HABITAT SELECTION OF MEADOW VOLES AND DIET OF VAGRANT SHREWS ON A WET PRAIRIE ECOSYSTEM IN WEIPPE, IDAHO

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AUTHORIZATION TO SUBMIT THESIS

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ABSTRACT

Wetlands are among the most productive ecosystems in the world, providing a variety of functions including water purification and habitat for fish and wildlife. Despite this importance, their presence has declined drastically due to modifications such as agricultural conversion and urban development. Those areas that remain are often not pristine, but subject to deliberate or accidental modification consequent from altered land-use patterns, e.g. agricultural conversion. To prevent further decline of wetlands, many of these modified areas are undergoing restoration efforts. However, determination of the species present, and how these interact with their environment must be understood in order for an area to be successfully restored. Small mammals play important roles in wetland ecosystems, such as wet prairies; they exert predatory pressure on invertebrates and other small mammals, provide a vital prey base, and have significant influences on vegetation and soil. The purpose of this study was to analyze habitat selection of meadow voles and the diet of vagrant shrews on a wet prairie ecosystem in northern Idaho projected to undergo restoration work. The focus of planned restoration efforts is to increase the presence of small camas (*Camassia quamash*), a native, facultative wetland species. Our specific goal was to determine how restoration activities might influence these small mammal species, in order to inform management decisions.

Results from three years of occupancy modeling indicated that meadow voles preferred areas containing meadow foxtail (*Alopecuris pratensis*), a nonnative pasture grass that dominates the site. Conversely, our results show that meadow vole presence is negatively associated with small camas, a relationship not previously recognized. The DNA barcoding method used for diet analysis revealed the occurrence of direct or indirect consumption of

plants by vagrant shrews. This method has not previously been used on vagrant shrews and provided more precise identification of diet components. In conclusion, our study provides an expansion of what is known about small mammals in a wet prairie ecosystem.

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DEDICATION

To my dad – for instilling in me a love for nature and science.

To my mom- for always being a voice of reason and source of strength.

To my girls – your unconditional love carries me through each day.

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INTRODUCTION

Wetlands are among the most productive ecosystems in the world (Mitch and Gosselink 2007). Their presence and productivity rely on specific relationships between hydrology, soil, and vegetation. They provide a variety of functions and values to surrounding ecosystems, such as water purification, flood protection, groundwater recharge and habitat for fish and wildlife (SWDE 2014). Wetlands enhance water quality by removing sediments, and retaining excess nutrients and other pollutants. Sediments, nutrients and toxins (e.g., pesticides and fertilizers) enter primarily through runoff. Excess nutrients are removed and absorbed by plants or converted to less harmful forms in the soil, which protect waters from problems associated with nutrient overload. Toxic chemicals are also held in the soil. (SWDE 2014) Wetlands are important for various bird and mammal species that depend on them for food, water and shelter; especially during migration and breeding (USEPA 2001a). Up to one-half of North American bird species nest or feed in wetlands (USEPA 2001a). Plants and small animals (especially insects) that inhabit wetlands are essential links at the bottom of the food chain, supporting the larger animals that feed on them (SWDE 2014).

Despite providing these vitally important functions and values, over half of the original wetlands in the lower 48 states have been lost or greatly degraded either due to human actions or natural threats (USEPA 2013). In Idaho, approximately 386,000 acres or 56% of the state's wetlands were lost by 1980, and ninety percent of the low-elevation wetlands are gone (Murphy et al. 2012). There are various types of wetlands, including freshwater marshes, tidal (coastal) marshes, playas and wet prairies (USEPA 2001b). The wet prairie ecosystem throughout the Pacific Northwest has been drastically reduced due to urbanization, agricultural conversion, irrigation and flood control development (Dahl 1990,

Rodhouse and Stucki 2013). Many wet prairies that remain are often structurally altered and are host to [many] non-native species.

Small mammals play important roles in wetland ecosystems, such as wet prairies; they exert predatory pressure on invertebrates and other small mammals, provide a vital prey base, and have significant influences on vegetation and soil (Sieg 1987). Effects on vegetation include alteration of primary productivity, species composition, and decomposition rates of plant materials. Small mammals also alter plant community composition and species distribution through their consumption and caching of seeds. In fact, some species may be dependent on small mammals for their seed and/or spore dispersal. Burrowing activities may influence the rate of plant succession; as burrowing tunnels provides bare soil for invasion of lower successional plants. (Sieg 1987)

Many predators including carnivorous and avian species feed on small mammals, whose population cycles exert significant influences on their life histories (Sieg 1987). Short-tailed weasels feed mostly on small rodents (EN 2015). Short-eared owls feed primarily on voles and shrews (PF 2014). Raptors also feed on small mammals and frequently establish in areas where *Microtus* is abundant (Tamarin 1985). For example, in the northern portion of Northern Harrier's range, voles (*Microtus* sp.) comprise the majority of their diet (HWR 2014). Lastly, small mammals influence other animals and arthropods by modifying their environment such that it provides habitat for other species; for example, burrows often provide nest sites and shelter for invertebrates and other vertebrates (Ryskowski 1975).

Weippe Prairie (WEPR), located in North Idaho, is representative of many of the issues discussed above. It was altered for agricultural purposes (such as haying and grazing) until the National Park Service purchased the property in 2003. Currently, non-native pasture

grasses dominate much of the site, and few wetland areas remain. The loss and degradation of sites like the Weippe Prairie has increased awareness of the valuable functions of wetland ecosystems, such as providing vital habitat for numerous wildlife species (Taft and Haig 2003). Given the apparent importance of small mammals in this ecosystem; the goal of this study was to assess the relationship between the small mammal communities and environmental variables within the wet prairie ecosystem on the WEPR. Nez Perce National Historical Park (hereafter NEPE) manages this site, and is interested in improving conditions for camas (*Camassia quamash*) and ecosystem function, as well as trying to reduce cover of the drier non-native pasture grasses. Because little is known about the small mammal community at this site and how this community might change as a result of restoration activities, this thesis contains two research chapters with two different objectives. In chapter 1, my objective was to determine the factors that influence Meadow vole (*Microtus pennsylvanicus*) distributions across the site by producing a habitat selection model. This model will enable managers to 1) predict presence or absence of these species at varying locations across the site and 2) predict how proposed management activities might influence these communities. This was done by creating presence/absence models using the R package 'unmarked' (Fiske & Chandler, 2011; R Core Team, 2013), and mapping predicted occurrence using ArcMap 10.3. In chapter 2, my objective was to 1) determine the diet of shrews to further understand their ecological role on the WEPR and 2) identify plant species that are contributing to the food web. These objectives were met using NGS and DNA barcoding methods. This study is important because it will inform NEPE management decisions on the WEPR, and can be used to address scientific issues that occur on similar wet prairie ecosystems. For instance, it can determine environmental factors influencing species

distributions and thus enable further understanding of their ecology. It can also provide insight into diet components and the species contributing to the food web within a system. Chapter 3 will provide a summary of my findings in addition to recommendations for future research directions.

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CHAPTER 1

THE INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON OCCUPANCY OF MEADOW VOLES (*MICROTUS PENNYSLVANICUS*) IN A WET PRAIRIE ECOSYSTEM

Abstract

Meadow voles (*Microtus pennsylvanicus*) can function as ecosystem engineers through their effects on vegetation composition and structure. They influence nutrient cycling and decomposition rates within their habitats, and serve as a prey item for many predators. Given their ecological importance in these meadow systems, distribution and abundance patterns of voles have often been used as a way to evaluate ecological conditions and effects of restoration on meadows. Studies analyzing habitat selection can address management and conservation concerns for small mammal populations and their preferred habitats. Typical approaches involve surveys estimating abundance, often utilizing mark-recapture methods, yet these are labor intensive. Alternatively, a more cost effect approach is through collection of detection/non-detection data to be used in occupancy modelling. Although occupancy is less informative than abundance, it is strongly and monotonically correlated with abundance and, as a truncated abundance metric (i.e. counts >1 = 1, else 0), does provide a way to measure variation in distribution. We used occupancy models and an information-theoretic approach to model meadow vole occupancy on a modified wet prairie in northern Idaho. Occupancy was modelled as a function of vegetation characteristics, distance to water, elevation and temperature. These covariates were chosen because we considered them most closely associated with wet meadow habitats and restoration activities. Meadow voles were

positively associated with the non-native pasture grass, meadow foxtail (*Alopecurus pratensis*) on our site. Conversely, meadow voles were negatively associated with small camas (*Camassia quamash*) and camas density, a native facultative wetland plant that is both culturally and ecologically significant. From these findings, we predict that continued effort towards increasing camas populations across the site may have a negative impact on meadow voles. Therefore, we suggest that management focus restoration efforts on wetter areas where grasses are not dominant (or not present). In this way, grasses can persist on the landscape and continue to provide benefits to small mammal species. Overall, our study provides insight into habitat selection of meadow voles on a wet prairie ecosystem, where management decisions are aimed at restoring these landscapes.

Introduction

Voles (*Microtus* spp.) are often abundant in grassland and wet meadow habitats, and can function as "ecosystem engineers" (Jones et al. 1994; 1997) through their effects on vegetation composition and structure (Huntzinger et al. 2011). Plant successional rates can be altered through selective herbivory of grasses, sedges, and forbs by voles, and up to 80% of the total annual primary plant production might be consumed by voles (Sieg 1987). In addition to influencing plant composition and production via herbivory, voles play an important role in nutrient cycling. Decomposition rates are influenced through reduction of plant matter to more easily decomposable form, and vole fecal deposition (Sieg 1987). Their burrowing and deposition of excrement affects the chemical composition of soils through changes in nutrient and mineral distribution, particularly via an increase in the level of

available nitrogen (Sieg 1987; Sirotnak & Huntly 2000). This increase in nutrient content of the soil may facilitate plant succession and increase survival of new plants (Ryskowski 1975).

In addition to their role as ecosystem engineers, voles are an important prey item for many avian and mammalian carnivores, and are consequently important in predator life histories (Sullivan 1996). For example, coyotes (*Canis latrans*), Red foxes (*Vulpes vulpes*) and short-tailed weasels (*Mustela erminea*) feed on small rodents (EN 2015). Owl species, such as short-eared owls (*Asio flammeus*), feed mostly on small mammals such as voles (ORI 2013). Almost all species of raptors take voles as prey and frequently establish in areas where voles are abundant (Tamarin 1985). For example, in the northern portion of Northern Harrier's (*Circus cyaneus*) range, meadow voles comprise the majority of their diet (HWR 2014).

Meadow voles (*Microtus pennsylvanicus*) are a common throughout N. America (Reich 1981), and their distributions are influenced by a variety of factors. Generally, meadow voles are found in moist grasslands, and previous studies have found meadow vole distribution to be largely determined by the relative ground cover of grasses and forbs (Birney et al. 1976); the temperature, moisture, and nutrient level of soils (Wrigley 1974); humidity (Getz 1971); and interspecific competition (Schramm & Clover 1994). They occur in areas with dense graminoid vegetation, substantial amounts of plant litter, and high soil moisture (Grant 1971; Tamarin 1985; Snyder 1988). They are often more abundant in wet meadow habitats due to increased moisture, which supports greater abundance and diversity of plants and insects, as well as soil conditions more suitable for burrowing (Sullivan 1996; Laubhan 2004).

Although studies cited above have identified general associations between meadow vole occupancy and vegetation communities, other more specific factors might influence occupancy. For example, in the Pacific Northwest, small camas (*Camassia quamash*) is a facultative forb species associated with seasonally inundated wet prairies (Gould 1942; Rodhouse et al. 2011). This plant is an indicator of the once extensive wet prairie ecosystems that dominated large areas of the region. Meadow voles are often the most abundant small mammals in these northern wetland camas prairies (Sullivan 1996). Because vegetation structure and composition are significant determinants of habitat occupancy (Morrison et al. 1998), the presence of camas in these areas preferred by meadow voles is likely to be a factor influencing their occupancy. Furthermore, it has been found that meadow voles are associated with moist areas across their range (Getz 1971; Reich 1981; Sullivan 1996) and that vole species are poorly adapted for conserving water (Tamarin 1985). However, to date, no studies have been conducted to determine if meadow vole presence is correlated with distance to major water sources, especially at fine site-level scales of specific meadow study and restoration sites. Addressing these fine-scale distribution patterns is important because it is at this scale (grain size) that protected-area management and restoration decisions are made. Too often managers are unwilling or unable to explicitly integrate scientific evidence into these local decisions (Cook et al. 2009; 2011) in part because of this misalignment in scale and subsequent (ir)relevancy of the contributed science.

Weippe Prairie (WEPR), located in Weippe, Idaho is an example of a wet meadow habitat where meadow voles are likely prevalent. The goal of planned restoration activities is to increase camas densities, and it is unknown how (or if) these modifications will impact the small mammal communities present on the WEPR. Habitat selection studies can offer an

alternative approach for addressing management and conservation concerns for small mammal populations and their preferred habitats e.g. Rodhouse et al. (2010). Knowledge of habitat selection strategies of wet prairie species such as meadow voles can help managers make informed decisions about restoration, particularly in light of the role that this species might play relative to plant succession, predator-prey relationships, and nutrient cycling. Meadow voles as dominant herbivores can at once be drivers of positive feedback loops on plant succession and weed invasion via facilitation and of negative feedback loops via predation. Understanding the fine-scale habitat associations is an important first step in anticipating if and whether vole populations will thwart or enhance restoration goals.

Our objective was to characterize resource relationships for meadow voles on the WEPR to inform management decisions. We used occupancy models to describe habitat selection across the landscape. It has been suggested that when using models to estimate species-habitat relationships, spatiotemporal variability in these factors must be accounted for, and should [ideally] reflect the unique community characteristics and environmental conditions in which management decisions are to be made (Rodhouse et al. 2010). Therefore, we evaluated hypotheses that took into consideration a subset of variables we considered to be most closely associated with the proposed restoration activities. Based on previous literature, our general hypothesis was that the most influential predictors of vole occupancy would include vegetation variables. We expected meadow voles to select habitat that incorporates plant species that provided maximal cover, i.e. protection from predators, and that vole presence would be negatively correlated with areas low in the amount of thatch. Because meadow voles prefer moist meadows and wet grassy areas near water (Pearson 1999), we further hypothesized that voles would occur closer to water. Overall, our goal was to

determine the environmental factors that affect meadow vole distributions across the site in order to predict how restoration activities, particularly focused on camas, might affect vole occupancy of WEPR.

Methods

Study Area

The study was conducted on the Weippe Prairie (WEPR), a National Historic Landmark managed by Nez Perce National Historical Park (NEPE). WEPR is a modified mountain meadow, located in the southern tip of Clearwater County, Idaho near Paunch Mountain. WEPR is approximately 2.4 km south of Weippe, Idaho (46°21'16.04" N, 115°55'17.66" W) and encompasses 111 ha (Fig. 1). Elevation ranges from 922 m in the southwest corner to 917 m in the north. This site receives approximately 106 cm of average annual precipitation excluding snowfall, as well as 300 cm of average annual snowfall (WRCC 2015). Average summer high temperatures reach 27˚C, and average winter high temperatures reach 2˚C (WRCC 2015). Small ponds and manufactured canals occur across the site. The northern section includes an approximately 1.6 km stretch of the perennial Jim Ford Creek (flowing southeast to northwest).

WEPR is part of the Palouse Grasslands of the shortgrass prairie ecoregion (Erixson & Cogan 2012). Due to the gentle slope of the site, mesic upland meadows and riparian shrubland streambanks occur along Jim Ford Creek. Agricultural settlement of the area in the late 19th century brought nonnative grass species to the site, including creeping bentgrass (*Agrostis stolonifera*), timothy (*Phleum pretense*), meadow foxtail (*Alopecurus pratensis*), and smooth brome (*Bromus inermis*). These nonnative grasses are scattered among various patches of native plants such as small camas (*Camassia quamash*), plantainleaf buttercup

(*Ranunculus alismifolius*), tufted hairgrass (*Deschampsia cespitosa*), and California oatgrass (*Danthonia californica*). The site was privately owned until 2003, and was used for agricultural crop production and livestock grazing until 2008. The major land use surrounding the WEPR is agricultural, and the fields are dominated by a mixture of agricultural plants, nonnative pasture grasses and native herbaceous plants. Current management on the WEPR is aimed towards restoring camas populations, as this species is the focal resource for managers of the site.

Capture Methods

For the purpose of this study, we established ten, 100 x 100 m sampling units (1 ha) across the study site (Fig. 1). The placement of plots was based on three environmental variables; distance to water, elevation, and camas density. Distance to water was classified as 1) near (<50 m) a permanent water source and 2) distant (>50 m) from a permanent water source. Elevation (m) was classified as low (913.5 - 916.4), medium (916.4 – 919.3), and high (919.3 – 922.2). Camas density (plants/m^2) was classified as low-mid density (0.33 – 27.75), and mid-high density (27.75 – 107.5), based on 2012 Camas monitoring data (see *Explanatory Variables* below). This was chosen as a variable because camas is the focal resource at the site, and future management actions regarding camas restoration should consider whether camas densities are likely to influence vole populations, or conversely, whether vole consumption of camas will affect success of camas explants. Upon placement of the plots, it was ensured that they all encompassed a combination of these variables.

Inside each plot, we established 11, 100-m transects running horizontally through the unit. We placed folding Sherman live traps (8 x 9 x 23 cm; H.B. Sherman Traps, Inc., Tallahassee, Florida) every 10 m along each transect (except at the 0-m or 100-m intervals on

each end of the transect) for a total of 99 traps inside each sampling unit. Traps were pre-baited (with oats and sunflower seeds), locked open for 7 days, and set to trap for three consecutive nights. We assumed pre-baiting would reduce some of the site-use bias inherent in a luring program by allowing animals to acclimatize to the presence of the new food supply. We baited with oats and sunflower seeds rolled in peanut butter on trapping nights. Traps were set in the evening, checked after dawn, and closed during the day. Captured animals were identified quickly to species, marked, and released. We used the binary pattern of species detections from captures to construct detection history matrices for each 3-night trapping session for subsequent occupancy modeling. All capture and handling procedures were approved by the University of Idaho Animal Care and Use Committee (protocol 2013-95) and followed guidelines approved by the American Society of Mammalogists as outlined by Sikes et al. (2011).

We trapped small mammals during the periods 7 July – 23 July 2013; 10 July – 26 July 2014; 13 July – 27 July 2015 (sessions 1, 2 and 3, respectively). Five sampling units were sampled for one week per sampling session, with a week of pre-baiting in between. We assumed that sufficient time existed between each of the 3 trapping sessions to allow for changes in site use. To prevent misidentification of target species in the field, we followed keys provided by Nagorsen (1996) and Verts and Carraway (1998). A small subset of voucher specimens were also collected to confirm species identification.

Explanatory Variables

We compiled five environmental metrics describing vegetation, hydrology, and topography, which included camas density, vegetation type, thatch depth, distance to water, and elevation (Table 1.1; 1.2). We selected our subset of variables due to their potential

ecological importance and correlation with restoration. Three of the five descriptors were derived from other research studies conducted on the WEPR. The elevation at each trap location was extracted from a light detection and ranging (LiDAR) derived digital elevation model at 1 m resolution, generated by Terrapoint USA Inc. (Woodlands Texas, unpublished report) in 2008. This high-resolution DEM provided unusually (high-resolution) information about meadow microtopography and swales shown to be highly predictive of patterns of camas density (Rodhouse et al. 2011). Camas density itself as an explanatory variable was extracted from interpolated density maps of camas across the WEPR (from 2013 to 2015), created by the Upper Columbia Basin Network (UCBN) using ordinary kriging (Rodhouse et al. 2007). Camas count data used to create these density values were acquired from the UCBN's camas monitoring program, which is conducted annually on the WEPR. This monitoring follows sampling methods detailed by Rodhouse et al. (2007), where camas plant density and flowering stem density are measured in a random sample of 4 m x 15 cm ($0.6m^2$) quadrats within a sampling frame that captures the entire WEPR camas population. Vegetation data was obtained from an inventory conducted by Erixson and Cogan (2012) across NEPE's park sites. This study involved classifying the vegetation on the landscape into plant associations and vegetation alliances using the National Vegetation Classification System (NVCS). Data was collected by establishing three to five classification plots in every vegetation alliance/plant association. Physiognomic class, leaf phenology, and type of dominant stratum were recorded in each plot (see Erixson & Cogan 2012 for more information). On the Weippe Prairie, vegetation was grouped into 6 classes (Table 1.2) based on the dominant species; 1) Meadow foxtail (ALPR, *Alopecurus pratensis*); 2) Kentucky bluegrass (POPR, *Poa pratensis*) and/or Quack grass (ELRE, *Elymus repens*); 3) Camas

(CAQU, *Camassia quamash*); 4) Smooth brome (BRIN, *Bromus inermis*); 5) Reed canary grass (PHAR, *Phalaris arundinacea*); 6) Plantainleaf buttercup (RAAL, *Ranunculus alismifolius*). We expected meadow voles to be positively associated with vegetation classified as graminoid species because their optimal habitat consists of moist, dense grassland with substantial amounts of plant litter (Sullivan 1996). Additionally, we hypothesized meadow voles to be negatively associated with camas density because high-density areas provide sparse (to no) graminoid cover.

Data for the other three descriptors were also collected during this study. Thatch depth was calculated by averaging measurements taken 20 cm to the N, S, E and W of each trap location, across all plots in 2013 and 2014. Ordinary kriging (Fortin and Dale 2009) was then used to produce an interpolated thatch density map across the WEPR. We hypothesized meadow vole occupancy would be positively associated with thatch depth; they are a litter dwelling Microtine and thicker thatch generally provides more protection from predators (Grant 1982). Distance to major water sources (i.e. creek or canals) from each trap was determined using the Near function in ArcMap 10.3 (ESRI, Redlands, CA, USA). Moisture and access to water have been implicated as determinants of vole habitat use (Sullivan 1996). We therefore predicted that meadow voles would prefer habitat closer to water.

Finally, estimates of habitat selection can be biased by detection error, particularly when cryptic species such as small mammals are involved (Rodhouse et al. 2010; MacKenzie et al. 2002). Although detection indicates that the species is present, non-detection does not equate to species absence (Mackenzie et al. 2003). To account for this, we included temperature as a potential effect on the probability of detection because meadow vole activity decreases when temperatures drop below 0°C (32°F; Reich 1981). Nightly temperature values

(°F) during each trapping session were obtained from The Climate Analyzer (http://www.climateanalyzer.org), where the closest weather station is located in Pierce, ID (approximately 18.67 km away).

Statistical Modeling

Prior to model development, we assessed correlation between our explanatory variables using the 'cor' function in R (R Core Team 2013). Results indicated that our variables were not correlated ($r < 0.75$), thus all were kept for building our meadow vole occupancy models. We implemented the approach of MacKenzie et al. (2002; 2006) to build our occupancy models, using the R package 'unmarked' (R Core Team 2013; Fiske & Chandler 2011). This approach allow us to maximize the likelihood of occupancy (ψ), while accounting for imperfect detection (p), given detection histories of the units surveyed.

We built null, subset and global models, and our subset and global models reflected our hypotheses about the influence of vegetation variables, hydrology and topography on occupancy of meadow voles. Because trapping was conducted across three years (2013-2015), we included an effect of trapping year in all models to account for an additive difference in occupancy among years. Modeled parameters were estimated using maximum likelihood, and we evaluated the influence of our standardized covariates on ψ using the logit link function. We standardized our covariates to assist with likelihood convergence. We assessed an effect of year on detection probability, but in all instances it was less supported relative to models with year effect on occupancy (based on Akaike's information criterion); thus we excluded a year effect on detection. We modeled detection probability assuming it was less than one, and constant among sites, surveys and years.

We evaluated our competing models using Akaike's information criterion adjusted for small sample size (AICc; Burnham & Anderson 2002) using the R package 'AICcmodavg' (Mazerolle 2014). The model with the smallest AIC_c is most supported, however, when $\Delta AIC_c < 2$ multiple models are supported by the data (Burnham & Anderson 2002). Because multiple models were supported in our model set (see Results), we display model averaged parameter estimates. Model averaged parameter estimates are simply a sum of the product of parameter estimates and their associated model weight across all models (Holbrook et al. 2015). Following the suggestions of MacKenzie and Bailey (2004), we evaluated fit of our global model using a parametric bootstrap approach where we compared our observed chi-squared statistic (X^2_O) to our bootstrapped value (X^2_B) to determine the probability of observing a larger value. We implemented this test using the 'AICcmodavg' package in R (Mazerolle 2014; R Core Team 2013), and used 1,000 bootstrapped samples. We considered p-values > 0.05, and overdispersion parameter values $\hat{c} = 1.0$, to indicate adequate model fit (MacKenzie & Bailey 2004).

Results

Trapping efforts resulted in the capture of 197 meadow voles in 2013, 121 in 2014, and 138 in 2015. Four other species were also caught across the 3 trapping sessions; vagrant shrew (*Sorex vagrans*), bushy-tailed woodrat (*Neotoma cinerea*), short-tailed weasel (*Mustela erminea*), and a mouse (*Peromyscus* spp.) Goodness-of-fit statistics suggested appropriate fit for our global model ($p = 0.29$, $\hat{c} = 1.21$). Of the eight models analyzed, three exhibited a $\Delta AIC_c < 2$ and all contained vegetation (camas density, vegetation type and thatch depth) (Table 1.4). The top model contained the year effect as well as effects of hydrology and vegetation on occupancy (Vegetation + Hydrology model). The second ranked model was the

global model, which included the effect of all variables on occupancy. The third ranked model included an effect of year and all effects of vegetation (Vegetation model). Model-averaged estimates of all vegetation types show that vole occupancy was positively associated with meadow foxtail, and negatively associated with smooth brome, Kentucky bluegrass, camas, reed canary grass and buttercup (Table 1.5). Odds ratios (Table 1.6) indicated the probability of meadow vole occupancy declined by approximately 90% in areas of smooth brome and by approximately 63% in areas of camas, when compared with areas containing meadow foxtail. Vole occupancy was also negatively associated with camas and distance to water, and year was an important variable influencing occupancy (Table 1.5).

Discussion

Understanding the factors that influence occupancy of ecosystem engineers is vital for assessing the current and future conditions of an ecosystem, and is critical for ecosystem conservation and management in this wet meadow system (Jones et al. 1994). The addition of year to our models was informative and differences in the effect on occupancy were strongly supported by our data. Our finding that meadow vole occupancy was positively associated with the first year of our study, 2013 (Year A) is consistent with other studies that have found meadow vole populations fluctuate annually, and tend to reach peak densities at 2 to 5 year intervals (Sullivan 1996; Reich 1981). Our results were mostly consistent with our expectations concerning the effect of vegetation on meadow vole occupancy. We first predicted that voles would be positively associated with graminoid vegetation. Our results generally support this prediction; the apparent selection for meadow foxtail may be due to its relative abundance across the site (Table 1.3). Alternatively, it is possible that meadow foxtail is preferred forage. Meadow foxtail is a non-native grass, and studies conducted by Howe

(2002; 2006) found that non-native pasture grasses in modified wet prairies are more palatable to voles than native prairie plants.

Second, we hypothesized that thatch depth would be positively associated with meadow vole occupancy because thicker thatch provides more protection from predators. In this study, thatch depth was not an important predictor of vole occupancy. Austin and Pyle (2004) found no significant effect of litter amount (thatch) on capture rates of meadow voles. Conversely, Snyder (1988) found that meadow voles were positively associated with litter and vegetation. Determining the relevant spatial scale important to animals is difficult (Levin 1992) and it is possible that we did not assess this characteristic at spatial scales that were relevant to meadow voles at this site. Thus, we suggest that future studies evaluating the effect of this variable, take measurements at the scale of the sampling plot.

Our results were consistent with our expectations concerning the effect of hydrology and topography on meadow vole occupancy. We predicted that as the distance to water increased, occupancy would decrease because of meadow vole preference for moist areas with free water (Sullivan 1996; Pearson 1999). Our results generally supported this prediction. Previous studies have found that *Microtus* is poorly adapted at conserving water (Tamarin 1985), and that their distributions may be limited by availability of free water throughout the summer (Hoffman 1960). Although elevation does not vary greatly (915.5 to 918.7 m) across trap locations, slight changes in elevation on the WEPR may result in changes in the amount of standing water and thus changes in soil moisture. Rodhouse et al. (2011) found a strong negative covariation of camas density with this high-resolution measure of site elevation (microtopography), suggesting that this does indeed reflect swales and soil moisture. Studies have found that meadow voles are most commonly associated with sites having high soil

moisture (Reich 1981; Sullivan 1996). It is plausible that the association of this variable with other factors (such as camas density and distance to water) lead to its support by the data. Overall, our results support the findings of previous studies that meadow voles are most associated with grasslands, preferring moister areas (Tamrin 1985; Sullivan 1996; Slane 2001; Howe 2006).

We predicted that camas density would be negatively associated with meadow vole occupancy because of the lack of graminoid cover and thus protection from predators. Our results supported this prediction. Our finding that meadow voles are negatively associated with areas classified as the vegetation type of camas further supports this. Currently no other studies have addressed this relationship. Therefore, this finding is significant to management activities that involve restoration of camas in other wet prairie ecosystems. Camas is both a culturally and ecologically significant plant. Camas was historically one of the most widely utilized indigenous foods in the Pacific Northwest, and played a key role in the society of the Nez Perce people (MNH 2010). This plant is also an ecologically significant wetland indicator species, strongly associated with the wet prairie ecosystems of the Pacific Northwest (Rodhouse & Stucki 2013). Much of this ecosystem type has been greatly degraded or lost due to agricultural conversion and other land use practices (Taft & Haig 2003; Dahl 1990). The areas that remain are often highly productive ecosystems and exhibit potential for restoration (Taft & Haig 2003; Rodhouse & Stucki 2013). Restoration of wet prairie habitat and conservation of camas populations is essential for preservation of the cultural practices of indigenous cultures (especially the Nez Perce), and the continued functionality of camas as a wetland indicator species. Therefore, restoration objectives are likely to focus on modifications that will facilitate the continued presence of this species. This is the goal of

restoration activities currently planned for the WEPR, as this site was a traditional Nez Perce gathering area for camas (Jason Lyon, National Park Service, personal communication).

This study adds to the literature addressing meadow vole responses to a variety of environmental variables. Although this study was conducted on a culturally important site, the findings have broad implications on other wet prairie ecosystems as well. Our finding of a negative association between meadow vole presence and camas density has implications for other restoration projects occurring where these species coexist. Our results also provide insight into the relationship of meadow voles with the non-native pasture grass meadow foxtail, which is often present on [modified] wet prairies (USDA, NRCS, 2015). The positive association between these species should be considered when restoration objectives include removal of non-native grasses. Additionally, meadow voles have been found to be a link between many primary producers and consumers (Ryszkowski 1975), and have both direct and indirect effects on other species in their environment (Huntzinger et al. 2011). Our study did not address demographic responses (survival and/or reproduction), but our results (in conjunction with other studies) can provide better understanding of the interactions of plants and animals in a wet prairie ecosystem.

Conclusion

The highly significant positive association we discovered between meadow vole occupancy and meadow foxtail is important to consider when planning for restoration. Because of this significance, we suggest maintaining areas currently dominated by meadow foxtail, even though this species is non-native. Although we did not analyze the particulars of this relationship, we predict that voles rely on this species for food as well as for shelter because of its dominance across the landscape. Although dominant, the occurrence of this

species does not strongly overlap with camas on the WEPR as camas prefers inundated swales. Therefore, managers should not consider meadow foxtail a detriment to present and future camas growth, or a negative influence on restoration efforts aimed at increasing camas populations. Furthermore, we believe efforts aimed at removing or greatly reducing this species have the potential to be unsuccessful. Rodents are known to alter grassland communities through seed predation and herbivory of seedlings and juvenile plants (Howe & Lane 2004). Meadow voles selectively forage on emerging vegetation and are significant grazers influencing species composition within their ecosystem (Howe 2002).

We further predict that an increase in camas will result in a negative impact on meadow voles. This is because of the significant negative relationship we found between vole occupancy and camas density. If meadow voles decrease in number and extent, it is likely that other species such as red foxes, short-eared owls and northern harriers present on the site will also decline, as meadow voles are a large portion of their diet in northern prairie wetlands (Sullivan 1996; PF 2014). This could result in an overall reduction in species diversity and a disruption to the ecosystem. In conclusion, because camas is the focus of restoration efforts on the WEPR, we propose that managers target wetter, low-elevation sites where grasses are not dominant (or not present) and camas already persists. We believe this approach will be most successful in maintaining small mammal communities. As restoration work progresses, managers should continue to monitor voles, and other small mammal species present by collecting presence/absence data. This continued monitoring will enable adaptive management of the WEPR.

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Table 1.1 Description of metrics used as explanatory variables for modeling occupancy of meadow voles (*Microtus pennsylvanicus*, Ord, 1815) during 2013–2015 at Weippe Prairie, Weippe, Idaho, USA. The ecological hypotheses associated with each variable are included.

Category	Variable (code - units)	Description	Hypothesis
Vegetation	Vegetation type (0.1 ha)	Data was obtained from Erixson & Cogan (2012) who classified the vegetation on the WEPR into plant associations using the NVCS. Data was collected in 2011-2012. See Table 1.2 for more detailed information.	Positive association with vegetation classified as graminoid species because their optimal habitat has been found to consist of moist, dense grassland with substantial amounts of plant litter.
	Camas density (plants/m^2)	Obtained from the Upper Columbia Basin Network (UCBN), who used ordinary kriging to produce an interpolated density map of camas across the site (Rodhouse et al. 2007). Camas count data was collected during the annual camas monitoring program.	Negative association with increasing camas density because these areas provide little (to no) graminoid cover.
	Thatch depth (cm)	Measurements were taken 20 cm to the N, S, E and W of each trap, across all plots, in 2013 and 2014. Ordinary kriging (Fortin and Dale 2009) was then used to produce an interpolated thatch density map across the site.	Positive association with increasing thatch depth because they are litter dwelling Microtines and thicker thatch can provide more protection from predators.
Hydrology	Distance to water (m)	Distance to nearest permanent water source from each trap, determined using the Near function in ArcGIS 10.3.	Negative association with increasing distance to water because moisture has been found to be a major aspect in habitat use.
Topography	DEM (m)	LiDAR derived digital elevation model (at 1 m resolution)	Negative association with increasing elevation because higher sites are further from water and have low soil moisture.

Table 1.2 Description of each vegetation type used as a (categorical) explanatory variable for modeling occupancy of meadow voles during 2013–2015 at Weippe Prairie, Weippe, Idaho, USA. The proportion of occurrences for each vegetation class across all trap sessions and the relative availability of each vegetation class across the entire Weippe site (shown as a percentage of the total acreage) are both shown. For most of the vegetation classes, their percentage of occurrence coincides with the actual percentage present across the study area.

Vegetation Class	Description	Proportion of Occurrence	Overall Occurrence
ALPR, meadow foxtail (*Alopecurus pratensis*)	Clearly dominates the plant community; other species may be present but do not contribute substantial cover	65%	52%
POPR-ELRE, Kentucky bluegrass (*Poa pratensis*), and/or quack grass *(Elymus repens*)	Dominates the plant community; ALPR ranges from absent to abundant (but does not dominate)	1.6%	7.4%
CAQU, camas (*Camassia quamash*)	Dominates the plant community; ALPR ranges from sparse to abundant (does not co-dominate)	25%	17%
BRIN, smooth brome (*Bromus inermis*)	Dominates the community; ALPR ranges from absent to abundant (does not dominate), POPR may range from sparse to abundant	2.1%	14%
PHAR, Reed canary grass (*Phalaris arundinacea*)	Dominates the community	1.8%	0.7%
RAAL, plantainleaf buttercup (*Ranunculus alismifolius*)	Dominates the community	5.1%	4.4%

Table 1.3 Mean (and range) of explanatory variables (across all three years) used in modeling occupancy of meadow voles during 2013–2015 at Weippe Prairie, Weippe, Idaho, USA. The vegetation type variable was not included on this table because it is categorical, see Table 1.2 for details.

Variable	Mean	Lower Range	Upper Range
Camas density (plants/m2)	21.807	0.00	122.928
Thatch depth (cm)	1.635	0.00	4.695
Distance to water (m)	46.65	0.00	143.34
Elevation (m)	916.6	915.5	918.7

Table 1.4 Hypotheses and model selection results for models assessing occupancy of meadow voles during 2013–2015 at Weippe Prairie, Weippe, Idaho, USA. The number of estimated parameters and Akaike weights for each model are indicated by *K* and w_i, respectively.

Hypothesis	Model	*K*	AIC_c	ΔAIC_c	w_i
Vegetation + Hydrology	ψ(Year+Camas+Veg+Thatch+Water), *p*(temp+shrew)	14	3388.18	0.00	0.43
Vegetation + Hydrology + Topography (global)	ψ(Year+Camas+Veg+Thatch+Water+DEM), *p*(temp+shrew)	15	3388.75	0.57	0.33
Vegetation	ψ(Year+Camas+Veg+Thatch), *p*(temp+shrew)	13	3390.09	1.92	0.17
Vegetation + Topography	ψ(Year+Camas+Veg+Thatch+DEM), *p*(temp+shrew)	14	3391.65	3.48	0.076
Topography	ψ(Year+DEM), *p*(temp+shrew)	7	3430.82	42.65	$2.4e^{-10}$
Hydrology + Topography	ψ(Year+Water+DEM), *p*(temp+shrew)	8	3432.04	43.86	$1.3e^{-10}$
Hydrology	ψ(Year+Water), *p*(temp+shrew)	7	3437.71	49.53	$7.4e^{-12}$
Null	ψ(.), *p*(.)	2	3524.49	136.31	$1.1e^{-30}$

Table 1.5 Model averaged parameter estimates (standardized) describing occupancy of meadow voles during 2013–2015 at Weippe Prairie, Weippe, Idaho, USA. Variable descriptions are as follows: Year – categorical variable (YearA=2013, YearB=2014, YearC=2015); VegXXXX– dominant vegetation type (VegALPR serves as master categorical variable); Camas – number of camas plants/m^2 (density); Thatch – depth of thatch layer (cm/cm^2); Water – distance (m) to nearest water source (stream or canal); DEM – elevation (m); temp – mean nightly temperature (°F).

	Standardized coefficient	*SE*	95% CI
Intercept	-0.27	0.19	-0.67, 0.08
YearB	-0.67	0.2	-1.05, -0.28*
YearC	-0.51	0.18	-0.86, -0.17*
VegBRIN	-2.29	1.06	-4.38, -0.21*
VegCAQU	-0.55	0.21	-0.95, -0.14*
VegPHAR	-0.32	0.64	-1.58, 0.94
VegPOPR	-0.97	0.61	-2.16, 0.23
VegRAAL	-0.29	0.34	-0.96, 0.35
Camas Density	-0.41	0.09	-0.59, -0.23*
Thatch Depth	0.02	0.08	-0.12, 0.17
Distance to Water	-0.16	0.08	-0.31, 0
DEM	-0.12	0.09	-0.3, 0.06
Temperature	-0.01	0.07	-0.14, 0.12

*95% CI does not overlap 0

Table 1.6 Calculated probability of occurence for categorical variables (vegetation type and year) used for assessing occupancy of meadow voles during 2013-2015 at Weippe Prairie, Weippe, Idaho, USA. Meadow foxtail (ALPR, *Alopecuris pratensis*) was used as the master categorical variable for the vegetation type covariate, and 2013 (Year A) was used as the master categorical variable for the year covariate, so probability of occurrence is compared to these parameters. The parameters that are starred were those found to have a 95% Confidence Interval not overlapping zero.

Parameter	Estimate	Probability of Occurence
VegBRIN (Smooth Brome)*	-2.29	9%
VegPOPR (Timothy)	-0.97	27%
VegCAQU (Camas)*	-0.55	37%
VegPHAR (Reed canary grass)	-0.32	42%
VegRAAL (Buttercup)	-0.29	43%
YearB (2014)*	-0.67	34%
YearC (2015)*	-0.51	38%

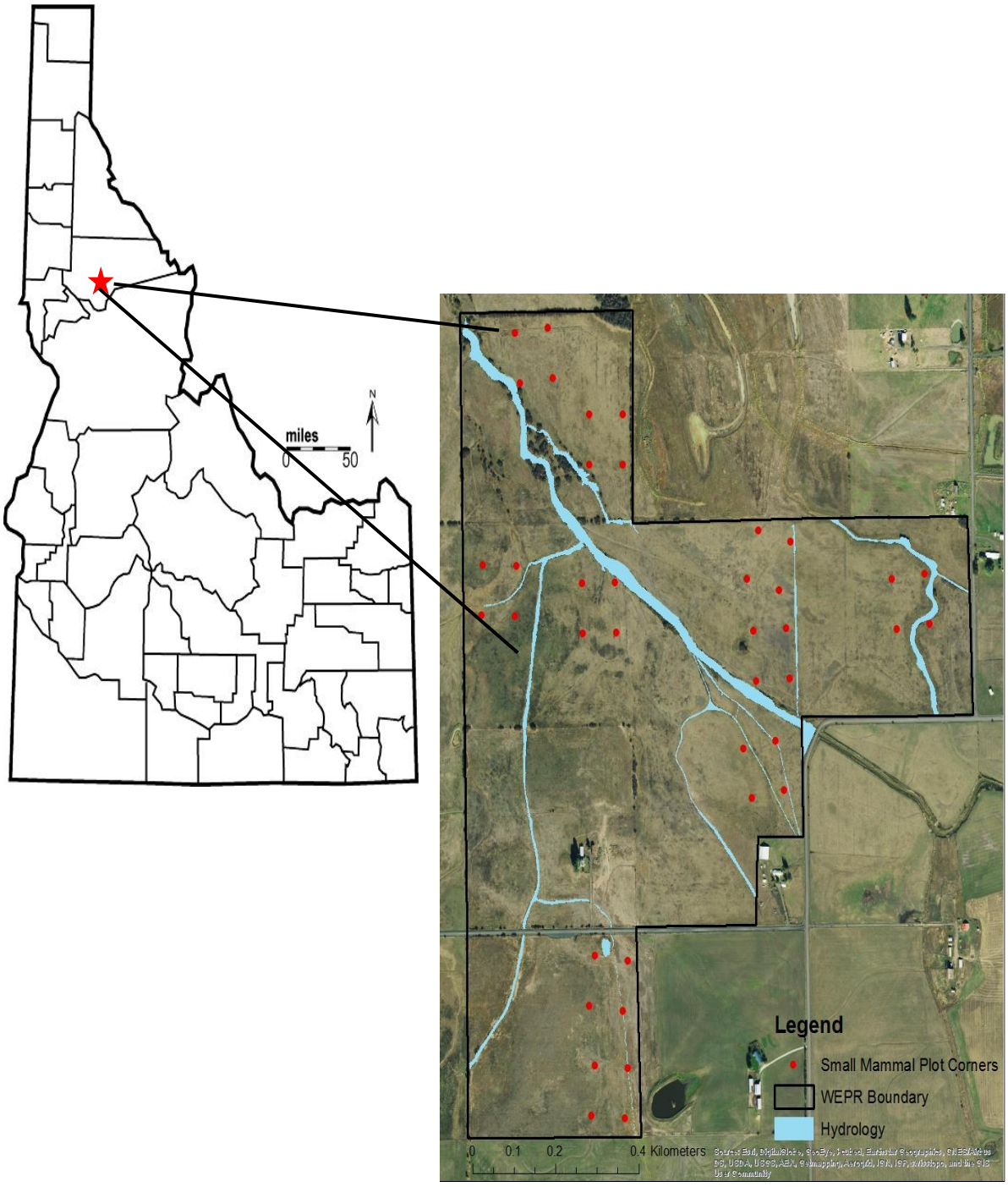

Figure 1.1 Location of Nez Perce National Historical Park's Weippe Prairie site near Weippe, Idaho, Clearwater County. Property boundary of the WEPR is outlined in black, and the corners of the small mammal plots where meadow voles were trapped are shown with red dots. The creek and canals that run across the site are highlighted in blue. The lines shown in the map of Idaho depict county boundaries.

CHAPTER 2

ANALYSIS OF VAGRANT SHREW (*SOREX VAGRANS*) DIET USING NON-INVASIVE GENETIC SAMPLING (NGS) AND DNA BARCODING

Abstract

Diet analysis is required in order to fully understand the biology of a species. Previous studies of shrew diets have used methods such as microhistology and direct observation of captive individuals, and found shrews to be a primarily insectivorous species. We analyzed DNA isolated from vagrant shrew (*Sorex vagrans*) fecal samples to reveal diet components. Our results identified plant species present in the diet of S. vagrans, revealing their presence in the food web on the Weippe Prairie. Nine plant families were identified from DNA analysis: Apiaceae, Asteraceae, Fabaceae, Fagaceae, Orobanchaceae, Pinaceae, Poaceae, Polygonaceae, and Ranunculaceae; with a predominance of Pinaceae, Poaceae and Asteraceae. Of our samples, DNA barcoding identified 10 plants to genus, and an additional 13 to genus and species. These results augment current knowledge of *S. vagrans* diet. With the use of DNA barcoding, the occurrence of direct or indirect consumption of plants by *S. vagrans* was revealed. This method allowed more precise identification of diet components, as well as insight into the food web present within our study system. The use of DNA-based methods for small mammal species' dietary analysis is underutilized. Future projects using this noninvasive methodology can assist the scientific community in improving our knowledge of small mammal diets.

Introduction

Diet analysis is necessary to fully understand the biology of a species (Shehzad et al. 2012; Valentini et al. 2009a). The quantity and quality of food eaten by an animal is related to multiple aspects of wildlife ecology, including population dynamics and regulation, mating systems, habitat use, and predator-prey interactions (Ortmann et al. 2006). Additionally, diet analyses can help to evaluate species' responses to environmental/anthropogenic stressors, as well as assist in the development of targeted strategies for conservation (Murray et al. 2011; Valentini et al. 2009b)

Numerous methods have been used to determine the composition of animal diets. The simplest of these is the direct observation of feeding, often of individuals in captivity. However, this method is time-consuming and typically precludes working on elusive animals and nocturnal species [2, 6]. Microscopic gut and/or feces examination is another technique that has been used but this approach also has limitations, which include high costs in time and labor, reliance on the examiner's identification skills, and disease transmission potential, as well as harm to the subject species (Pompanon et al. 2012; Valentini et al. 2009a). Other diet analysis studies have used stable isotope-based approaches, exploiting the carbon-isotopic distinction between C_3 or C_4 photosynthesis, to determine the diets of herbivores (Chabers & Doucett 2008). Although this method provides information on long-term diets, isotopic enrichment is subject to many variables, and the isotopic signatures of different food items are often not sufficiently distinct for clear resolution of family, genus or species identification (Pompanon et al. 2012).

More recently, DNA-based analysis has been used in dietary studies. This method involves noninvasive genetic sampling (NGS), where nucleic acids are obtained from sources such as the animals' hair or feces (Waits & Paetkau 2005), without the need to harm or

capture the animal being investigated. Because fecal samples contain the DNA of dietary components, as well as DNA of the depositor, they can be used to identify what an animal has eaten. Multiple methods have been developed to extract DNA from fecal material. Currently, the most frequently used technique involves using commercially available silica-binding extraction kits (Qiagen) (Waits & Paetkau 2005). The extracted DNA is used in a polymerase chain reaction (PCR) to amplify DNA of dietary components, using general or taxon-specific primers, followed by sequencing of amplicons to identify individual taxa via DNA sequence, i.e., taxon identification using a standardized DNA sequence (Pompanon et al. 2012). Sequence databases have expanded dramatically from DNA barcoding of animals and plants, enabling accurate taxon identification via the sequences generated by NGS (Pompanon et al. 2012).

DNA barcoding not only provides higher taxonomic resolution, but also indicates species present in the diet which are not readily visible from observational or microhistological methods (Hibert et al. 2013)--these features allow this method to extend and refine the list of items recognized in a species' diet. This is of importance because studies of interactions between predators and prey or herbivores and plants using past methods, such as macro- or microhistology, have always been time-consuming, inexact, and more expensive (Yoccoz 2012).

To date, the combination of NGS and DNA barcoding has seldom been used in dietary studies (Hibert et al. 2013; Yoccoz 2012). However, recently this method has enabled authors to conduct diet analyses on carnivores and herbivores (Hibert et al. 2013). Shehzad et al. (2012) studied the diet of the elusive, nocturnal leopard cat (*Prionailurus bengalensis*) and found that the NGS method indicated a broader diet that included larger prey than had been

noted before. Shehzad et al. (2012) also provided more precise information about diet by taking major diet groups to a more specific taxonomic level (i.e. genus or species versus family), which has not been previously possible with conventional methods. Characterizing the diet of herbivores is also a difficult task in wild species (Rayé et al. 2011). However, studies have used this novel DNA-based method to analyze herbivory by a broad range of taxa including birds, molluscs, insects and mammals such as tapirs, marmots or voles (Hibert et al. 2013; Valentini et al. 2009a; Soininen et al. 2009), and domestic and wild ruminants (Pompanon et al. 2012; Rayé et al. 2011; Pegard et al. 2009).

The use of this approach is increasingly common, but generally biased towards larger animals and uncommon with small mammal diet analysis. The majority of studies on small mammal diets have used other methods, such as microhistology (Whitaker et al. 1983; Clothier 1955). Most recently, DNA barcoding was used to determine the diet of subterranean rodents of the genus *Ctenomys* in southern Brazil (Lopes et al. 2015). Soininen et al. (2009) compared the efficacy of the traditional microhistological method to DNA barcoding for diet analysis of two subarctic vole species. They determined that DNA barcoding provided far more taxonomically detailed results on what each species ate (Soininen et al. 2009). Their study represents an important advancement for understanding small mammal diets, which are elusive and difficult to observe directly (Hibert et al. 2013). Moreover, their scat is often morphologically indistinguishable among species, and, species assignation based on morphology can be difficult to do in the field, especially among closely related taxa. Therefore, DNA barcoding is also a useful tool to effectively identify small mammal species as well as what they are feeding on (Moran et al. 2009).

Studies conducted on small mammals enable better understanding of ecosystems as a whole. Small mammal communities often respond rapidly to changes in their habitat structure and plant composition, and occupy significant positions in food webs; this makes them important biological indicators of ecosystem health and change (Leis et al. 2008). Wetlands are one such ecosystem where small mammals are an important component for proper functioning. Small mammals such as shrews prefer these moist environments because their high metabolic rates require ample amounts of moisture to prevent dehydration. Wetlands, riparian areas and moist forests and fields are the preferred habitat of shrews, supporting their greatest diversity and abundance, and these environments tend to have diverse and abundant invertebrate fauna, offering a rich food supply (Nagorsen 1996). Shrews, as members of Insectivora, are generally assumed to feed primarily on arthropods and other invertebrates (Gillihan & Foresman 2004). However, considerable diversity in shrew diets has been reported. Information on shrew diet comes from past studies conducted on stomach contents of trapped animals, fecal pellets recovered from traps, or through observations made on captive individuals (Nagorsen 1996).

The vagrant shrew (*Sorex vagrans*, subsequently referred to as *S. vagrans*) is most commonly associated with grassy fields and rich, moist soils near water, and they prefer rich soils where there is more soft-bodied prey (Nagorsen 1996). Whitaker and Maser (1976) found that the five most important foods exhibited in the stomach contents of over 30 individuals were insect larvae, slugs and snails, unidentified invertebrates, Endogone (fungi) and spiders. Although some vegetative material was also found, it was not highlighted as significant. Other studies concur with these findings (Clothier 1955; Broadbooks 1939). Captive individuals have also been observed caching, eating the seeds of trees, as well as

seeds and fruits of herbaceous, and shrub species (Gillihan & Foresman 2004; Saarikko 1989; Terry 1978). However, no studies have been conducted on wild *S. vagrans* using the NGS DNA barcoding method to investigate shrew herbivory, and little is known about which plant taxa are present in fecal samples from *S. vagrans*. Given the apparent importance of small mammals in this ecosystem, determining the diet of shrews in greater detail will allow better understanding of shrews' role in the Weippe ecology. The objective of this study is to use NGS and DNA barcoding approaches on this small nocturnal mammal. We use these methods to obtain information on 1) species identification of small mammal fecal samples not clearly distinguishable morphologically, and 2) determine the plant species contributing—directly or indirectly—to the diets of vagrant shrews on a wet prairie ecosystem in northern Idaho.

Methods

Study Area

The study was conducted on the Weippe Prairie (WEPR), a National Historic Landmark managed by Nez Perce National Historical Park (NEPE). WEPR is a disturbed mountain meadow, located in the southern tip of Clearwater County, Idaho near Paunch Mountain. WEPR is approximately 2.4 kilometers south of Weippe, Idaho (46°21'16.04" N, 115°55'17.66" W) and encompasses 111 ha (Fig 1). Elevation ranges from 922 meters in the southwest corner to 917 meters in the north. Small ponds and manufactured canals occur across the site. The northern section includes an approximately 1.6 kilometer stretch of the perennial Jim Ford Creek (flowing southeast to northwest).

WEPR is part of the Palouse Grasslands of the shortgrass prairie ecoregion (Erixson & Cogan 2012). Agricultural settlement of the area in the late 19th century brought nonnative grass species to the site, including creeping bentgrass (*Agrostis stolonifera*), timothy (*Phleum*

pretense), meadow foxtail (*Alopecurus pratensis*), and smooth brome (*Bromus inermis*). These nonnative grasses are scattered among various patches of native plants such as small camas (*Camassia quamash*), plantainleaf buttercup (*Ranunculus alismifolius*), tufted hairgrass (*Deschampsia cespitosa*), and California oatgrass (*Danthonia californica*). The major land use surrounding the WEPR is agricultural, and the fields are dominated by a mixture of agricultural plants, nonnative pasture grasses and native herbaceous plants. Current management on the WEPR is aimed towards restoring camas populations, as this species is the focal resource of the site.

Scat Sample Collection

Scat samples were collected from ten, 100x100 meter grids during late May/early June, July, and October 2014. Samples were collected by randomly placing 10 Sherman live traps along transects within each grid. Traps were locked open and baited with oats and sunflower seeds (which are not plant species that occur on the site). Traps were randomly checked and samples were collected from the first six traps to have scat. All scat samples were placed into an Eppendorf tube and labeled with the date, plot and collection location within the plot. Samples were then frozen until DNA extractions were conducted. Trapping methods were approved by the Animal Care and Use Committee of the University of Idaho (protocol 2013-95), and trapping was conducted following appropriate guidelines of the American Society of Mammalogists as outlined by Sikes et al. (2011).

Small Mammal Species Identification

Various small mammal species are known to occur in Clearwater County on WEPR, including *Peromyscus, Microtus, Sorex, Mus* and *Zapus* species. NEPE staff have conducted a

small mammal inventory for three consecutive years and determined that the resident populations on WEPR consist primarily of two species: meadow voles (subsequently referred to as *M. pennsylvanicus*) and *S. vagrans*. Although these species are physically distinct, unambiguous species identification from their scat is problematic because it is not morphologically distinguishable. In fact, visual identification of scat by experienced naturalists can have error rates approaching 50% (Foran et al. 1997). To avoid misidentification of the species whose scat samples were collected, sequence-based identification using polymerase chain reaction (PCR) technology was conducted with species-specific primers (designed specifically for *M. pennsylvanicus* and *S. vagrans*) to amplify short regions of the cytochrome b (*cytb*) sequence. The mitochondrial DNA (mtDNA) *cytb* gene has only moderate intraspecific variation, but clear differences between voles and shrews, and is thus well suited for use in species identification (McWilliam et al. 2013). Total DNA (nuclear, mtDNA and cholorplast DNA) was extracted from samples using a commercial QIAamp Fast DNA Stool Mini Kit (Qiagen), following manufacturer's protocol except for two modifications: 1) samples sat in Inhibit EX buffer for 10-15 minutes, and 2) incubation time at 70°C was extended to 2 hours. DNA was eluted in a total volume of 200 µl of Buffer ATE.

All available *cytb* sequences of *M. pennsylvanicus* and *S. vagrans* were retrieved from GenBank and were aligned using ClustalW2 – Multiple Sequence Alignment tool (Kohn & Wayne 1997). Conserved, species-diagnostic regions were identified by eye and were targeted as potential regions for primer design. Species-specific PCR primers were designed using PrimerQuest (Integrated DNA Technologies, Inc. 2015); primer sequences used in this study are shown in Table 1.

Cytb amplicons were generated in 20 µl reactions containing 1.0 µl (50-100 ng/µl) undiluted DNA, 1 µl of each primer (10pM/µl), 0.4 µl 10 mM dNTPs, 2 µl 25 mM $MgCl_2$, 2 µl 10X reaction buffer supplied by the polymerase manufacturer, and 1 unit of *Taq* DNA polymerase. Reactions were loaded in 0.2 mL thin-walled PCR tubes and placed into a DNA Engine Tetrad2 (MJ Research) thermal cycler and amplified using the following conditions: incubate at 94°C for 3 min; 6 cycles of 94°C for 30 s, 55°C for 30 s, followed by 72°C for 45 s; 36 cycles of 94°C for 30 s, 51°C for 30 s, followed by 72°C for 45 s; 1 cycle of 72°C for 3 minutes, and 10°C for 3 minutes. Negative controls consisting of 1.0 µl deionized water (in place of undiluted DNA) and 19 µl of cocktail (from reagents listed above) were run with all of the samples containing mtDNA. This was done to insure that successful PCR results were from either *M. pennsylvanicus* or *S. vagrans*, not from contamination of reagents.

Plant Species Identification

Amplification and sequencing of a region of chloroplast DNA (cpDNA) present in scat can be used to identify plant species' DNA present in the sample (McWilliam et al. 2013). In this study, plant chloroplast sequences were amplified using non-specific primers of the *rbcL* and *trnH-psbA* loci (Table 1). Total DNA that was extracted following the procedure described above was also used for these analyses. cpDNA amplicons were generated in 20 µl reactions. The *rbcL* locus was amplified using the following conditions: incubate at 94°C for 2 minutes, 6 cycles of 94°C for 30 s, 55°C for 1 min, followed by 72°C for 1 min; 31 cycles of 94°C for 30 s, 51°C for 1 min, followed by 72°C for 1 min; 1 cycle of 72°C for 3 min. The *trnH-psbA* region was amplified using the following conditions: incubate at 94°C for 3 min, 6 cycles of 94°C for 30 s, 55°C for 30 s, followed by 72°C for 45 s; 36 cycles of 94°C for 30 s,

51°C for 30 s, followed by 72°C for 45 s; 1 cycle of 72°C for 3 minutes, and 10°C for 3 minutes.

A standard recipe for 20 µl reactions used to amplify plant DNA consisted of 1.0 µl (50-100 ng/µl) undiluted DNA (1.5µl for *trnH-psbA* primers), 1 µl of 10pM/µl primers, 0.8 µl 10 mM dNTPs, 2 µl 25 mM $MgCl_2$ (3µl for *trnH-psbA* primers), 4 µl 5X reaction buffer supplied by the polymerase manufacturer, and 1.5 units of *Taq* DNA polymerase (Promega). Table 2 summarizes PCR reaction conditions. Negative controls consisting of 1.0 µl deionized water (in place of undiluted DNA) and 19 µl of cocktail (from reagents listed above) were run with all of the samples containing plant DNA. All of the extractions were done in a laboratory where plant materials are not normally present; more particularly, the plant species identified in the course of this study have not been present in the laboratory where this work was carried out. Because PCR will not always generate a representative sample of the DNA types present, replicate reactions were conducted on a randomly selected subset of samples (n=5) in order to determine if PCR and subsequent sequencing results identified the same specific taxa each time, or gave evidence of PCR bias.

DNA Sequencing

Both mtDNA and cpDNA amplicons were sequenced to identify the small mammal species whose scat was collected, and plant components present in the scat samples. PCR products were treated with ExoSAP-IT (USB, Cleveland, Ohio, USA) according to the manufacturer's instructions, to remove leftover primer and dNTPs. Once purified PCR products were obtained they were sequenced in one primer direction; using either rbcLa-F or psbA3_f for cpDNA sequencing, and *S. vagrans* FWD or *M. pennsylvanicus* FWD for mtDNA sequencing. A standard recipe for 10 µl reactions consisted of 3 µl of product from

purification step, 1 µl 2.5pM/µl primer (one direction only), 2 µl 5x Sequencing buffer, and 2 µl BigDye. Excess primers and nucleotides were then removed using the following procedure: 1) 10 µl of product from Sequence reaction mixed with 1 µl 3M NaAcetate, pH 5.5; 2) Addition of 22 µl ethanol, resulting in a 33 µl end volume; 3) Incubate at room temperature for 20 minutes; 4) Centrifuge at top speed (14,000 X *G*) for 20 minutes; 5) Discard liquid and add 100 µl 70% ethanol; 6) Incubate at room temperature for 2 -3 minutes; 7) Centrifuge for 2 minutes at top speed; 8) Remove liquid and air dry pellet 10-15 minutes; 9) Re-suspend pellet in 12 µl BigDye® Formamide (Life Technologies).

Fluorescently labelled DNA products from the sequencing reactions were electrophoresed and read on an ABI 3130 *xl* automated sequencer (Applied Biosystems), and then aligned to other sequences using the program Sequencher 4.5 (Gene Codes, Ann Arbor, Michigan, USA). Further identification was done using the reference database GenBank (Bethesda, Maryland, USA), using the BLAST (blastn) tool on the National Center for Biotechnology Information (NCBI) web site. Results with >95% identity to known taxa were used as family designation for the small mammal species ID and plant species ID. Some sequenced taxa were successfully assigned to the rank of genus and/or species using the same criteria.

Results

Small Mammal Species Identification

Positive identification of the species (i.e., shrew or vole) from which the scat originated could not be done upon collection in the field. This is due to the difficulty of visually distinguishing small mammal scat because it is often similar in size, shape, and color. Therefore, both species-specific primer sets (*S. vagrans* and *M. pennsylvanicus*, Table 1) were

used during PCR amplification and subsequent sequencing. A total of 136 scat samples were collected in the field. A subsample of 68 were randomly chosen to be sequenced for small mammal identification. Of these 68 samples sequenced, 65 were identified to have originated from *S. vagrans*.

Plant Species Identification

After conducting PCR amplification on all 136 samples, utilizing *rbcL* and *trnH-psbA* gene primers, a total of 46 samples produced strong bands of amplified DNA, which were then sequenced. Of these, 28 resulted in plant family designations (Table 3). With the remaining 18, the sequences generated by the ABI 3130 *xl* automated sequencer were not strong enough to produce positive identification. All of the 28 samples with positive plant species identification were from the samples identified as shrew scat. These sequences indicated nine plant families (Table 3): the parsley family (Apiaceae), aster family (Asteraceae), legume family (Fabaceae), beech or oak family (Fagaceae), broomrape family (Orobanchaceae), pine family (Pinaceae), grass family (Poaceae), buckwheat family (Polygonaceae), and the buttercup family (Ranunculaceae). Of these, we were able to identify 10 to genus: marigold species (*Flaveria*), pine species (*Pinus* x7), switchgrass species (*Panicum),* and buttercup species (*Ranunculus*). For an additional 13 samples, we were able to identify genus and species: western yarrow (*Achillea millefolium* x2), white panicle aster (*Symphotrichum lanceolatum*), string bean (*Phaseolus vulgaris* x2), garden pea (*Pisum sativum*), quackgrass (*Elymus repens* x2), California oatgrass (*Danthonia californica* x2), and American bistort (*Polygonum bistortoides* x3). Furthermore, all of the samples that underwent replicate reactions produced the same sequencing results each time i.e. they identified the same specific taxon.

These data indicate a degree of seasonal variation in selection of plant species between July and September (Table 4). Only two samples collected in June produced positive plant species identification, therefore a strong correlation could not be made to other collection times. In July, plants from the Asteraceae, Poaceae, and Polygonaceae families occurred most frequently. Although detected in July, the Polygonaceae and Ranunculaceae families were not present in September. Furthermore, the plant families Apiaceae, Fagaceae, and Orobanchaceae were present in scat samples collected in September, but were not present in July. The Pinaceae family was present both months, occurring most frequently in September.

Discussion

This is the first analysis conducted on shrew scat using DNA barcoding. This study has important methodological implications, underlining the usefulness of this technology, especially for species which are elusive, and whose scat is morphologically indistinguishable from other small mammal species (such as shrews). Furthermore, our study reveals a potentially important ecological implication for wetland ecosystems where *S. vagrans* is abundant; the species may not only influence insect and predator populations in these systems, but plant populations as well, possibly through direct herbivory. While we cannot rule out that the plant DNA present in shrew scat comes from the insects these animals consume, our results suggest that *S. vagrans* may augment its diet by consuming plants. It is possible that plant DNA amplified in this study is from the gut contents of the prey item, for example, grass eaten by a grasshopper. Nevertheless, the plants we identified are contributing to the food web associated with *S. vagrans*. Identifying the plants that make up the food web is important because it allows us to interpret how energy and nutrients are moving through the system (WWF 2015). This leads to better understanding of ecosystem structure and function.

Our results provide novel information about the plants contributing to *S. vagrans*--no studies to date have collected such taxonomically detailed information. Previous studies suggested that *S. vagrans* may eat plant material such as roots, shoots and probably seeds (Nagorsen 1996). Saarikko (1989) suggested that *S. vagrans* might supplement their diet with plant material such as seeds and proposed that they may be an important part of their diet in winter. However, unlike our study, the family, genus and/or species were not determined. *S. vagrans* was observed caching and eating conifer seeds (in captivity), specifically from the Pinaceae family (Terry 1978), and Pinaceae was the most common species found in scat samples in our study (n=7). The presence of Pinaceae species in and around the WEPR site is limited to a few locations >100 meters away from the nearest trap site (as can be seen in Fig. 1). Although it is not definitively known whether shrews were caching and eating conifer seeds or whether the DNA detected came from insects that had eaten pine and then been eaten by vagrant shrews, it suggests that this family is of importance, given its limited abundance and distribution on the landscape.

Similarly, the presence of string beans (*Phaseolus vulgaris*) and peas (*Pisum satinum*) in the samples may also indicate the importance of these species to the food web, as these vegetable plants are not readily available. The closest distance between garden and trap site in our study area is roughly 300 meters. These results warrant a follow up study. With a larger sample size and the addition of primers designed to amplify invertebrate DNA, the role and importance of these plant species can be further investigated.

WEPR is dominated by non-native pasture grasses, interspersed with a few native grass species. Due to the level of abundance of these species, it seems logical that they would contribute to the food web. Furthermore, because replicated PCR identified the same species

each time, we conclude that PCR artifact was likely not influencing species identification. Thus, these species are well represented in the DNA recovered from scat. Overall, our results strongly suggest that shrews may augment their diet by consuming plants opportunistically. The plants detected in this study exhibit some seasonal variation in the food web on the WEPR. Phenology of the plant species present, their distribution, seed availability, root and/or bulb depth, and/or what was locally abundant are all possible sources for this variation. However, in order to determine if this variation is real and meaningful further collection and analysis needs to be conducted. Unfortunately, the data set in this study is not sufficient to draw such a conclusion.

DNA barcoding does not provide information on what part of the plant was eaten; for example, it cannot distinguish a diet of leaves versus seeds. Although different plant parts have different potential nutritional values (presumably affecting the likelihood of them being actively sought out) we believe that this becomes relevant when (or if) we know that herbivory is occurring; which can be accomplished using DNA barcoding. As demonstrated in this study, this method can provide accurate determination of the plant family, as well as genus and/or species of some samples. This is an advantage compared to other diet analysis methods, including microhistology and stable isotope analysis, neither of which provides as taxonomically precise results (Rayé et al. 2011; Soininen et al. 2009). The improved resolution in plant determination obtained through DNA barcoding has general application for investigating the diets of small mammals, or other larger species. For example, it can be used to explore temporal shifts in diet, as well as inter-individual variability in diet composition (Rayé et al. 2011). It can also be used to more precisely answer questions about plant-herbivore interactions and diet selection (the link between use and availability (Soininen et al.

2009). This method can further be used to identify a single species from remains that are not visually identifiable or for more broad applications such as biodiversity assessments (Valentini et al. 2009b).

The NGS method, as with all methodology, has limitations of its own. For example, DNA cannot be obtained from all scat, and DNA degrades when exposed to the elements for extended periods (Foran et al. 1997). Furthermore, the low quantities of DNA in noninvasively collected samples (such as scat) can contain high concentrations of PCR inhibitors (Valentini et al. 2009a; Foran et al. 1997). However, if DNA from scat is too degraded to amplify, no results will be produced, as opposed to incorrect results (Foran et al. 1997). This is significant because, unlike with the other methods, it limits false identification and subsequent misrepresentation of an animal's diet. Overall, these limitations could explain why the majority of the scat samples collected during this study did not successfully produce amplified plant species DNA.

In conclusion, the results of this study provide evidence of the plant species that contribute to S. vagrans diet, either directly or indirectly. The results also indicate that there is seasonal variation in the food web on the WEPR . Because small mammal species' scat is difficult to distinguish visually, NGS was an appropriate tool with which to examine trophic relationships. This methodology allowed acquisition of useful information without harming the subject species, and efficient use of resources. It also allowed us to accurately identify the species (i.e. shrew versus vole) depositing the scat in the traps, and enabled us to obtain a higher resolution of the plant species found in scat samples, through accurate classification of family, genus and/or species. This study, in conjunction with future studies utilizing this

methodology can assist the scientific community in gaining a better understanding of the diet of *S. vagrans*, as well as other small mammal species.

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Table 2.1 Sequences of the primer pairs used for determining plant and animal species identity, with respective references. *S. vagrans* FWD and REV were designed for GenBank ACC# KF302838.1. *M. pennsylvanicus* FWD and REV primers were designed for GenBank ACC# KJ556623.1.

Primer	Sequence	Reference
Plant		
psbA3_f	GTTATGCATGAACGTAATGCTC	Sang et al. (1997)
trnHf_05	CGCGCATGGTGGATTCACAATCC	Tate and Simpson (2003)
rbcLa-F	ATGTCACCACAAACAGAGACTAAAGC	Levin et al. (2003)
rbcLa-R	GTAAAATCAAGTCCACCRCG	Kress and Erickson (2009)
Animal		
S. vagrans FWD	TCCTAGGCGTCTGCTTAATTG	NA – designed for study
S. vagrans REV	CTAACCCGATTCTTCGCCTT	NA – designed for study
M. pennsylvanicus FWD	CCTACACGAAACAGGGTCAAA	NA – designed for study
M. pennsylvanicus REV	TTATCCTCACATGAATCGGCG	NA – designed for study

Table 2.2 End concentrations of PCR reagents in 20 µl reactions.

Reagent	**End Concentration**
Primer(s)	0.5 µM
$MgCl_2$ (for rbcLa reactions)	2.5 mM
$MgCl_2$ (for trnH reactions)	3.75 mM
DNA	ca. 100ng/reaction
dNTPs	0.4 mM
Taq Polymerase	1.5 units/reaction

Table 2.3 The plant families, genus and/or species designated from scat samples collected during June, July and September 2014 on the Weippe Prairie. A total of 28 samples resulted in positive plant identification.

Family		**Collection month**			
Genus	*Species*	June	July	Sept	Total
Apiaceae					
NA	NA	0	0	1	1
					1
Asteraceae					
Achillea	*millefolium*	0	2	1	3
Symphyotrichum	*lanceoloatum*	0	1	0	1
Flaveria	NA	0	1	0	1
					5
Fabaceae					
Pisum	*satinum*	0	0	1	1
Phaseolus	*vulgaris*	2	1		3
					4
Fagaceae					
NA	NA	0	0	1	1
					1
Orobanchaceae					
NA	NA	0	0	1	1
					1
Pinaceae					
Pinus	NA	0	2	5	7
					7
Poaceae					
Danthonia	*californica*	0	1	1	2
Elymus	*repens*	0	2	0	2
Panicum	NA	0	0	1	1
					5
Polygonaceae					
Polygonum	*bistortoides*	0	3	0	3
					3
Ranunculaceae					
Ranunculus	NA	0	1	0	1
					1

Table 2.4 Comparison of the plant families that occurred in July and/or September scat samples.

Plant Family	July Samples	September Samples
Apiaceae	0	1
Asteraceae	4	0
Fabaceae	1	1
Fagaceae	0	1
Pinaceae	2	5
Poaceae	3	2
Polygonaceae	3	0
Ranunculaceae	1	0
Orobanchaceae	0	1

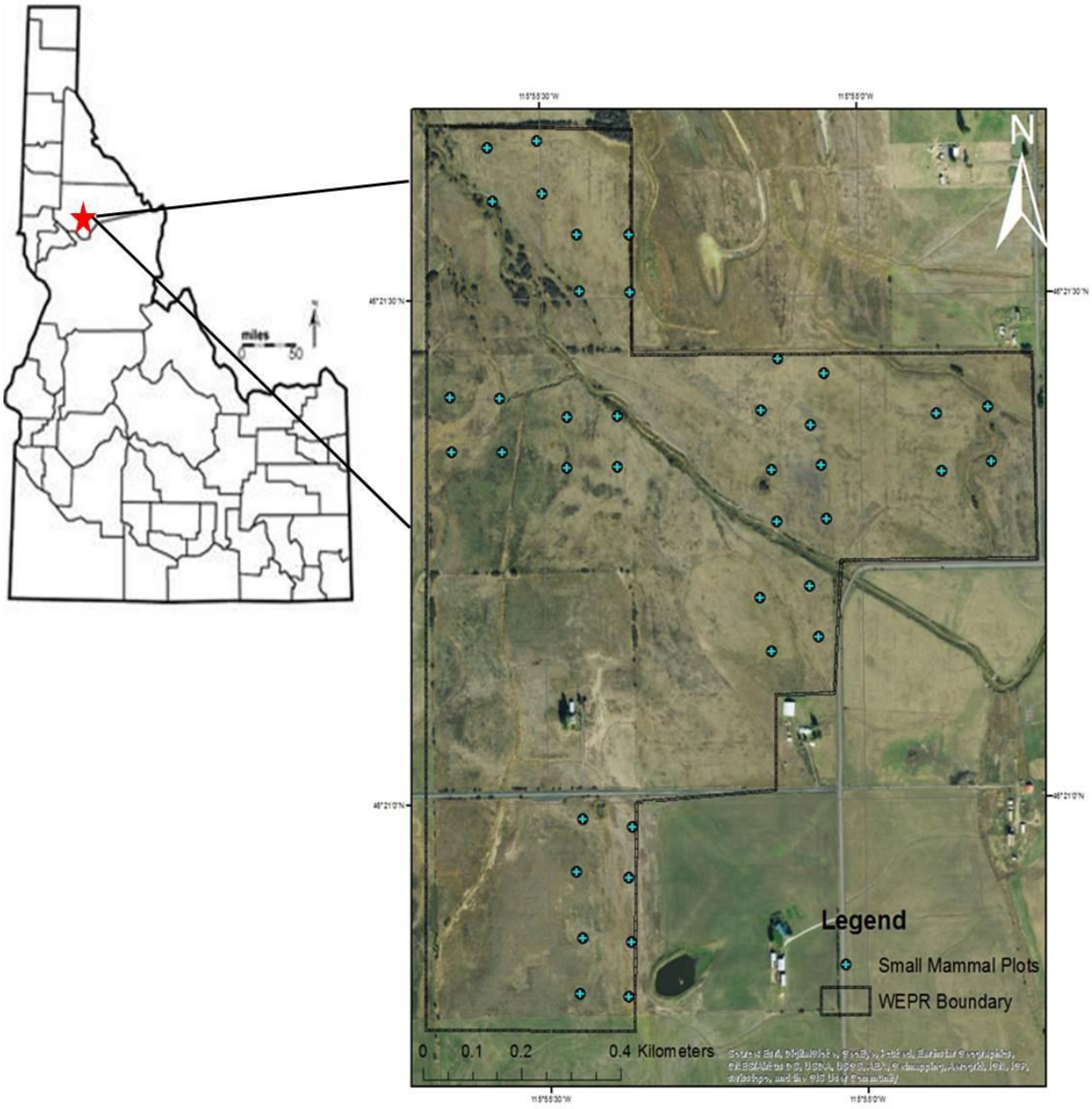

Figure 2.1 Location of Nez Perce National Historical Park's Weippe Prairie site near Weippe, Idaho, Clearwater County. Property boundary of the WEPR is outlined in black, and the corners of the small mammal plots where scat samples were collected are shown with blue crosses.

CONCLUSIONS AND FUTURE WORK

This thesis examined habitat selection of meadow voles through occupancy modeling and the diet of vagrant shrews through the utilization of non-invasive genetic sampling (NGS) and DNA barcoding methods on a wet prairie ecosystem in northern Idaho. The management implications and future work from my chapters include:

Chapter 1:

Prior to initiation of this study, a full census of the small mammal populations present on the Weippe Prairie, located in Clearwater County, Idaho, had not been conducted. Therefore, managers did not know if there were any threatened and endangered (T&E) species present, something that needed to be determined before restoration of the wet prairie could begin. Our results indicate that no T&E species are present, and the most common small mammal species are meadow voles (*Microtus pennsylvanicus*) and vagrant shrews (*Sorex vagrans*). Based on our finding that meadow vole occupancy is negatively associated with camas density, we predict meadow vole populations may be adversely affected if camas density greatly increases across the Weippe Prairie as a consequence of restoration efforts. This impact on meadow voles could also indirectly affect predator populations. Northern Harriers (*Circus cyaneus*) and Short –eared owls (*Asio flammeus*) are prime examples, because meadow voles are a large portion of their diet on northern prairie wetlands (PF 2014, Sullivan 1996). Furthermore, our finding of a strong association with a non-native pasture grass, meadow foxtail, is informative. Restoration plans aimed at reducing the number/extent of this species may also adversely affect meadow voles. However, future studies should evaluate whether meadow voles will switch to a native grass species if meadow foxtail is slowly phased out of the

landscape. We suggest that camas growth should be promoted in such a way that grass species are maintained and co-dominate the plant community. In this way, management can ensure that the benefits of graminoid vegetation (i.e. cover and food) persist. In addition, many studies have found that meadow voles are significant grazers and can change plant community composition and species distributions (Sieg 1987; Thompson 1965). Therefore, a change in their population status could alter certain vegetation types that they had previously heavily grazed. Lastly, although we did not collect data on meadow vole density because their populations cycle every three to five years (and our study was limited to 3 years), managers could better analyze the influence of restoration on meadow vole populations if future studies incorporated population density.

Chapter 2:

The results of Chapter 2 highlight the application of NGS and DNA barcoding methods as a means of identifying species and their diet components. Our results indicate that vagrant shrews are consuming plants, either directly or indirectly. The plant families identified in scat samples included parsley (Apiaceae), aster (Asteraceae), legume (Fabaceae), oak (Fagaceae), broomrape (Orobanchaceae), pine (Pinaceae), grass (Poaceae), buckwheat (Polygonaceae), and buttercup (Ranunculaceae). A portion of these samples were further identified to genus including marigold (*Flaveria*), pine (*Pinus*), switchgrass (*Panicum*) and buttercup (*Ranunculus*) species. Lastly, seven specific species were identified; western yarrow, white panicle aster, string bean, garden pea, quackgrass, California oatgrass and American bistort. Some samples were also successfully identified to genus and species Because we cannot say with certainty that consumption is direct, further studies should determine the invertebrate

species present in vagrant shrew diet. These results will not only indicate whether vagrant shrews exhibit herbivory, but can also highlight the invertebrate species of importance on the Weippe Prairie. Future studies should also analyze the diet of meadow voles to determine if they consume any of the species consumed by vagrant shrews in our study.

In conclusion, our study provides valuable information about habitat selection of meadow voles and the diet of vagrant shrews in a [modified] wet prairie ecosystem, and can therefore usefully inform management decisions aimed at restoring these landscapes.