*Collinsia*
	Collinsia parviflora	*Collinsia parviflora*
	Penstemon	*Penstemon*
	Penstemon deustus	
	Penstemon gairdneri	
	Penstemon globosus	
	Penstemon wilcoxii	
	Plantago lanceolata	*Plantago lanceolata*
	Veronica biloba	*Veronica biloba*
Poaceae	*Achnatherum*	
	Achnatherum nelsonii	*Achnatherum nelsonii*
	Agropyron cristatum	
	Agropyron spicatum	
	Agrostis	*Agrostis*
	Alopecurus pratensis	*Alopecurus pratensis*
	Anthoxanthum	*Anthoxanthum*
	Bromus	*Bromus*
	Bromus arvensis	
	Bromus briziformis	

	Bromus carinatus	
	Bromus commutatus	*Bromus commutatus*
	Bromus inermis	*Bromus inermis*
	Bromus japonicus	*Bromus japonicus*
	Bromus marginatus	
	Bromus rubens	
	Bromus tectorum	*Bromus tectorum*
	Calamagrostis	
	Calamagrostis rubescens	*Calamagrostis rubescens*
	Dactylis glomerata	*Dactylis glomerata*
	Danthonia	*Danthonia*
	Danthonia unispicata	
	Deschampsia	
	Deschampsia danthonioides	
	Deschampsia elongata	
	Elymus	*Elymus*
	Elymus elymoides	
	Elymus multisetus	
	Festuca	*Festuca*
	Festuca idahoensis	*Festuca idahoensis*
	Festuca ovina	*Festuca ovina*
	Festuca rubra	
	Koeleria macrantha	
	Leymus cinereus	
	Melica	*Melica*
	Melica bulbosa	
	Melica spectabilis	
	Melica subulata	*Melica subulata*
	Pascopyrum smithii	
	Phalaris arundinacea	*Phalaris arundinacea*
	Phleum pratense	
	Poa	*Poa*
	Poa bulbosa	*Poa bulbosa*
	Poa compressa	*Poa compressa*
	Poa nervosa	
	Poa pratensis	*Poa pratensis*
	Poa secunda	*Poa secunda*
	Pseudoroegneria spicata	*Pseudoroegneria spicata*
	Thinopyrum intermedium	
	Ventenata dubia	
Polemoniaceae	*Collomia*	*Collomia*
	Collomia grandiflora	*Collomia grandiflora*
	Collomia linearis	*Collomia linearis*

	Ipomopsis aggregata	*Ipomopsis aggregata*
	Leptosiphon harknessii	*Leptosiphon harknessii*
	Microsteris gracilis	*Microsteris gracilis*
	Navarretia	*Navarretia*
	Navarretia capillaris	
	Navarretia divaricata	*Navarretia divaricata*
	Navarretia intertexta	*Navarretia intertexta*
	Phlox	*Phlox*
	Phlox hoodii	
Polygonaceae	*Eriogonum*	*Eriogonum*
	Eriogonum douglasii	
	Eriogonum heracleoides	*Eriogonum heracleoides*
	Eriogonum sphaerocephalum	
	Eriogonum umbellatum	*Eriogonum umbellatum*
	Polygonum	*Polygonum*
	Polygonum douglasii	*Polygonum douglasii*
	Polygonum majus	*Polygonum majus*
	Polygonum polygaloides	*Polygonum polygaloides*
	Rumex	*Rumex*
	Rumex acetosella	*Rumex acetosella*
	Rumex utahensis	
Primulaceae	*Dodecatheon*	
	Primula cusickiana	*Primula cusickiana*
Ranunculaceae	*Anemone piperi*	
	Clematis hirsutissima	*Clematis hirsutissima*
	Delphinium	*Delphinium*
	Delphinium bicolor	*Delphinium bicolor*
	Ranunculus	*Ranunculus*
	Ranunculus glaberrimus	
	Ranunculus uncinatus	
	Thalictrum occidentale	
Rhamnaceae	*Ceanothus*	
	Ceanothus velutinus	*Ceanothus velutinus*
Rosaceae	*Amelanchier alnifolia*	
	Crataegus douglasii	*Crataegus douglasii*
	Drymocallis	*Drymocallis*
	Drymocallis arguta	
	Drymocallis glandulosa	*Drymocallis glandulosa*
	Fragaria	*Fragaria*
	Fragaria vesca	
	Fragaria virginiana	*Fragaria virginiana*
	Geum triflorum	*Geum triflorum*
	Physocarpus malvaceus	

	Potentilla	*Potentilla*
	Potentilla gracilis	*Potentilla gracilis*
	Potentilla recta	
	Poteridium annuum	*Poteridium annuum*
	Prunus	*Prunus*
	Prunus emarginata	
	Prunus virginiana	
	Purshia tridentata	*Purshia tridentata*
	Rosa	*Rosa*
	Rosa woodsii	
	Rubus parviflorus	
	Spiraea betulifolia	*Spiraea betulifolia*
Rubiaceae	*Galium*	*Galium*
	Galium aparine	*Galium aparine*
	Galium bifolium	
	Galium triflorum	
	Kelloggia galioides	
Salicaceae	*Populus tremuloides*	
	Salix scouleriana	
Saxifragaceae	*Lithophragma*	*Lithophragma*
	Lithophragma glabrum	*Lithophragma glabrum*
	Lithophragma parviflorum	*Lithophragma parviflorum*
	Micranthes integrifolia	
	Saxifraga	
Scrophulariaceae	*Verbascum thapsus*	
Violaceae	*Viola*	*Viola*
	Viola adunca	*Viola adunca*
	Viola nuttallii	
	Viola purpurea	*Viola purpurea*

Table S2. Number of the 188 northern Idaho ground squirrel fecal samples that included each genera within each gene region sampled.

Genus	ITS3_ITS4	ITS5_ITS2	trnLi_9_189
Abies			10
Achillea	2	38	2
Achnatherum	9		
Agoseris	28	30	10
Agrostis			1
Allium	47	63	67
Alopecurus			1
Alyssum	14	17	20
Antennaria	1		2
Anthoxanthum			1
Arachis			1
Arenaria		1	
Arnica	10	16	1
Artemisia	5	10	3
Astragalus			1
Balsamorhiza	2	18	
Bromus	43	67	43
Calamagrostis	2	2	
Calochortus			45
Camassia		1	2
Carex	2	3	2
Castilleja	1	6	22
Ceanothus	1	1	1
Cirsium			24
Clarkia	2	2	
Claytonia	9	16	13
Clematis		3	2
Collinsia	20	38	24
Collomia	27		41
Cordylanthus		10	
Coriandrum			1
Crataegus			1
Crepis	3	3	7
Cryptantha	2	6	3
Cynoglossum	1	2	
Dactylis		2	
Danthonia	1		
Delphinium			9
Dianthus		1	
Draba	5	4	6
Drymocallis	9	9	
Elymus	12	28	
Epilobium	11	45	29
Eremogone	3	6	2
Erigeron	33	36	
Eriogonum	10	18	18
Erodium			1
Festuca	10	13	1
Fragaria			6
Frasera	23	29	29
Fritillaria	1	2	6
Galium	1	2	2

Gayophytum	34	22	22
Geranium		4	
Geum	2	2	1
Gnaphalium	1		
Grindelia	1		
Hackelia		2	1
Helianthella	2	2	
Hieracium	4	5	6
Hydrophyllum			2
Ipomopsis	1		1
Juncus		2	1
Lathyrus			1
Lepidium	1	1	1
Leptosiphon	5	4	
Linanthus		1	
Lithophragma	29	35	
Lomatium	118	81	1
Lonicera		2	
Lotus	5	9	13
Lupinus	41	58	65
Madia	20		5
Medicago			2
Melica		1	3
Mertensia	5	6	
Microseris	21	33	
Microsteris		57	
Moehringia			2
Montia	6	5	6
Navarretia	18	58	22
Nemophila	3		
Nothocalais		1	
Olsynium		3	3
Orobanche	6		9
Orogenia		2	
Orthocarpus		1	1
Paxistima		1	1
Penstemon	3	11	11
Perideridia	60	74	
Phacelia	1	2	
Phalaris		1	
Phlox	46		58
Pinus	5		41
Plagiobothrys	10	11	
Plantago	1	1	1
Poa	57	95	21
Polygonum	1	38	6
Potentilla	11	9	4
Poteridium	1	1	
Primula	2	3	3
Prunus	1	1	1
Pseudoroegneria	1		
Pseudotsuga			9
Purshia		2	
Ranunculus	2		3
Ribes	1	1	1
Rosa	1		

Rumex		2	
Sedum	22	28	31
Senecio	5	9	
Sidalcea	11	11	3
Silene	1	1	1
Solidago	3	5	
Spergularia	6	7	
Spiraea			2
Symphoricarpos			10
Taraxacum	7	5	
Toxicoscordion	1	6	4
Tragopogon	25	3	
Trifolium	14	19	20
Triteleia			18
Vaccinium		1	
Veratrum	1		
Veronica			2
Vicia			2
Viola	3	7	17
Wyethia	21	10	
Total number of genera	**83**	**89**	**84**

Table S3. The 196 univariate generalized linear regression models we ran to explore the relationships between 35 predictor variables and 5 hibernation behaviors. Only those with a P-value less or equal to 0.007 were considered significant after accounting for false discovery rate. The 12 significant relationships are in bold.

	Emergence Date		Hibernaculum Depth		Duration of heterothermal period		Immergence date		Number of euthermic periods	
Predictors[1]	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*
Sex	-9.50	0.070	-0.06	0.756	-16.78	0.012	1.70	0.670	-2.81	0.257
Residual body mass[2]	0.03	0.838	0.00	0.774	0.31	0.048	**-0.27**	**0.001**	**0.14**	**0.007**
Elevation	0.03	0.037	0.00	0.772	**0.06**	**0.007**	-0.01	0.395	0.01	0.213
Elevation*Sex	0.04	0.132	0.00	0.322	0.06	0.06	-0.02	0.436	0.00	0.996
Canopy	0.09	0.316	0.00	0.498	0.07	0.551	0.06	0.297	0.03	0.476
Slope	**0.97**	**0.002**	0.02	0.226	0.61	0.249	0.27	0.355	0.07	0.719
Sin(aspect)	-0.07	0.986	0.08	0.576	5.91	0.264	-0.98	0.746	2.26	0.234
Cos(aspect)	-4.80	0.247	-0.21	0.364	-6.67	0.182	2.27	0.420	-0.55	0.75
Number of trees (0-4)	1.30	0.397	0.01	0.78	1.83	0.379	1.09	0.348	0.98	0.161
DBH of closest tree	-3.52	0.711	0.54	0.381	-2.20	0.877	4.25	0.524	2.06	0.673
Distance to closest tree	0.38	0.459	-0.01	0.71	-0.20	0.761	-0.01	0.987	-0.02	0.940
Log present (Y/N)	8.33	0.118	**0.36**	**0.003**	10.67	0.149	0.04	0.992	-0.10	0.971
Nearest log width	19.71	0.229	**1.73**	**0.007**	23.15	0.336	4.57	0.736	-2.05	0.806
Distance to nearest log	3.68	0.142	0.18	0.079	6.59	0.072	-0.78	0.701	1.35	0.297
T_a at hib[3] before emergence	-1.97	0.308								
Average winter T_a	-2.33	0.383	0.18	0.355	-3.28	0.539			-0.23	0.903
T_a before immergence							-0.88	0.059		
Minimum T_a before immergence[4]							-0.79	0.043		
Minimum T_b in January	1.46	0.632	0.07	0.405	6.33	0.131			0.37	0.793
Minimum T_b	5.24	0.162	0.09	0.553	7.22	0.151			-1.12	0.528
Average minimum T_b	-2.61	0.074	-0.05	0.528	-2.18	0.295			0.72	0.314
Date of lowest T_b after Nov	0.06	0.472	0.00	0.529	0.15	0.155			0.01	0.478
Date of lowest T_b after Nov*Sex	-0.10	0.571	-0.01	0.16	0.08	0.666			-0.05	0.384
Average minimum T_b*Sex	0.28	0.926	0.14	0.798	0.58	0.885			0.63	0.671
Day T_s increases in spring	0.29	0.175	0.00	0.583	0.15	0.690			0.10	0.428
Duration of snow pack	**0.36**	**<0.001**	0.00	0.824	0.38	0.013			0.03	0.631
Duration of snow pack*Sex	0.19	0.244	0.02	0.068	0.17	0.517			-0.17	0.219
Maximum snow depth	**0.31**	**0.003**	0.00	0.775	0.26	0.058			0.04	0.422
Maximum snow depth *Sex	0.40	0.045	0.01	0.062	0.07	0.783			-0.14	0.212

Snowmelt date	**0.50**	**<0.001**	0.00	0.874	0.49	0.019			0.05	0.538
Snowmelt date*Sex	0.10	0.458	0.03	0.083	0.20	0.547			-0.18	0.258
Immergence date	0.00	0.992	0.00	0.845	**-1.14**	**0.003**			-0.31	0.028
Longest torpor length (days)	0.56	0.406	0.03	0.325	-0.56	0.559			-0.48	0.138
Longest torpor*Sex	0.05	0.293	0.00	0.721	0.01	0.913			0.00	0.917
Hibernaculum depth	47.32	0.139			10.52	0.811			-9.32	0.526
Average torpor length (days)	0.81	0.523	0.03	0.377	-2.86	0.073			**-2.26**	**<0.001**
Duration heterothermal (days)			0.00	0.811					0.19	0.011
Duration heterothermal*Sex			0.03	0.203					-0.26	0.237
Number of euthermic periods	-0.03	0.959	-0.01	0.526	1.65	0.011				
Hrs aboveground pre-emergence							-2.01	0.062		
Hrs of light before immergence[5]							-6.81	0.017		
Avg euthermic period length (days)	-27.10	0.203			-24.47	0.385			-9.67	0.316
Total days euthermic			-0.01	0.82					**0.89**	**0.006**
Total days euthermic*Sex	-1.04	0.619	0.11	0.181	-0.68	0.720			-0.37	0.512

[1] T_a = Air temperature, T_b = Body temperature, T_s = Soil temperature

[2] Residual body mass represents the squirrel’s body mass (corrected for capture date) in the summer prior to hibernation.

[3] Hibernacula

[4] Logger was placed in the forest

[5] Logger was placed in open canopy

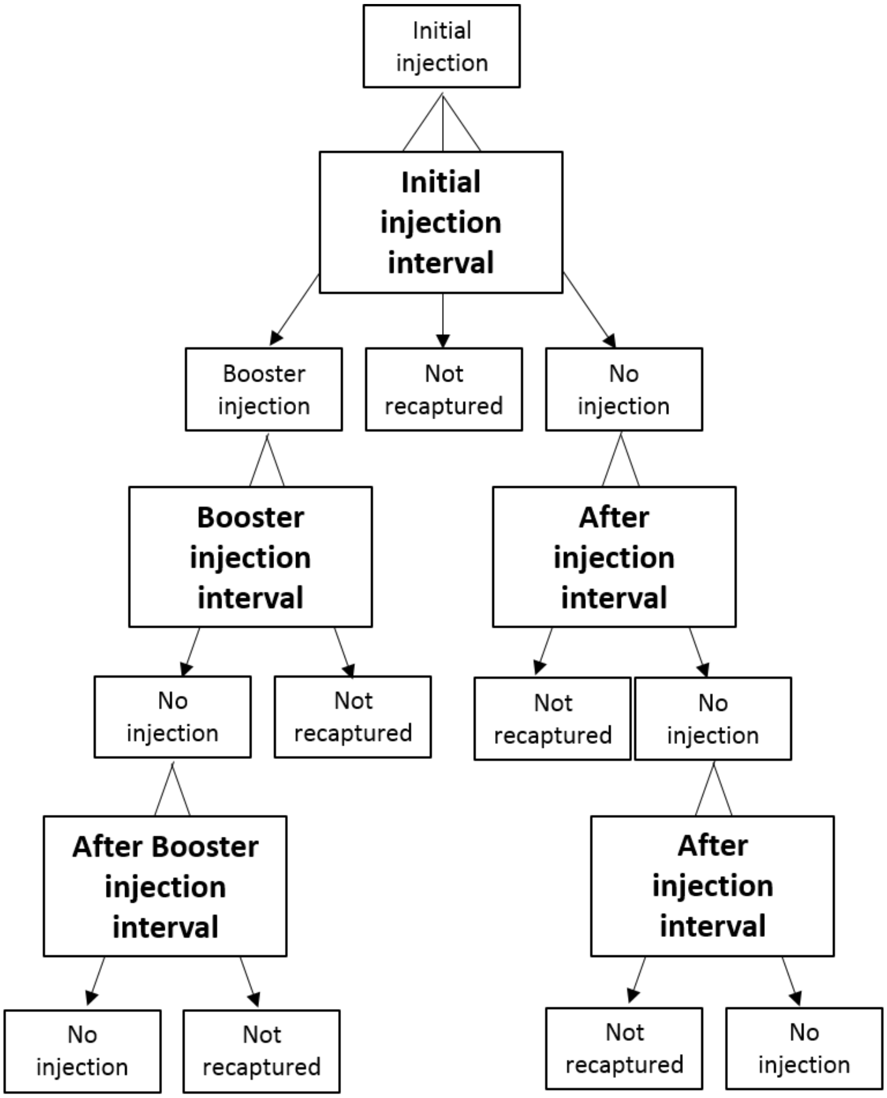


Figure S1. Diagram outlining names for vaccine treatment state. Animals upon first trapping session are given an initial shot (vaccine or placebo).