Ecology and Conservation of the Western Gray Squirrel (*Sciurus griseus*) in the North Cascades

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Abstract

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The western gray squirrel (*Sciurus griseus*) was classified as a Washington State threatened species by the Washington Department of Fish and Wildlife in 1993 due to a decline in range and number. The North Cascades population is geographically and genetically isolated from others in Washington, Oregon, and California, and may be ecologically unique as it exists in a mixed-conifer forest habitat that lacks oak (*Quercus spp.)*: a source of forage and maternal nests in most other portions of the range. The North Cascades are also distinguished by high average annual snowfall and cold temperatures, frequent wildfire and dynamic forest management. Land management agencies have initiated fire fuel reduction plans that may have potentially adverse effects on western gray squirrels. Local populations in Stehekin and the Methow Valley are likely small, making them susceptible to stochastic threats including genetic drift and inbreeding, which reduce evolutionary fitness and increase extinction risk. We studied distribution, life history, and response of squirrels to fire fuel treatments in the North Cascades from 2008-2011 using live trapping, radiotelemetry, and genetic and fecal sampling. Scientific communication between researchers and the general public was evaluated with interviews and an experimental study on the effectiveness of alternate communication methods. Squirrels used fire fuel treated areas disproportionately within their home ranges indicating that recent treatments and wildfires have not negatively affected western gray squirrel habitat at the home range scale. We also found no evidence that treatments and wildfire have negatively affected western gray squirrel diet. Areas used for nesting were characterized by large, tall trees, high levels of dwarf mistletoe infection, high canopy cover and connectivity; all characteristics that can decrease with fire fuel reduction treatments. Future treatments can focus on retaining patches of large trees with some mistletoe infection, and moderate levels of canopy cover and connectivity to conserve western gray squirrel nesting habitat in the North Cascades. Average home range size, degree of overlap, and effective population size indicate that the North Cascades may support a larger population of western gray squirrels than previously thought. Understanding and support for wildlife research increased significantly through science communication.

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DEDICATION

To my Grampa Charles G. Caddey who always believed I could get my doctoral degree,
and my daughter Ellinor who made it happen on time

Introduction

Conservation and management of endangered species requires knowledge of the life history of the species and its associated habitats (Meffe and Carroll 1997). The western gray squirrel (*Sciurus griseus*),the largest native tree squirrel to Washington, Oregon, and California, was classified as a state threatened species in 1993 by the Washington Department of Fish and Wildlife (WDFW) (WAC 232.12.011). Once ranging nearly continuously around the Columbia River gorge on both sides of the Cascade mountains (Dalquest 1948, Ingles 1965; Figure 1), western gray squirrels in Washington are now limited to three geographically isolated areas: in Pierce and Thurston Counties in the southern Puget Trough, in southern Washington in Klickitat, Yakima, and Skamania Counties, and in north-central Washington in Chelan and Okanogan Counties (Figure 1). Causes for decline over the past century include over-hunting, automobile accidents, disease, predation, potential competition with introduced squirrels, and habitat loss (Linders and Stinson 2007).

Habitat loss can occur naturally through forest succession and disturbance, or anthropogenically through land conversion and management practices. Development and urbanization has severely reduced habitat of the central Puget Trough population in Washington (Linders and Stinson 2007), while wildfire and forest management practices are the driving forces of habitat loss for the North Cascades population. A policy of fire suppression combined with logging from 1910 until the late 1960s led to changes in species composition, increases in tree density, often accompanied by decreases in individual tree diameter; an accumulation of dead woody debris; and an increase in forest disease and ladder fuels, particularly dwarf mistletoe brooms. Forest changes have facilitated an increase in the intensity and scale of wildfires in recent years and subsequent implementation of fire fuel reduction management plans including prescribed burning, thinning, and removal of ladder fuels. Management plans focus on protecting human life and property, decreasing the risk of stand-replacing wildfire, and maintaining and restoring wildlife habitat

The North Cascades region represents a unique habitat for western gray squirrels, composed primarily of Douglas-fir (*Pseudotsuga menziesii*), ponderosa pine (*Pinus ponderosa*), and bigleaf maple (*Acer macrophyllum*) (Franklin and Dyrness 1973, Hamer et al. 2005). Oak (*Quercus spp.*), used as a food source and for maternal nests in most other portions of the western gray squirrel's range (Linders and Stinson 2007), is not present in this habitat. The WDFW has a set a recovery goal of 1000 western gray squirrels in the North Cascades (Linders and Stinson 2007), and local land managers including the National Park Service (NPS) and United States Forest Service (USFS) have agency mandates and missions that make conservation of western gray squirrel habitat a high priority. However, little information existed on the distribution and ecology of the western gray squirrel in this northern extent of their range.

Research Questions

The primary set of questions driving this study addressed the main natural and anthropogenic habitat disturbances in North Cascades: wildfire and fuel reduction treatments. Key questions included: How does wildfire and fuels management affect western gray squirrel space use and habitat selection in the North Cascades? For example: do western gray squirrels use burned, treated, or untreated areas more frequently? Do western gray squirrels select burned, treated, or untreated areas for nesting? In addition, because so little knowledge existed on this population of western gray squirrels, several other research questions focused on basic ecology, such as: where are western gray squirrels distributed within the North Cascades and Lake Chelan

National Recreation Area? What habitat variables are important for western gray squirrel nesting and daily use? What do western gray squirrels eat in the North Cascades; is the diet consistent across seasons? How do western gray squirrels adapt to winter in the North Cascades? How large are the populations in Stehekin and the Methow Valley and what are their genetic variabilities? How inter-related are western gray squirrels in the North Cascades and how does that influence social dynamics? Additionally, what is the best way to communicate western gray squirrel research results to the general public to promote understanding and conservation?

General Methods

We used live trapping and radiotelemetry to understand basic ecology and the effects of wildfire management on western gray squirrels in the North Cascades. Capturing, marking, and monitoring individuals allows measurement of fine-scale habitat selection as well as estimation of fitness correlates: reproductive success and survival, which can be more telling of habitat quality and population status than abundance or habitat use (Van Horne 1983). We also collected genetic tissue and fecal samples from marked individuals to analyze genetic diversity, abundance, distribution, social behavior, reproductive success, and diet.

Study Area

Field work took place at two study sites in north central Washington State, USA: the Stehekin Valley and Squaw Creek in the Methow Valley. Comparative analysis also used previous data (Gregory 2005) from Black Canyon Creek in the Methow Valley (Figure 2, 3). See Gregory (2005) for a full site description of Black Canyon Creek.

Stehekin is a remote town at the northernmost end of Lake Chelan within the Lake Chelan National Recreation Area, which is administered as part of the North Cascades National Park Complex. It is only accessible by boat, float plane, or a 20 mile hike from the Pacific Crest Trail and the 80-100 year round residents do not have access to phone service. One main road provides access to the nine miles of the valley inhabited by year-round and summer residents. The forest type is mixed conifer dominated by Douglas-fir with smaller amounts of ponderosa pine, bigleaf maple, and black cottonwood and is managed by the NPS. This area experiences cool, wet winters with hot, dry summers; temperatures range from -15 °C in winter to 38 °C in summer with average annual precipitation of 82.8cm: 75% of this falls as snow on average (2005-2010 NCDC NOAA Climatological Data). Elevations range from 348 to 700m. The Stehekin Valley Watershed comprises numerous tributary streams; the main ones in our study area include Bridge Creek, Rainbow Creek, Boulder Creek, Imus Creek, Purple Creek, Hazard Creek, 4-mile Creek, Flick Creek, and Fish Creek, all of which flow into the Stehekin River and Lake Chelan. Other common mammalian species include the red squirrel (*Tamiasciurus hudsonicus*), the Douglas squirrel (*Tamiasciurus douglasii*), yellow pine chipmunk (*Tamias amoenus*), mule deer (*Odocoileus hemionus*), black bear (*Ursus americanus*), bobcat (*Lynx rufus*), and marten (*Martes martes*).

Squaw Creek is a class III stream that flows into the Methow River. The Squaw Creek Watershed is part of the Okanogan National Forest managed by the USFS. The forest type is mixed conifer dominated by ponderosa pine with smaller amounts of Douglas-fir, lodgepole pine (*Pinus contorta*), black cottonwood, and trembling aspen (*Populus tremuloides*). Temperatures range from -23 °C in winter to 41°C in summer (also cool, wet winters, hot, dry summers) with average annual precipitation of 24.4cm: about 50% of this falls as snow on average. Elevations range from 330 to 1060m. Other mammalian species commonly observed in this area include the red squirrel, yellow pine chipmunk, snowshoe hare (*Lepus americanus*), mule deer, white tailed-deer (*Odocoileus virginianus*), black bear, and moose (*Alces alces*). The Methow Valley, which extends from Mazama (north) to Brewster (south), WA has approximately 5,000 year round residents. Both study sites have similar forest management plans including recent prescribed burns and thins and have had documented western gray squirrel presence prior to 1950 (WDFW Heritage Database).

Significance

Western gray squirrels provide many ecological, social, and economic services. As preferential mycophagists, western gray squirrels aid in the propagation of hypogeous fungal (truffle) spores. Many truffle species share mycorrhizal associations with trees vital for forest health and function and can be prized food items for human consumption (Maser et al. 2008). Squirrels also encourage tree establishment through scatter hoarding of seeds and nuts (Carraway and Verts 1994). Squirrels share close associations with people; they are seen as both pests and objects of observation and photography. Western gray squirrels are also hunted for recreation in California and Oregon. Because the general public has a vested interest in the western gray squirrel, it is important for researchers to communicate scientific proposals and results to them. This study took extra steps to ensure that information on the western gray squirrel was appropriately transferred to stakeholders through frequent, multi-modal presentations to scientists, land managers, and local communities, and a quantitative analysis on the efficacy of alternate educational strategies. It is my hope that the additional information provided from this 5-year study will encourage accommodating management practices to facilitate recovery of the western gray squirrel in Washington State.

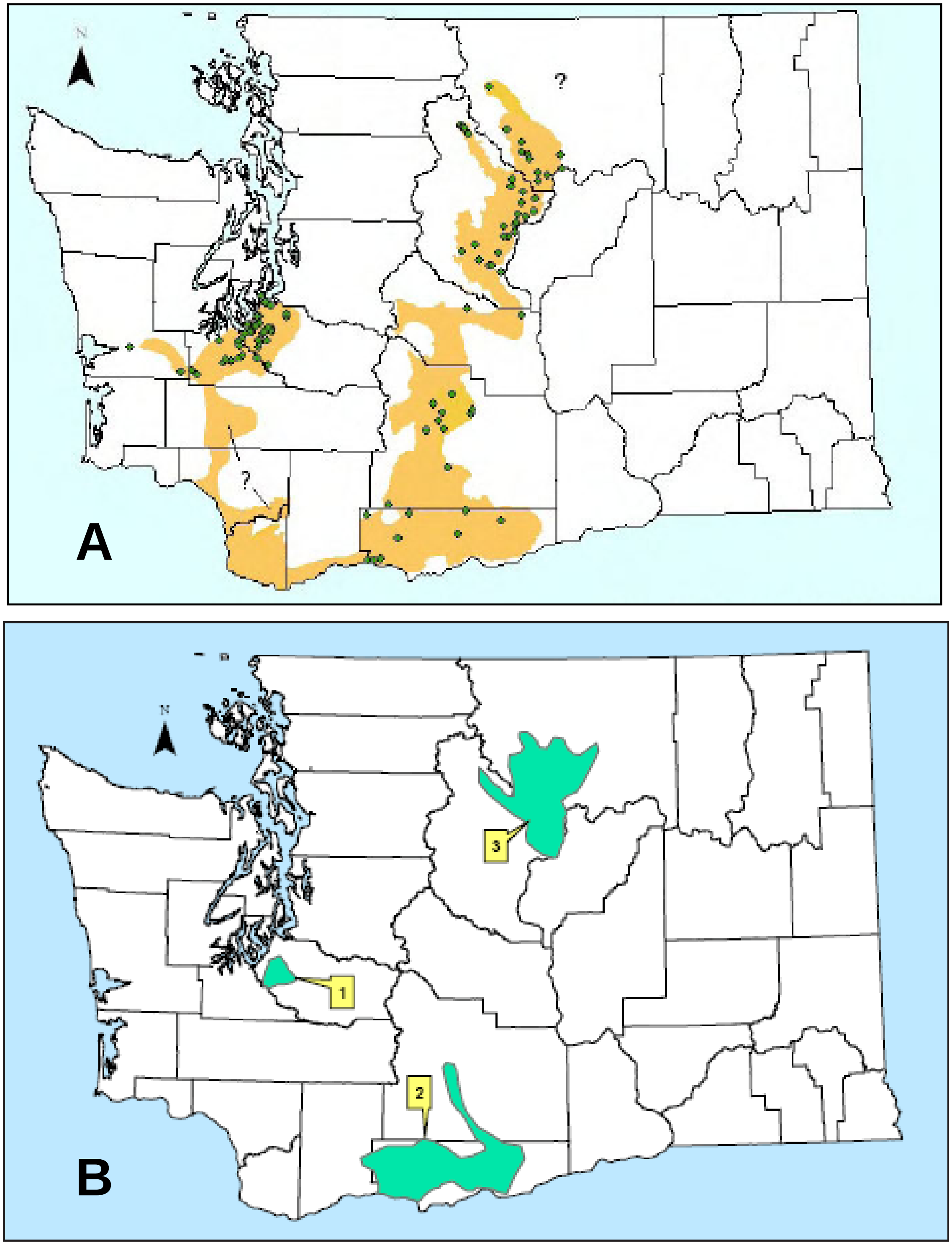


Figure 1. Historic (A) and current (B) distribution of the western gray squirrel (*Sciurus griseus*) in Washington State. The three remaining populations in map B are found in 1: the Puget Trough on Joint Base Lewis-McChord, 2: south central Washington, and 3: the North Cascades. Modified from Linders and Stinson (2007).

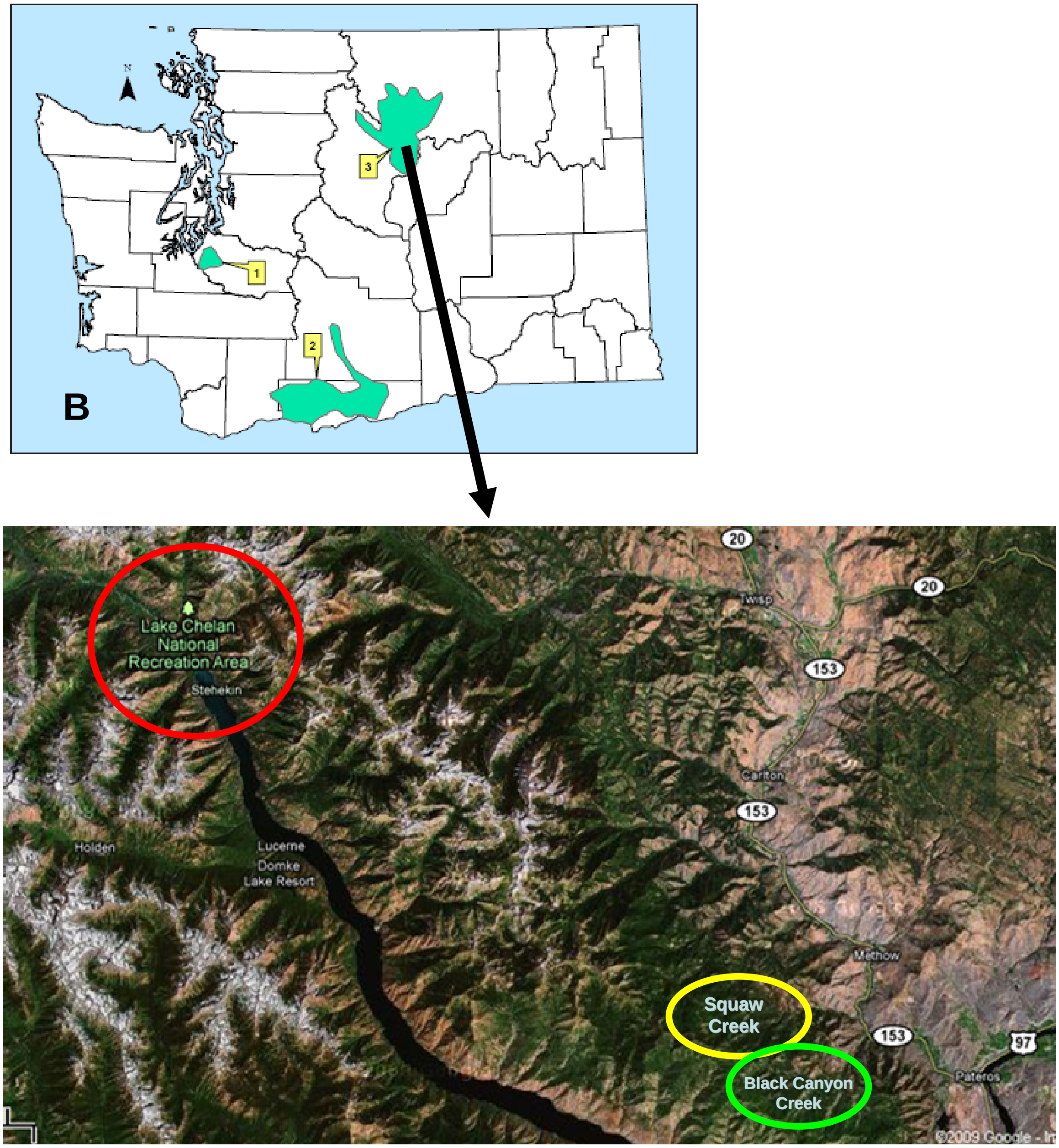


Figure 2. North Cascades study sites.

Figure 3. North Cascades study sites, top: Stehekin Valley, middle: Squaw Creek, Methow Valley, bottom: Black Canyon Creek, Methow Valley.

Chapter 1: Resource Selection of Western Gray Squirrels in Relation to Fire and Land Management Practices

Abstract

The Western gray squirrel (*Sciurus griseus*) was listed as a Washington State threatened species in 1993 and is confined to three geographically isolated areas: the southern Puget Trough of Pierce and Thurston Counties, southern Washington in Klickitat, Yakima and Skamania counties, and north-central Washington in Chelan and Okanogan counties. Recovery of the species has become a priority, however, distributional and life history data on the North Cascades population are limited. This population is ecologically unique as it exists in a mixed-conifer forest habitat composed primarily of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*) that lacks oak (*Quercus spp.*), an important source of forage and maternal nests elsewhere in the range. The North Cascades are also distinguished by frequent wildfire and dynamic forest management. A history of logging and fire suppression has created dense, diseased, and fire-prone forest stands, leading to several large-scale, high-intensity wildfires in recent years. In response, land management agencies have initiated fire fuel reduction plans that may have adverse effects on western gray squirrels. We investigated resource selection of 46 western gray squirrels in response to fuel management treatments at multiple spatial scales at three study sites in the North Cascades using radiotelemetry. Squirrels used fire fuel treated areas more intensively than non-treated areas. However, areas used for nesting by squirrels were characterized by large, tall trees, high levels of dwarf mistletoe infection, high canopy cover and connectivity, and a high percentage of live trees within stands; all characteristics that can decrease with wildfire and fire fuel reduction treatments. Although this study provides no evidence that fire fuel reduction treatments have negatively affected

western gray squirrel habitat selection, future treatments can focus on retaining patches of large trees with some mistletoe infection, and moderate levels of canopy cover and connectivity to conserve elements of western gray squirrel habitat in the North Cascades region.

Introduction

Wildlife populations are influenced by many types of natural disturbances. On the eastern slopes of the Washington North Cascades, wildfire is the dominant natural disturbance that has continually shaped the landscape. Wildfires naturally burn in mosaics of intensity based on the energy and moisture content of fuel, mass of fuel consumed, and the rate of spread of fire (Agee 1993). Low intensity fires burn close to the ground with flame lengths <1m, medium intensity or understory fires have flame lengths of 1-3m, and high intensity "crown" fires burn with flame lengths in excess of 3m (Agee 1993). In response to several large-scale, high intensity forest fires in the early 1900s natural resource managers followed a strict policy of fire suppression from 1910 until the late 1960s, excluding and quickly containing all forest fires whenever possible. The absence of fire has significantly changed the structure of forest stands in many areas of the North Cascades. Key changes include: altered species composition from ponderosa pine (*Pinus ponderosa*) dominant to mixed conifer and/or Douglas-fir (*Pseudotsuga menziessii*) dominant; increase in tree density, often accompanied by decreases in individual tree diameter; an accumulation of dead woody debris; and an increase in forest disease, particularly dwarf mistletoe infection.

Dwarf mistletoe affects both ponderosa pine and Douglas-fir (*Arceuthobium campylopodum* and *A. douglasii* respectively) and is parasitic, deriving water, nutrients, and carbohydrates from host trees. Host trees respond to higher demands for photosynthesis with increased branching, usually in the lower and mid crown; these distorted structures are called mistletoe brooms and can act as ladder fuels, drawing flames higher into the canopy, increasing fire intensity and spread (Hadfield et al. 2000, Beatty and Mathiasen 2003). Dwarf mistletoe combined with increased fuels, including both standing small diameter trees and dead woody debris on the forest floor, have facilitated an increase in the intensity and scale of wildfires in recent years and subsequent implementation of fire fuel reduction management plans in the North Cascades.

Fire fuel reduction treatments are designed to reduce the probability of high intensity “stand-replacing” crown fires through silvicultural activities such as prescribed burning, which generally mimics low intensity fire; mechanical thinning, which also helps remove added fuels and increase tree vigor and resistance to fire; and removal of ladder fuels including mistletoe brooms (Figure 1.1). Fire fuel reduction plans may focus on protection of human life and property maintaining late successional forest structure and wildlife habitat, and/or restoring natural fire regimes. Fire fuel treatments have the potential to positively or negatively affect wildlife populations, including the western gray squirrel.

The Washington Department of Fish and Wildlife listed the western gray squirrel as a Washington state threatened species in 1993 (WDFW; WAC 232.12.011). Populations of western gray squirrels have declined in range and number and are now limited to three geographically isolated areas: in Pierce and Thurston Counties in the southern Puget Trough, in southern Washington in Klickitat, Yakima, and Skamania counties, and in the North Cascades of north-central Washington in Chelan and Okanogan counties (Figure 1). Causes for decline over the past century include over-hunting, automobile accidents, disease, predation, potential competition with introduced squirrels, and habitat loss (Linders and Stinson 2007).

Fire fuel treatments alter habitat by reducing canopy connectivity and cover which can be negative for arboreal squirrels. Reduced connectivity of canopy trees and thick understory in recently thinned areas impedes traveling and foraging of flying squirrels (Carey 2000, 2001) and could similarly restrict arboreal travel of western gray squirrels. Prior study in the Methow Valley indicated that western gray squirrels prefer to nest in large diameter trees, with well connected canopies (Gregory 2005). Similarly, Hamer et al. (2005) concluded that western gray squirrels in Stehekin prefer to nest in trees with higher levels of interlocking crowns and larger mean diameters, that are dominant or co-dominant members of the canopy community and within a group of trees (all characteristics that may aid travel through the canopy). However, decreasing the density of trees also allows remaining trees to increase in diameter over time, which would likely be positive for western gray squirrels. Reduced canopy cover in treated areas also leads to increased snow cover in winter which may seasonally restrict western gray squirrel travel and foraging ability. However, fire fuel treatments also reduce understory cover, which could facilitate ground travel and foraging ability in spring and summer; studies have indicated that western gray squirrels select for areas of lower shrub cover (Gregory 2005, A. Johnston pers. comm.). Pasch and Koprowski (2011) found that shrub cover and understory volume best explained differences between fire-suppressed and fire-prescribed areas in Arizona and that Mexican fox squirrels (*Sciurus nayaritensis chiricahuae*) traveled shorter distances and had smaller home ranges in fire-prescribed areas, indicating that treated areas are higher quality habitat for this species. Leonard and Koprowski (2010) also found that home range sizes of endangered Mount Graham red squirrels (*Tamiasciurus hudsonicus grahamensis*) were smaller in areas that had experienced recent low intensity wildfire.

Dwarf mistletoe treatments as outlined in several fire fuel reduction plans could reduce preferred nesting sites for western gray squirrels. Gregory et al. (2010) found 46% of all nests (n=64) in mistletoe brooms. Mistletoe total broom volume (TBV; Parker and Mathiasen 2004) was also the most influential predictive variable for nest tree selection (Gregory et al. 2010). Western gray squirrels build nests out of branches, needles, and vegetation for resting sites and raising young. Resting nests are typically flat, platform nests, while natal nests and nests used over winter are spherical shelter nests known as dreys. Cavities are also used frequently for natal nests (Ingles 1947, Cross 1969, Gilman 1986) but are less available in the North Cascades. Mistletoe brooms are likely desirable to squirrels because they provide additional structure and vegetative cover for both platform nests and dreys. Garnett et al. (2004) found trees with mistletoe brooms were used more frequently than trees without brooms for many wildlife activities including foraging/caching, nesting, and roosting/resting sites, with mammal use in 80% of broomed Ponderosa pine trees in Northern Arizona (n=42). Evidence of use (presence of nests, cone scales, seed casings, or cone cores) by Abert squirrels (*Sciurus aberti*), the closest relative of the western gray squirrel, was found in 39 (~93%) of these. Similarly, Parks et al. (1999) found significantly more mammalian use, predominantly foraging and nesting, in trees with mistletoe brooms, relative to trees without mistletoe brooms in Douglas-fir forests in Northeast Oregon. Bull et al. (2004) suggest, however, that the type of mistletoe reduction treatment affects impacts to wildlife and that retaining patches of mistletoe infected trees may still provide enough nesting/resting opportunities.

To determine effects of fire fuel reduction treatments in the North Cascades on western gray squirrels we used radiotelemetry to study habitat use at three spatial scales: the nest-tree, nest-site, and the squirrel home range, in the Lake Chelan National Recreation Area (LCNRA) of the North Cascades National Park Complex and the southern Methow Valley.

Methods

Study Area

I studied resource selection of western gray squirrels at two study sites in north central Washington State, USA: the Stehekin Valley, part of the Lake Chelan National Recreation Area (LCNRA) of the North Cascades National Park Complex, and in the Squaw Creek drainage in the Methow Valley. I also used data collected previously as part of a colleague's Master's thesis (Gregory 2005) from the Black Canyon Creek drainage in the Methow Valley for comparative analysis. All study sites are mixed conifer/deciduous habitats composed primarily of Douglas-fir and ponderosa pine. Summers are hot and dry; winters are cool and wet with temperatures ranging from -20 to 40°C and average rain/snowfall of 50 cm (2005-2010 NCDC NOAA Climatological Data). Additional study site information: p.4.

Four fire fuel treatments across our study sites were included in analysis: the Buckner Orchard in Stehekin, a 48-ha area that has been thinned and prescribed burned in a step-wise fashion over small spatial scales at approximate two-year intervals starting in 1996 as part of the Lake Chelan National Recreation Area Fire Fuel Reduction Area (Figure 1.1, 1.2), the 440-ha East Douglas treatment of the Hungry Hunter Ecosystem Management Project at Squaw Creek which was prescribed burned and thinned as part of a timber sale over a large spatial scale in 2008, and the Black Canyon Creek treatment, a 430-ha area that was prescribed burned and thinned once in 1995 (Figure 1.3). We also included an approximately 690-ha area surrounding the Stehekin Landing as a treated area, because it has been thinned for fire fuel reduction and hazard trees since 1995 and was naturally burned at medium intensity in the Flick Creek fire of 2006 (Figure 1.2).

Study area extents were delineated using all squirrel relocations surrounded by a 500-m buffer, representative of average breeding male movements and consistent with Gregory (2005). In Stehekin, the 500m buffer only included the northwestern to northeastern side of the lake, not encompassing or crossing the water because western gray squirrels have never been observed on the south side of Lake Chelan. Total study area sizes were 3,468 and 5,773 hectares in Stehekin and Squaw Creek respectively. Fire fuel treated areas covered 37.4% and 17.8% of the Stehekin and Squaw Creek study areas respectively. The fire fuel treatment at Black Canyon covered 43.2% of the total study area.

Field Methods

Trapping

Between April 2008 and September 2011 we live-trapped squirrels using wire mesh 15 x 15 x 48-cm Tomahawk live traps (Tomahawk Live Trap Co. Tomahawk WI, USA). Traps were spaced between 50m and 80m apart and placed on the North side of the base of large diameter trees to maximize shade coverage. Traps were baited with whole English walnuts and wired open with metal 3.2-cm book rings for a pre-baiting period to “train” squirrels to enter the traps. Trapping began when bait was removed from approximately half of the traps which took 1 to 4 weeks of pre-baiting on average. Once set, traps were checked approximately every 2 hours, with animals left in traps for no longer than 4 hours. Traps were opened just prior to sunrise, with most trap checks conducted before noon to minimize squirrel exposure during the hottest parts of the afternoon; trapping was not conducted on days with rain, snow, or extreme high or low temperatures.

Handling

Captured animals were processed in a handling bag (Koprowski 2002) modified with an additional ventral opening. Squirrels were weighed to the nearest 5g with a 1000-g or 2500-g Pesola spring scale, sexed, and examined for reproductive status: on males this is the measurement and position (scrotal or abdominal) of testes, for females enlarged teats, presence of nuzzle marks and/or vulvar swelling. The hind foot was measured from the hind edge of the heel to the distal edge of the toe pad end using a clear mm ruler. All captured squirrels were marked with uniquely numbered ear tags (model 1005-3, National Band and Tag Co., Newport, KY, USA) and most were also fitted with radiocollars (Holohil Model SC-2C). We targeted females for collaring when possible because they provide more information on reproductive success, habitat use, and nest site selection. Females generally have small home ranges and exhibit territoriality, whereas males inhabit larger home ranges of potentially lower quality (Linders 2004, Gregory 2005, Vander Haegen et al. 2005), allowing travel between multiple females to maximize reproductive opportunity (Steele and Koprowski 2001). Radiocollars weighed approximately 12g and consisted of a metal cable protected with a thick plastic coating and covered with Tygon tubing to prevent abrasion to the squirrel. Only squirrels weighing $\geq$ 600g were fitted with radiocollars. Guidelines of the American Society of Mammalogists recommend that radiocollars weigh less than 5% of an animal's body weight; in this case the collar to weight ratio was approximately 2% to allow for an extra margin of caution. All trapping, handling and radiocollaring methods were approved by the University of Washington Institutional Animal Care and Use Committee (IACUC). Protocol number: 2479-29.

Radiotelemetry

Radio-collared squirrels were located with ground-based homing techniques using two-element, hand-held directional antennas and portable TR-4 receivers (Telonics, Mesa, Arizona, USA). Activity of the squirrel was monitored prior to homing based on the consistency of the signal: changes in volume and pulse rate indicated active squirrels, whereas a consistent volume and pulse rate for 3 minutes indicated inactive squirrels. Tracking was discontinued if signal strength decreased abruptly more than once during pursuit, indicating the animal was “running from observer” and movements were being influenced. Squirrels were tracked to the tree (or location on the ground) when possible and locations confirmed with visual sightings using 10 X 40 binoculars. The condition of the animal and behavior, such as running on ground, traveling through canopy, foraging, or sitting in tree, was also documented for most telemetry locations. Universal Transverse Mercator (UTM; NAD 1983) coordinates were recorded on hand-held GPS units and plotted on Geographic Information Systems (GIS) maps.

Tree locations were examined for nests for a minimum of 10 minutes using 10 X 40 binoculars. For each nest we recorded UTM coordinates (NAD 1983), tree species, tree diameter at breast height (DBH), tree height, height of the nest in the tree, condition of the nest, color of the nest, whether mistletoe was present in the tree, and which squirrels had been found in the nest and how frequently. We noticed that nests were often located near each other in patches and quantified average nest patch size and distance between patches by delineating ellipses connecting the outermost nests in each patch and measuring the average distance between adjacent nest patches (nearest edge to nearest edge) in ArcMap (Esri 2009).

Squirrels were located at least every 5 days by a field researcher; on average 3-6 days per week which increased data consistency and timely discovery of mortalities. To ensure independence between observations (White and Garrott 1990, Swihart and Slade 1997, Otis and White 1999), relocations were spaced across the diurnal period and individual squirrels were located 3 times per day at maximum with a minimum lapse of 2 hours: a period adequate for a squirrel to traverse its home range (Linders et al. 2004). All squirrels were located with approximate equal frequency. Field forms are located in Appendix B.

Analytical Methods

Calculating Home Range Size

We computed home ranges for each squirrel with a minimum of 30 locations across the season of interest (Kernohan et al. 2001). Home range sizes were calculated with Hawth's Analysis Tools for GIS (Beyer 2004) using a fixed kernel estimator (Worten 1989), which is considered most accurate along the outer portions of the home range (Seamen et al. 1999). We defined the breeding season in the North Cascades as March 1-July 31 and non-breeding as August 1-February 28 based on temperature and precipitation data (2005-2011 NCDC NOAA Climatological Data) and field observations of squirrels. We removed repeated observations of squirrels in the same nest to correct for spatial autocorrelation. We used the bivariate plug-in smoothing parameter calculated with package ks (Duong 2012) in R version 2.7.2 (R Development Core Team 2008) because home range sizes reached relative asymptotes at smaller sample sizes using this smoothing parameter compared to least-squares cross-validation (LSCV; Figure 1.4). The bivariate plug-in smoothing parameter was also most consistent across varying sample sizes and created kernel distributions that fit observed movement patterns and spatial configuration of telemetry points best (Gitzen et al. 2006).

Resource Selection

We examined resource selection of western gray squirrels at three spatial scales: nest-trees, nest-sites, and home ranges. For the analysis of nest-trees we compared characteristics of used nest trees to characteristics of randomly selected unused nest trees within plots inscribed around the nest tree. For the analysis of nest-sites we compared average characteristics of habitat variables measured within plots around nest-trees to plots distributed throughout each squirrel's home range. At the home range scale, we compared plot-based habitat measurements between high and low-use areas within individual squirrel home ranges. Temporal scales were not examined directly due to sample size limitations, but were accounted for by stratifying analyses by season (breeding vs. non-breeding). Measuring resource selection at only one scale introduces bias through the researcher's definition of what resources are available with arbitrary selection of the study area or time (Johnson 1980) and disturbance processes and management activities (ie. wildfire and fuel reduction treatments) may affect squirrels at different spatial scales.

We measured habitat variables in nested 25.25m (0.2 ha), 10.6m (0.035 ha), and 5.6m (0.01 ha) vegetation plots (Figure 1.5). At the scale of the nest-tree, variables were measured at 50 randomly selected nest sites in each study area identified through radiotelemetry. Tree characteristics (Table 1.1) were measured for nest-trees and eight randomly selected trees within a 25.25m radius plot centered around the nest tree. Random trees were selected through the procedure described by Skalski (1987) which corrects for bias toward the center of circular plots. Following Parker (2001) and (Parker and Mathiasen 2004), we measured mistletoe TBV by visually dividing the entire height of the tree from ground level to treetop into thirds, with each rated between 0 and 3: 0 = no brooms, 1 = 1-33%, 2 = 34-66% and 3 = ≥ 67% occupied by brooms; the sum of the ratings had a maximum of 9. We used maximum scorch height as an indicator of fire (both wildfire and prescribed) presence and intensity.

At the scale of the nest-site, measurements from the nest tree and random trees were averaged to create corresponding average tree variables representing the site. Average DBH at sites was summarized by Quadratic Mean Diameter (QDBH) which corresponds to the DBH of a tree of average basal area for the stand. Additional site variables were measured in the 10.6m and 5.6m radius plots (Figure 1.5). GIS layers were also included in nest-site analysis (Table 1.2).

Elevation, aspect, and slope were summarized from map layers created from 10m Digital Elevation Models (DEMs) provided by USFS. Aspect was converted to a categorical variable: N: 315 to 360°, 0 to 45°, E: 45 to135°, S: 135 to 225°, W: 225-315°. We also included forest type as a categorical variable for analysis using the NCLEVEL2 vegetation data created using Landsat imagery in 1993 for the North Cascades grizzly bear ecosystem evaluation effort (Almack et al. 1993) provided by Pacific Biodiversity Institute. A statistical accuracy assessment as part of this report using polygon analysis (Dicks and Lo 1990) revealed that this layer was 93.2% accurate, however, we noticed some inconsistencies with our ground vegetation data, particularly in Stehekin. Most of the habitat in Stehekin was classified as ponderosa pine under NCLEVEL2, however, the tree species composition summarized from our vegetation sampling plots following Gregory (2005; Table 1.2) was predominately mixed conifer. Sample sizes for many of the vegetation categories were too low for meaningful comparisons, therefore we combined the 18 vegetation types identified at our study sites into 6 categories: Ponderosa pine, mixed conifer, deciduous, herbaceous, shrub-steppe, and other (Table 1.3). We also calculated the distance from each vegetation plot to the nearest water source and road in ArcMap. Fire fuel treatment was represented as a binary variable (treated or untreated) based on whether sampling points fell in or outside fire fuel treatment polygon layers provided by USFS and NPS.

At the scale of the squirrel home range we evaluated core-area selection by dividing each squirrel's fixed kernel (1m raster cell size) into polygons representing the upper and lower 25% of their home range to increase our ability to detect potential differences between high and low-use areas. For each squirrel we randomly selected 9 plots for vegetation sampling: 3 in high-use areas (upper 25%) and 6 in low use (lower 25%) on average. Vegetation sampling methods for high and low-use areas followed the same protocol as for nest-sites. More habitat plots were required in low-use areas because these habitats are larger and more variable. For squirrels with smaller home ranges it was not always possible to sample 3 high-use and 6 low-use areas without overlap among 25.25-m plots or nest sites; alternately, we increased the number of high and low use-plots for squirrels with very large home ranges so that plots sampled at least 10% of each squirrel's high-use and 1% of each squirrel's low-use area at minimum. High and low-use areas with a high degree of overlap between squirrels were combined and habitat plots randomly selected from pooled kernels. This resulted in the repeated use of some vegetation plots for analysis to represent several individual squirrels. On average, each high-use vegetation plot was used to represent 2.72 ± 0.11 [SE] squirrels (range 1- 8); each low-use vegetation plot represented 1.91 ± 0.09 [SE] squirrels (range 1-6). Depending on the degree of overlap and the size of each squirrel's high and low-use areas each squirrel shared between 0% and 75% of high-use, and 0% and 100% of low-use vegetation plots with one or more other squirrels. We consider each squirrel's resource selection to be statistically and biologically independent because western gray squirrels are solitary, and we infrequently radio-collared individuals from the same family group (Millspaugh et al. 1998). A random selection of high and low-use sites which did not contain a nest within 25.25m were also used for comparison with used nest-sites at the scale of the nest-site. Because repeated observations of squirrels to the same nest were removed prior to home range calculation, core-area analysis represents a separate level of habitat selection, more indicative of non-nesting activities such as foraging.

We analyzed nest-tree and nest-site selection for Stehekin and Squaw Creek only. For core-area analysis we also sampled vegetation in high and low use areas for squirrels from Black Canyon Creek (using radio-telemetry information provided by Sara Gregory 2005).

We also evaluated resource selection across each squirrel's home range with Utilization Distributions (UDs), and Resource Utilization Functions (RUFs; Marzluff et al. 2004). Utilization Distributions are probability density functions (PDFs) which describe the relative frequency distribution for the location data over a specific time period (Van Winkle 1975). The height of a UD:

$$\hat{f}_{UD}(x,y) \text{ at location } (x,y)$$

represents the amount of use at that location relative to other locations in the plane (Silverman 1986). Resource Utilization Functions measure resource use as a continuous response variable, an argued improvement over the more commonly used Resource Selection Functions (RSF) (Manly et al. 1993) which rely on dichotomous characterization of response variables (used vs. available) and use of relocation points as the experimental unit (Keating and Cherry 2004). We believe that enough squirrels were radio-tracked in both areas to encompass variation between individuals and represent a random sample of the population (Manly et al. 2002), allowing individual animals to be treated as independent experimental units (Aebischer et al. 1993). Treating individual squirrels as the experimental unit minimizes pseudoreplication: artificially high estimates of precision generated through dependence or correlation of sample units

(Hurlbert 1984). RUFs have also been found to be more useful for species with small home range sizes, such as the western gray squirrel (Long et al. 2009). We used the UDs to create RUFs for each squirrel and an average for all squirrels collectively. We defined the spatial extent of space use as the 99% fixed-kernel home range boundary which limited inference about resource use to the area inhabited by the animal based on relocation points (Marzluff et al. 2004). Kernels were created using a 10 X 10m pixel size which matched the scale of GIS layers used for nest-site, core-area, and RUF analysis.

Statistical Methods

To identify groups of variables that contribute to western gray squirrel nest-tree, nest-site, and core-area selection we used conditional logistic regression with the information theoretic approach (Burnham and Anderson 1998, 2002). Logistic regression is appropriate because the dependent variables are binary: trees are either used or available for nests, sites are either used or available for nesting, and represent either high or low use for squirrels. Using habitat variables summarized from GIS maps and measured at vegetation sampling plots (Table 1.2) we created a set of *a priori* candidate models for each level of resource selection analysis (Tables 1.4, 1.5) based on previous work in the North Cascades (Gregory 2005, Hamer et al. 2005, Gregory et al. 2010), observations from pilot field work in 2008, and variables of interest to natural resource managers. For example, Model 1 for nest-tree selection (treest) was the most parsimonious model for western gray squirrel nest-tree selection at Black Canyon Creek; model 4 (nest-tree univariates) included the most influential variables from *post-hoc* AICc (Aikake's Information Criterion) analysis (Gregory 2005). Variables describing fire fuel treatments and wildfire were of particular interest to this study and included in multiple models. Additionally, we were interested in comparing models with variables collected from ground measurements with models using only existing GIS layers (e.g. models 7, 8; Table 1.5) to see how well western gray squirrel habitat selection could be predicted in other areas without additional habitat sampling. Models were similar across spatial scales to facilitate comparisons.

Collinearity among discrete variables was tested with Pearson's correlations and scatter plots. Variables with a coefficient >0.7 were considered correlated and one was removed from final analysis. Continuous variables were screened by calculating their individual variance inflation factors (VIF). Those with a VIF score >10 were considered closely related, and one was removed from analysis to facilitate final interpretation of coefficients (Neter et al. 1996). We decided which variable to remove based on field observations of habitat selection by squirrels and land management questions.

We calculated AIC values for all *a priori* models with the second-order bias adjustment for small sample size relative to the number of parameters. We ranked the models by AIC_c values in ascending order, calculated the differences (ΔAIC_c), and used their weights (w_i) to obtain the probability that each model was the best in the set. Models within two AIC units of the best model were considered to be competitive. The best model(s) at each scale were then compared to a null model using a likelihood ratio test.

We estimated regression coefficients and odds ratios using a generalized linear mixed model (GLMM) stratified by squirrel for nest-site and core-area selection, and nest-site for nest-tree selection using package lme4 (Bates et al. 2011) in R version 2.14.2 (R Development Core Team 2008). GLMM takes into account the random effects of variables induced by the non-random sampling structure (nest-trees and comparison trees were located within nest-sites identified through radio-telemetry, high and low-use plots were randomly selected by squirrel).

Odds ratios were calculated by exponentiating the coefficient, 95% confidence intervals were calculated with the formula: $e^{(\beta \pm 1.96SE)}$.

We calculated averages of continuous variables and counts of categorical variables between used and available nest-sites, high and low squirrel use areas, and fire fuel treated and untreated areas (a random selection of high and low use vegetation plots post-stratified to represent each fire fuel treatment and adjacent untreated site). We then compared the number of nest sites in treated and untreated areas, and averages of continuous variables potentially affected by fire fuel reduction treatments between treated and untreated sites with Wilcoxon Signed Rank tests in R version 2.14.2 (R Development Core Team 2008).

For RUF analysis we used multiple regression to evaluate the seven GIS layers simultaneously and calculated maximum likelihood estimates using both unstandardized and standardized coefficients using package ruf (Handcock 2004) in R version 2.10 (R Development Core Team 2008). Unstandardized regression coefficients represent the raw spatial scales of variables, and are necessary for predicting expected use of resources, while standardized coefficients allow comparisons of the relative influence of resources on animal use, regardless of the measurement scale (Zar 1986, Marzluff et al. 2004). To create average RUFs across groups and study sites we averaged the coefficients and associated standard errors from the individual squirrel regressions using equation 1 for unstandardized coefficients, and equation 2 for standardized coefficients. Equation 1 assumes that each animal is independent, equation 2 is more conservative (generates wider confidence intervals) and accounts for inter-animal variation (Marzluff et al. 2004).

Eq. 1 $$\mathrm{Var}(\hat{\bar{\beta}}_j^{*}) = \frac{1}{n^2}\sum_{i=1}^{n} \mathrm{SE}^2\,\hat{\beta}_{ij}^{*}.$$

Eq. 2 $$\mathrm{Var}(\hat{\bar{\beta}}_j) = \frac{1}{n-1}\sum_{i=1}^{n} (\hat{\beta}_{ij} - \hat{\bar{\beta}}_j)^2.$$

Spatial autocorrelation between the deviations in neighboring kernel pixels was addressed by fitting a Matern regression model (Handcock and Stein 1993) to the UD with spatial correlation as a function of the distance between pixels (Marzluff et. al 2004).

Results

Over 4 field seasons we captured a total of 61 squirrels: 24 in Stehekin, 37 at Squaw Creek. We collared 12 females and 10 males in Stehekin, and 12 females and 12 males at Squaw Creek. Of those, 12 females and 5 males from Stehekin, and 11 females and 10 males from Squaw Creek (total sample size: 38 squirrels) had greater than 30 relocations and were used for home range and habitat use analysis. We were also able to use data on 12 squirrels: 8 females, 4 males, from Sara Gregory's 2003-2004 research at Black Canyon Creek (Gregory 2005) which created a total sample size of 50 squirrels: 31 females, 19 males. Fourteen (3 male, 11 female) squirrels in Stehekin, 11 (6 male, 5 female) squirrels at Squaw Creek, and 12 (4 male, 8 female) squirrels at Black Canyon had home ranges encompassing fire fuel treated and untreated areas.

We collected a total of 3813 radiotelemetry locations of collared squirrels: 1690 in Stehekin, 2124 at Squaw Creek, in addition to Gregory's 1020 from 2003-2004. Of these new relocations we were able to visually confirm 22% of the locations in Stehekin and 44% at Squaw Creek. Another 57% percent of locations in Stehekin and 37% of locations at Squaw Creek were confidently identified to the tree allowing accuracy within 5m for 92% of locations at both study areas. The number of fixes used to calculate home range sizes for the 38 squirrels at Stehekin and Squaw Creek with greater than 30 relocations ranged from 30 to 192. Squirrels were tracked on average for 6 months, range 3-17 months.

Nest Characteristics

We located a total of 54 nests in Stehekin and 118 nests at Squaw Creek in the Methow Valley. Each squirrel used multiple nests; 4.60 ± 0.69 [SE] on average in Stehekin (range 1-12), 9.29 ± 1.01 [SE] on average at Squaw Creek (range 1-19), so that each nest was used by at least 2 ± 0.1 [SE] squirrels on average (range 1-9). Nests were often found in clusters of 3 to 10 (average 5.23 ± 0.74 [SE]) within an average patch size of 3.44 ± 0.96 [SE] hectares (range 0.02-12.46 ha). We identified 5 nest clusters at Squaw Creek and 8 in Stehekin. The average distance between adjacent nest clusters (nearest edge to nearest edge) was 254.33m ± 24.08 [SE] (range 144-310m). Most nests (136/172) were spherical shelter nests or dreys, with a smaller number of platform nests (34/172; Figure 1.6). One cavity nest was found at each study site, in a ponderosa pine snag at Squaw Creek and a bigleaf maple (*Acer macrophyllum*) snag in Stehekin. Many nest trees contained dwarf mistletoe brooms and there was a higher percentage of mistletoe infected nest trees in Stehekin (56%) compared to Squaw Creek (30%) and Black Canyon Creek (47%) (Gregory et al. 2010; Figure 1.6). Consistent across study areas, nests were found most frequently on the south side of the tree followed by west, east, and north (Figure 1.6) in trees that were 51 ± 1.7cm [SE] and 65 ± 3cm [SE] DBH on average at Squaw Creek and Stehekin, respectively, compared to 45 ± 1.8cm [SE] DBH at Black Canyon Creek. In Stehekin nests were most commonly found in Douglas-fir (43/56) followed by ponderosa pine (11/56); at Squaw Creek there were equal numbers of nests in Douglas-fir and ponderosa pine. Both of these findings contrast with Gregory's (2005) findings at Black Canyon Creek where squirrels predominately nested in ponderosa pine (Figure 1.6). The mean ratio of nest height to tree height was 0.57 ± 0.01 [SE] at Squaw Creek, very similar to Black Canyon (Gregory et al. 2010), and 0.50 ± 0.02 [SE] in Stehekin, however, trees were taller in Stehekin on average (Figure 1.7). In

Stehekin 62% of nest sites were located in areas with recent fire fuel treatments or wildfire, compared to only 36% at Squaw Creek and 54% at Black Canyon. Thirty percent of nest trees at Squaw Creek and 47% of nest trees in Stehekin showed evidence of recent wild or prescribed fire with scorch marks of average heights 3.28 ± 0.4m [SE] and 7.15 ± 0.8m [SE]. Eight nests were found in dead snags.

Nest-Tree Selection

There were no significant correlations or multicollinearity between nest-tree predictor variables. Two models were within 2 AIC_c units carrying 91% of the AIC_c weight combined (Table 1.6). The likelihood ratio test determined that the residual deviances of both models were significantly less than the residual deviance of the null model (P<0.001). The coefficients for mistletoe total broom volume (TBV), connectivity, and tree DBH all had positive associations with nest-tree selection, meaning that holding all other variables constant, the odds of a squirrel choosing a tree for nesting increase as mistletoe TBV, connectivity, and DBH increase from their reference levels of zero (Table 1.7). The odds of a squirrel selecting a tree for nesting decreased as scorch height (an indication of burn severity) increased. Average scorch heights were significantly higher in treated areas compared to untreated areas (W= 649, P<0.001; Table 1.8). The 95% confidence intervals for the odds ratios did not include 1.0 for mistletoe TBV and connectivity, indicating that these habitat variables are strong predictors of western gray squirrel nest-tree selection.

Nest-Site Selection

We found no significant correlations or multicollinearity between nest-site predictor variables. Model 6 (nest-tree comparison) had the highest likelihood of being the best model with a weight of 64% (Table 1.9). The likelihood ratio test determined that the residual deviance of this model was significantly lower than that of the null model (P<0.001). Similar to nest-tree selection, coefficients for average TBV, average connectivity and QDBH had positive associations with western gray squirrel nest-site selection, however only connectivity had a 95% odds ratio confidence interval that did not include 1.0 (Table 1.10). Strong predictor variables for nest-site selection included percent mistletoe and canopy cover. Canopy connectivity was lower on average in treated areas, however differences were not statistically significant across treatments (W= 2231, *P*= 0.122; Table 1.8). At both study sites there were fewer nest-sites in fire fuel treated areas than untreated areas, however this difference was also not significant (W= 4700, *P*= 0.397; Table 1.11).

Core-Area Selection

There was no significant correlation or multicollinearity between variables used to examine core-area selection. The full model had the highest AIC_c rank and weight, with an almost 100% likelihood of being the best model (Table 1.12). The likelihood ratio test determined that the residual deviance of the full model was significantly lower than the null model ($P<0.001$). Significant coefficients with positive relationships to core-area selection included: North facing aspects (in relation to all other aspects), mixed conifer forest in relation to ponderosa pine forest, canopy cover, lowest live crown (high-use areas were more likely to have higher lowest live crowns on average), and average tree height. Mixed conifer forests have a stronger association with high use areas than estimated due to the misclassification of forest types in Stehekin with the NCLEVEL2 vegetation layer. Additionally, high-use areas were more likely to have been recently fire fuel treated or burned at moderate intensity in wildfire, while also having higher average scorch heights and higher percentages of live trees than low-use areas within 10.6-m radius plots. Mistletoe TBV and connectivity had the opposite relationship to core-area selection as observed for nest-tree and nest-site selection: squirrel high-use areas were more likely to have lower TBV and connectivity, however, connectivity was higher on average in high use plots. The odds of a squirrel selecting an area as a core-area also decreased as counts of coarse woody debris and the number of understory species increased (Table 1.13, 1.14).

Resource Utilization Functions

Table 1.15 provides estimates of unstandardized RUF coefficients for squirrels by study site and for females only (overall and by study site), as females provide more consistent estimates of resource selection across seasons. The RUF from the pooled analysis of squirrels across our three study areas indicates highest use of areas within their home range that are lower in elevation, away from roads, and have been recently fire fuel treated and/or burned in wildfire. Relative to ponderosa pine forest (the most abundant forest type across study sites and included in the RUF intercept coefficient estimate), squirrel use was highest in mixed conifer forest types; lowest use was in "other" forest types including water, bare rock, wet soil and gravel, fallow land and pasture, and orchards and crops. South facing aspects close to water also had higher use within the home range. Treatment was the most significant and consistent resource attribute related to concentration of western gray squirrel use across all study sites ($P<0.001$, confidence interval does not contain zero, 87% of squirrels had use positively associated with treated areas; Table 1.16).

In Stehekin, distance to nearest water ($P= 0.025$) and treatment (treated/burned, $P= 0.001$) were both positively related to utilization by western gray squirrels (Table 1.17). The confidence intervals for both of these habitat variables did not include zero, and resource selection patterns were consistent among the majority of squirrels (79% and 93% of squirrels exhibited positive associations with distance to water and treatment respectively). Use by squirrels in Stehekin was also positively related to west-facing slopes and negatively to "other" types of forests (relative to north aspects, ponderosa pine forest; Table 1.17).

In Squaw Creek, use by squirrels was positively associated with fire fuel treatments (*P*<0.001) and negatively with distance to roads (*P*=0.006) (Table 1.18). Neither confidence interval for these resources included zero and patterns were consistent across individual squirrels (100% of squirrels with home ranges encompassing fire fuel treated areas showed a positive association with treated areas, 81% of squirrels showed a negative association with distance to nearest road). Elevation was negatively related (high use areas within squirrel home ranges were at lower elevations), and mixed-conifer forests were positively related to utilization by squirrels in relation to ponderosa pine forests (Table 1.18).

Squirrels at Black Canyon showed similar associations with resource attributes, however most confidence intervals encompassed zero, so *P*-values were not significant. There was also less consistency among squirrels at this study site (Table 1.19).

Discussion

Western gray squirrels used fire fuel treated areas disproportionately within their home ranges indicating that recent fire fuel reduction treatments and moderate intensity wildfires have not negatively affected western gray squirrel habitat at the home range scale. In addition to the strong, significant correlation between fire fuel treated and high use areas from the Resource Utilization Function, the treated/untreated GIS variable was positively associated with squirrel high-use areas in combination with other ground-based habitat variables for AIC_c logistic regression analysis at the core-area scale (Table 1.13). The treated/untreated variable was also positively associated to squirrel nest-site selection in model 12, which was just outside the competitive range of AIC_c values (Table 1.9, Table 1.20). This demonstrates that there is some consistency among all measured scales of western gray squirrel resource selection analyses related to fire fuel treatment history.

Causation of western gray squirrel resource selection in response to fire fuel reduction treatments cannot be assessed without baseline information on western gray squirrel habitat selection at study sites prior to fire fuel treatments. If the opportunity arises to conduct a radio-telemetry study before and after future fire fuel treatments more definitive cause and effect conclusions could be made regarding fuel treatments and western gray squirrel habitat selection. An ideal experimental design would involve randomly assigning treatments to evenly divided portions of existing squirrel home ranges. Our results are limited to analysis of habitat use after treatments by squirrels with home ranges unevenly encompassing treated and untreated areas (Figure 1.8). In Stehekin, most of the squirrels we collared had home ranges near fire fuel treated areas. However, in Squaw Creek, trapping success was much higher outside of recently treated areas (especially for females) indicating that squirrels may be avoiding treated areas at a larger spatial scale than we examined, for example, through initial selection of their home range site. The large area of the East Douglas treatment at Squaw Creek relative to squirrel home range size (see Chapter 2 for home range sizes) also decreased the ability of squirrels to travel in and out of fire fuel treated areas on a daily basis. It was more common for squirrels to spend the majority (or all) of their time in either treated or untreated areas at this study site. Suitable habitat in Stehekin seems more limited due to sharp elevation and vegetation gradients, giving squirrels less of a choice in where to center their home range. The 2006 Flick Creek fire also affected a large portion of the Stehekin study site, reducing the ability of squirrels to use untreated areas. Additionally, our research questions and study design led to target trapping of squirrels in and on the fringe of fire fuel treatment areas, which could have biased our sample.

There are also limitations in drawing conclusions on the effects of fire fuel treatments on squirrels based solely on the binary classification of treated versus untreated areas derived from

GIS layers. Considerable differences existed between wildfire and fire fuel treatments across study sites including when the treatment was completed, the geographic extent, and the intensity of thinning and fire (prescribed and wildfire); and none of these attributes were represented in the GIS classification. For example, it had been nearly 10 years since the Black Canyon fire fuel treatment when Gregory monitored resource selection patterns of western gray squirrels. Fifteen years had passed by the time we sampled vegetation at this site which is likely why we did not observe marked differences in habitat variables between treated and untreated areas (Table 1.8). In contrast, squirrels were monitored just one season after the East Douglas treatment at Squaw Creek.

The general agreement of results from RUF analysis based on GIS variables and core-area analysis based on GIS and ground-measured habitat variables suggests that existing GIS layers can provide valuable information on resource selection when detailed measurements from ground sampling are not available, however. Our average RUF can be used to extrapolate habitat quality for western gray squirrels in additional areas. The only significant inconsistency within GIS variables within the home range scale of selection was related to aspect: North facing aspects (relative to all other aspects) were significantly associated with squirrel high-use areas under core-area selection analysis, while RUF analysis indicated higher use of all other aspects (especially west) within squirrel home ranges. We believe this discrepancy is an artifact of different sampling and statistical methods associated with two ways of identifying high use areas within home ranges and does not represent a meaningful biological difference. Aspect is not a critical component of western gray squirrel habitat and is not manipulated through land management activities. Sampling and statistical methods may also have contributed to the observed difference in sign of the coefficient for distance to roads between Stehekin and the Methow Valley, or may represent meaningful differences between study sites.

A limitation of GIS-based RUF analysis, and all analyses that only examine resource selection at one scale, is that inferences about resource use are dependent on the estimated spatial extent of the area used by animals (Johnson 1980, Marzluff et al. 2004). By evaluating resource selection at multiple spatial scales, we gained a more complete understanding of essential habitat variables for multiple life history requirements and daily activities of western gray squirrels (e.g. foraging, nesting, resting, and reproduction). Different habitat variables are important for nesting and non-nesting activities (foraging) of western gray squirrels because models from the same set ranked differently among scales.

At the scale of the core-area, the full model ranked highest, indicating that the *a priori* model set was weak for this scale of resource selection analysis, which makes it difficult to draw conclusions about the relative importance of habitat variables within this scale. One surprising influential variable for core-area selection warranting further investigation is canopy cover, which was not significantly related to western gray squirrel resource selection in previous studies (Gillman 1986, Foster 1992, Linders 2000, Gregory 2005) and therefore was not included in many *a priori* candidate models. Our findings suggest that canopy cover may be more significant than previously reported and could be an important variable to monitor as it can be significantly reduced with fire fuel reduction treatments.

Nest-tree characteristics and selection in Stehekin and Squaw Creek closely fit patterns observed by Gregory et al. (2010) at Black Canyon. The only key difference between our study sites, Black Canyon, and southern Washington (Linders 2000) was the higher proportion of nests built in Douglas-fir trees compared to ponderosa pine, which is likely a result of the higher ratio of Douglas-fir at our sites. More nests were found in Douglas-fir trees at study sites in Klickitat County where Douglas-fir was the dominant species (M. Vander Haegen, WDFW, pers. comm.). This study strengthens conclusions that dwarf mistletoe total broom volume (TBV) has a strong influence on western gray squirrel nest-tree selection in the North Cascades, in addition to DBH and connectivity (Gregory et al. 2010). Of note, the majority of squirrel nests, including those built within mistletoe brooms, were in the top 1/3 of the tree. Retaining high mistletoe brooms can protect western gray squirrel nest-sites while still reducing ladder fuels. The same tree variables were also strongly associated with nest-site selection. Nest-sites contained a larger number of mistletoe broomed trees, all with higher average TBV scores, and higher average QDBH and connectivity, indicating that one large well-connected tree with mistletoe brooms in an area may not be sufficient to encourage nesting.

It is clear that fire fuel reduction treatments are essential for preventing large-scale, high intensity wildfires, restoring natural and ecological processes, and protecting human life and property and may be beneficial to western gray squirrels at certain scales of habitat selection. However, we encourage land managers to also consider the effects of future fire fuel reduction treatments on the smaller scale ground-based habitat variables we found influential for western gray squirrel nest-site, and nest-tree selection. When managing for optimal squirrel nesting habitat, patches containing numerous potential nest trees with these characteristics should be maintained in forest stands. Average patch size and spatial configuration of nest clusters, and home range and overlap of breeding females (Chapter 2) at our study sites support recommendations outlined in the WDFW Management Recommendations of Washington's Priority Habitats and Species for western gray squirrels (Linders et al. 2010). We did not observe significant differences in average QDBH, TBV, canopy cover and connectivity between fire fuel treated and untreated areas across study sites, indicating that previous/current fire fuel reduction treatment protocols have retained an adequate number of suitable habitat patches for western gray squirrel nesting and foraging in the North Cascades.

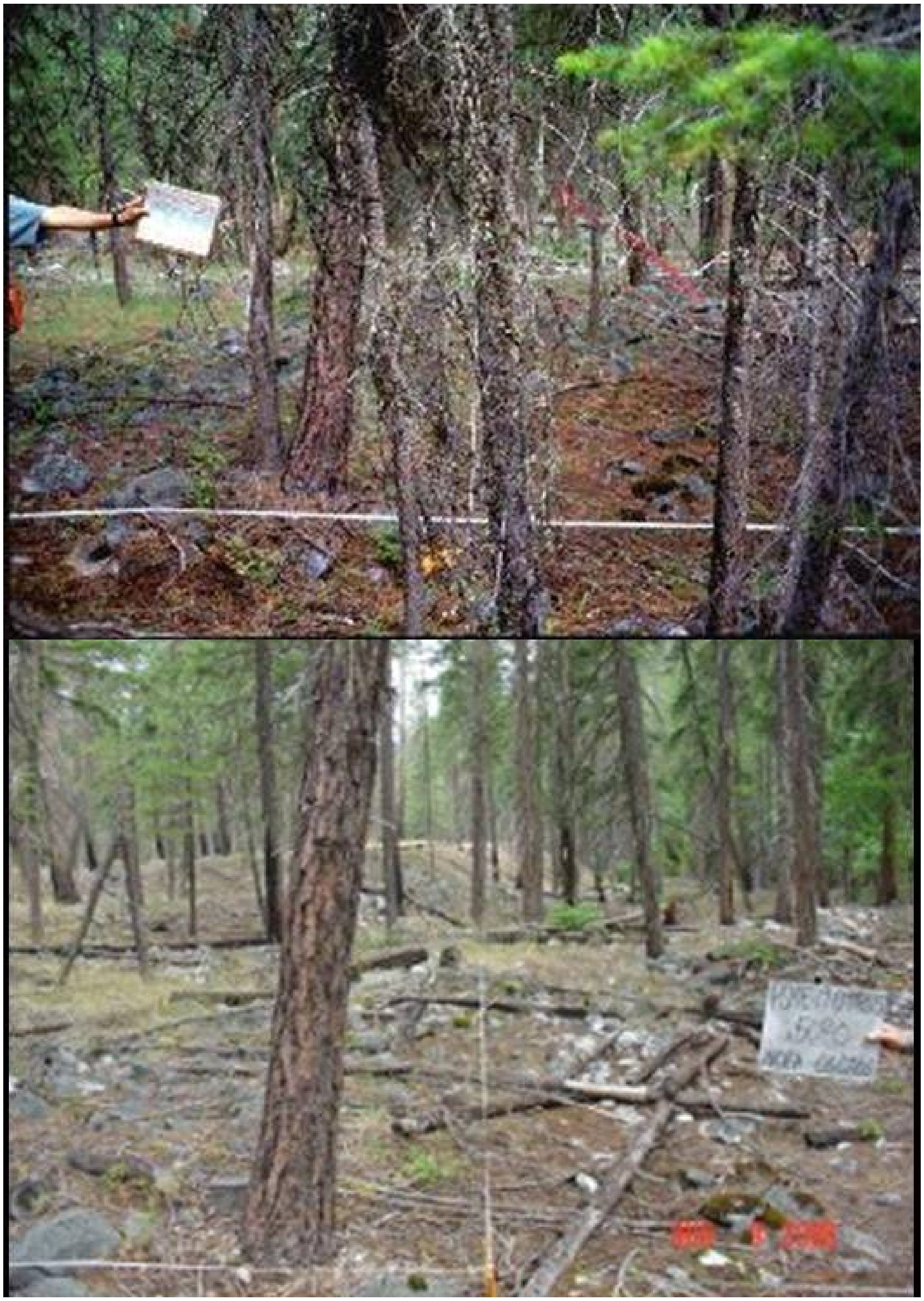


Figure 1.1. Example of a fire fuel treatment area in Stehekin. Top: before treatment, bottom: after prescribed burning and thinning.

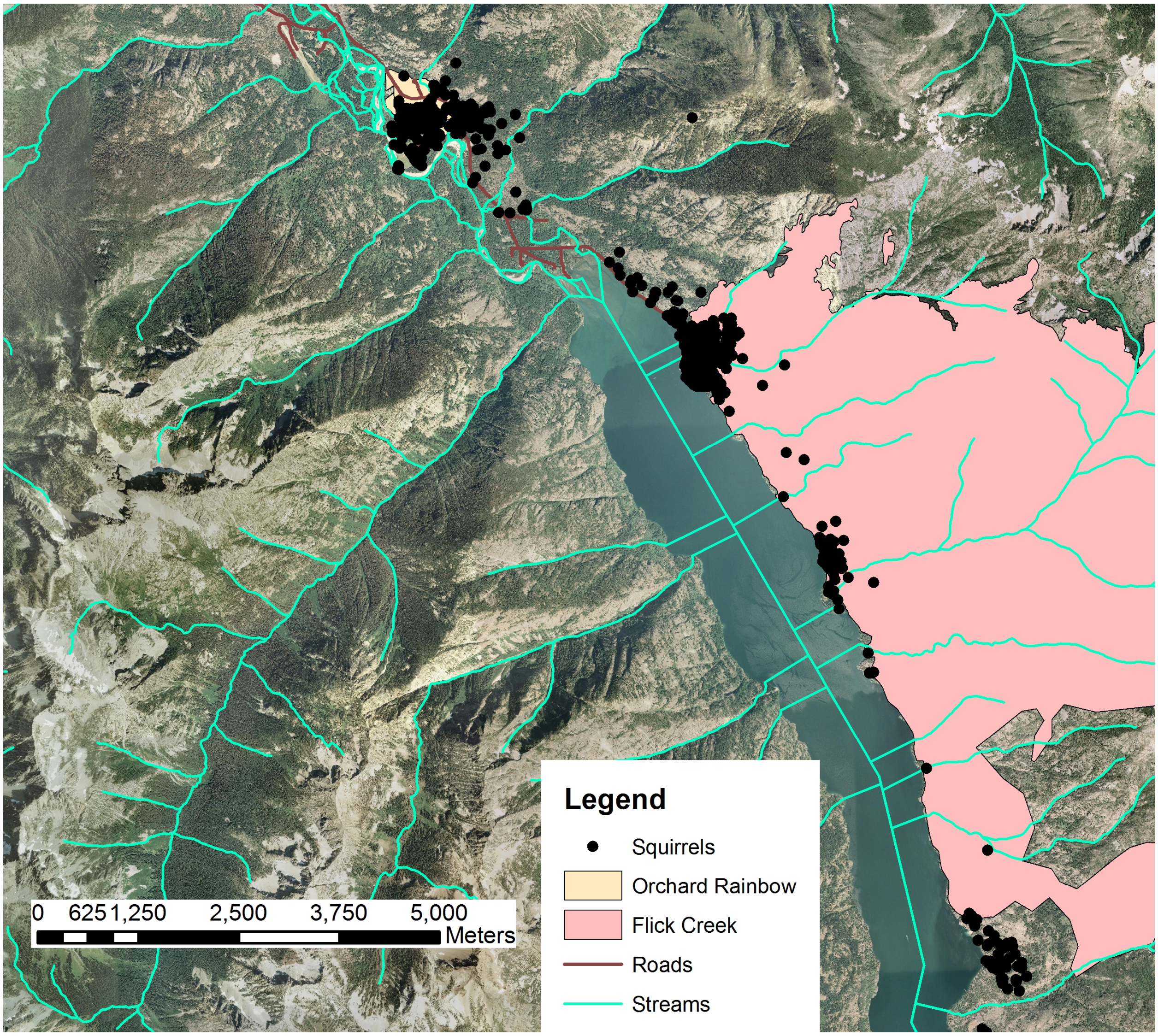


Figure 1.2. Fire fuel treatment areas used for analysis in the Stehekin Valley of the Lake Chelan National Recreation Area of North Cascades National Park, Washington.

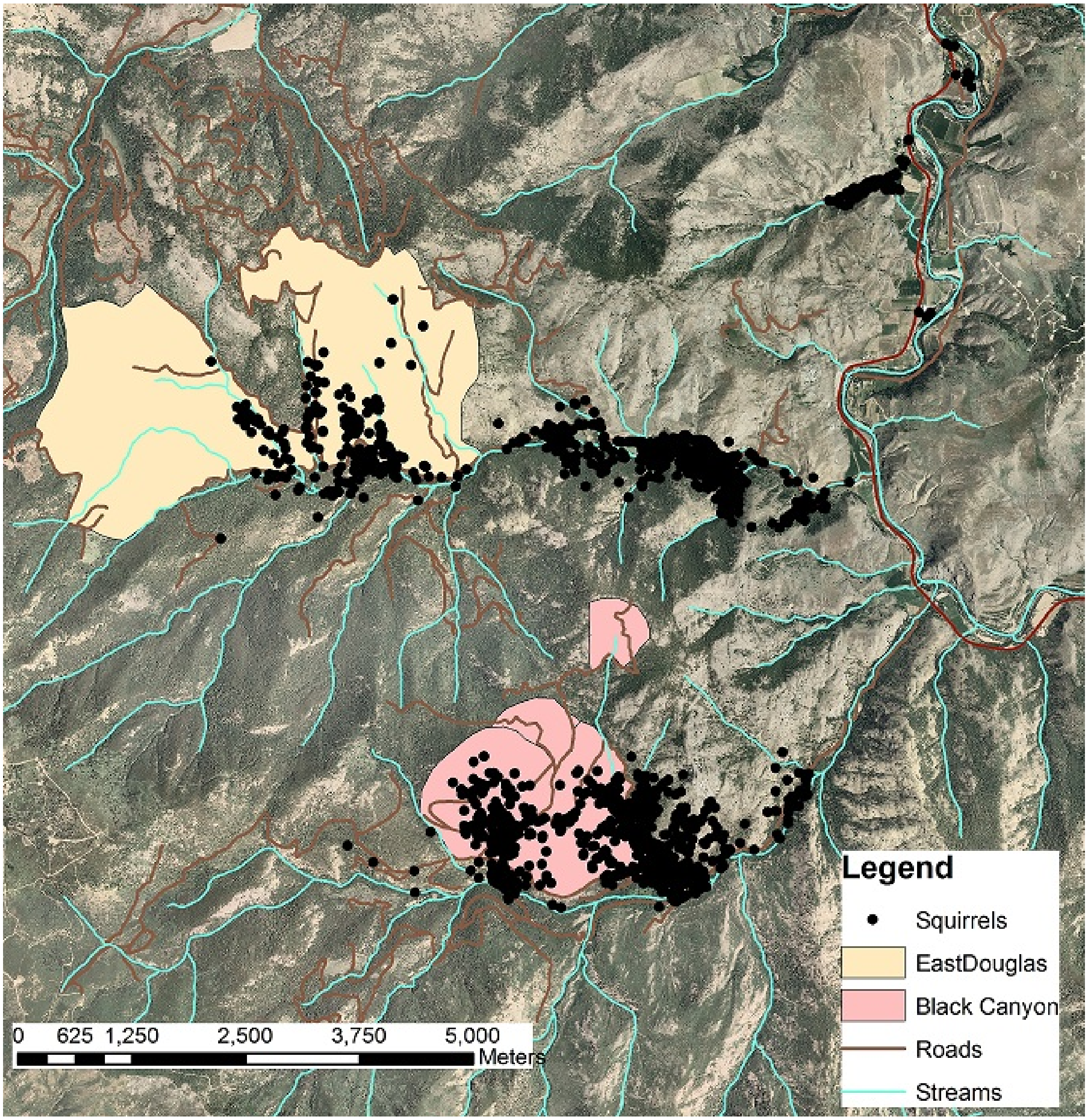


Figure 1.3. Fire fuel treatment areas used for analysis in the southern Methow Valley, Washington.

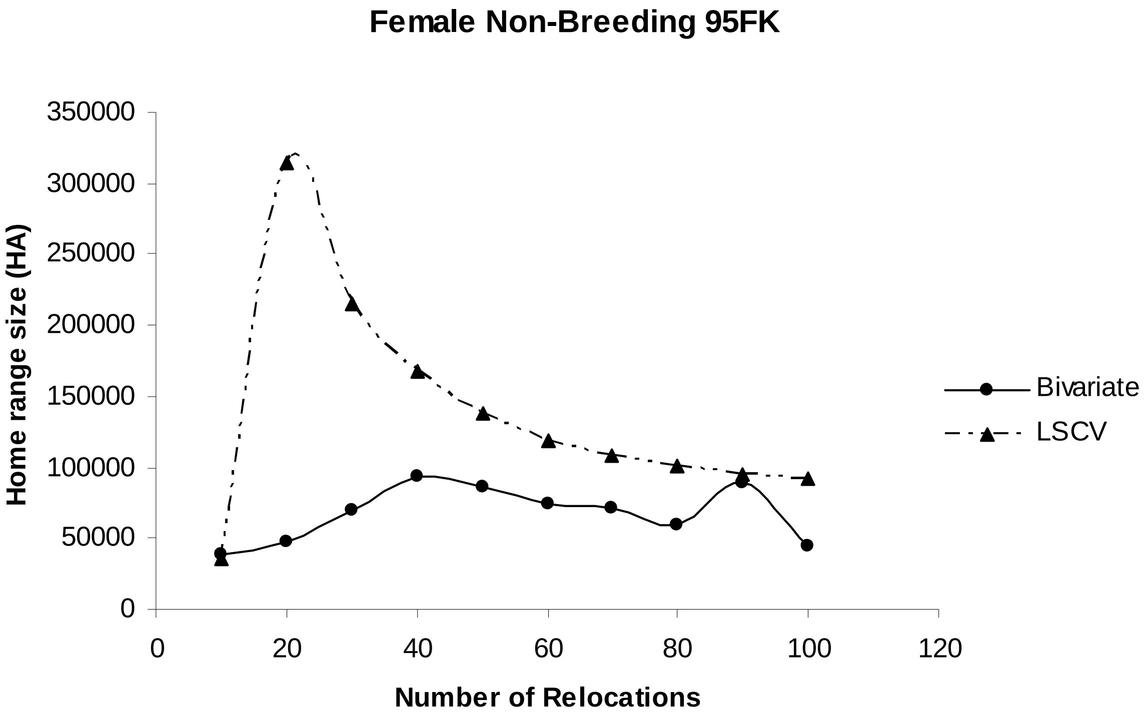


Figure 1.4. Comparison of bivariate plug-in and least-squares cross-validation smoothing parameter calculations of 95% fixed kernel home range sizes for two female squirrels with the largest number of relocations (mean of two squirrels represented with each curve).

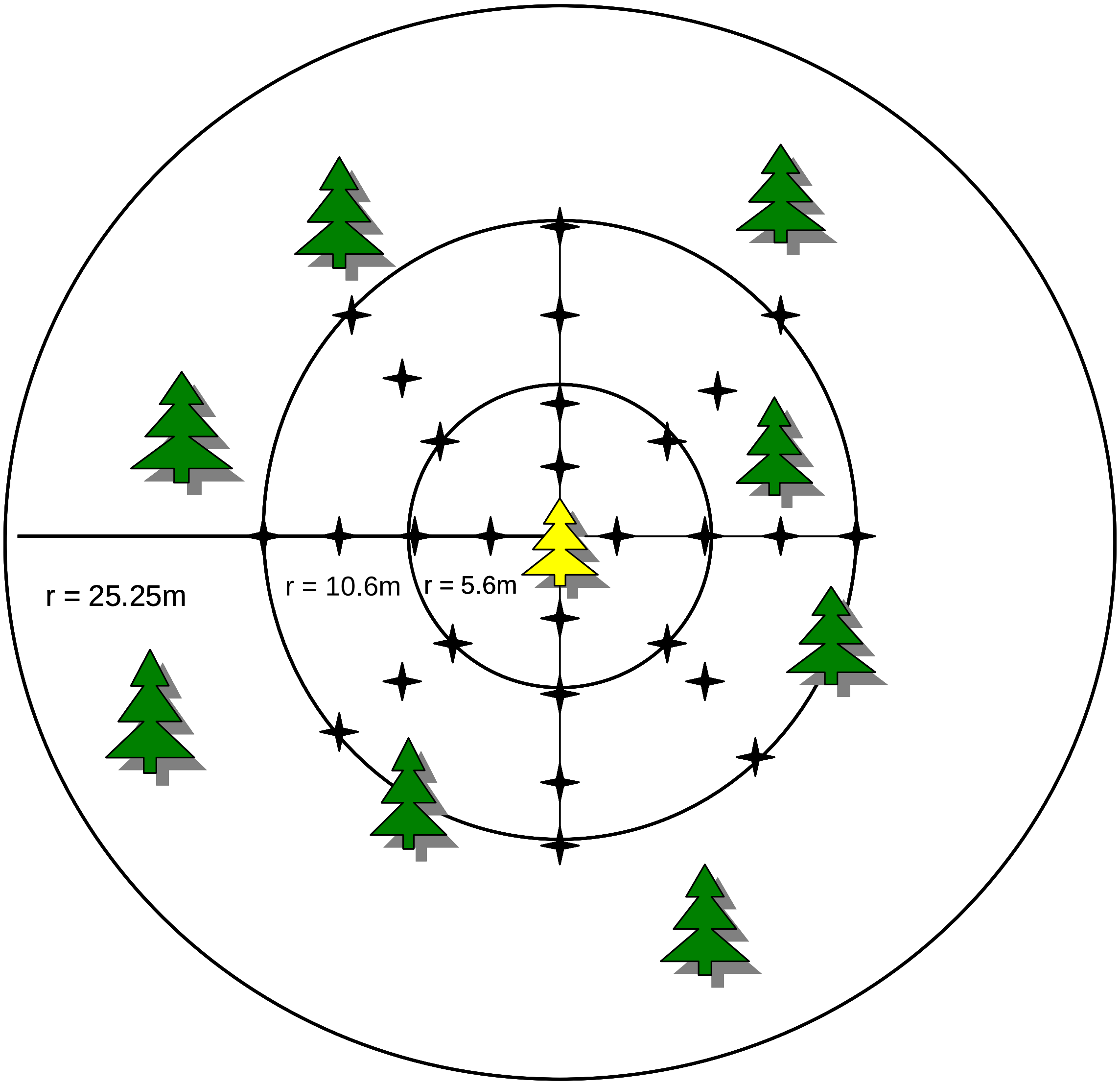


Figure 1.5. Diagram of vegetation sampling plots. The yellow tree is the nest-tree at nest-sites or tree closest to the randomly selected UTM coordinate (NAD83) for high and low use plots. The large circle (0.2ha) represents the area for tree variable measurements of 8 random trees (selected with the procedure described by Skalski 1987). The middle circle (0.035ha) represents the area for coarse woody debris counts, and tree species classifications and DBH measurements. The small circle (0.01ha) represents the area for understory and ground cover measurements. The stars represent locations of densitometer measurements of canopy cover.

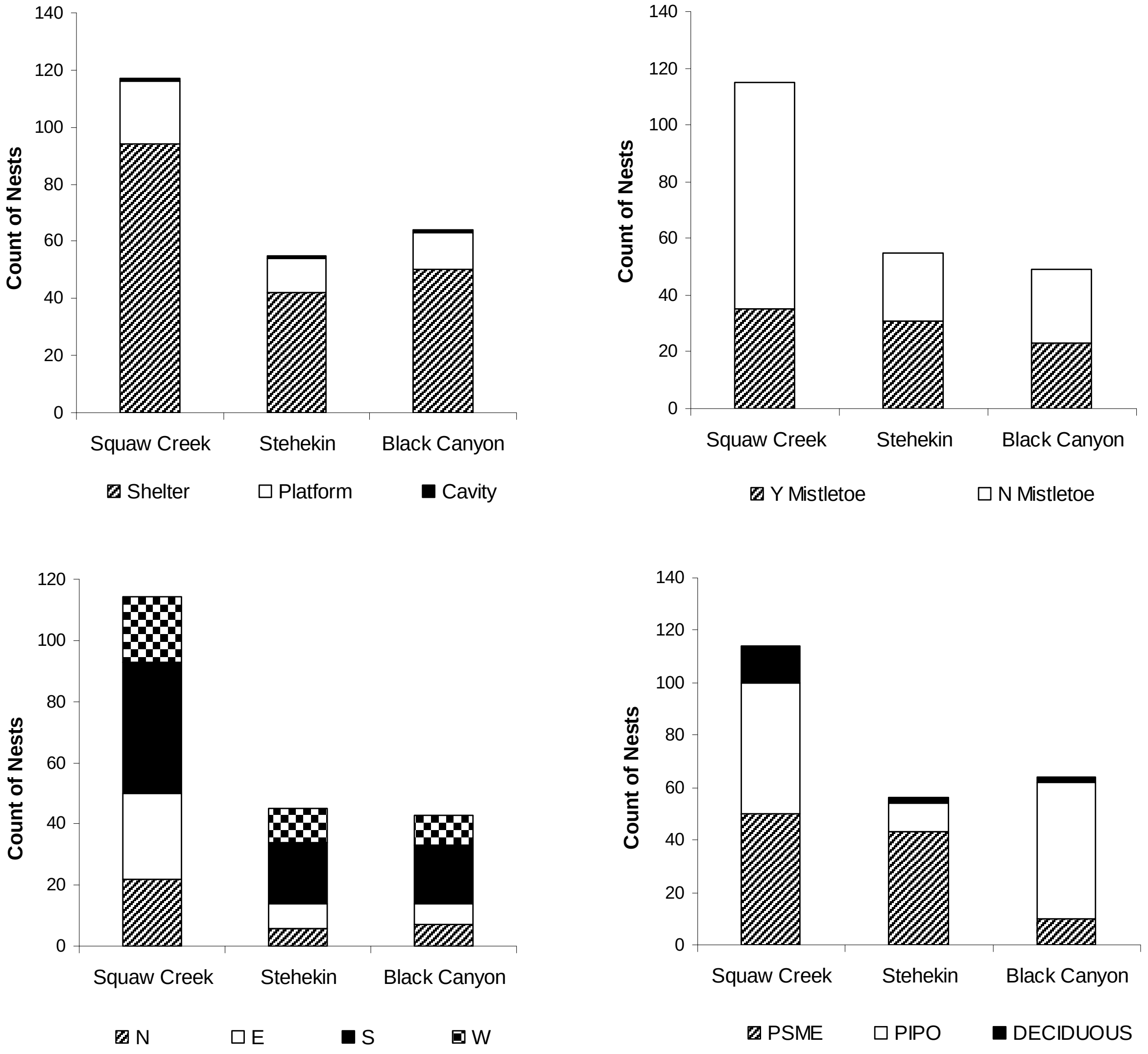


Figure 1.6. Nest characteristics by study area. Clockwise from upper left: counts of type of nests: shelter, platform, and cavity, count of nests with and without mistletoe brooming, count of nests facing North, East, South, and West, count of nests by species.

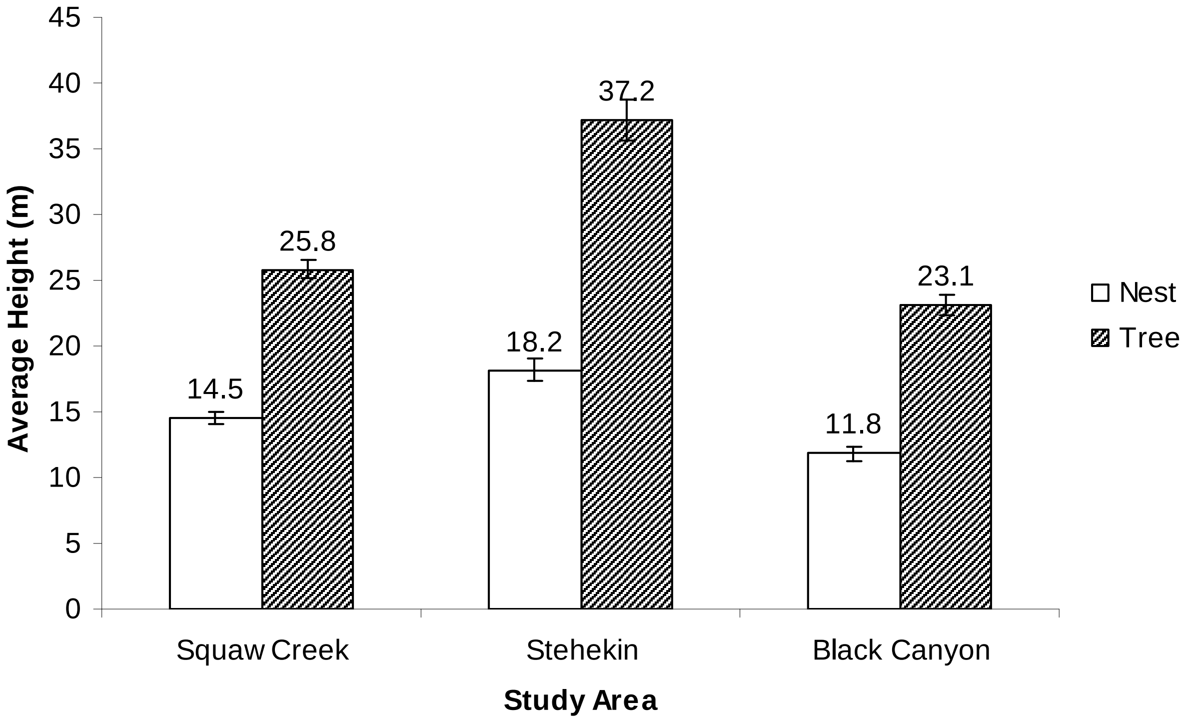


Figure 1.7. Average heights of nests and nest trees by study area. Error bars represent one standard error of the mean estimate.

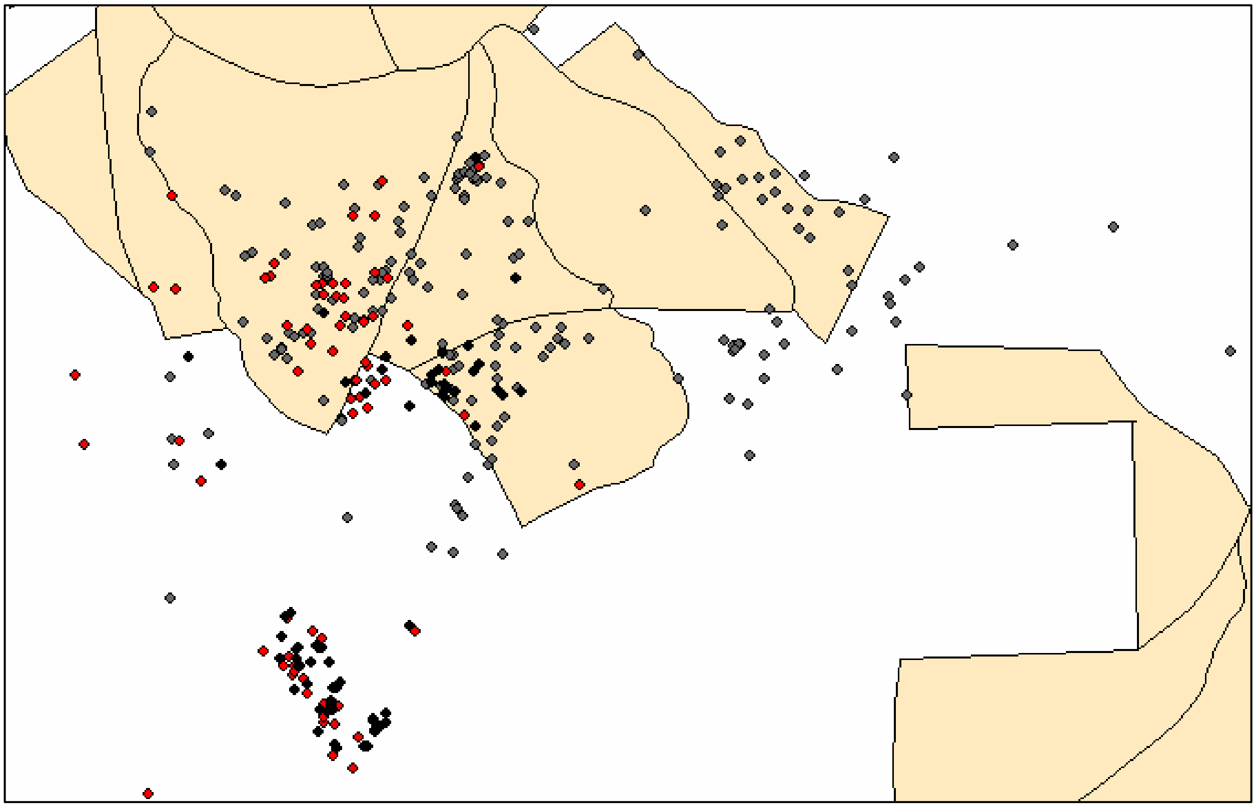


Figure 1.8. Telemetry relocations of three squirrels: represented by red, black, and gray dots, surrounding the Buckner/Rainbow fire fuel treatment area in Stehekin (beige polygon).

Table 1.1. Habitat variable codes and collection methods for western gray squirrel nest-tree selection in the North Cascades, Washington. Habitat variables were measured on nest-trees and 8 randomly selected trees within a 25.25-m radius plot following Skalski (1987) and Gregory (2005). See Figure 1.5 for habitat sampling plot schematic.

Variable	Description	Method
tbv	Degree of mistletoe infestation	Total Broom Volume (Parker and Mathiasen 2004)
llc	Lowest live crown	Height from ground to lowest live brances (m)
cond	Tree condition	Categorical 1: Alive, 2: Dead
ht	Tree height	Height (m)
spp	Tree species	Categorical 1: Douglas-fir, 2: Ponderosa Pine, 3: Other
scorch	Wildfire/prescribed burn intensity	Maximum scorch height (m)
connect	Canopy connectivity	Number of tree crowns $\leq$ 1m from the crown
dbh	Diameter at Breast Height	DBH (cm)
dom	Tree relative height	Categorical 1: Dominant, 2: Codominant, 3: Subdominant

stratification variable: nest-site located with radiotelemetry

Table 1.2. Habitat variable codes and collection methods for western gray squirrel nest-site, core-area, and resource utilization function (RUF) selection in the North Cascades, Washington. See Figure 1.5 for habitat sampling plot schematic.

Variable	Description	Method
qdbh	Quadratic Mean Diameter	DBH (cm) of all trees in 10.6m plot
trees	Tree species composition expanded from Gregory (2005)	Categorical classification in 10.6m radius plot: 0: no live trees 1:>90% Ponderosa Pine, 2: Mixed Conifer, 3: Mixed Conifer/Deciduous, 4:>90% Douglas-fir
ba	Basal area	DBH (cm) of all trees in 10.6m plot
avgscorch	Wildfire/prescribed burn intensity	Average scorch height (m) of focal and 8 random trees in 25.25m radius plot
avgtbv	Degree of mistletoe infestation	Average Total Broom Volume rating (Parker and Mathiasen 2004) of focal and 8 random trees in 25.25m radius plot
avgllc	Lowest live crown	Average lowest live crown (m) of focal and 8 random trees in 25.25m radius plot
avght	Tree height	Average tree height (m) of focal and 8 random trees in 25.25m radius plot
avgconnect	Canopy connectivity	Average Number of tree crowns ≤ 1m from the crown of focal and 8 random trees in 25.25m radius plot
live	% Live trees	Percent live trees in 10.6m radius plot
tx*	Fuel treatment history	GIS layer: binary untreated = 0, fire fuel treated = 1
elev*	Elevation	GIS layer (10m DEM)
asp*	Aspect	GIS layer (10m DEM) categorical N, E, S, W
slope*	Slope	GIS layer (10m DEM)
water*	Distance to nearest water body	GIS layer calculation (m)
road*	Distance to nearest road/trail	GIS layer calculation (m)
forest*	Forest type	GIS layer nclevel2 (Almack et al. 1993): 1: Ponderosa Pine, 2: Mixed Conifer, 3: Deciduous, 4: Herbaceous, 5: Shrub-Steppe, 6: Other
coveg	Ground cover	Occular estimation of ground cover category in 5.6m radius plot: 0, 1:>0-1%, 2: 2-5%, 3: 6-25%, 4: 26-50%, 5: 51-75%, 6: >76%
cntveg	Understory species	Count and Identification of understory tree and shrub species in 5.6m radius plot
cwd	Coarse woody debris	Total count in 10.6m radius plot >10cm, decay classes 1 and 2 (Maser 1979)
permis	% Mistletoe infected trees	% of trees in 10.6m radius plot with dwarf mistletoe brooms
cc	Canopy cover	% from 28 Densitometer readings: 10.6, 8.1, 5.6, 3.1m cardinal directions; 10.6, 8.1, 5.6m ordinal directions

stratification variables:
nest-site selection: squirrel
core-area selection: squirrel, sex, season (breeding vs. non-breeding)
* also used for resource utilization function (RUF) analysis

Table 1.3. Original forest type categories from the NCLEVEL2 vegetation layer, and collapsed forest categories used for analysis of western gray squirrel resource selection at three study sites: Stehekin, Squaw Creek, and Black Canyon in the North Cascades, Washington.

NCLEVEL2* Vegetation Category	Collapsed Category
Ponderosa Pine	Ponderosa Pine
Eastside Douglas-fir/Mixed Conifer	Mixed Conifer
Ponderosa Pine/Douglas-Fir	Mixed Conifer
Eastside Riparian Deciduous Forest	Deciduous
Eastside Upland Deciduous Forest	Deciduous
Riparian Deciduous Forest	Deciduous
Upland Deciduous Forest	Deciduous
Eastside Montane Herbaceous	Herbaceous
Eastside Montane Mosaic	Herbaceous
Herbaceous Shrub-Steppe	Herbaceous
Low Elevation Lush Herbaceous	Herbaceous
Big Sagebrush Shrub-Steppe	Shrub-Steppe
Bitterbrush Shrub Steppe	Shrub-Steppe
Bare Rock	Other
Fallow Land and Pasture	Other
Wet soil and gravel	Other
Orchards and crops	Other
Water	Other

* Almack et al. 1993

Table 1.4. *A priori* candidate models for western gray squirrel nest-tree selection in the North Cascades, Washington. Model variables are described in Table 1.1

	Model	Variables	Data Type
1	treest (Gregory 2005)	tbv + llc + cond + ht + spp	ground
2	treest scorch	tbv + llc + cond + ht + spp + scorch	ground
3	treat/fire effects	scorch	ground
4	nest-tree univariates (Gregory 2005)	tbv + connect + dbh	ground
5	nest-tree univariates + fire effects	tbv + connect + dbh + scorch	ground
6	nest-tree univariates - mistletoe	connect + dbh	ground
7	mistletoe	tbv	ground
8	full	all	ground

Stratification variable: nest-site

Table 1.5.*A priori* candidate models for western gray squirrel nest-site and core-area selection in the North Cascades, Washington. Variables are described in Table 1.2.

	Model	Variables	Data Type
1	stand	qdbh + trees + ba	ground
2	stand + treat	qdbh + trees + ba + tx	ground + GIS
3	stand + treat + scorch	qdbh + trees + ba + tx + scorch + live	ground + GIS
4	treat	tx	GIS
5	trees	qdbh + trees + avgtbv + avgllc + avght + avgconnect + live	ground
6	nest-tree comparison	qdbh + avgtbv + avgconnect	ground
7	site	elev + asp + slope + water + road + forest	GIS
8	site + treat	elev + asp + slope + water + road + forest + tx	GIS
9	ground + treat	coveg + cntveg + cwd + tx	ground + GIS
10	ground + treat + scorch	coveg + cntveg + cwd + tx + scorch	ground + GIS
11	treatment effects	qdbh + ba + avgtbv + avgconnect + permis + cwd + cc	ground
12	treatment effects + GIS	qdbh + ba + avgtbv + avgconnect + permis + cwd + cc + tx	ground + GIS
13	treatment + fire effects	qdbh + ba + avgtbv + avgconnect + permis + cwd + cc + tx + scorch + live	ground + GIS
	Full	all	ground + GIS

Stratification variables:
Nest-site: squirrel
Core-area: squirrel, sex, season (breeding vs. non-breeding)

Table 1.6. Results of logistic regression analysis of western gray squirrel nest-tree selection across two study sites: Stehekin and Squaw Creek in the North Cascades, Washington. Models are defined in Table 1.4.

	Model	[Log(*L*)]	K	AICc	Δ AICc	wi
4	nest-tree univariates*	-275.00	4	559.94	0.00	0.489112
5	nest-tree univariates + fire effects	-274.10	5	560.27	0.32	0.416268
	Full	-268.50	12	563.25	3.31	0.093538
7	mistletoe	-283.60	2	573.11	13.17	0.000676
1	treest*	-279.50	7	575.23	15.28	0.000235
2	treest scorch	-278.90	8	575.86	15.92	0.000171
6	nest-tree univariates -mistletoe	-298.60	3	605.33	45.38	6.84E-11
3	treat/fire effects	-310.30	2	626.61	66.67	1.63E-15
	Null	-311.40	1	626.80	66.86	1.48E-15

* Gregory 2005

Table 1.7. Weighted, untransformed coefficients incorporating the two highest ranked models: 4 (nest-tree univariates), and 5 (nest-tree univariates + fire effects) describing western gray squirrel nest-tree selection across two study sites: Stehekin and Squaw Creek in the North Cascades, Washington, using a generalized linear mixed model stratified by nest-site. Odds ratios are calculated by exponentiation of the coefficient. The 95% confidence interval= $e^{(\beta \pm 1.96SE)}$. Models are defined in Table 1.4; variables are defined in Table 1.1.

Model 4

Variable	Coefficient	Odds ratio	Std. Error	95% C.I.
Intercept	-3.5388	0.0290	0.3156	0.02 to 0.05*
tbv	0.3510	1.4205	0.0501	1.29 to 1.57*
connect	0.1719	1.1876	0.0534	1.07 to 1.32*
dbh	0.0108	1.0108	0.0049	1.00 to 1.02

*C.I. does not contain 1.

Model 5

Variable	Coefficient	Odds ratio	Std. Error	95% C.I.
Intercept	-3.5464	0.0288	0.3171	0.02 to 0.05*
tbv	0.3678	1.4446	0.0521	1.30 to 1.60*
connect	0.1590	1.1724	0.0549	1.05 to 1.31*
dbh	0.0130	1.0131	0.0052	1.00 to 1.02
scorch	-0.0385	0.9622	0.0307	0.91 to 1.02

*C.I. does not contain 1.

Table 1.8. Means of continuous variables and counts of categorical variables for characteristics of fire fuel treated and untreated or recently burned (wildfire) and unburned areas used by western gray squirrels in the North Cascades at three study sites in Chelan and Okanogan Counties. Sample sizes for each category = 12. Variables are defined in Table 1.2.

	Black Canyon		East Douglas		Flick Creek		Orchard/Rainbow	
Variable	Treated	Untreated	Treated	Untreated	Burned	Unburned	Treated	Untreated
QDBH	31.61	27.86	29.29	33.07	44.74	44.95	43.94	35.9
Trees >90% Ponderosa Pine	10	7	2	5	0	0	0	0
Trees Mixed Conifer	2	4	9	5	6	4	5	2
Trees Mixed Conifer/Deciduous	0	1	0	0	0	6	4	9
Trees >90% Douglas-fir	0	0	1	2	6	2	3	1
Basal area	0.60	0.70	0.53	0.65	1.23	1.22	1.22	1.44
Average scorch height	2.04	1.18	3.00	0.45*	5.51	1.10*	1.16	0.57*
Average TBV	0.88	1.60	0.25	0.27	0.19	1.20*	0.20	0.01
Average LLC	4.45	4.05	5.62	4.05	12.87	7.50	8.20	8.11
Average tree height	18.59	17.24	20.41	18.78	29.14	26.67	27.28	28.78
Average connectivity	2.10	2.28	1.32	1.40*	1.99	2.95	2.91	3.20
% Live trees	0.97	0.90	0.73	0.98*	0.73	0.91	0.94	0.96
Elevation	788.50	713.08	820.83	527.00	437.58	358.75	362.50	394.50
Aspect N	2	0	3	2	0	0	0	0
Aspect E	9	10	6	5	0	0	2	0
Aspect S	1	2	1	4	4	5	11	8
Aspect W	0	0	2	1	6	7	1	4
Slope	101.42	91.00	100.00	79.42	25.40	19.84	2.71	10.14
Distance to nearest water body	239.89	186.10	190.41	163.28	220.98	217.40	222.63	352.22
Distance to nearest road/trail	182.92	159.15	190.68	293.22	540.80	293.33	155.65	141.96
Forest type Ponderosa Pine	1	6	6	8	12	12	9	2
Forest type Mixed Conifer	7	1	0	0	0	0	0	0
Forest type Deciduous	0	1	0	0	0	0	1	4
Forest type Herbaceous	2	3	4	4	0	0	1	5
Forest type Shrub-Steppe	2	1	2	0	0	0	1	1
Cover total vegetation	5.00	5.33	4.50	5.25	3.83	4.33	3.75	4.25
Count understory species	3.25	3.58	2.67	2.50	0.50	1.50	0.34	1.42*
% Mistletoe infected trees	0.25	0.41	0.16	0.01*	0.06	0.36*	0.03	0.01
Count coarse woody debris	2.08	3.00	3.67	1.25	2.67	2.25	2.33	2.10
Canopy cover	0.49	0.50	0.43	0.45	0.50	0.56	0.59	0.64

* Significant differences at $\alpha = 0.05$. Wilcoxon Signed Rank Tests

Table1.9. Results of logistic regression analysis of western gray squirrel nest-site selection across two study sites: Stehekin and Squaw Creek in the North Cascades, Washington. Models are defined in Table 1.5.

Model		[Log(L)]	K	AICc	Δ AICc	wi
6	nest-tree comp	-130.10	4	274.30	0.00	0.643736
12	treatment effects + GIS	-124.90	9	276.80	2.50	0.184433
11	treatment effects	-126.60	8	277.30	3.00	0.143637
	Null	-138.63	1	279.28	18.61	0.000080
13	treatment + fire effects	-124.70	11	282.50	8.20	0.010668
5	trees	-123.90	11	282.90	8.60	0.008735
4	treat	-137.80	2	283.50	9.20	0.006471
1	stand*	-132.00	7	287.00	12.70	1.12E-03
2	stand + treat	-130.70	8	287.30	13.00	9.68E-04
9	ground + treat	-137.60	5	292.10	17.80	8.78E-05
3	stand + treat + scorch	-130.50	10	293.10	18.80	5.33E-05
10	ground + treat + scorch	-137.50	6	295.10	20.80	1.96E-05
	Full	-100.80	31	296.70	22.40	8.80E-06
7	site	-131.60	13	304.20	29.90	2.07E-07
8	site + treat	-131.20	14	306.50	32.20	6.55E-08

*Gregory 2005

Table 1.10. Weighted, untransformed coefficients incorporating the highest ranked model: 6 (nest-tree comparison) describing western gray squirrel nest-site selection across two study sites: Stehekin and Squaw Creek in the North Cascades, Washington, using a generalized linear mixed model stratified by squirrel. Odds ratios are calculated by exponentiation of the coefficient. The 95% confidence interval= $e^{(\beta \pm 1.96SE)}$. Models are defined in Table 1.5; variables are defined in Table 1.2.

Model 6

Variable	Coefficient	Odds ratio	Std. Error	95% C.I.
Intercept	-1.4060	0.25	0.4523	0.10 to 0.59*
conn	0.3653	1.44	0.1171	1.15 to 1.81*
qdbh	0.0089	1.01	0.0099	0.99 to 1.03
tbv	0.2973	1.35	0.1562	0.99 to 1.83

*C.I. does not contain 1.

Table 1.11. Means of continuous variables and counts of categorical variables for characteristics of used and available nest-sites of western gray squirrels in the North Cascades at two study sites: Stehekin and Squaw Creek, in the North Cascades, Washington. Variables are defined in Table 1.2.

	Stehekin		Squaw Creek	
Variable	Used n= 45	Available n= 45	Used n= 55	Available n= 55
QDBH	47.20	40.08	29.46	28.02
Trees >90% Ponderosa Pine	0	0	7	15
Trees Mixed Conifer	6	15	29	28
Trees Mixed Conifer/Deciduous	27	18	15	6
Trees >90% Douglas-fir	11	11	4	6
Basal area	1.57	1.25	0.99	0.74
Fuel treatment/Wildfire Yes	9	5	20	16
Fuel treatment/Wildfire No	36	40	35	39
Average scorch height	3.13	2.56	1.17	1.31
Average TBV	0.92	0.60	0.45	0.29
Average LLC	10.16	10.02	7.32	5.94
Average tree height	32.56	27.10	22.87	20.62
Average connectivity	3.37	2.76	2.65	1.78
% Live trees	0.82	0.82	0.89	0.88
Elevation	392.16	400.47	610.60	609.58
Aspect N	0	0	19	25
Aspect E	0	3	17	21
Aspect S	21	15	11	4
Aspect W	24	27	8	5
Slope	14.38	17.69	69.20	88.24
Distance to nearest water body	274.91	294.90	89.36	133.64
Distance to nearest road/trail	65.36	87.43	232.03	239.99
Forest type Ponderosa Pine	34	33	28	26
Forest type Mixed Conifer	0	0	6	12
Forest type Deciduous	3	3	0	2
Forest type Herbaceous	7	7	11	10
Forest type Shrub-Steppe	0	1	10	5
Forest type Other	1	1	0	0
Cover total vegetation	4.22	3.80	4.20	4.64
Count understory species	1.53	1.11	3.02	3.64
% Mistletoe infected trees	0.24	0.13	0.14	0.04
Count coarse woody debris	3.07	2.40	3.22	3.20
Canopy cover	0.69	0.52	0.69	0.56

Table 1.12. Results of logistic regression analysis of western gray squirrel core-area selection across three study sites: Stehekin, Squaw Creek, and Black Canyon in the North Cascades, Washington. Models are defined in Table 1.5.

	Model	[Log(*L*)]	K	AICc	Δ AICc	wi
	Full	-460.70	33	994.94	0.00	1.000000
8	site + treat	-513.30	16	1068.61	73.66	1.01E-16
7	site	-528.60	15	1089.53	94.59	2.89E-21
5	trees	-547.40	13	1123.40	128.46	1.27E-28
13	treatment + fire effects	-565.10	12	1156.35	161.40	5.72E-36
12	treatment effects + GIS	-567.20	10	1157.24	162.30	8.96E-36
3	stand + treat + scorch	-568.70	12	1162.35	167.40	4.46E-37
2	stand + treat	-571.40	10	1164.24	169.30	1.73E-37
11	treatment effects	-573.00	9	1166.20	171.25	6.49E-38
6	nest-tree comp	-580.40	6	1175.09	180.15	7.61E-40
1	stand*	-578.70	9	1177.20	182.25	2.65E-40
9	ground + treat	-589.30	7	1194.12	199.18	5.61E-44
4	treat	-593.20	4	1195.04	200.10	3.54E-44
10	ground + treat + scorch	-589.30	8	1195.16	200.21	3.34E-44
	Null	-598.67	1	1199.30	204.36	4.20E-45

*Gregory 2005

Table 1.13. Weighted, untransformed coefficients incorporating the full model describing western gray squirrel core-area selection across three study sites: Stehekin, Squaw Creek, and Black Canyon in the North Cascades, Washington using a generalized linear mixed model stratified by squirrel. Odds ratios are calculated by exponentiation of the coefficient. The 95% confidence interval= $e^{(\beta \pm 1.96SE)}$. Models are defined in Table 1.5; variables are defined in Table 1.2. The intercept represents female squirrels in the breeding season, north facing aspects, ponderosa pine forests (forest = 1,) with no live trees (tree=0), that have not been fire fuel treated or recently burned in wildfire.

Variable	Coefficient (β)	Odds ratio	Std. Error	95% C.I.
Intercept	-1.4057	0.2452	1.8409	0.01 to 9.05
asp East	-0.7602	0.4676	0.2577	0.28 to 0.77*
asp South	-1.2232	0.2943	0.3259	0.16 to 0.58*
asp West	-1.0875	0.3371	0.3527	0.17 to 0.67*
cc	1.4669	4.3359	0.5103	1.59 to 11.79*
cntveg	-0.1098	0.8960	0.0438	0.82 to 0.98*
llc	0.0909	1.0951	0.0381	1.02 to 1.18*
conn	-0.2587	0.7720	0.0917	0.65 to 0.92*
tbv	-0.2985	0.7419	0.1189	0.59 to 0.94*
cwd	-0.0554	0.9461	0.0256	0.90 to 0.99*
tx	0.9272	2.5275	0.2267	1.62 to 3.94*
live	2.4552	11.6487	0.6852	3.04 to 44.62*
forest Mixed Conifer	1.1234	3.0753	0.3348	1.60 to 5.93*
forest Deciduous	-0.7314	0.4813	0.3640	0.24 to 0.98*
sex Male	-0.1461	0.8641	0.1775	0.61 to 1.22
season Non-Breeding	0.1354	1.1450	0.1747	0.81 to 1.61
elev	-0.0038	0.9962	0.0010	0.99 to 1.00
slope	0.0070	1.0070	0.0023	1.00 to 1.01
water	-0.0022	0.9978	0.0006	1.00 to 1.00
road	-0.0036	0.9964	0.0007	1.00 to 1.00
tree >90% Ponderosa Pine	-0.0595	0.9423	1.6406	0.04 to 23.48
tree Mixed Conifer	-0.5453	0.5797	1.6180	0.02 to 13.82
tree Mixed Conifer/Deciduous	-0.2189	0.8034	1.6303	0.03 to 19.62
tree >90% Douglas-fir	-1.5280	0.2170	1.6394	0.01 to 5.39
ba	0.1600	1.1735	0.2282	0.75 to 1.84
qdbh	-0.0024	0.9976	0.0089	0.98 to 1.02
covveg	0.1413	1.1518	0.0725	1.00 to 1.33
permis	0.5716	1.7711	0.4055	0.80 to 3.92
scorch	0.0220	1.0222	0.0541	0.92 to 1.14
ht	0.0471	1.0483	0.0239	1.00 to 1.10
forest Herbaceous	-0.4026	0.6686	0.2643	0.40 to 1.12
forest Shrub-Steppe	0.0882	1.0922	0.3948	0.50 to 2.37
forest Other	0.0372	1.0379	0.7857	0.22 to 4.84

* C.I. does not contain 1

Table 1.14. Means of continuous variables and counts of categorical variables for characteristics of high and low use areas of western gray squirrels at three study sites: Stehekin, Squaw Creek, and Black Canyon in the North Cascades, WA. Variables are defined in Table 1.2.

	Stehekin		Squaw Creek		Black Canyon	
Variable	High n= 100	Low n= 138	High n= 133	Low n= 255	High n= 97	Low n= 193
QDBH	44.27	38.19	30.43	27.35	28.07	28.61
Trees >90% Ponderosa Pine	0	0	47	81	51	104
Trees Mixed Conifer	26	49	48	111	33	37
Trees Mixed Conifer/Deciduous	57	43	26	36	13	52
Trees >90% Douglas-fir	17	45	12	27	0	0
Basal area	1.50	1.07	0.80	0.62	0.70	0.68
Fuel treatment/Wildfire Yes	62	73	35	87	77	84
Fuel treatment/Wildfire No	38	65	98	168	20	109
Average scorch height	1.78	2.72	1.09	1.07	1.72	0.90
Average TBV	0.31	0.63	0.38	0.58	0.57	0.85
Average LLC	10.57	7.67	6.27	4.91	5.38	4.44
Average tree height	30.58	25.48	21.74	19.19	18.79	18.45
Average connectivity	3.19	2.41	1.88	1.63	2.01	2.29
% Live trees	0.89	0.81	0.88	0.90	0.97	0.91
Elevation	382.32	420.41	574.37	655.11	750.73	770.11
Aspect N	0	0	54	64	23	23
Aspect E	3	8	66	115	53	140
Aspect S	45	73	7	48	21	30
Aspect W	52	57	6	28	0	0
Slope	100.00	138.00	133.00	255.00	97.00	193.00
Distance to nearest water body	210.74	283.73	95.91	136.49	227.30	297.52
Distance to nearest road/trail	66.68	98.55	150.43	253.83	106.24	166.56
Forest type Ponderosa Pine	80	95	74	98	39	49
Forest type Mixed Conifer	0	0	12	44	50	53
Forest type Deciduous	8	18	6	15	3	20
Forest type Herbaceous	10	17	27	78	3	42
Forest type Shrub-Steppe	2	5	10	16	1	29
Forest type Other	0	3	4	4	1	0
Cover total vegetation	4.13	4.12	4.65	4.70	4.86	5.19
Count understory species	1.24	1.26	3.59	3.47	4.13	4.69
% Mistletoe infected trees	0.10	0.12	0.06	0.11	0.24	0.24
Count coarse woody debris	2.40	3.06	2.06	3.22	3.14	2.50
Canopy cover	0.66	0.55	0.60	0.49	0.55	0.55

Table 1.15. Resource utilization functions (RUFs) for western gray squirrels at three study sites in the North Cascades, Washington. RUFs for female squirrels are listed separately because females have more consistent resource selection across seasons and drive population health. Positive coefficients indicate that use increases with increasing values of the resource. β_0 (intercept) represents ponderosa pine (*Pinus ponderosa*) forest and N aspect areas that have not been recently fire fuel treated or burned in wildfire. The RUF at location x is modeled as: RUF(x) = C(x)β + Z(x) where β is the vector of unstandardized RUF coefficients corresponding to C(x), the vector of resource utilization characteristics. The final term Z(x) measures the spatial variation in RUF induced by the kernelling modeled as a mean-zero Gaussian random field with empirically estimated Matern correlation function. Standard errors were calculated using Eq. 2 which quantifies uncertainty by individual squirrel.

		Mean estimates of unstandardized RUF coefficients (1 SE)						
Squirrel group	n	βo	βtx	$\beta\mathrm{forest}_{\mathrm{SS}}$	$\beta\mathrm{forest}_{\mathrm{Herb}}$	$\beta\mathrm{forest}_{\mathrm{Decid}}$	$\beta\mathrm{forest}_{\mathrm{Mixed}}$	$\beta\mathrm{forest}_{\mathrm{Other}}$
All	46	51.92 (2.36)	7.92 (0.47)	0.31 (0.61)	1.37 (0.55)	1.20 (0.77)	4.29 (0.66)	-6.56 (0.43)
All Stehekin	14	8.06 (2.00)	7.55 (0.30)	-5.39 (0.40)	3.63 (0.63)	0.51 (0.38)	NA	-8.55 (0.39)
All Squaw Creek	21	102.85 (2.44)	7.78 (0.58)	-0.36 (0.55)	-3.07 (0.37)	1.82 (0.64)	3.30 (0.59)	-4.57 (0.48)
All Black Canyon	11	44.84 (2.64)	8.42 (0.53)	6.69 (0.87)	3.56 (0.67)	1.26 (1.28)	5.28 (0.72)	NA
All Females	29	53.56 (2.33)	8.71 (0.37)	1.93 (0.44)	2.44 (0.36)	2.39 (0.53)	5.11 (0.53)	-0.33 (0.19)
Stehekin Females	12	1.01 (1.98)	7.39 (0.18)	-4.70 (0.28)	-0.05 (0.28)	0.96 (0.23)	NA	-7.72 (0.12)
Squaw Creek Females	10	123.27 (2.27)	12.11 (0.36)	3.66 (0.33)	0.54 (0.25)	5.21 (0.37)	3.12 (0.41)	7.06 (0.26)
Black Canyon Females	7	36.38 (2.74)	6.63 (0.58)	6.84 (0.70)	6.84 (0.56)	2.91 (0.98)	7.10 (0.65)	NA

Squirrel group	n	$\beta\mathrm{aspect}_{\mathrm{E}}$	$\beta\mathrm{aspect}_{\mathrm{S}}$	$\beta\mathrm{aspect}_{\mathrm{W}}$	βslope	βelevation	βwater	βroad
All	46	2.16 (0.69)	3.45 (0.56)	2.78 (0.62)	-0.10 (0.02)	-0.05 (0.00)	-0.01 (0.00)	0.19 (0.01)
All Stehekin	14	2.18 (0.83)	6.37 (0.37)	5.68 (0.41)	-0.35 (0.02)	0.02 (0.01)	0.06 (0.00)	-0.03 (0.00)
All Squaw Creek	21	0.15 (0.34)	-2.49 (0.47)	-2.63 (0.51)	-0.06 (0.01)	-0.13 (0.01)	-0.06 (0.00)	0.63 (0.03)
All Black Canyon	11	4.14 (0.90)	6.46 (0.85)	5.30 (0.93)	0.11 (0.02)	-0.04 (0.00)	-0.01 (0.00)	-0.03 (0.00)
All Females	29	0.23 (0.44)	3.35 (0.40)	2.50 (0.47)	-0.13 (0.01)	-0.05 (0.00)	-0.03 (0.00)	-0.02 (0.00)
Stehekin Females	12	-0.44 (0.29)	7.02 (0.15)	7.22 (0.19)	0.35 (0.01)	(0.04 (0.01)	0.05 (0.00)	-0.03 (0.00)
Squaw Creek Females	10	0.34 (0.26)	-1.51 (0.31)	-2.02 (0.41)	-0.06 (0.01)	-0.17 (0.00)	-0.13 (0.00)	-0.01 (0.00)
Black Canyon Females	7	0.80 (0.77)	4.55 (0.75)	2.30 (0.80)	0.02 (0.02)	-0.03 (0.00)	0.00 (0.00)	-0.03 (0.00)

Codes: tx= fire fuel treated, $\mathrm{forest}_{\mathrm{SS}}$= Shrub-Steppe forest type, $\mathrm{forest}_{\mathrm{Herb}}$= Herbaceous forest, $\mathrm{forest}_{\mathrm{Decid}}$= Deciduous forest, $\mathrm{forest}_{\mathrm{Mixed}}$= Mixed Conifer forest, $\mathrm{forest}_{\mathrm{Other}}$= "other" forest type, $\mathrm{aspect}_{\mathrm{E}}$= East, $\mathrm{aspect}_{\mathrm{S}}$= South, $\mathrm{aspect}_{\mathrm{W}}$= West, water= distance to nearest water, road= distance to nearest road

Table 1.16. Estimates of standardized RUF coefficients for 46 western gray squirrels across three study sites in the North Cascades, Washington. Relative importance of resources is indicated by the magnitude of β. Consistency in selection at the population level is indicated by significance of and the number of squirrels whose use was either positively or negatively associated with each attribute. *P* values test the null hypothesis that the average β is zero with a Wilcoxon signed rank test. Confidence intervals were calculated with the conservative Eq. 3, which includes inter-animal variation in the calculation of variance.

Resource Attribute	Mean standardized β	95% confidence interval	*P* (β = 0)	No. squirrels with use significantly associated with attribute	
				+	-
Fire treatment history	+3.88	2.10 to 5.69	<0.001	26	4
Forest Deciduous	+0.23	-0.54 to 0.89	<0.001	18	18
Forest Shrub-Steppe	+0.15	-1.65 to 0.94	<0.001	17	20
Forest Herbaceous	+0.06	-0.49 to 1.12	<0.001	17	14
Forest Mixed	+2.15	0.22 to 4.24	<0.001	7	5
Forest Other	-2.19	-3.61 to 0.77	<0.001	3	15
Aspect S	+1.71	-0.20 to 1.67	<0.001	20	15
Aspect W	+1.17	0.21 to 2.25	<0.001	24	19
Aspect E	+0.71	-0.06 to 1.94	0.362	19	21
Elevation	-4.22	-7.45 to -1.03	0.012	15	29
Slope	-0.60	-1.80 to 0.43	0.232	17	25
Distance to nearest water	+1.80	-1.79 to 4.33	0.551	26	19
Distance to nearest road	-3.26	-6.81 to -0.08	0.045	15	29

Table 1.17. Estimates of standardized RUF coefficients for 14 western gray squirrels at Stehekin in the North Cascades, Washington. Relative importance of resources is indicated by the magnitude of β. Consistency in selection at the population level is indicated by significance of and the number of squirrels whose use was either positively or negatively associated with each attribute. *P* values test the null hypothesis that the average β is zero with a Wilcoxon signed rank test. Confidence intervals were calculated with the conservative Eq. 3, which includes inter-animal variation in the calculation of variance.

Resource Attribute	Mean standardized β	95% confidence interval	*P* (β = 0)	No. squirrels with use significantly associated with attribute	
				+	-
Fire treatment history	+3.99	2.24 to 6.17	0.001	13	1
Forest Deciduous	-0.59	-1.64 to 0.23	0.001	3	6
Forest Shrub-Steppe	+0.50	-1.98 to 2.98	0.001	5	3
Forest Herbaceous	+0.54	-0.58 to 2.01	0.001	7	4
Forest Mixed	NA	NA	NA	NA	NA
Forest Other	-3.40	-5.26 to -1.99	0.001	0	9
Aspect S	-0.17	-1.41 to 1.07	0.001	2	3
Aspect W	+2.74	0.97 to 4.64	<0.001	10	2
Aspect E	+2.03	0.03 to 4.13	0.104	9	5
Elevation	-7.09	-13.07 to 2.96	0.217	4	10
Slope	-2.17	-4.65 to 0.28	0.091	5	9
Distance to nearest water	+5.10	1.37 to 9.37	0.025	11	3
Distance to nearest road	+1.81	-9.15 to 9.07	0.808	9	5

Table 1.18. Estimates of standardized RUF coefficients for 21 western gray squirrels at Squaw Creek in the North Cascades, Washington. Relative importance of resources is indicated by the magnitude of β. Consistency in selection at the population level is indicated by significance of and the number of squirrels whose use was either positively or negatively associated with each attribute. *P* values test the null hypothesis that the average β is zero with a Wilcoxon signed rank test. Confidence intervals were calculated with the conservative Eq. 3, which includes inter-animal variation in the calculation of variance.

Resource Attribute	Mean standardized β	95% confidence interval	*P* (β = 0)	No. squirrels with use significantly associated with attribute	
				+	-
Fire treatment history	+4.48	2.11 to 6.85	<0.001	6	0
Forest Deciduous	+0.16	-1.05 to 1.36	<0.001	9	9
Forest Shrub-Steppe	-1.46	-3.28 to 0.35	0.128	7	13
Forest Herbaceous	+0.47	-0.86 to 1.81	<0.001	7	7
Forest Mixed	+1.85	0.22 to 3.47	<0.001	4	1
Forest Other	-0.76	-3.18 to 1.66	<0.001	3	6
Aspect S	+0.55	-0.57 to 1.67	0.257	13	8
Aspect W	-0.66	-1.59 to 0.27	0.140	8	13
Aspect E	-0.18	-0.84 to 0.48	0.434	5	11
Elevation	-4.06	-8.04 to -0.09	0.040	7	13
Slope	-0.53	-2.10 to 1.05	0.412	7	12
Distance to nearest water	-1.72	-5.74 to 2.31	0.495	10	11
Distance to nearest road	-5.09	-8.39 to -1.80	0.006	3	17

Table 1.19. Estimates of standardized RUF coefficients for11 western gray squirrels Black Canyon in the North Cascades, Washington. Relative importance of resources is indicated by the magnitude of β. Consistency in selection at the population level is indicated by significance of and the number of squirrels whose use was either positively or negatively associated with each attribute. *P* values test the null hypothesis that the average β is zero with a Wilcoxon signed rank test. Confidence intervals were calculated with the conservative Eq. 3, which includes inter-animal variation in the calculation of variance.

Resource Attribute	Mean standardized β	95% confidence interval	*P* (β = 0)	No. squirrels with use significantly associated with attribute	
				+	-
Fire treatment history	+3.18	-1.37 to 7.73	0.206	7	3
Forest Deciduous	+1.01	-0.28 to 2.31	0.001	6	3
Forest Shrub-Steppe	+1.14	-1.70 to 3.98	0.413	5	4
Forest Herbaceous	-0.50	-2.24 to 1.25	0.004	3	3
Forest Mixed	+2.46	-0.68 to 5.61	0.001	3	4
Forest Other	NA	NA	NA	NA	NA
Aspect S	+1.74	-0.89 to 4.36	0.365	5	4
Aspect W	+2.97	-0.30 to 6.25	0.148	6	4
Aspect E	+1.61	-1.59 to 4.81	0.638	5	5
Elevation	-3.55	-8.75 to 1.66	0.320	4	6
Slope	+0.92	-1.04 to 2.89	0.577	5	4
Distance to nearest water	+1.74	-7.63 to 11.11	1.000	5	5
Distance to nearest road	-4.63	-10.52 to 1.25	0.120	3	7

Table 1.20. . Weighted, untransformed coefficients incorporating the next highest ranked model from AIC$_c$ logistic regression: Model 12 (treatment effects + GIS) describing western gray squirrel nest-site selection across two study sites: Stehekin and Squaw Creek in the North Cascades, Washington, using a generalized linear mixed model stratified by nest-site. Odds ratios are calculated by exponentiation of the coefficient. The 95% confidence interval= $e^{(\beta \pm 1.96SE)}$. Models are defined in Table 1.5; variables are defined in Table 1.2.

Model 12

Variable	Coefficient	Odds ratio	Std. Error	95% C.I.
Intercept	-2.8548	0.06	0.6851	0.02 to 0.22*
ba	-0.2420	0.79	0.3070	0.43 to 1.43
qdbh	0.0074	1.01	0.0137	0.98 to 1.03
conn	0.1869	1.21	0.1315	0.93 to 1.56
tbv	0.0443	1.05	0.2170	0.68 to 1.60
permis	2.5875	13.30	1.0622	1.66 to 106.64*
cwd	0.0092	1.01	0.0341	0.94 to 1.08
cc	2.9245	18.62	0.8697	3.39 to 102.42*
txY	0.4128	1.51	0.3433	0.77 to 2.96

*C.I. does not contain 1.

Chapter 2: Western Gray Squirrel Space Use in the North Cascades

Abstract

Habitat quality influences spatial use patterns of wildlife populations. We evaluated home range size, interfix distance, and spatial overlap of western gray squirrels in the Lake Chelan National Recreation Area of the North Cascades National Park Complex, and the southern Methow Valley, Washington. Female average home range sizes across both sites were statistically similar to Klickitat County, Washington, and significantly smaller than previous estimates for the North Cascades (Gregory 2005). Maximum interfix distance of males and pairwise overlap among all sexes was larger than previously reported. Our calculations of smaller home range size and larger overlap indicate that the North Cascades may be a higher quality habitat than previously hypothesized.

Introduction

Habitat quality influences movements, home range size, and territoriality within wildlife populations (Burt 1943). When habitat quality and population density is low animals must travel greater distances to obtain resources and find mates (Don 1983, Kenward 1985). Overlap of individuals can also be expected to decrease when resources are limited, as animals defend high quality habitat patches from con-specifics (Gurnell 1987). Population density is generally lower at the edge of a species' distribution (Sagarin et al. 2006).

The Washington North Cascades represent the northern-most documented range of the western gray squirrel (*Sciurus griseus)*, which was classified as a Washington state threatened species by the Washington Department of Fish and Wildlife (WDFW) in 1993 due to a decline in range and number. The North Cascades represent a unique mixed-conifer forest habitat composed primarily of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*) that lacks oak (*Quercus spp.*), an important source of forage and maternal nests in most other portions of the range. Previous research in the area has suggested the North Cascades is a low quality habitat with a low population density of western gray squirrels (Gregory 2005).

We examined space use including interfix distance, home range size, and spatial overlap of western gray squirrels between sexes and seasons (breeding v. non-breeding) at two study sites in the North Cascades: the Lake Chelan National Recreation Area (LCNRA) of the North Cascades National Park Complex, and the southern Methow Valley. We compared our results with data from Klickitat County, Washington, the largest western gray squirrel population in the state, in addition to Gregory's (2005) research in the North Cascades, expecting to find similar patterns: larger average interfix distances and home range sizes, and lower average overlap (particularly in the LCNRA) because the North Cascades represent lower quality habitat at the edge of the species range.

Methods

Study sites: p. 4.

Field methods: p.16.

Analysis

Study area extents were delineated using all squirrel relocations surrounded by a 500m buffer, representative of average breeding male movements and consistent with Gregory (2005). In Stehekin, the 500m buffer only included the northwestern to northeastern side of the lake, not encompassing or crossing the water because western gray squirrels have never been observed on the south side of Lake Chelan. Total study area sizes were 3,468 and 5,773 hectares in Stehekin and Squaw Creek respectively.

We calculated interfix distance between every location for each squirrel using Hawth's Tools (Beyer 2004) in ArcMap (Esri 2009) and compared average and maximum interfix distance between sexes and study sites using the Wilcoxon Signed Rank test.

Home range sizes were calculated for squirrels with greater than 30 relocations per season (Kernohan et al. 2001) in Hawth's Tools using a fixed kernel estimator (Worten 1989), which is considered most accurate along the outer portions of the home range (Seamen et al. 1999). We defined the breeding season in the North Cascades as March 1-July 31 and non-breeding as August 1-February 28 based on temperature and precipitation data (2005-2011 NCDC NOAA Climatological Data) and field observations of squirrels. We removed repeated observations of squirrels in the same nest to correct for spatial autocorrelation. We used the bivariate plug-in smoothing parameter because home range sizes reached relative asymptotes at smaller sample sizes using this smoothing parameter compared to least-squares cross-validation (LSCV; Figure 1.4). The bivariate plug-in smoothing parameter was also most consistent across varying sample sizes and created kernel distributions that best fit observed movement patterns and spatial configuration of telemetry points (Gitzen et al. 2006). We compared home range sizes between sexes, seasons (breeding vs. non-breeding), and study sites, including raw data from Black Canyon Creek from 2003-2004 provided by Sara Gregory (2005), and raw data from Klickitat county from 2003-2005 provided by Matt Vander Haegen (Washington Department of Fish and Wildlife, unpublished data). Home range sizes for all areas were calculated using the same methods and smoothing parameter.

We calculated and compared two-dimensional home-range overlap between pairs of squirrels across sexes using polygons created from 95% Fixed Kernels (95FK) and Minimum Convex Polygons (MCP), and core area overlap with polygons created from 50% Fixed Kernels (50FK). We also calculated three-dimensional overlap or Volume of Intersection (V.I.; Seidel 1992) for each squirrel pair. We used the non-parametric conservative Kruskal-Wallis rank sum chi-square test with multiple comparison after Kruskal-Wallis ($P= 0.05$), for home range size and overlap comparisons because we could not assume normality and homogeneity of variance for either set of data.

Statistical tests were conducted in R version 2.7.2 (R Development Core Team 2008) with packages ks (Duong 2012), and pgirmess (Giraudoux 2008).

Results

Over 4 field seasons we captured a total of 61 squirrels: 24 in Stehekin, 37 at Squaw Creek. Average weight at capture in Stehekin was 777g for males, range 685-835g (n= 12). Average female weight was 845g, range 715-950g (n=13). At Squaw Creek males weighed 813g on average, range 685-900g (n= 22). Females averaged 810g, range 730-865g (n= 15). We radio-collared 12 females and 10 males in Stehekin, and 12 females and 12 males at Squaw Creek. Of those, 12 females and 5 males from Stehekin, and 11 females and 10 males from Squaw Creek (total sample size: 38 squirrels) had greater than 30 relocations and were used for home range and habitat use analysis. We were also able to use data on 12 squirrels: 8 females, 4 males, from Sara Gregory’s 2003-2004 research at Black Canyon Creek which created a total sample size of 50 squirrels: 31 females, 19 males.

Interfix Distance

Average distance between fixes was 189m ± 16m and 207m ± 21m for female squirrels in Stehekin and Squaw Creek, respectively. The maximum distance traveled by a female between two consecutive relocations was 1009m in Stehekin and 1168m in Squaw Creek. Male squirrels traveled larger distances than females between fixes on average and also had significantly larger maximum distances traveled (W = 73 for average distance, 89 for maximum distance, P<0.001 for both). The average distance between fixes for males in Stehekin was 864m ± 168m and was 541m ± 72m for males at Squaw Creek. One male squirrel in Stehekin averaged 1,883m between fixes, as he regularly traveled between the Stehekin Landing and Moore Point on a daily basis (Figure 2.1). We also observed three male relocations over 2-day periods: 5,176m when a male relocated from Squaw Creek to the mouth of McFarland Creek in July of 2011, 7,646m when another male relocated from Squaw Creek to a privately owned orchard at the base of Bray Canyon in June of 2010 (Figure 2.2), and a 15,413m trek made by a male squirrel from the Stehekin Landing to Cascade Creek (Figure 2.1) in April of 2010. The two squirrels from Squaw Creek remained in their new areas until we removed their collars at the end of the study. The male from Stehekin was predated a month after he relocated to Cascade Creek. Maximum distances between fixes for males that did not relocate averaged 8,408m in Stehekin and 5,257m at Squaw Creek.

Average and maximum movements for females were consistent with data from Black Canyon Creek, which were significantly larger than females in Klickitat County (Gregory 2005). Average distance between fixes for males at Squaw Creek and Stehekin were also similar to Black Canyon; however, males in Stehekin and Squaw Creek both had significantly larger maximum distances between fixes than at Black Canyon (χ^2= 14.725, P<0.001).

Home Range Size

The number of fixes used to calculate home range sizes for the 38 squirrels at Stehekin and Squaw Creek with greater than 30 relocations ranged from 30 to 192. Squirrels were tracked on average for 6 months, range 3-17 months. Consistent with other studies on western gray squirrels in Washington (Linders 2000, Gregory 2005, Vander Haegen et al. 2005) males in Stehekin and Squaw Creek had significantly larger 50FK core use areas and 95FK and MCP home ranges than females in both the breeding and non-breeding seasons (50FK: χ^2= 28.414, 95FK: χ^2= 32.123, MCP: χ^2= 31.8293; P<0.001 for all). Home ranges of males in Stehekin and Squaw Creek were both significantly larger in the breeding season than in the non-breeding season (50FK: χ^2=10.732, P= 0.005, 95FK: χ^2=11.075, P= 0.003, MCP: χ^2= 7.4003, P = 0.025). Home ranges of females were slightly larger in the breeding season compared to the non-breeding season, however, differences were not significant (50FK: χ^2= 3.521, P= 0.061, 95FK: χ^2= 1.688, P= 0.194, MCP: χ^2= 0.75, P= 0.387). Consequently, breeding and non-breeding females were grouped together for further analyses (Table 2.1, Figure 2.3).

There were no significant differences in home range size between study areas (Stehekin, Squaw Creek, Black Canyon, Klickitat) for males in either the breeding or non-breeding seasons. Females had significantly larger 95FK home range sizes at Black Canyon Creek (χ^2= 11.102, P=0.011); differences between Black Canyon and Stehekin, Black Canyon and Squaw Creek, and Black Canyon and Klickitat were all significant. Differences between Stehekin and Squaw Creek, Stehekin and Klickitat, and Squaw Creek and Klickitat were not significant. Core-area 50FK areas at Black Canyon Creek were also significantly larger than at Stehekin (χ^2= 8.554, P=0.036). There were no statistically significant differences in female MCP home range sizes between study sites (Table 2.2). Results were consistent regardless of smoothing parameter, and when standardized by the number of locations (Figure 2.4).

Spatial Overlap

Sample sizes were not large enough to run separate statistical analyses of spatial overlap for each study area. Across study areas, there were significant differences in two-dimensional percent overlap between one or more sex groupings (female/female, female/non-breeding male, female/breeding male, non-breeding male/non-breeding male, breeding male/breeding male) at all scales (50FK: χ^2= 14.794, 95FK: χ^2= 29.700, MCP: χ^2= 33.938; P>0.001 for all). Average percent overlap between females and breeding males was significantly larger than overlap between females at all scales (50FK: observed difference = 34.81, critical difference = 25.73, 95FK: observed difference = 53.24, critical difference = 30.64, MCP: observed difference = 44.47, critical difference = 26.13, P=0.05 for all). Average 95FK and MCP female/breeding male overlap was also significantly larger than the average percent overlap between non-breeding males (95FK: observed difference = 48.98, critical difference = 40.61, MCP: observed difference = 45.17, critical difference = 34.24). There were no significant statistical differences in V.I. between any group of squirrels (χ^2= 4.149, P= 0.386). In general, average V.I.s were lower than average 50FK overlap, with the exception of female/breeding male and female/non-breeding male overlap in Stehekin which had the two highest V.I. averages. Average V.I.s were also smaller at Squaw Creek (Table 2.3). Overlap was also examined in relation to genetic relatedness in Chapter 4.

Discussion

Average and maximum interfix distances for squirrels at our two study sites in the North Cascades were larger than for squirrels in southern Washington, Oregon, and California, and similar to those recorded by Gregory (2005). We recorded the highest maximum interfix distances for male western gray squirrels, including three long distance relocations. These trends are generally indicative of low population density (Kenward 1985), and potentially lower habitat quality (Don 1983) as male squirrels travel longer distances to find mates and resources. However, the lack of significant difference in average home range sizes of females, who are more consistent predictors of squirrel resource selection (Linders 2004, Gregory 2005, Vander Haegen et al. 2005), between our North Cascades study sites and southern Washington indicates that habitat quality in the North Cascades may not be as low as previously hypothesized (Gregory 2005).

We were surprised to find significant differences in female home range sizes between Black Canyon and Squaw Creek because these two sites are in adjacent drainages. Habitat differences that distinguish Black Canyon from Squaw Creek and could result in larger squirrel home range size at Black Canyon include higher average elevation, further average distance from water, and closer average proximity to roads. The Resource Utilization Function reported previously (Chapter 1), indicated that squirrel high use areas were more likely to be at lower elevations, closer to water, and further from roads. Additionally, Black Canyon had higher average counts of understory species which were negatively related to squirrel high use areas. Through field work at both sites we also noticed that Black Canyon was a more patchy habitat with clumps of forest habitat interspersed with rocky outcroppings. Future research could look more specifically at land cover patchiness using metrics similar to those used by Marzluff et al. (2004). Despite geographic proximity, these landscape differences between Squaw Creek and Black Canyon may be substantial enough to explain the significant differences observed in home range size, and classify Black Canyon as a lower quality habitat within the North Cascades ecosystem.

The large-scale daily movements and relocations by male squirrels indicate that there may be some connection between sub-populations of western gray squirrels in the North Cascades. The 37 km relocation of one male squirrel from Stehekin down the Lake Chelan corridor also challenges the previous theory that western gray squirrels were introduced to this area (Linders and Stinson 2007).

Home-range (95FK) and core use (50FK) overlap between squirrels at our study sites (all sex groupings) were larger than reported by Gregory (2005) for the North Cascades, Linders (2000) for southern Washington, and Gilman (1986) for California. The smaller home range sizes, higher average degree of overlap, and recent identification of additional sites of western gray squirrel presence within the northern Methow Valley (Yamamuro et al. 2011) indicate that the North Cascades may be a higher quality habitat, potentially able to support a larger population of western gray squirrels than previously estimated in the Western Gray Squirrel Recovery Plan (Linders and Stinson 2007).

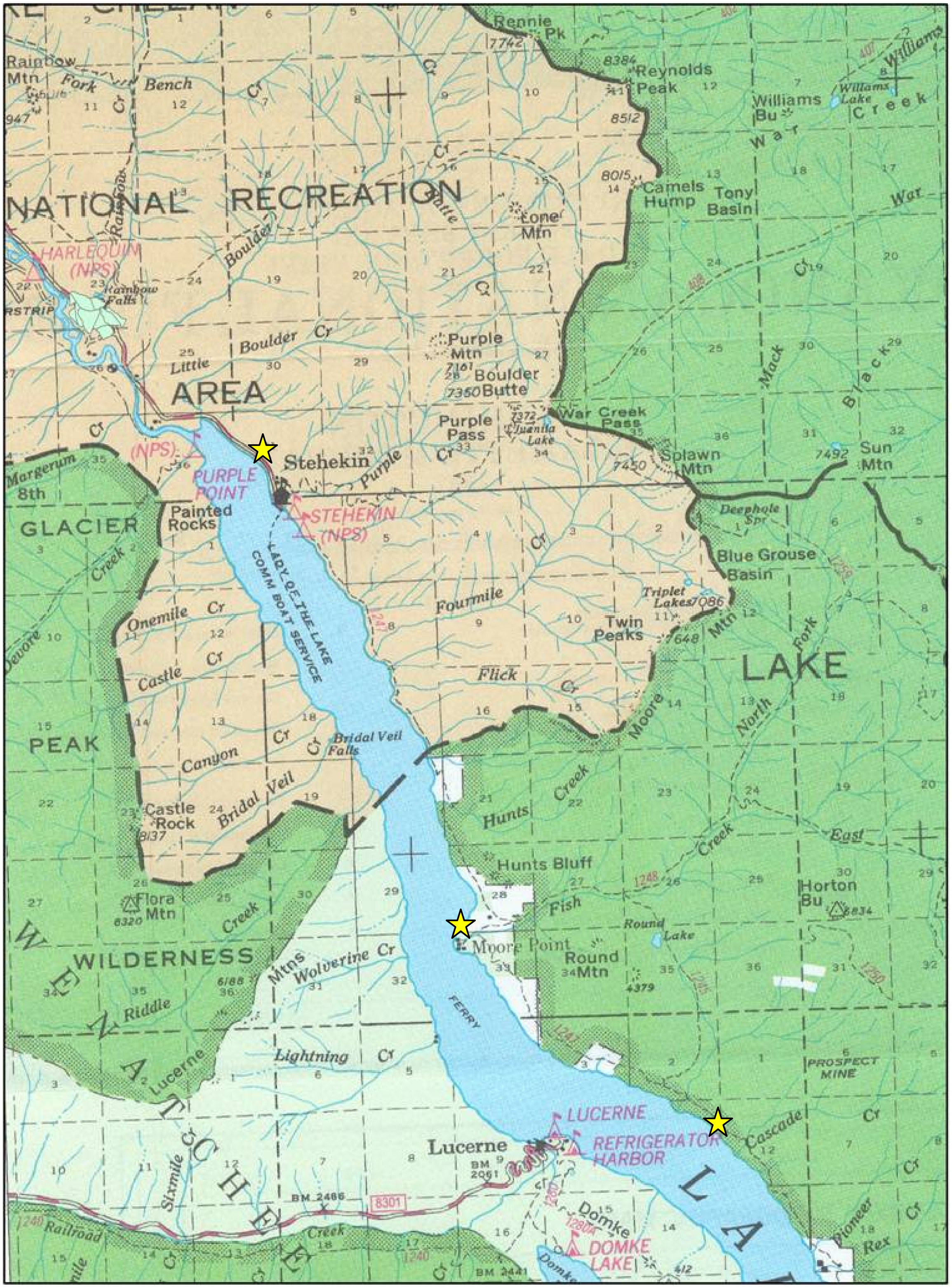
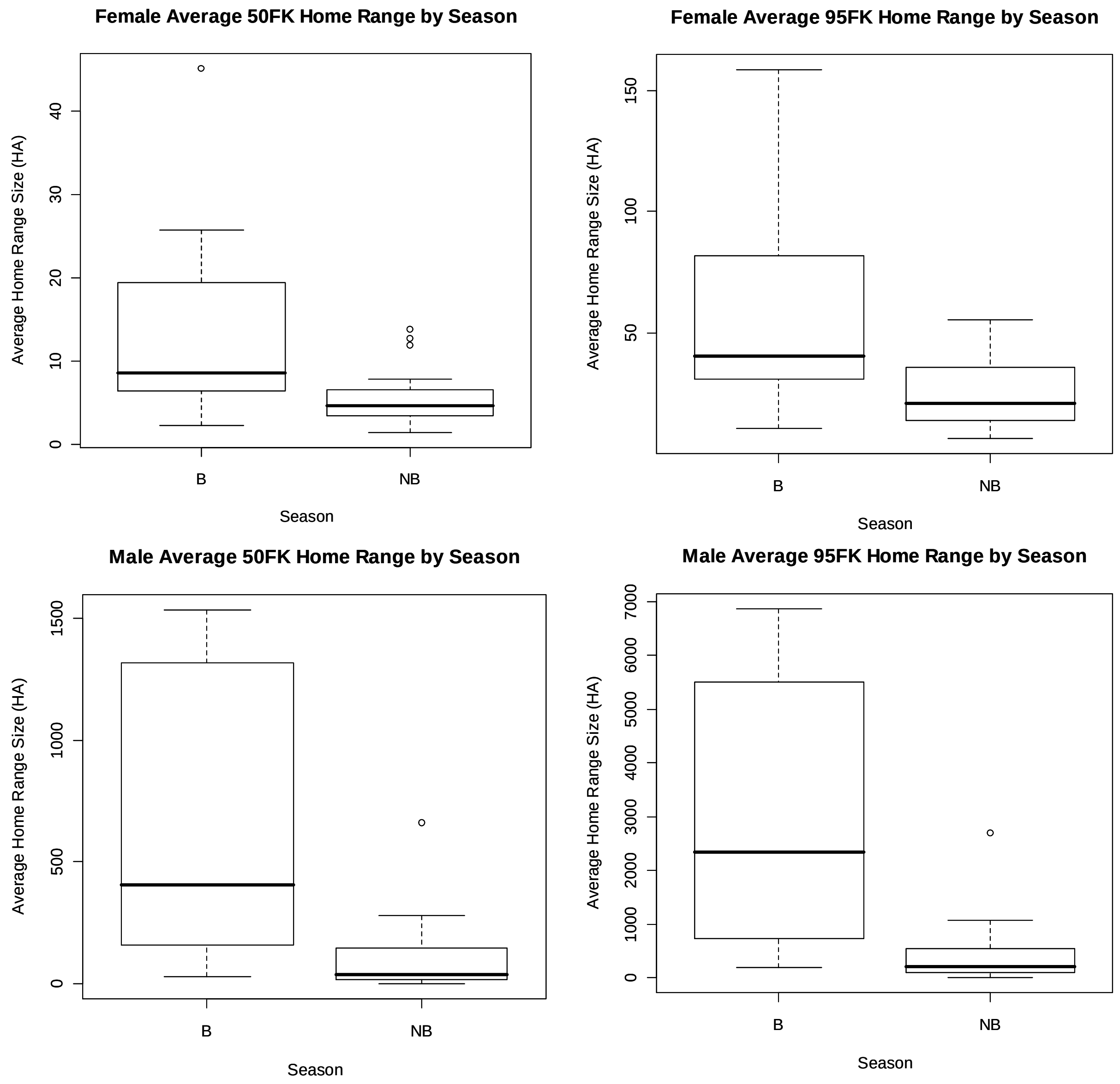


Figure 2.1. Map of Stehekin showing interfix and relocation areas: Stehekin Landing, Moore Point, and Cascade Creek.

Figure 2.2. Map of the southern Methow Valley showing interfix and relocation areas: Squaw Creek, Bray Canyon, and McFarland Creek.


Female Average 50FK Home Range by Season

Female Average 95FK Home Range by Season

Male Average 50FK Home Range by Season

Male Average 95FK Home Range by Season

Figure 2.3. Average home range size of male and female squirrels at Stehekin and Squaw Creek by season calculated with 50% and 95% Fixed Kernel home range estimators (Worten 1989) using the bivariate plug-in smoothing parameter to represent core use and overall home range sizes. Error bars represent one standard error from the mean. Seasonal differences for males were statistically significant (50FK: χ^2=10.732, *P*= 0.005, 95FK: χ^2=11.075, *P*= 0.003). Differences between females were not statistically significant (50FK: χ^2= 3.521, *P*= 0.061, 95FK: χ^2= 1.688, *P*= 0.194) therefore all females were grouped together for further analysis.

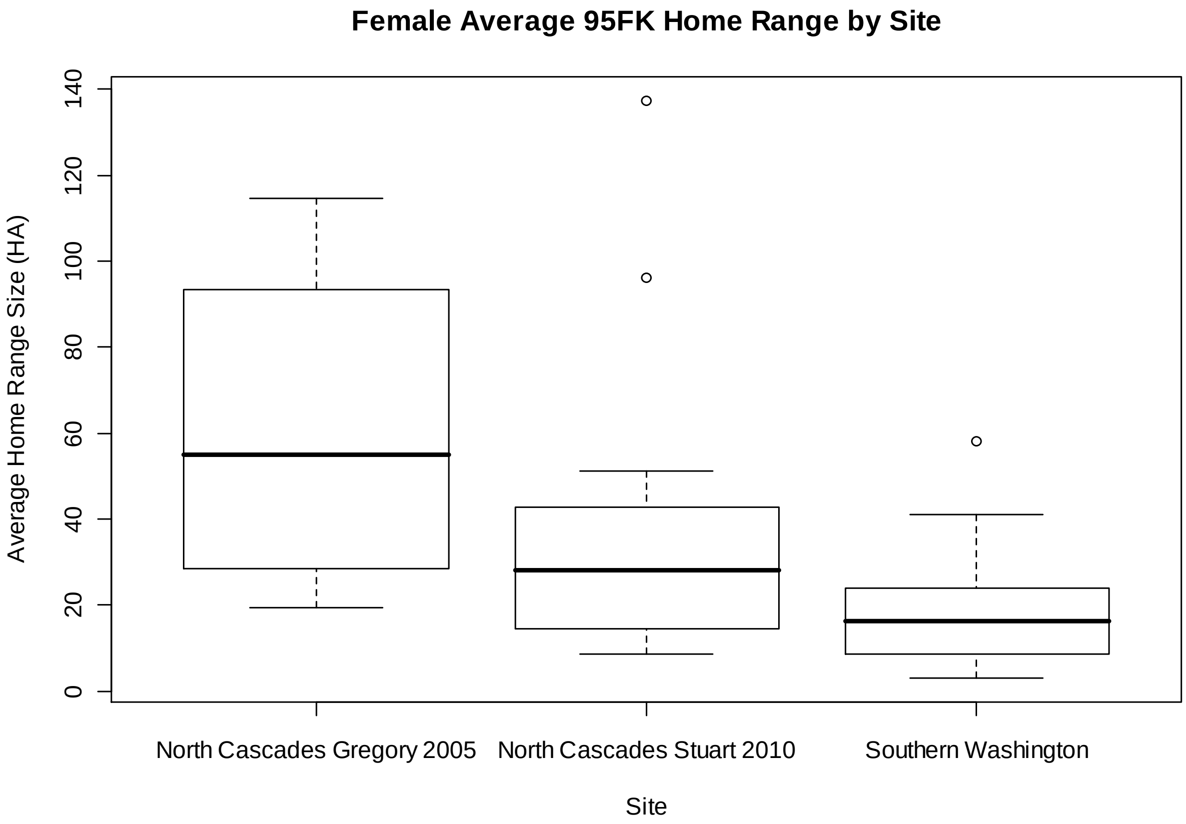


Figure 2.4. Comparison of home range sizes in the North Cascades from 2005 (Gregory), 2010 (Stuart), and southern Washington (Vander Haegen) using only the first 40 relocations for each female squirrel with a 95% Fixed Kernel estimator (Worten 1989) and the bivariate smoothing parameter. Error bars represent one standard error from the mean. North Cascades home range sizes as calculated in 2005 are significantly larger than those calculated by Stuart in 2010 and those reported for Southern Washington (Vander Haegen 2005) χ^2= 11.102, *P*=0.011; multiple comparison *P*= 0.05.

Table 2.1. Average core-use (estimated with a 50% fixed kernel estimator: FK; Worten 1989) and home range sizes (95% FK, and Minimum Convex Polygon: MCP) for western gray squirrels at Stehekin and Squaw Creek, in the Washington North Cascades. Fixed kernel estimates calculated with least-squares cross-validation (LSCV) and the Bivariate Plug-in smoothing parameters.

		Stehekin				Squaw Creek			
		LSCV		Bivariate Plug-In		LSCV		Bivariate Plug-In	
Squirrel Group	Size	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Female Non-Breeding	50FK	4.38	0.83	5.751	1.07	3.87	0.89	5.65	1.32
n = 12, 9	95FK	19.72	3.56	26.307	4.61	16.60	3.47	23.87	5.11
	MCP	23.60	5.72	23.602	5.72	17.76	3.86	17.76	3.86
Female Breeding	50FK	5.04	1.37	10.10	1.52	11.24	5.17	19.39	14.25
n =3, 5	95FK	23.88	6.60	40.31	4.04	50.67	21.28	78.29	50.24
	MCP	31.31	12.43	31.31	12.43	53.09	28.39	53.09	28.39
Female Total	50FK	4.42	0.75	5.85	1.05	6.92	2.40	9.89	4.64
n= 12, 11	95FK	19.81	3.53	26.46	4.57	31.39	10.32	41.03	16.19
	MCP	26.13	6.99	26.13	6.99	32.21	11.32	32.21	11.32
Male Non-Breeding	50FK	32.35	21.07	21.27	7.77	14.05	5.14	17.40	6.67
n= 4, 10	95FK	164.77	118.28	134.32	52.14	57.90	19.01	74.02	30.18
	MCP	124.31	53.57	124.31	53.57	89.29	44.31	89.29	44.31
Male Breeding	50FK	74.27	10.49	95.67	64.64	126.86	19.05	99.74	21.22
n= 2, 7	95FK	436.85	62.50	459.65	276.91	663.75	101.57	429.72	86.57
	MCP	773.91	35.77	773.91	35.77	547.58	193.91	547.58	193.91

Table 2.2. Comparison of North Cascades and Klickitat County, Washington core-use and home range sizes (ha), calculated with Minimum Convex Polygons (MCP) and fixed kernel estimators (FK; Worten 1989) with the bivariate plug-in smoothing parameter. Statistical significance calculated with the conservative Kruskal-Wallis rank sum chi-square test and multiple comparison after Kruskal-Wallis, *P*= 0.05.

	Study Area	n	50FK ± SE	95FK ± SE	MCP ± SE
Female	Stehekin	12	5.85 ± 1.05†	26.46 ± 4.57†	26.13 ± 6.99
	Squaw Creek	11	9.33 ± 3.79	37.76 ± 13.39†	29.63 ± 9.11
	Black Canyon Creek[a]	7	18.86 ± 3.13^	79.65 ± 12.37^*~	53.72 ± 8.38
	Klickitat[b]	12	7.81 ± 1.66	35.90 ± 5.22†	66.88 ± 18.84
Male Non-Breeding	Stehekin	4	21.27 ± 7.77	134.22 ± 52.14	124.31 ± 53.57
	Squaw Creek	10	17.40 ± 6.67	74.02 ± 30.18	89.29 ± 44.31
	Black Canyon Creek[a]	3	26.06 ± 11.75	120.33 ± 45.66	97.30 ± 32.71
	Klickitat[b]	6	18.75 ± 6.97	100.66 ± 40.57	134.61 ± 60.12
Male Breeding	Stehekin	2	95.67 ± 64.64	459.65 ± 276.91	773.91 ± 35.77
	Squaw Creek	7	99.74 ± 21.22	429.72 ± 86.57	547.58 ± 193.91
	Black Canyon Creek[a]	4	64.30 ± 20.23	274.96 ± 82.42	210.26 ± 58.57
	Klickitat[b]	9	61.13 ± 15.36	282.37 ± 57.69	334.89 ± 75.00

[a] Gregory (2005)

[b] Vander Haegen (2005)

^Statistically different from Stehekin

*Statistically different from Squaw Creek

†Statistically different from Black Canyon Creek

~Statistically different from Klickitat

Table 2.3. Average two and three-dimensional overlap of Minimum Convex Polygons (MCP) and fixed kernels (FK; Worten 1989) between groups of squirrels at two study sites: Stehekin and Squaw Creek in the North Cascades, Washington. Sample size (n) represents the number of overlapping pairs in each category. Fixed kernels calculated with the bivariate smoothing parameter.

Stehekin

Type of Overlap	MCP	n	95FK	n	50FK	n	V.I.*	n
F-F	40.8% ± 5.1	37	43.5% ± 4.1	38	33.2% ± 5.0	30	29.3% ± 5.2	13
MNB-MNB	25.9% ± 7.0	2	13.1% ± 4.0	2	NA	0	5%	1
MB-MB	47.0% ± 2.1	2	48.8% ± 29.4	2	NA	0	20%	1
F-MNB	76.3% ± 16.3	5	60.0% ± 14.6	10	55.2% ± 12.3	6	78.4% ± 1.1	11
F-MB	52.0% ± 11.1	12	74.8% ± 8.6	12	76.4% ± 11.5	6	90.0% ± 4.9	8

Squaw Creek

Type of Overlap	MCP	n	95FK	n	50FK	n	V.I.*	n
F-F	35.2% ± 7.0	16	27.8% ± 6.3	17	21.0% ± 8.1	12	19.7% ± 5.8	10
MNB-MNB	38.8% ± 6.1	20	44.0% ± 6.6	20	37.2% ± 6.9	20	18.6% ± 8.0	10
MB-MB	69.0% ± 4.8	20	47.1% ± 6.4	20	40.8% ± 6.9	20	34.1% ± 6.9	24
F-MNB	68.7% ± 10.2	10	50.3% ± 5.6	25	37.7% ± 7.6	15	28.1% ± 4.7	25
F-MB	74.0% ± 4.9	44	69.5% ± 5.4	45	55.2% ± 7.1	27	24.7% ± 5.7	38

*V.I. = volume of intersection (three-dimensional overlap; Seidel 1992)

Chapter 3: Notes on Winter Ecology of the Western Gray Squirrel in the North Cascades

Abstract

We studied winter ecology of the threatened western gray squirrel (*Sciurus griseus)* at its northern-most documented range between 2009 and 2011 at two study sites in the Washington North Cascades. We followed 14 squirrels with radiotelemetry and compared activity patterns between season, time of day, temperature, precipitation type (none, rain, snow), wind velocity (none, slight, moderate, severe) and cloud cover using logistic regression. We also recorded observations on snow depth and presence of tracks and food cache retrieval digs at squirrel radiotelemetry location sites and monitored squirrel mortality by season. We compared winter and non-winter core-use (50% fixed kernel) and home range (95% fixed kernel) sizes for two squirrels with adequate relocation information per season. Activity significantly decreased in winter, especially in the afternoon, however it was not due to lower average temperatures or active snowfall. Core use and home range sizes also decreased in winter. We observed a large number of mortalities during fall and winter at one study site. Identification of winter nest sites and core use areas could help prioritize western gray squirrel habitat conservation efforts with land management.

Introduction

Winter: characterized by cold temperatures and presence of snow, challenges wildlife with scarcity of food, increased heat loss to the surrounding environment, and reduction of mobility. Challenges can be magnified for small populations and species at the northern edge of their range, where densities and reproductive rates are generally lower than in the core of a species' range. The Washington North Cascades represent the northern-most documented range of the western gray squirrel (*Sciurus griseus)*, which was classified as a Washington state threatened species by the Washington Department of Fish and Wildlife in 1993 due to a decline in range and number. Average annual snowfall in the North Cascades is 317cm with temperatures ranging from -30 to 10°C in winter months (November-February; 2005-2011 NCDC NOAA Climatological Data).

We began a radiotelemetry study in 2008 to evaluate habitat use and resource selection of western gray squirrels in the North Cascades at two study sites: Stehekin, WA, part of the Lake Chelan National Recreation Area of the North Cascades National Park Complex, and the Squaw Creek drainage in the Methow Valley near Pateros, WA. Early accounts of squirrels in these areas report small populations unable to thrive due to harsh weather (Stream 1993, Walker pers. comm.), however, museum records of western gray squirrels in Chelan County date back to 1918 (Manson), and 1921 (Lakeside; Linders and Stinson 2007), and residents report that sizeable populations have been in existence since at least the 1960s, and 1980s in Stehekin and Squaw Creek respectively. We monitored squirrels throughout winter when possible to evaluate changes in activity patterns that could provide insight to behavioral adaptations that have allowed squirrels to persist in these areas.

Western gray squirrels do not hibernate (Cross 1969), but may alter activity levels and resource selection patterns in response to temperature and snow quantity (depth) and quality (density), which alters mobility and access to food. Previous research on western gray squirrels has suggested that during the shorter days of winter activity may be reduced to a single period (Cross 1969, Gurnell 1987). Koprowski and Corse (2005) reported unimodal, and shortened periods of activity in Mexican fox squirrels (*Sciurus nayaritensis*) in Arizona during winter compared to longer, bimodal periods of activity in summer, and an increase in communal nesting in winter. Hicks (1949) also noted substantial declines in western fox squirrel (*Sciurus niger rufiventer*) activity in winter in Iowa with fewer observations of squirrels and tracks per hour compared to other seasons. Fox squirrels were also less active when snow was falling compared to clear weather and reduced activity considerably when snow depth exceeded 25cm (Hicks 1949). Red squirrels (*Sciurus vulgaris L.*) in Finland also decreased activity, and traveled on the snow covered ground more frequently than through the tree canopy in winter (Pulliainen 1973).

We hypothesized that western gray squirrels would be less active in winter months during cold periods and periods of active snowfall, spending more time in nests and co-nesting more frequently than in other seasons. We also hypothesized that squirrels might spend more time traveling through the canopy than on the ground during periods of heavy snow cover or that they may alter their home range and/or core use sizes and move to areas with less snow cover to ease movement and access to food.

Winter can also be a time of high mortality: the severity of winter weather was negatively associated with survival of eastern gray squirrels (*Sciuris carolinensis*) in southern England (Gurnell 1996), and survival was significantly lower and inversely related to duration of snow cover for Abert’s squirrel’s (*Sciurus aberti*) in North-Central Arizona (Dodd et al. 2003). Further, survival of Eurasian red squirrels was also lower during autumn-winter in a high elevation edge habitat compared to more productive low elevation habitats in Italy (Rodrigues et al. 2010, Romeo et al. 2010). We documented mortality and its causes by season to evaluate whether winter challenges may have played a role in the decline of the western gray squirrel in Washington.

Methods

Field

We studied winter ecology of western gray squirrels at two sites in the Washington North Cascades: Stehekin, , which is part of the Lake Chelan National Recreation Area of the North Cascades National Park Complex, and along Squaw Creek road in the Methow Valley. Both are mixed conifer/deciduous habitats composed primarily of Douglas-fir (*Pseudotsuga menziessi*) and ponderosa pine (*Pinus ponderosa*). Typically winter weather is cool and wet; summers are hot and dry. Additional study site information: p.4. Between April 2008 and September 2011 we live-trapped western gray squirrels using wire mesh 15 x 15 x 48-cm Tomahawk live traps (Tomahawk Live Trap Co. Tomahawk WI, USA). Traps were baited with whole English walnuts and spaced between 50m and 80m apart placed on the North side of the base of large diameter trees. Traps were wired open with metal 3.2-cm book rings for a pre-baiting period to train squirrels to enter the traps; trapping began when bait was removed from approximately half of the traps which took 1 to 4 weeks of pre-baiting. Traps were opened just prior to sunrise and checked approximately every 2 hours, with animals left in traps for no longer than 4 hours. Trapping was not conducted on days with rain, snow, or extremely low temperatures.

Captured animals were processed in a handling bag (Koprowski 2002) modified with an additional ventral opening. Squirrels were weighed to the nearest 5g with a 1000g or 2500g Pesola spring scale, sexed, examined for reproductive status, and marked with uniquely numbered ear tags (model 1005-3, National Band and Tag Co., Newport, KY, USA). Most were also fitted with radio collars (Holohil Model SC-2C), weighing approximately 12g and consisting of a metal cable protected with a thick plastic coating and covered with Tygon tubing to prevent abrasion to the squirrel. Only squirrels weighing $\geq$ 600g were fitted with radio-collars. All trapping, handling and radio-collaring methods followed guidelines of the American

Society of Mammalogists (Gannon and Sikes 2007) and were approved by the University of Washington Institutional Animal Care and Use Committee (IACUC) protocol number: 2479-29.

Radio-collared squirrels were relocated with homing using two-element, hand-held directional antennas and portable TR-4 receivers (Telonics, Mesa, Arizona, USA). Activity of squirrels was monitored prior to homing based on the consistency of the signal: changes in volume and time between pulses indicated active squirrels; a consistent volume and pulse for 3 minutes indicated inactive squirrels. Tracking was discontinued if signal strength decreased abruptly more than once during pursuit, indicating the animal was “running from observer” and movements were being influenced. Squirrels were tracked to the tree (or location on the ground) when possible and locations confirmed with visual sightings using 10 X 40 binoculars. Tree locations were examined for nests for a minimum of 10 minutes using 10 X 40 binoculars. The condition of the animal and behavior, such as running on ground, traveling through canopy, foraging, or sitting in tree, was also documented for most relocations. Universal Transverse Mercator (UTM) (NAD 1983) coordinates were recorded on hand-held GPS units and plotted on Geographic Information Systems (GIS) maps. Habitat characteristics were assessed categorically for each radiotelemetry location and included the following information:

- Date and Time
- Substrate (type of tree, nest, or on ground)
- Categorical data regarding size, structure, and composition of stands
- Weather (categorical for sky and wind; temperature to 1 ºC using 2005-2011 NCDC NOAA Climatological Data)

Additionally, during winter, we recorded observations on snow quantity and quality, and presence and depth of tracks and recent digs. Snow depth was measured to the nearest cm with a clear ruler or marked stick in several locations within the 5-m radius surrounding the relocation to obtain an average. Snow variation at relocation sites dictated the specific number of measurements taken. Track and dig depth were measured using the same instrument.

Squirrels were located at least every 5 days by a field researcher; on average 3-6 times per week throughout the year and 6-12 times per week during winter to measure activity patterns. To ensure independence between observations (White and Garrott 1990, Swihart and Slade 1997, Otis and White 1999), relocations were spaced across the diurnal period and individual squirrels were located three times per day at maximum with a minimum lapse of two hours: a period adequate for a squirrel to traverse its home range (Linders et al. 2004). All squirrels were located with approximate equal frequency.

Analysis

To evaluate squirrel activity patterns across seasons we used logistic regression in a generalized linear mixed model fit by the Laplace approximation with a binary dependent variable (active or inactive), and predictor variables: squirrel (random effect used for stratification), precipitation type (categorical: none, rain, snow), wind velocity (categorical: none, slight, moderate, severe), cloud cover (categorical % by 10 from 0-100), temperature (°C), time (categorical: early morning= 0600-0859, mid-morning= 0900-1159, early afternoon= 1200-1459, afternoon/evening= 1500-1900, and season (Fall= September-November, Winter= December-February, Spring= March-May, Summer= June-August). Odds ratios were calculated by exponentiating the coefficient, 95% confidence intervals were calculated with the formula: $e^{(\beta \pm 1.96SE)}$.

For squirrels with greater than 30 relocations per season (Kernohan et al. 2001), we calculated home range and core use areas using Hawth's Analysis Tools for GIS (Beyer 2004) in ArcMap 9.3.1. (Esri 2009) with a fixed kernel estimator (Worten 1989), which is considered most accurate along the outer portions of the kernel (Seamen et al. 1999). Winter (November-February) home range and core-use areas were compared to non-winter with approximately the same number of telemetry locations used per season for each squirrel. We used the bivariate plug-in smoothing parameter because it was most consistent across varying sample sizes and created kernel distributions that best fit observed movement patterns in the field (Gitzen et al. 2006). We then examined seasonal shifts in overall home range size (95% Fixed Kernel; 95FK) and core use (50% Fixed Kernel; 50FK) areas by comparing two dimensional overlap of polygons and three dimensional overlap or volume of intersection (V.I.; Seidel 1992) between winter and non-winter for each squirrel.

Statistical tests were conducted in R version 2.7.2, and 2.14.2 (R Development Core Team 2008), using package lme4 (Bates et al. 2011), and ks (Duong 2012).

Results

We observed 14 squirrels: 8 female, 6 male, during the winters of 2009-2011 (November-February) with radiotelemetry for an average of 2 months each. Mortality was high in Stehekin in fall and we were only able to monitor two female squirrels through the duration of the winter season. Complete winter field work was also not possible at Squaw Creek due to logistical challenges; however, we did obtain regular observations of two female squirrels throughout the winter season from two local landowners we trained in radiotelemetry.

Squirrels were tracked in temperatures down to -25°C in snow depths up to 93cm in Stehekin and 50cm at Squaw Creek, with active squirrels observed in snow depths up to 30cm at both study sites. The minimum temperature associated with observations of active squirrels was -19° C at Squaw Creek and -13° C in Stehekin. Fresh western gray squirrel tracks were observed in snow depths up to 28cm with maximum track depths of 12cm. The distance between prints ranged from 10 to 55cm with shorter distances in deep, light density snow. On several occasions we observed squirrels dragging their bellies through deep, light snow. When there was a 2-3cm hard crust layer squirrels were able to run more efficiently on top of the snow without breaking through. Squirrels were also observed traveling along snow covered logs and vegetation both in the canopy and close to the ground, however, we did not observe any clear preference for traveling above or on the ground during periods of snow cover: active squirrels were observed traveling on the ground 36% of the time in winter, compared to 34% during all other seasons. Snow did not seem to restrict access to cached food stores in the ground. We located several fresh digs in snow at Squaw Creek ranging from 20 to 33cm deep with 8-15cm of the dig through snow, and the remaining 12-18cm through ground. Several digs were surrounded by shelled bigleaf maple (*Acer macrophyllum*) samaras, indicating that this may be an important winter food cache as there were no bigleaf maple trees in the surrounding areas.

Squirrel activity was not restricted during periods of active snowfall. Squirrels were active during approximately half of telemetry locations recorded when snow was falling (0.54 active, 0.46 inactive) which was similar to periods of no precipitation (0.53 active, 0.47 inactive). Activity decreased during periods of rain (0.41 active, 0.59 inactive; Figure 3.1). The regression coefficient for rain had a negative association with squirrel activity level, with a confidence interval that did not encompass 1, indicating that rain is a strong predictor of activity. Activity also decreased slightly during periods of moderate to severe wind: squirrels were active 43% of the time compared to 52% during periods of slight or no wind, however only 89 (5%) of relocations occurred during periods of moderate and severe wind, reducing power of this conclusion (Figure 3.2). None of the regression coefficients for wind were significant. Cloud cover was also not a significant predictor of squirrel activity. Low temperatures in winter did not significantly decrease squirrel activity until temperatures dropped below -12° C. However, only 35 (2%) of relocations occurred during these low temperatures, suggesting that low sample sizes may influence power of this conclusion. Squirrel activity was also reduced during periods of very warm temperatures above 24° C (number of relocations = 268; 15%); squirrel activity was greatest when temperatures ranged from 7 to 21° C (Figure 3.3). Overall, the regression coefficient for temperature had a significant positive association with squirrel activity, with a confidence interval that did not encompass 1. Time of day had a negative relationship with squirrel activity. Irrespective of temperature, squirrels were more active in the morning between 0600 and noon: 60% of relocations were active, compared to 50% in the afternoon/early evening (noon-1900). Local residents consistently observed squirrels feeding in the early morning by from December through March at Squaw Creek. Squirrels were much less likely to be active in the afternoon and evening in winter: squirrels were active during 39% of telemetry locations after noon, and during only 10% of locations after 1500 (Figure 3.4). Seasonally, squirrels were most active in fall, followed by spring (0.65 active, 0.35 inactive and 0.56 active, 0.44 inactive respectively) and less active in winter and summer (0.42 active, 0.58 inactive and 0.41 active, 0.59 inactive, respectively; Figure 3.5). All seasonal categories were significantly associated with squirrel activity (Table 3.1).

Squirrels were almost always (71%) found in nests during periods of inactivity in winter. Squirrels used an average of 4 nests in winter months compared to 7 during spring, summer, and fall months, and squirrels moved nests less frequently during winter than in summer. We found squirrels using the same nest a maximum of 13 times on average in winter, compared to 8 times in spring, summer, and fall combined (squirrel n = 11). The same nest was also more likely to be used multiple days in a row in winter. On several days during winter we radiotracked squirrels to the same nest throughout the day, indicating that squirrels likely did not leave the nest the entire day. This did not occur in spring, summer, or fall months. Moreover, nests that were used most frequently by squirrels in spring, summer and fall months were not always the same as those used in winter months. Additionally, all of the co-nesting we observed at both study sites occurred between October and January during periods of colder temperatures often when snow was present.

We observed 31 co-nesting events in Stehekin: 4 times by a hypothesized mother/daughter pair from October-December 2008/2009, 17 times by three young of the year hypothesized female siblings from 2009/2010 (14 times between squirrels 2699 and 2700, 2 times between squirrels 2699 and 2663, 1 time between squirrels 2700 and 2663), and 10 times by a hypothesized mother/son pair from December-January 2009/2010. Average temperature during co-nesting events was -1.2 ± 1.8°C [SE] (range -12 to 7°C); snow was present on the ground during 77.4% of these events. At Squaw Creek co-nesting was observed 11 times between a mother and 3 hypothesized offspring (2 juvenile females, 1 juvenile male), and 4 times between another mother/hypothesized daughter pair from October-January 2010/2011. The average temperature during co-nesting events was 1.4 ± 3.4°C [SE] (range -4 to 13°C); snow was present on the ground during 72.7% of events.

Only two female squirrels in Stehekin had a sufficient number of telemetry locations to compare seasonality of core use and home range sizes. For both squirrels winter 50FK and 95FK home range sizes were reduced to approximately half the size of non-winter ranges. Core-use areas (50FK) were 2.2 and 4.8ha in winter compared to 8 and 9.5ha in non-winter. Home range sizes (95FK) were 19.6 and 22.6ha in winter compared to 36.7 and 41.4ha in non-winter. Two dimensional overlap of winter core use areas with non-winter core use areas was 0.09 and 0.55; overlap of winter home range sizes with non-winter home range sizes was 0.85 and 0.99. VI was 0.34 and 0.60.

We observed a large number of mortalities between fall and winter at both study sites. In Stehekin, 2 of 6 (33.3%) radio-collared squirrels died during fall and winter 2008/2009 compared to 3 of 8 (37.5%) during spring and summer 2008; 8 of 11 (72.7%) radio-collared squirrels died during fall and winter 2009/2010, compared to 1 of 7 (14.3%) during spring and summer 2009. Two of the squirrels in Stehekin died of a combination of starvation and disease immediately following a particularly cold period in November of 2009 with temperatures hovering below freezing (average -7°C) for a week. Another two squirrels died sometime between February and March of 2009 in Stehekin (months that were slightly colder, with slightly more snow than average years in this area), however we do not know the exact date or cause of mortality because these squirrels were not regularly monitored with radiotelemetry during that time. At Squaw Creek, 3 of 18 (16.7%) radio-collared squirrels died between fall and winter 2010/2011; 1 death was observed in summer 2010 (8.3%; Tables A1.1, A1.2).

Discussion

The seasonal and daily activity patterns we observed are consistent with previous research on western gray squirrels (Cross 1969, Gilman 1986, Foster 1992, Ryan and Carey 1995). Although we observed a significant decrease in activity during winter it was not directly related to low temperatures or active snowfall. Low temperatures did not restrict or alter activity patterns except below -12°C which only occurred 12 days in Stehekin and 29 days at Squaw Creek during the winters of 2009-2011. Snowfall, the more ubiquitous characteristic of winter in the North Cascades (occurring a total of 57 days in Stehekin and 39 days at Squaw Creek during our study), also did not restrict overall squirrel activity. As suggested by Hicks (1949), snowfall may be less of a stressor for tree squirrels than rain because snow does not wet pelage as quickly as rain. Wind and rain significantly decreased core body temperature in Abert's squirrels outside the nest (Golightly and Ohmart 1978) in Arizona, leading to avoidance of these conditions (retreat to the nest). We observed similar patterns in western gray squirrels in the North Cascades across all seasons, also consistent with previous accounts of western gray squirrels throughout their range (Grinnell and Storer 1924, Ingles 1947). The only inconsistency in results from this and previous studies was the lack of association between squirrel activity and cloud cover: previous researchers have reported an increased level of activity on cloudy days with low wind (Ingles 1947, Packard 1956, Ryan and Carey 1995), whereas we found no relationship between cloud cover and squirrel activity.

Although active western gray squirrels were observed traveling through trees more often than on the ground, we did not detect an increase in tree canopy travel when the ground was snow covered as hypothesized. Presence of snow on the ground did not affect mobility until it exceeded 25cm, which we observed on only 3 days in Stehekin and 5 days at Squaw Creek during our study. This snow depth threshold was consistent with Hicks (1949), who also noted that snow texture was an important factor for mobility and foraging behavior of fox squirrels. Western gray squirrels may favor ground travel during snowy conditions because they are better able to maintain core body temperature by running and foraging on the ground than by traveling and foraging in the tree canopy, as reported for Abert's squirrels in Arizona (Golightly and Ohmart 1978). Ground travel is also necessary for retrieval of caches; we documented successful cache recovery by western gray squirrels even during times of maximum snow depth.

The most noticeable change in western gray squirrel winter activity was the decrease in activity after 1500. We were surprised to find that activity was still highest in the early morning hours (consistent with the rest of the seasons) despite lower temperatures. The decreased overall activity in winter may be because squirrels restrict activity only to foraging during this time as observed for red squirrels in Finland (Puilliainen 1973). Western gray squirrels were often observed feeding in the early morning; this may be the preferred foraging time across all seasons.

Increased time spent in a smaller number of nests in winter may indicate that squirrels invest more time in building fewer, warmer nests for winter, however winter and non-winter nest structure must be compared to verify this. Spending more time in nests is an efficient adaptation to winter as temperatures can be 20-30 degrees warmer inside dreys (Puilliainen 1973). Co-nesting further increases thermogenic capacity, therefore we were not surprised to find an increase in co-nesting occurrence during winter. Gregory (2005) also reported some nest sharing by western gray squirrels in the North Cascades in winter.

It is unclear whether winter challenges in the North Cascades have contributed to the overall decline of the western gray squirrel in Washington State. Squirrels showed behavioral adaptations for conserving energy and heat during periods of cold and high snow cover. However, we did observe a large proportion of mortalities in Stehekin between fall and winter. We speculate that the relatively large number of mortalities between fall and winter may reflect the greater activity of squirrels during fall, which may in turn be related to winter preparation (eg: storing food, building/modifying winter nests).

Results also indicated potentially significant decreases in core-use and overall home range sizes of squirrels in winter, as well as seasonal shifts in location. Increased radiotelemetry of male and female western gray squirrels in the North Cascades across seasons, with a particular increase in winter information, would help verify these results. Winter core-use areas may have higher concentrations and/or greater availability of resources, allowing squirrels to travel less, conserve energy and heat, and reduce predation risk. Identification of winter core-use areas, and winter nest-sites could help prioritize habitat conservation efforts with land management.

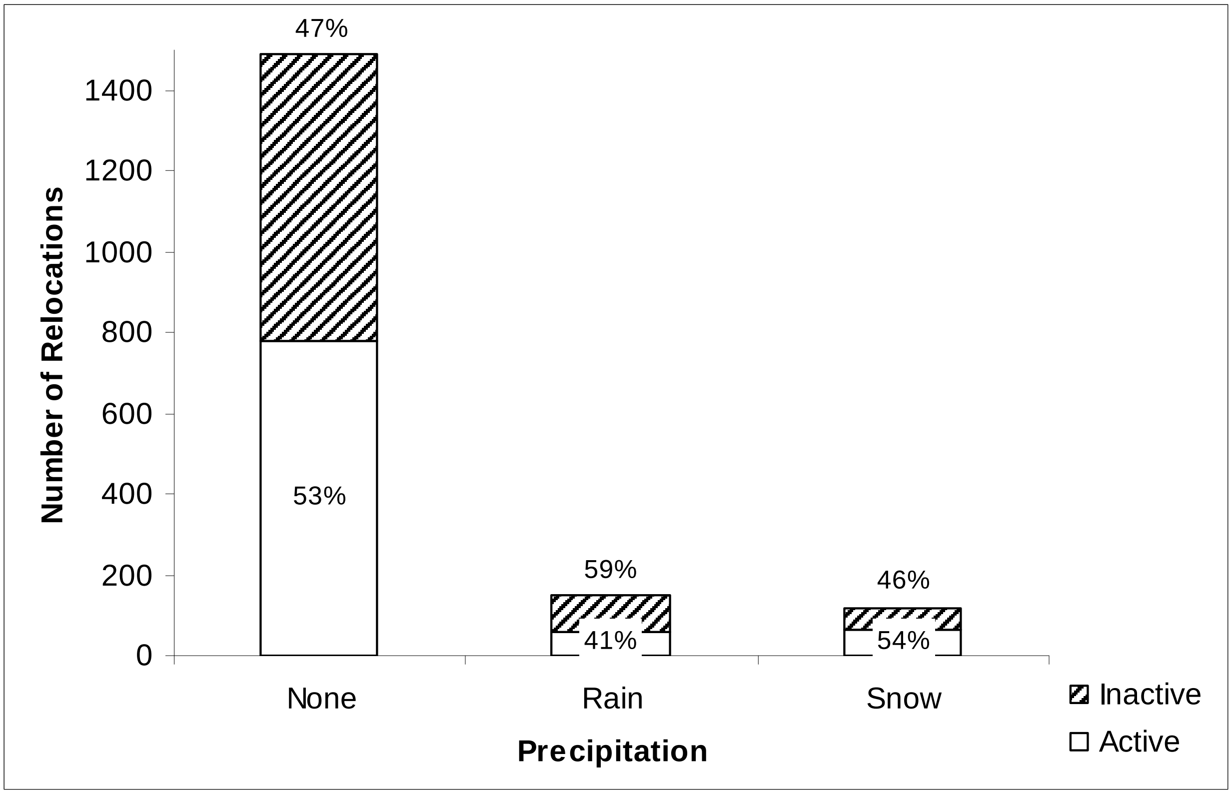


Figure 3.1. Squirrel activity by precipitation type. Percentages represent the ratios of radiotelemetry relocations in which squirrels were active or inactive.

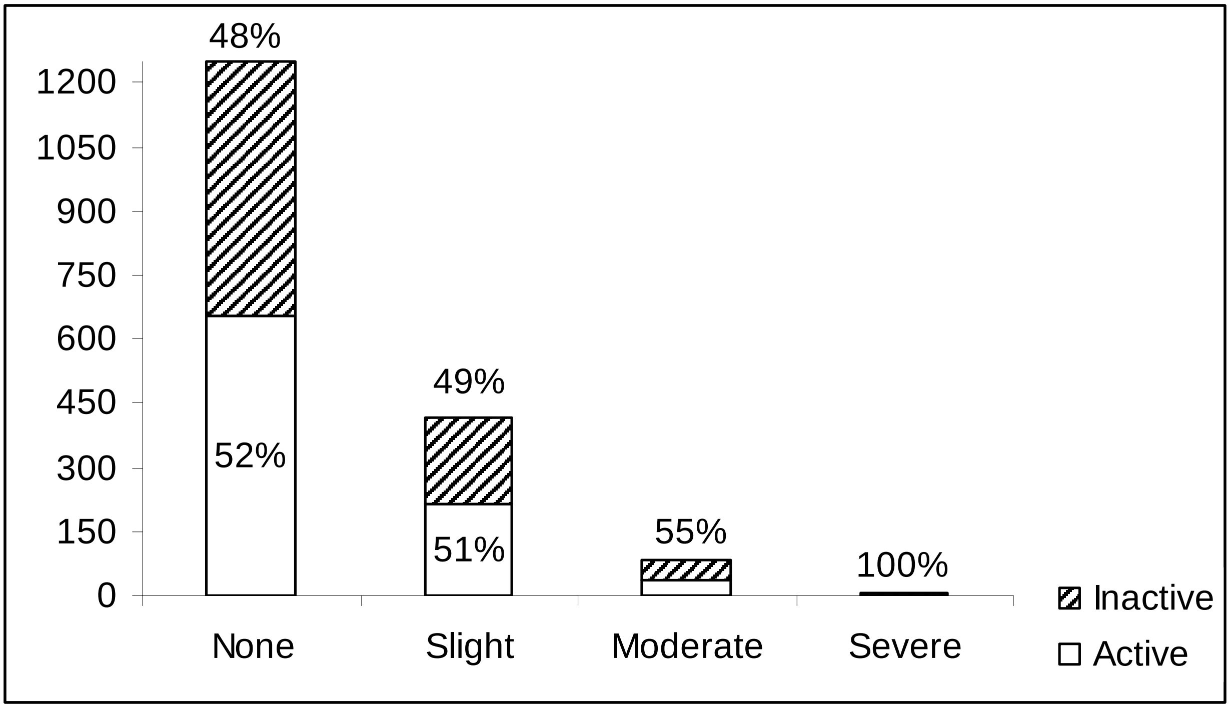


Figure 3.2. Squirrel activity by wind category. Percentages represent the ratios of radiotelemetry relocations in which squirrels were active or inactive.

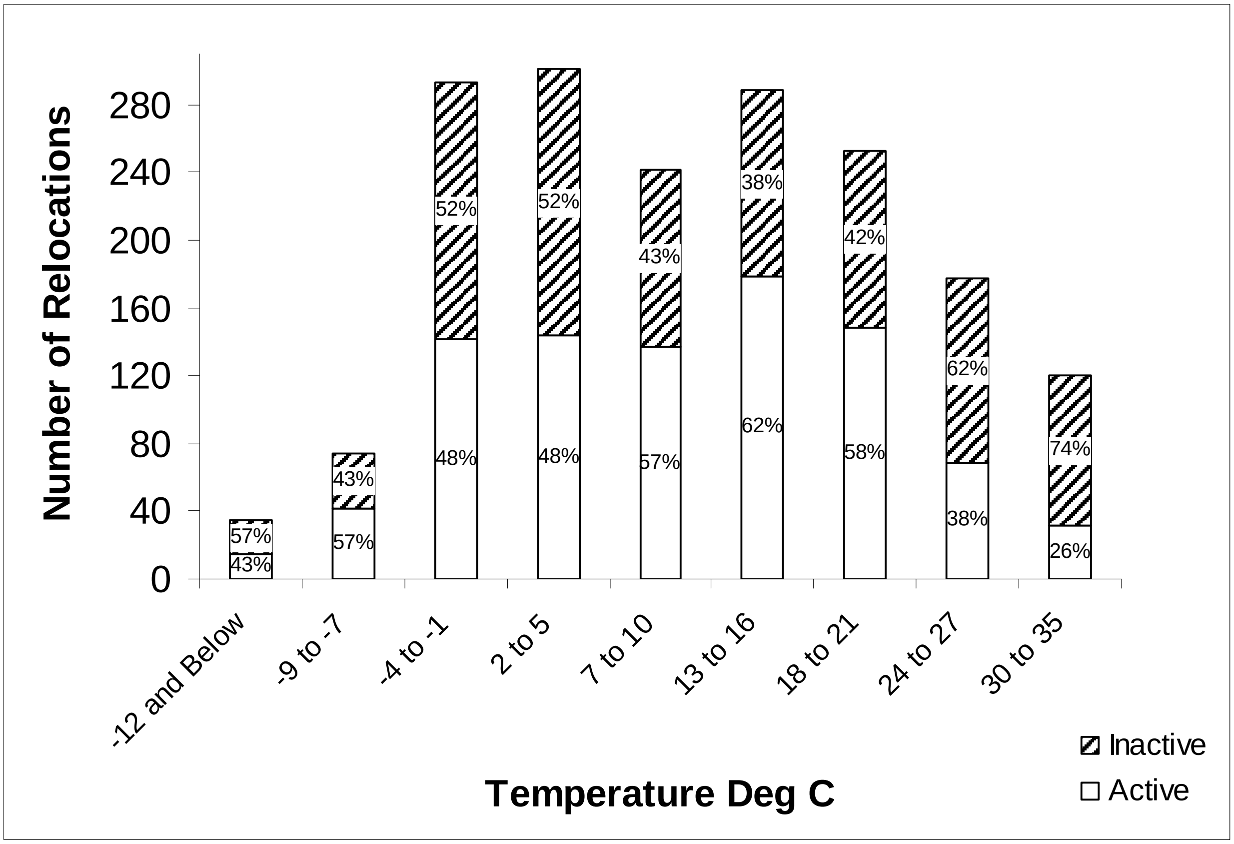


Figure 3.3. Squirrel activity by temperature (°C). Percentages represent the ratios of radiotelemetry relocations in which squirrels were active or inactive.

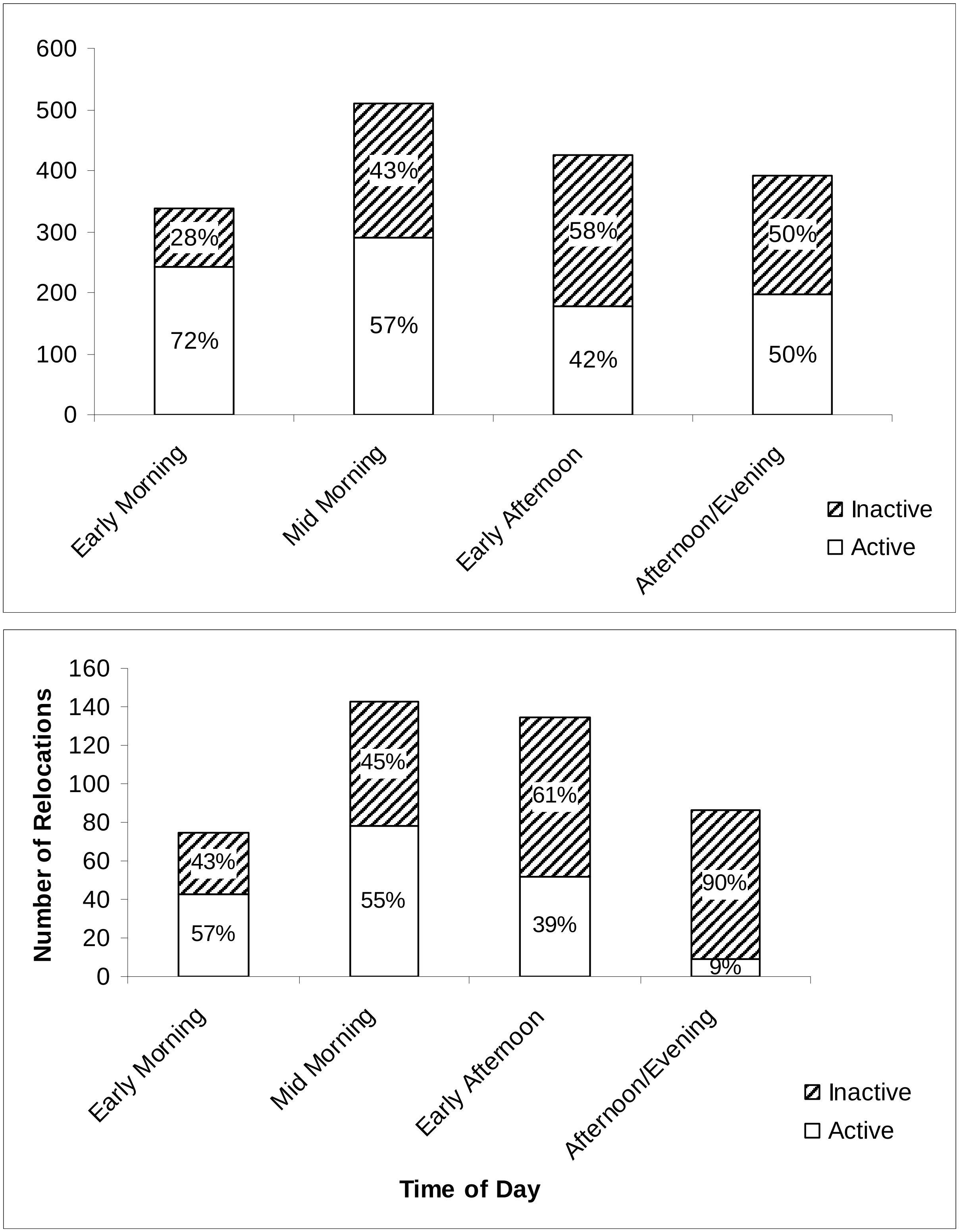


Figure 3.4. Squirrel activity by time: early morning= 0600-859, mid morning= 0900-1159, early afternoon= 1200-1459, afternoon/evening= 1500-1900, in spring, summer and fall (top), and in winter only (bottom). Percentages represent the ratios of radiotelemetry relocations in which squirrels were active or inactive.

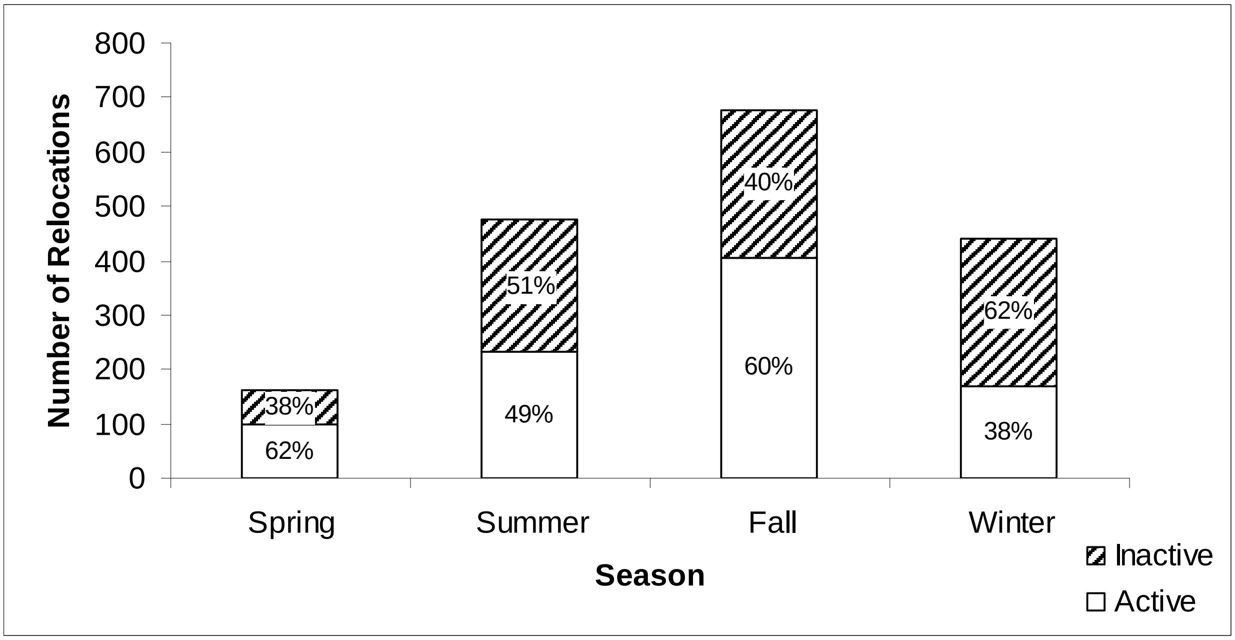


Figure 3.5. Squirrel activity by season: spring= March-May, summer= June-August, fall= September-November, winter= December-February. Percentages represent the ratios of radiotelemetry relocations in which squirrels were active or inactive.

Table 3.1. Weighted, untransformed coefficients describing western gray squirrel activity patterns across two study sites: Stehekin and Squaw Creek in the North Cascades, Washington, using a generalized linear mixed model stratified by squirrel. Odds ratios are calculated by exponentiation of the coefficient. The 95% confidence interval= $e^{(\beta \pm 1.96SE)}$. The intercept represents season: fall.

Variable	Coefficient (	Odds ratio	Std. Error	95% C.I.
Intercept	1.346	3.842	1.050	0.49 to 30.08
seasonSpring	-0.553	0.575	0.194	0.39 to 0.84*
seasonSummer	-1.614	0.199	0.179	0.14 to 0.28*
seasonWinter	-0.765	0.465	0.164	0.34 to 0.64*
Time	-0.417	0.659	0.053	0.59 to 0.73*
Cloud Cover	0.000	1.000	0.001	1.00 to 1.00
precipNone	-0.402	0.669	1.566	0.03 to 14.40
precipRain	-0.606	0.546	0.205	0.37 to 0.81*
precipSnow	0.065	1.067	0.222	0.69 to 1.65
windModerate	0.160	1.174	1.060	0.15 to 9.37
windNone	0.281	1.324	1.035	0.17 to 10.07
windSevere	-14.770	0.000	614.700	0 to ∞
windSlight	0.344	1.411	1.039	0.18 to 10.81
Temperature	0.028	1.029	0.009	1.01 to 1.05*

*C.I. does not contain 1

CHAPTER 4: Diversity, Relatedness, and Spatial Distribution Patterns of a Small Western Gray Squirrel Population at the Northern Range Periphery

Abstract

The genetics of threatened and endangered populations on the edge of their range is an important component of conservation. The western gray squirrel was classified as a Washington State threatened species in 1993 due to a decline in range and number and now exists in three geographically and genetically isolated populations within the state. We evaluated genetic diversity, population structure, relatedness, and spatial distribution of three populations of western gray squirrels (*Sciurus griseus*) in the North Cascades, Washington. Based on 13 microsatellite loci isolated for western gray squirrels, we found higher average heterozygosity and allelic richness than previously reported in this area. We also found significant genetic differentiation between populations of western gray squirrels in Stehekin, and the Methow Valley, Washington. The Stehekin population had lower genetic diversity and a smaller effective population size. The census population size of western gray squirrels in the North Cascades estimated from effective population size is between 500 and 1000, which was larger than previous estimates (Linders and Stinson 2007). Estimated inbreeding levels fit expectations based on effective population sizes and we did not find evidence of recent population bottlenecks as measured by heterozygosity excess. Mantel tests and spatial autocorrelation analysis did not reveal an isolation by distance pattern. Pairwise relatedness between individual squirrels was not related to pairwise overlap of home ranges or frequency of co-nesting. Low variability of genetic markers and small sample sizes limit power of our conclusions. Further analysis

including additional populations of squirrels in Washington, Oregon, and California will be conducted in collaboration with the Washington Department of Fish and Wildlife.

Introduction

The genetics of threatened and endangered populations on the edge of their suitable range is an important component of conservation. Effective population size tends to be lower in peripheral populations leading to more pronounced effects of genetic drift. Inbreeding, mating between relatives, also occurs frequently in small, isolated populations because most individuals are related (Allendorf and Luikart 2007). Many of these populations have experienced recent population bottlenecks from habitat loss and fragmentation or may have been established initially through a founder event (Frankham 1997, 1998). Loss of genetic variation due to genetic drift, inbreeding, and population bottlenecks can lead to decreased fitness of individuals by affecting survival and reproductive success. A reduction in genetic variability, even without reductions in individual fitness, can sometimes reduce population viability (Conner and White 1999) due to life history variations (age at sexual maturity, clutch size etc.), mating types and sex determination (Allendorf and Luikart 2007). Evolutionary potential, the ability of a population to respond to future environmental changes through natural selection, also decreases with decreasing heterozygosity and allelic diversity (Reed and Frankham 2003).

The western gray squirrel (*Sciurus griseus*) was listed as a Washington state threatened species in 1993 by the Washington Department of Fish and Wildlife (WDFW) due to a decline in range and number. The three remaining populations in the state, at the Joint Military Base Lewis-McChord in the Puget Trough in Pierce and Thurston Counties, southern Washington in Klickitat Country, and the North Cascades in Okanogan and Chelan Counties (Figure 4.1 inset), have been geographically and genetically isolated from each other, and from the larger populations of western gray squirrels in Oregon and California for some time (Warheit 2003, 2007). Little research has been conducted in the North Cascades, the northernmost population isolate, which represents a unique habitat that lacks oak trees as a food resource, experiences severe winters and frequent wildfire, and has a dynamic history of forest management practices including recent fire fuel reduction treatments composed of prescribed burning and thinning.

One specific western gray squirrel population of interest in the North Cascades is located in Stehekin, WA, part of the Lake Chelan National Recreation Area of the North Cascades National Park Complex. Due to unique habitat conditions and possible long-term isolation, Stehekin western gray squirrels in Stehekin may be a genetically distinct population, which could be of special concern to wildlife managers (Hughes et al. 1997). Colonization of Stehekin may have originated from a founder event through natural migration or intentional introduction from another Washington population (Linders and Stinson 2007). Genetic analysis can help determine if a population augmentation program similar to that occurring on Fort Lewis, WA (Warheit 2003, 2007, Vander Haegen et al. 2005) is warranted in Stehekin (goal 3.2.1 of the 2007 State of Washington Western Gray Squirrel Recovery Plan; Linders and Stinson 2007).

The effect of drift and inbreeding on the populations of western gray squirrels in the North Cascades could be very strong because of small census and effective population sizes. Previously reported genetic diversity in this area is low relative to all other western gray squirrel populations throughout the species range (Warheit 2003, 2007). Distribution and connectivity between western gray squirrel populations within the North Cascades is also uncertain, as there are many potential geographic barriers to migration. Under an isolation by distance pattern gene flow decreases and genetic differentiation increases with geographic distance; the scale of this pattern is dependent on the mobility of the species (Wright 1943). However, if gene flow is low, and populations are small, drift can operate separately of any geographic pattern (Slatkin 1993). Immigration across the North Cascades could increase genetic variability and alleviate potential effects of inbreeding by genetic rescue: the recovery in average fitness of individuals though increased gene flow into small populations (Allendorf and Luikart 2007). However, immigration also has the potential to cause outbreeding depression: the relative reduction in fitness of offspring from matings between genetically divergent individuals, owing to dilution of local adaptation and/or disruption of epistasis (Tallmon et al. 2004). Outbreeding depression is of particular concern in the North Cascades, as it represents a unique habitat for the western gray squirrel, which may require specialized squirrel adaptations. Genetic differentiation and population structure analysis can be used to help identify genetic barriers and isolation by distance to determine if the North Cascades is functioning as a metapopulation (Levins 1970) which could suggest different management objectives (e.g. corridors). Molecular pedigree analysis is an excellent tool for understanding genetic differentiation, spatial distribution and dispersal, and population viability of western gray squirrels in the North Cascades. Population level genetic analyses have been used to evaluate genetic diversity and guide management of endangered populations of European ground squirrels (*Spermophilus citellus*; Slimen et al. 2011), and red squirrels (*Sciurus vulgaris*; Ogden et al. 2005).

Individual-level analyses, such as kinship analysis, can also be used as a measurement of reproductive success and an index of inbreeding to complement traditional population level analyses when studying genetic structure over small spatial scales. The small population size and suspected high degree of relatedness among individuals in the North Cascades could make social patterns in overlap and co-nesting more apparent than within larger populations.

Individuals can increase their own genetic potential by helping relatives produce successful offspring following inclusive fitness theory (Hamilton 1963) and reciprocal altruism, or kin selection (Maynard Smith 1964). Following inclusive fitness we would expect a higher degree of overlap and co-nesting occurrence between related individuals. Significantly more amicable than agonistic interactions between related individuals have been documented for eastern gray squirrels (*Sciurus carolinensis*; Koprowski 1993, 1996), with higher degrees of overlap between mother-daughter pairs (Gurnell et al. 2001), however relatedness has not been consistently correlated with spatial distance and overlap across sexes (Spritzer and Brazeau 2003). Female eastern gray squirrels also often co-nest with other related females (Koprowski 1996), and kinship plays a significant role in the formation of overwinter nesting aggregations of southern flying squirrels (*Glaucomys volans*; Winterrowd et al. 2005, Thorington et al. 2010, Thorington and Weigl 2011). Increased aggregation of close kin in small, isolated populations can also threaten population persistence, however, through increased loss of genetic variation and inbreeding depression (Keller and Waller 2002). To reduce this threat, western gray squirrels might also show a lower degree of overlap and co-nesting occurrence among related individuals, as juveniles disperse to reduce the likelihood of inbreeding. Relatedness did not influence overlap or co-nesting in fox squirrels (*Sciurus niger*), because juveniles always dispersed from their natal area (Koprowski 1996). However, red squirrels (*Tamiasciurus hudsonicus*) in southwest Yukon did not show similar inbreeding avoidance mechanisms: genetic relatedness did not predict patterns of parentage, and effects of inbreeding depression on this population were minimal (Lane et al. 2006).

Our objectives were to examine population structure, genetic diversity, and relatedness of three populations of squirrels in the North Cascades and estimate effective population sizes. For the Stehekin population in particular, we expected to find low genetic diversity and allelic richness, a small N_e, and high degree of inbreeding, indicative of population isolation and a recent population bottleneck or founder event. We observed a large proportion of mortalities in Stehekin and also hypothesized a negative correlation between the number of days survived after radio-collaring and individual inbreeding estimates. Genetic relatedness of western gray squirrels in the North Cascades was also compared to geographic location, home range overlap, and co-nesting occurrence, to test for an isolation by distance pattern, and higher degree of home range overlap and co-nesting between related individuals following inclusive fitness theory.

Methods

Field

Western gray squirrel tissue samples were collected from three main study sites in the Washington North Cascades: Black Canyon Creek and Squaw Creek in the Methow Valley, and Stehekin, WA, part of the Lake Chelan National Recreation Area of North Cascades National Park (Figure 4.1). Study sites are mixed conifer/deciduous forests composed primarily of ponderosa pine (*Pinus ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*), and experience cool, wet winters and hot, dry summers. Additional study site information: p.4. Most tissue samples were collected from squirrels captured from 2003-2011 for radiotelemetry studies of resource selection (Gregory 2005, K. Stuart Chapter 1) and a genetic augmentation project for the Fort Lewis population of squirrels in the Washington Southern Puget Trough (Vander Haegen et al. 2007). Tissue samples from the ear were collected with sterile, disposable biopsy punches and preserved in 100% ethanol. Samples were also collected opportunistically from freshly killed specimens (usually due to road kill). UTM coordinates were recorded at each trap or kill site when possible; squirrels that were radio-collared also had habitat use, home range size, and spatial overlap information. Sexes of squirrels were also recorded in most cases, as well as life stage (adult or juvenile) when possible. A total of 92 samples were collected from the North Cascades: 30 from Black Canyon, 24 from Stehekin, 26 from Squaw Creek, and 12 road kills throughout and between study areas.

Lab

DNA extraction and microsatellite genotyping were performed by the Washington Department of Fish and Wildlife's Molecular Genetics Laboratory. Genomic DNA was extracted following standard Qiagen DNeasy® recommendations for single tube silica-membrane kits. Microsatellite genotyping of samples used 19 primer sets, which were multiplexed into seven PCR reactions and then pooled into 5 final sets for electrophoresis by Kenneth I. Warheit (WDFW unpublished data; Table 4.1). Instead of fluorescent labeling the forward primer, an unlabeled oligonucleotide was attached to the 5’ end of the PCR primers, following a modification of the method described in Schuelke (2000). This oligonucleotide was complementary to a fluorescently labeled probe included separately in the PCR reaction. Inclusion of the probe in PCR resulted in fluorescent label incorporation in the final PCR products.

Each PCR reaction was conducted within a total volume of 10 µl, of which 1 µl was un-quantitated DNA. PCR reactions were conducted with a thermal profile as follows: an initial denaturation step of 3 min. at 94^{o}C, 30 cycles of denaturation at 94^{o}C for 15 s., annealing at 50^{o}C for 30 s., and extension at 72^{o}C for 1 min., plus a final extension at 72^{o}C for 10 min and final holding step at 10 ^{o}C. PCR reactions were conducted with Applied Biosystem 9700 thermal cyclers. PCR products were visualized using an ABI-3730 DNA Analyzer with internal size standards (GS500LIZ 3730) and scored using GeneMapper 3.7 software.

Analysis

We analyzed genetic characteristics of squirrels within each study site separately, and across the pooled North Cascades population. Data were checked for null alleles, stuttering and large allele dropout using Microchecker (Oosterhout et al. 2004). A maximum likelihood estimate of null allele frequency was estimated with ML-NULL (Kalinowski and Taper 2006). We tested for Hardy-Weinberg equilibrium and linkage disequilibrium, and calculated observed and expected heterozygosities, allelic richness and Wright's F-statistics for each locus with programs GENEPOP (Raymond and Rousset 1995, Rousset 2008) and FSTAT (Goudet 2001). Average heterozygosity and allelic richness were compared among populations with the conservative Kruskal-Wallis chi-square test in R version 2.7.2 (R Development Core Team 2008), $\alpha = 0.05$.

We determined the number of populations of western gray squirrels in the North Cascades and assigned individuals to populations using a Monte-Carlo Markov Chain clustering method in STRUCTURE v2 (Pritchard et al. 2000) which minimizes Hardy-Weinberg disequilibrium and gametic phase disequilibrium between loci. Baseline populations were classified by collection site with spatial geographic information (Hubisz et al. 2009). The most likely number of populations (K) was estimated by running a range of K's from 1-10 with 3 iterations each and evaluating the LnP(D) or probability of $(X|K)$, and ΔK (Evanno et al. 2005). Iterations were performed using the admixture ancestry model and correlated allele frequencies with a burn-in period of 100,000 and 100,000 Monte-Carlo Markov Chain repetitions after burnin. We verified that these values were sufficient by ensuring that parameters including F_{ST}, alpha and likelihood, had stabilized before data were produced. Individuals with $q > 0.79$ and a 90% confidence interval (C.I.) between 0.1 and 1 were assigned to their original sampling area, while individuals with a $q < 0.2$ and a 90% C.I. between 0 and 0.9 were rejected from their sampling area. The remaining individuals were assigned according to the highest q value, with a wide 90% C.I. of 0-1 (Vähä and Primmer 2006, Andersen et al. 2011). We also used the Bayesian method of Rannala and Mountain (1997) with the Monte-Carlo probability method of Paetkau (2004) for a population mixture to assign individuals and identify first generation migrants in GENECLASS 2 (Piry et al. 2004). This method does not assume that all populations were sampled. Spatial geographic information was used for reference populations with an assignment threshold of 0.05.

Accurate pedigrees of at least four generations were not available for calculating the inbreeding coefficient *F* (Balloux et al. 2004) for western gray squirrels in the North Cascades, so we used Monte-Carlo simulations in program COANCESTRY v1 (Wang 2010) accounting for inbreeding to select the most appropriate estimator of *F*. Based on these simulations we selected Ritland's moment estimator (1996) which had a correlation of 0.41 with the true simulated value of *F*, within the range reported in previous studies (Van de Casteele et al. 2001). The maximum likelihood estimators had higher overall correlations with the simulated true value of *F* ($r^2 = 0.56$ and 0.55 for the dyadic estimator: Milligan 2003, and triadic estimator: Wang 2007 respectively), however both significantly overestimated *F* when the true value was zero (Figure 4.2). Ritland's *F* estimates for individuals in Stehekin were compared to days of survival after radio-collaring (a fitness correlate) using linear regression in R version 2.7.2 (R Development Core Team 2008) at $\alpha = 0.05$. The relationship between *F* and cause of death (disease or predation) was compared with a two-tailed t-test assuming unequal variances, also in R. Survival was higher in the Methow Valley, with most squirrels outliving the time frame of our study therefore corresponding survival information for this area was not available. We used the permutation test of difference in COANCESTRY with Ritland's moment estimator and 1000 bootstraps to compare individual inbreeding coefficients between study areas.

Effective population sizes were estimated from linkage disequilibrium using LDNe v1.31, (Waples and Do 2007), which includes the bias correction method of Waples (2006) for sample size. We used a random mating model assuming neutral markers in a single, closed population. Alleles at frequency of less than 0.05 were not included in analysis. To investigate the possibility of recent population bottlenecks at study sites we used BOTTLENECK v1.2 (Piry et al. 1999) with the two-phase mutation model (TPM; Di Rienzo et al. 1994) and 3,000 replicates. The variance among multiple steps was set at 30 with 90% of mutations assumed to be single-steps. Heterozygote excess was evaluated using a one-tailed Wilcoxon test.

We explored relationships between geographic and genetic distances of western gray squirrels in the North Cascades with Mantel tests and spatial autocorrelation analysis in GenAlex: a Microsoft Excel (© Microsoft Corporation) Add-in (Peakall and Smouse 2006). For spatial autocorrelation analysis the maximum geographic distance between squirrels was evenly divided into ten distance classes with genetic and geographic correlation evaluated within classes.

To evaluate the reliability of our data for relatedness analysis and select an appropriate estimator, we used another simulation in COANCESTRY. Again, the maximum likelihood estimators had the highest correlation with the true values of relatedness, but overestimated low levels of relatedness (Figure 4.3). We selected Ritland's moment estimator (Ritland 1996) to estimate pairwise relatedness within and among populations of squirrels in the North Cascades ($r^2 = 0.35$ for Stehekin, 0.40 for the Methow Valley). Average relatedness among populations was compared with the permutation test of difference with 1000 bootstraps. Associations between pairwise relatedness of squirrels within populations were compared to three dimensional spatial overlap (also known as volume of intersection: V.I.; Seidel, 1992) and co-nesting occurrence between pairs of squirrels using two-sided Spearman's Rank Correlation Coefficient in R version 2.7.2 (R Development Core Team 2008) at $\alpha = 0.05$. The question of interest was whether V.I. and co-nesting occurrence were associated with relatedness (either positively or negatively, alternative hypothesis = two sided). For these analyses we only used genetic information from pairs of squirrels that were monitored during the same time frame with radiotelemetry at Stehekin and Squaw Creek. Volume of Intersection for squirrel pairs was calculated with Hawth's Tools (Beyer 2004) in ArcGIS (Esri 2009) following Kertson and Marzluff (2009).

We also used program COLONY (Wang 2004, Wang and Santure 2009, Jones and Wang 2010) to infer sibship among individuals across populations using their multi-locus genotypes. Because we had overlapping generations and incomplete sampling of candidate parents and offspring, we ran COLONY analyses for populations without identifying sexes or generations (all individuals were classified as offspring). Our small sample size, relatively large number of loci, and relatively large expected family sizes (due to low squirrel densities and potentially high levels of inbreeding) suggest that this method is still powerful enough to accurately identify sibship relationships (Wang & Santure 2009). The mating system was classified as polygamous (both genders) for dioecious, diploid species, for the full-likelihood analysis method with medium precision, and one medium length run.

We evaluated the power for relationship inference (PW_R) across loci using program KinInfor v1.0 (Wang 2006) assuming the primary hypothetical relationship was full-sibs, compared to the null hypothetical relationship of unrelated squirrels. We set the prior Dirichlet distribution to (1,1,1); significance level of 0.05.

Results

We omitted five of the 18 loci: Sgr-D108, Sgr-D119, Sgr-D2, Sgr-227, and Sgr-D7 from analyses due to evidence of null alleles in Microchecker and significant deviations from Hardy Weinberg Equilibrium (HWE) in two or more populations (Table 4.2). Null allele frequencies estimated from ML-NULL for the remaining loci ranged from 0.025 to 0.3 and were incorporated into analysis programs accommodating genotyping error rates. Significant pairwise linkage disequilibrium within the 13 loci used for analysis was less than 10% for all populations (2.6%, 6.5%, and 9.2% for Black Canyon, Stehekin, and Squaw Creek respectively; Table 4.3). Linkage was not consistent across populations indicating that significant *P*-values were a result of low sample size, rather than physical linkage of loci on the chromosome.

As predicted, genetic diversity of squirrels, as measured with average allelic richness and expected heterozygosity, was lowest in Stehekin, however, differences between populations were not statistically significant ($\chi^2 = 1.560$, $P=0.458$; $\chi^2 = 5.383$, $P= 0.068$ for allelic richness and heterozygosity respectively). Allelic diversity of squirrels from Stehekin was a subset of diversity observed in the rest of the North Cascades. Only one private allele was present in the Stehekin population; seven alleles not found in Stehekin were found at both Black Canyon and Squaw Creek. Black Canyon had six private alleles, Squaw Creek had one. All population groups had significant deviations from HWE at one to three loci. Global tests identified significant deviation from HWE at 3 loci: Sgr-A101, Sgr-B208, and Sgr-C222, however all differences were driven by one population only (Table 4.2). All deviations were due to a deficiency of heterozygotes, likely a result of null alleles.

F_{ST} and R_{ST} values were significant ($P < 0.001$) between Stehekin and both Methow populations; there was no significant differentiation between Black Canyon and Squaw Creek ($P = 0.622$; Table 4.4). The Bayesian clustering analysis in STRUCTURE suggested the presence of two populations with only Stehekin significantly differentiated (Figure 4.4). Ln Pr(X|K) scores from STRUCTURE did not clearly discriminate between the optimal number of two or four genetic groups [$k = 2$, average (over three runs) = 1595.9; $k = 3$, average ln Pr(X|K) = 1573; $k = 4$, average ln Pr(X|K) = 1579.3, $k = 5$, average ln Pr(X|K) = -1662.8], however ΔK (Evanno et al. 2005) clearly peaked at two populations (Figure 4.5). Without geographic information, STRUCTURE assigned 75% of individuals from Stehekin to one cluster with a *q* value greater than 0.79; only 20% of individuals from Black Canyon and Squaw Creek respectively were clustered with their pre-defined sampling populations. Results improved slightly when using geographic information: 92% of individuals from Stehekin were assigned to the Stehekin sampling area, 78% from Black Canyon, and 9% from Squaw Creek. Because STRUCTURE results confirmed that there were only two clear populations within the North Cascades, we ran an additional analysis with only two pre-defined populations to assign individuals to either Stehekin or the combined Methow Valley sampling areas using spatial geographic information. Eighty out of the 92 individuals were assigned to their original sampling areas with a *q* value greater than 0.79: 88% from Stehekin, 80% from the Methow Valley. Two individuals from Stehekin clustered more closely with the Methow Valley group with lower confidence *q* values of 0.74 and 0.55. These may be recent migrants to the Stehekin population. Ten of the 12 road kills, including the samples collected farthest north near Twisp and Winthrop, WA, were assigned to the Methow Valley population with *q* values greater than 0.79. One other road-killed individual collected between Squaw Creek and Black Canyon was most closely assigned to the Methow Valley population with lower confidence: $q = 0.61$. The remaining individual collected near Manson, WA did not clearly cluster into either group: $q = 0.59, 0.41$, for the Methow Valley and Stehekin respectively. GENECLASS 2 assigned 95% of individuals to their sampling population: 71% with 95% confidence, 76% at 90% confidence. Eleven first generation migrants were identified: 2 were road-killed individuals, 3 were from the sampled Stehekin population and 7 from the Methow Valley population. Four of these squirrels (including two in Stehekin) were females. Seven of the 11 migrants identified with GENECLASS 2 were consistent with clustering results from STRUCTURE.

The mean estimate of inbreeding using Ritland's moment estimator for *F* was 0.077 ± 0.130 in Stehekin and 0.066 + 0.120 in the Methow Valley. This difference was not statistically significant as the observed difference fit within the 2.5 and 97.5% permuted percentiles of simulated differences (Table 4.5). *F* estimates for individuals in Stehekin had a significant positive correlation with days of survival ($r^2 = 0.622$, $P < 0.001$, $n = 15$). This relationship is opposite to the expected one, indicating that more inbred individuals lived longer in Stehekin. There were no differences in *F* estimates for squirrels killed by predators and those that died of disease ($t = 0.430$, $P = 0.683$, $n_{predation} = 9$, $n_{disease} = 6$). The average level of inbreeding at both sites was as expected based on effective population size calculations ($F = 1/2N_e$; Allendorf and Luikart 2007; Table 4.6). Using Frankham's (1995) estimate of the relationship between effective population size and census population size (N_e = 10% of N_c), there are 72 squirrels in Stehekin and 953 in the Methow Valley. Waples' (2002) more conservative estimate accounting for temporal changes (N_e= 20% of N_c), estimates census sizes as 36 and 476 for Stehekin and the

Methow Valley respectively. Effective population size was lowest in Stehekin, consistent with estimations of low relative abundance in this area (Appendix A). We found no evidence of a recent population bottleneck in either Stehekin or the Methow Valley with no evidence of significant heterozygosity excess (Wilcoxon test Stehekin, *P* = 0.689, Methow Valley *P* = 0.420). This indicates that both populations are in mutation-drift equilibrium.

Mantel tests showed little relationship between geographic and genetic distance for either population (combined Methow Valley r^2= 0.001, *P*= 0.480; Stehekin r^2= 0.003, *P*= 0.380). Spatial autocorrelation analysis also did not show isolation by distance patterns: all correlations between geographic and genetic distance within size classes were within the bootstrapped 95% confidence intervals (Figure 4.6).

Average pairwise relatedness using Ritland's moment estimator was 0.007 $\underline{+}$ 0.029 in Stehekin, -0.043 $\underline{+}$ 0.025 in Squaw Creek, and -0.022 $\underline{+}$ 0.091 in the Methow Valley. This difference was not statistically significant as the observed difference fit within the 2.5 and 97.5% permuted percentiles of simulated differences (Table 4.7). There were no significant monotonic associations between pairwise relatedness (Ritland's moment estimator) and volume of intersection or co-nesting at Stehekin or Squaw Creek (rho= 0.125, *P*= 0.325 for V.I., rho= -0.050, *P*= 0.702 for co-nest in Stehekin, rho= 0.039, *P*= 0.690 for V.I., rho= 0.140, *P*= 0.154 for co-nest at Squaw Creek).

We identified 6 maximum likelihood family clusters in Stehekin; 16 in the Methow Valley with COLONY, again indicating lower genetic diversity and a higher level of relatedness in Stehekin. In Stehekin, 12.5% of full-sib dyads were assigned with 95% confidence; 27.3% in the Methow Valley. All full-sib dyads were clustered closely to their respective collection sites, imparting confidence in the power of our data to accurately detect levels of relatedness of 0.5

and above. COLONY also identified many half-sib dyads with 95% confidence: 17.1%, and 48.6% in Stehekin and the Methow Valley respectively. The distribution of half-sib dyads showed less of a pattern across populations, however, implying lower confidence in the power of our data to detect relatedness values of 0.25 or less (Figure 4.7). Power for relationship values from KinInfor were 0.18 and 0.23 for Stehekin and the Methow Valley respectively.

Discussion

All results from this study suffer from small sample size and low variability and power of genetic markers. Therefore, results and implications should be interpreted with caution. Average allelic richness across all loci was higher than previously reported for western gray squirrels in Chelan and Okanogan counties Warheit (2003, 2007), likely due to the use of species-specific markers. Previous study used loci isolated from red squirrels. The lack of statistical difference in average heterozygosity and allelic richness between Stehekin and the Methow Valley fails to support our original hypothesis that genetic diversity is lower in Stehekin. We also failed to detect significant differences in average inbreeding and relatedness among populations and inbreeding levels did not negatively affect survival of individuals in Stehekin. In this case, we did not find a clear link between heterozygosity, allelic richness, and population fitness following Reed and Frankham (2003). Our results do not suggest that the higher number of mortalities observed in Stehekin compared to the Methow Valley was related to genetic factors. Extinction risk may be driven more by demographic factors in this area including reproduction, immigration, and predation. Further study on factors affecting survival rates of western gray squirrels in the North Cascades is needed. The lack of private alleles in Stehekin is consistent with the lower effective (and census) population size, and suggests a higher rate of genetic drift in Stehekin. Most migrants were assigned to the Methow Valley population (including two individuals collected in Stehekin) suggesting that migration is mostly unidirectional into Stehekin, and that Stehekin may be functioning as a sink population (Pulliam 1988) within a North Cascades metapopulation (Levins 1970). The higher number of mortalities in Stehekin compared to the Methow Valley is better explained by this hypothesis of lower quality habitat in Stehekin. Although Stehekin is a unique habitat for the western gray squirrel within the North Cascades ecosystem and managed by an agency focused on preservation of natural resources (National Park Service), the population of squirrels in this area does not represent a reservoir of unique genetic and phenotypic variation and therefore does not warrant status as separate management unit in a genetic sense (Moritz 1994, Hughes et al. 1997).

However, the small effective and census population size in Stehekin could make this population of squirrels more vulnerable to extinction through stochastic events like stand-replacing wildfire or extreme winter weather. Keller and Waller (2002) suggest a higher risk of "mutational meltdown" for populations with an N_e <100 due to a simultaneous loss of genetic variation necessary for adaptive evolution (Lande 1995), and an accumulation of deleterious mutations through ineffective selection (Lynch et al. 1999). Genetic augmentation for the Stehekin population may be a management option worth investigating in the future if genetic diversity continues to decline and the population size remains small (Griffith et al. 1989). To reduce the likelihood of outbreeding depression with augmentation, the Methow Valley would be the best fit as a source of individuals because the habitat is most similar. Klickitat County, and the Oregon Willamette Valley may also be viable source populations with higher genetic variabilities (Vander Haegen et al. 2007).

Our calculation of effective population size in the combined Methow Valley suggests a potentially larger number of squirrels in the North Cascades than previously estimated based on home range size by WDFW in the western gray squirrel recovery plan (Linders and Stinson 2007). This is further supported by our additional research indicating that average home range sizes in the North Cascades are smaller than previously estimated by Gregory (2005), thus currently identified habitat may be able to support a larger number of squirrels (Chapter 2), and recent identification of additional areas of western gray squirrel presence within the North Cascades (Yamamuro et al. 2011). However, the true relationship between effective population size and census population size in the North Cascades is unknown.

Because we did not detect spatial autocorrelation and isolation by distance, we could not identify potential barriers to migration. The squirrel collected near Manson was not closely aligned with either population cluster we identified in the North Cascades and may be from a separate population that was not sampled and also genetically differentiated from the Methow Valley. Additional sampling in this area could provide more insight into potential habitat corridors currently connecting populations of squirrels within the North Cascades. We found evidence that the western gray squirrel population throughout the Methow Valley from Winthrop (north) to Pateros (south) is connected, which is further supported by our observations of occasional long distance migrations of individual squirrels over a four year period of field work in the North Cascades (Chapter 2). Because female western gray squirrels tend to be philopatric, with dispersal by juvenile males (Linders 2000, Vander Haegen et al. 2005), an isolation by distance pattern could emerge with the use of mtDNA for phylogeographic studies (Peakall et al. 2003, Banks and Peakall 2012).

We also did not find evidence for recent population bottlenecks in either of the North Cascades populations as indicated by an excess of heterozygosity, tested for with program BOTTLENECK. We had more than the recommended number of polymorphic loci for the program, but fewer than the recommended number of typed individuals in Stehekin (Piry et al. 1999). BOTTLENECK did not find a genetic signature of a demographic population bottleneck of golden-mantled ground squirrels reduced to 14 individuals over a four year intermediate period within a 10 year study in Colorado (McEachern et al. 2011). Often only allelic richness, not heterozygosity, declines markedly after a bottleneck (Nei et al. 1975, Leberg 1992). BOTTLENECK also assumes mutation-drift equilibrium prior to bottlenecks, which can be hard to maintain for fluctuating rodent populations. This assumption is also violated through immigration and metapopulation dynamics. Even a small amount of immigration can maintain and recover genetic variation relatively quickly. The low levels of inbreeding observed in Stehekin provide some evidence of immigration and genetic rescue (Tallmon 2004). Finally, it takes between $2N_e$-$4N_e$ generations depending on the severity and mutation rate of loci being studied to detect a population bottleneck after it occurs (Cornuet and Luikart 1996); our data included information from approximately 3 generations at each study site: 2002-2004 at Black Canyon, 2008-2011 at Stehekin, and 2009-2011 at Squaw Creek. More recent declines due to events such as the 2006 Flick Creek fire and subsequent severe winter of 2007 in Stehekin, for example, would not be seen with genetic analysis for several more years.

Our inability to detect a relationship (positive or negative) between relatedness and overlap and co-nesting is likely due to the low variability and power of our genetic markers, in addition to small sample sizes. The fundamental kin relationship in tree squirrels (for both overlap and co-nesting) is the female-female dyad, especially mother-daughter pairs (Gurnell et al. 2001, Koprowski 1996), however sample sizes were too small to restrict analyses to female-female and/or hypothesized mother-daughter pairs. All results from this study suffer from small sample size and low variability and power of genetic markers. Combining and comparing our analyses with genetic analyses of the other populations within Washington State (currently underway with WDFW) could make patterns more apparent.

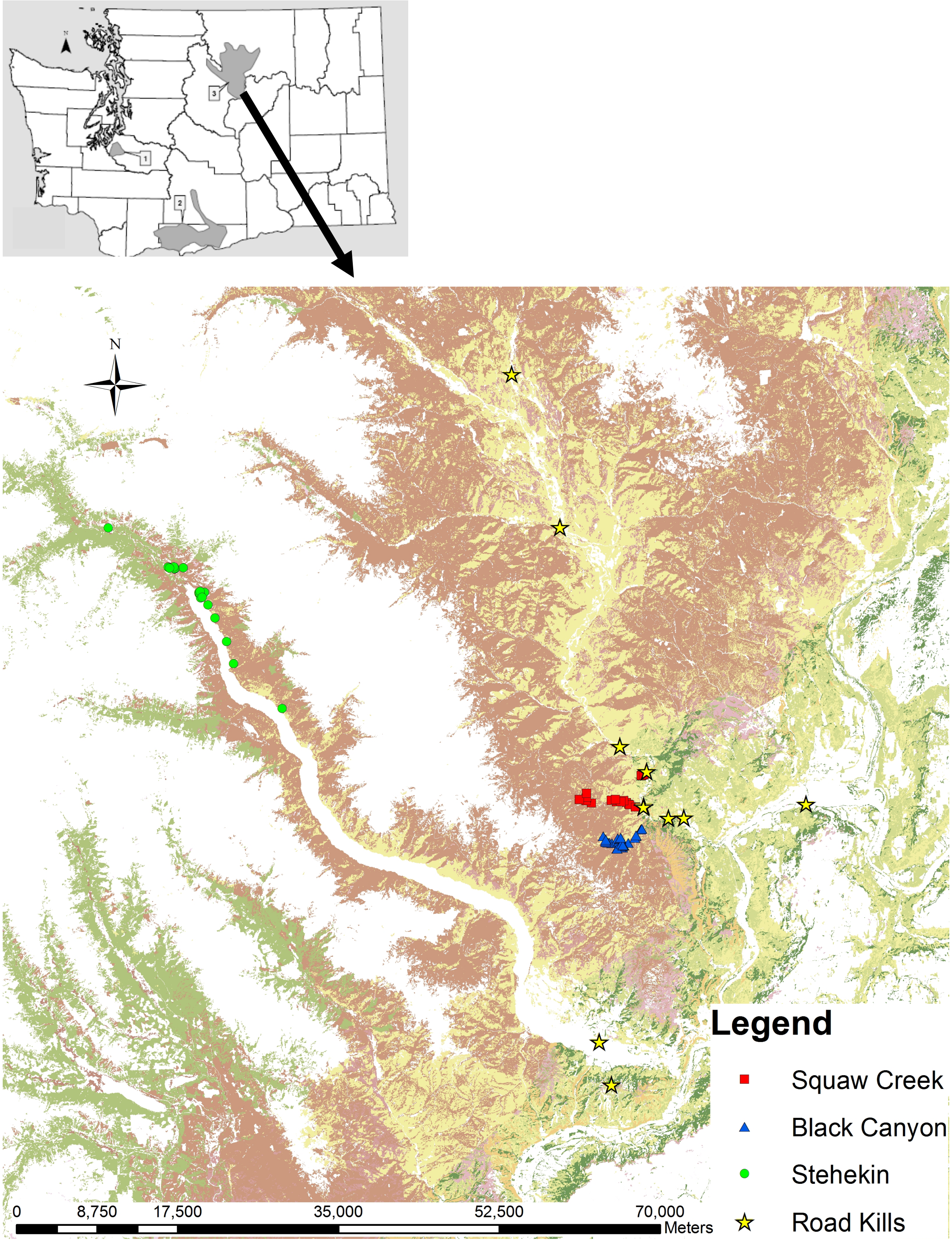


Figure 4.1. Collection sites for western gray squirrel genetic samples in the North Cascades.

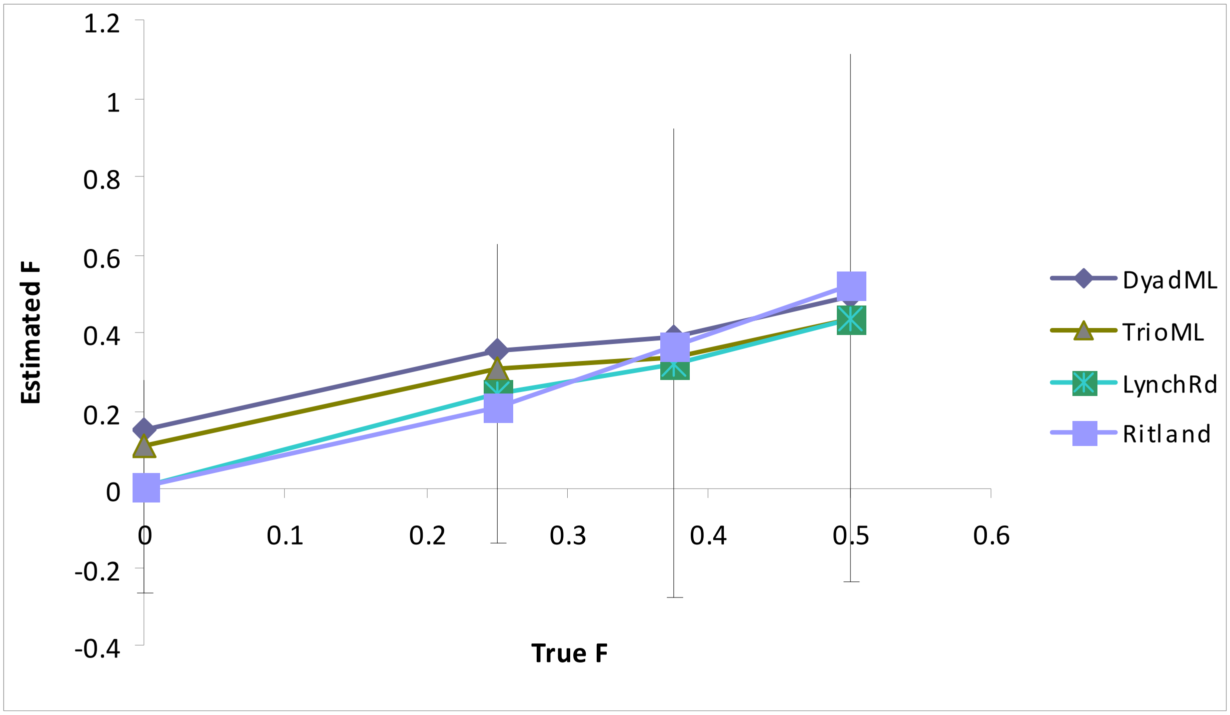


Figure 4.2. Estimates of the inbreeding coefficient *F* compared to the true *F* calculated with simulations in program COANCESTRY (Wang 2010) using four methods: moment estimators from Ritland (1996), and Lynch et. al (1999; LynchRd), a triadic likelihood estimator which uses a third individual as a reference for estimating pairwise relatedness between individuals (Wang 2007; TrioML), and a dyadic likelihood estimator described in Milligan (2003; DyadML).

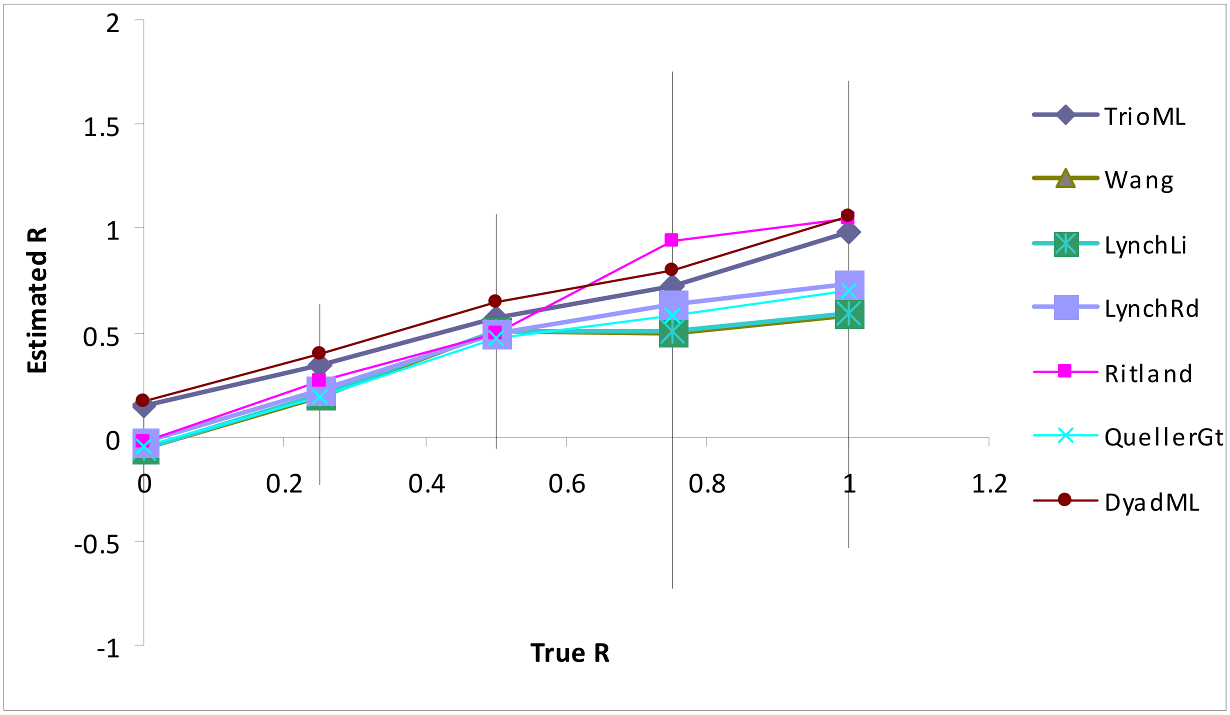


Figure 4.3. Estimates of the relatedness compared to true values of relatedness calculated with simulations in program COANCESTRY (Wang 2010) using seven methods: moment estimators from Ritland (1996), Lynch et. al (1999; LynchRd), Wang (2002; Wang), Lynch (1988; LynchLi), and Queller and Goodnight (1989; QuellerGt), a triadic likelihood estimator which uses a third individual as a reference for estimating pairwise relatedness between individuals (Wang 2007; TrioML), and a dyadic likelihood estimator described in Milligan (2003; DyadML).

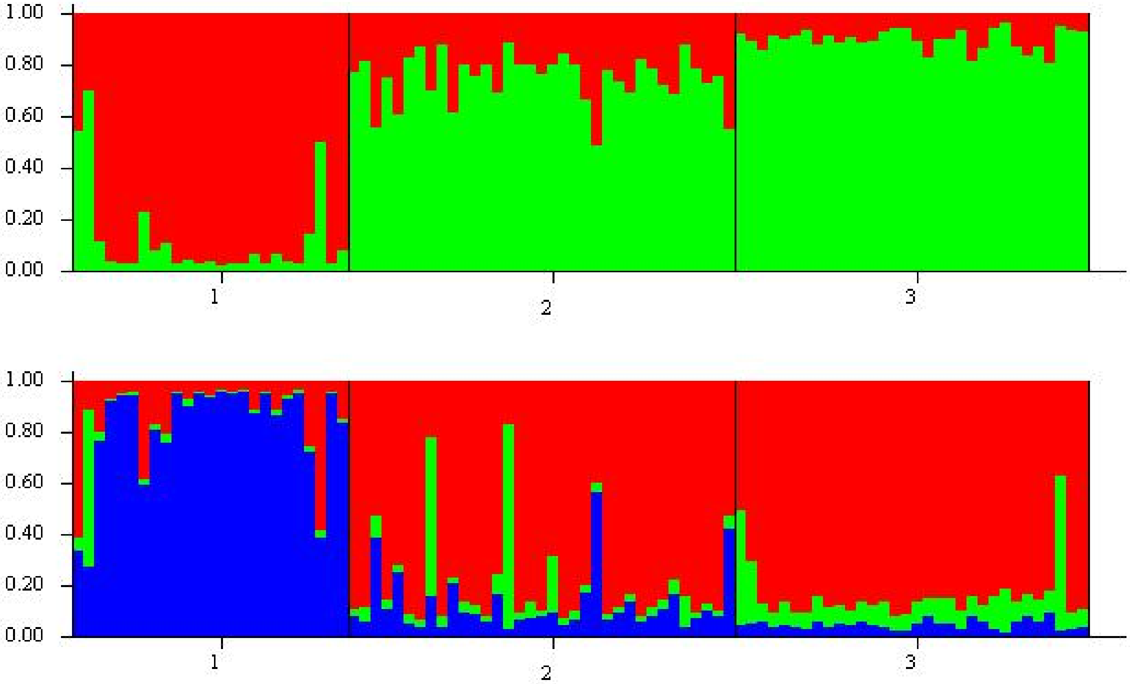


Figure 4.4. Population clustering by program STRUCTURE (Pritchard et al. 2000) using geographic information for two (red and green) and three (blue, red, and green) genetic clusters. Prior population assignments are represented on the x-axis as 1: Stehekin, 2: Squaw Creek, and 3: Black Canyon. Road kills were assigned to the closest geographic population.

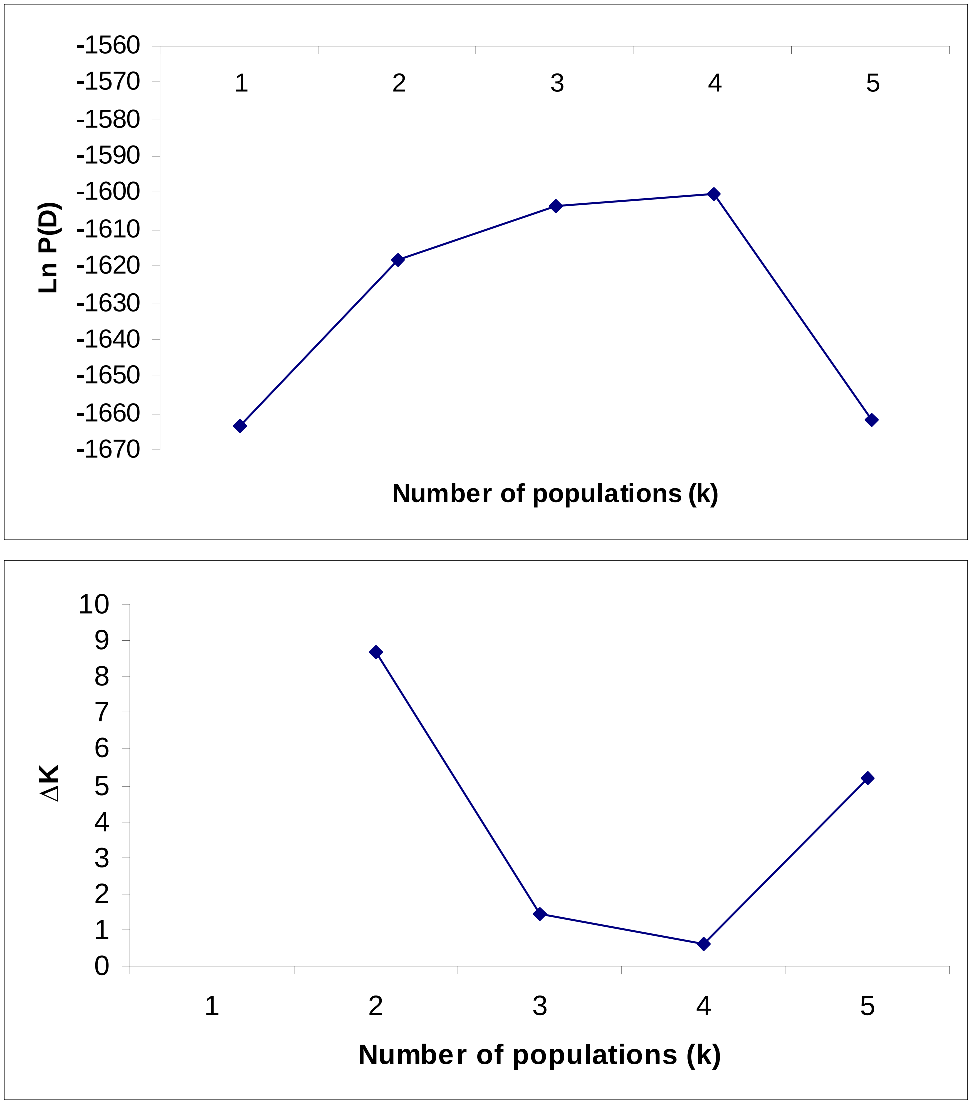


Figure 4.5. Two methods for determining the most likely number of populations using program STRUCTURE with spatial geographic information (Pritchard et al. 2000). Top: LnP(D), or probability of (X|K), versus the likely number of populations (k), Bottom: ΔK by the number of likely populations (Evanno et al. 2005).

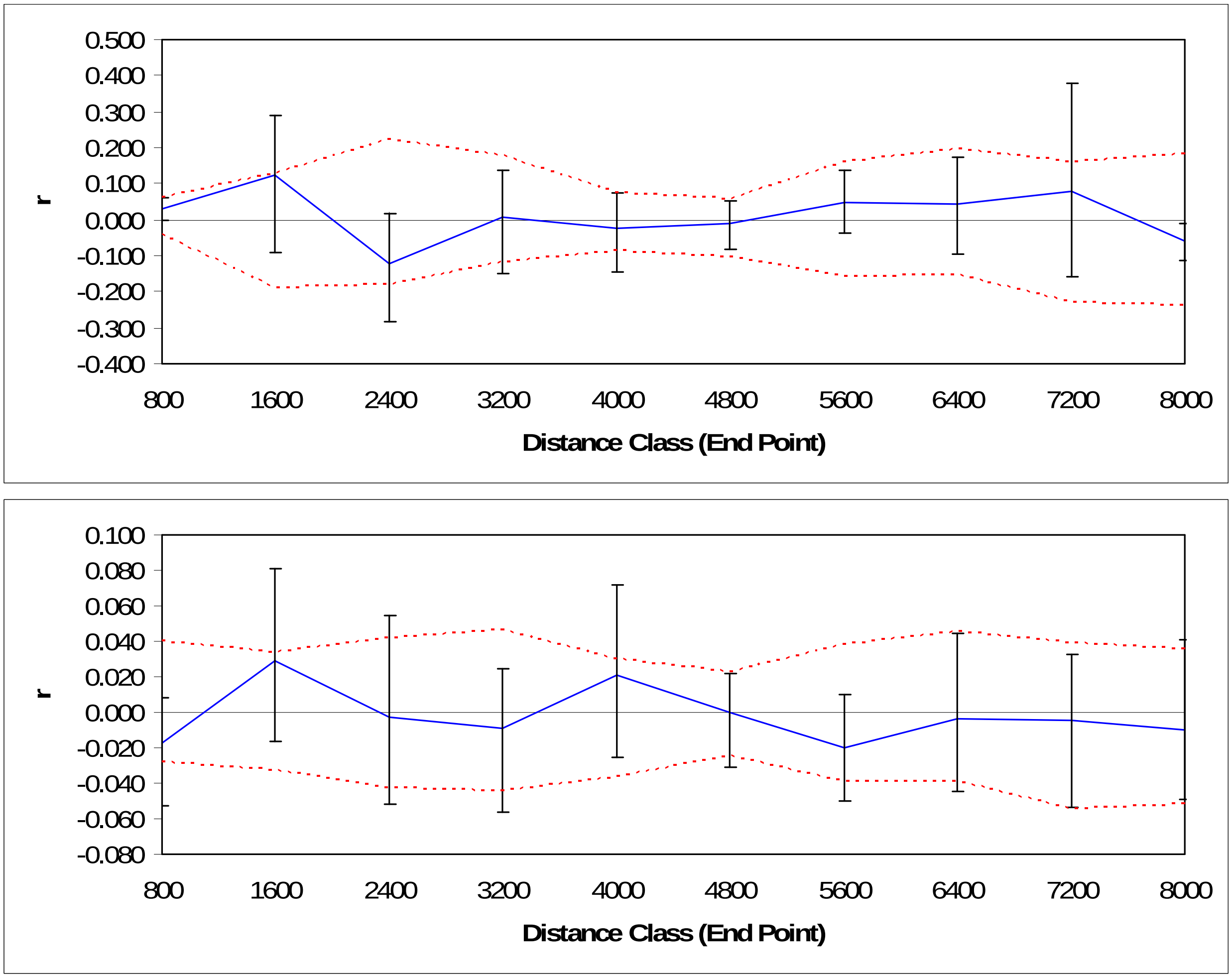


Figure 4.6. Spatial Structure Analysis for Stehekin (top), and the Methow Valley (bottom) using program GenAlex (Peakall and Smouse 2006) using spatial autocorrelation with 10 distance classes of 800m each. The black line is the null hypothesis of no relationship between geographic and genetic distance, the blue line is the calculated correlation between genetic distance and geographic distance, the red lines are the upper and lower confidence limits of the random expectations, and the black error bars represent the confidence limits for r calculated through bootstrapping.

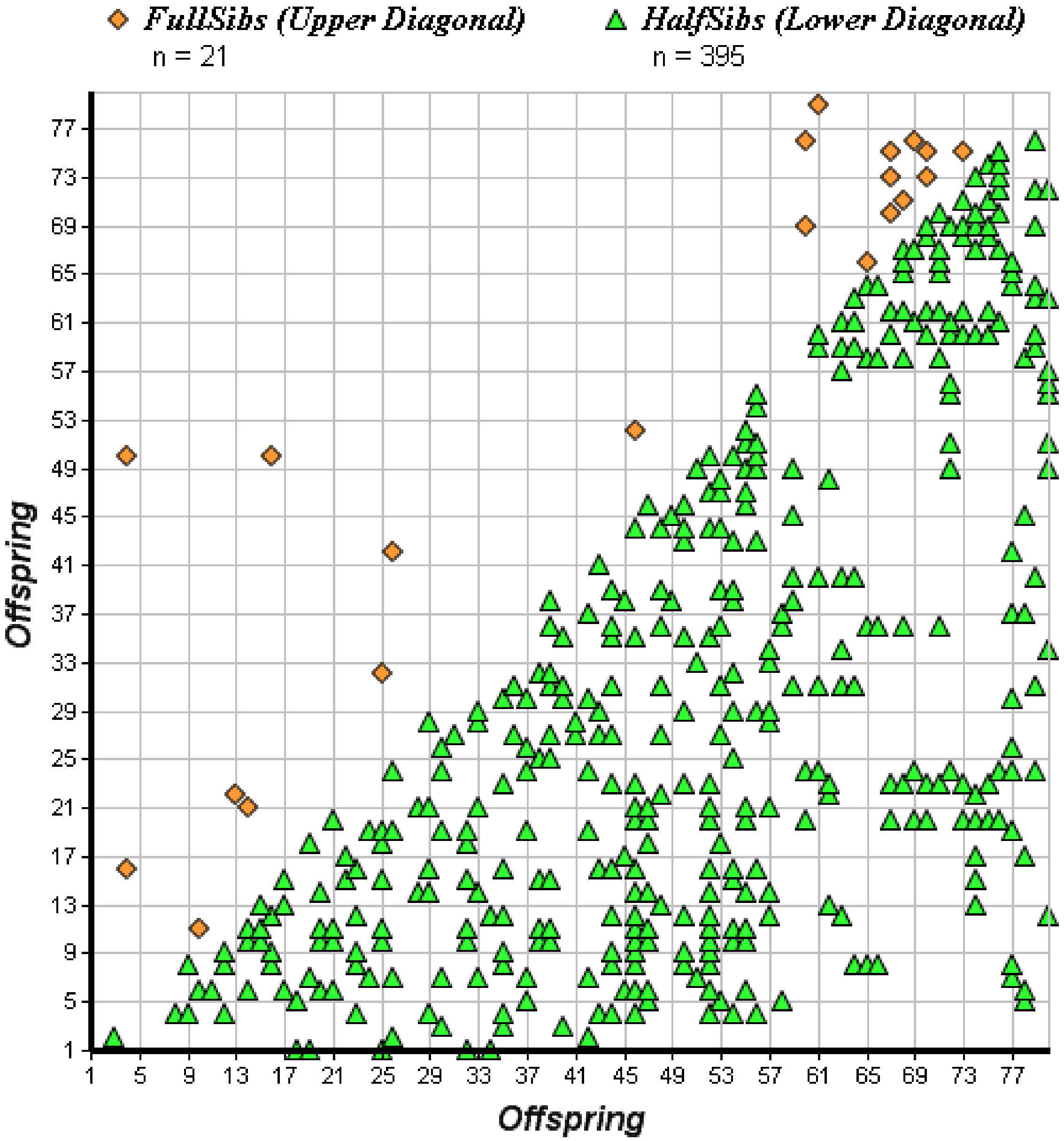


Figure 4.7. Best maximum likelihood sibship for the combined North Cascades population calculated with program COLONY (Wang 2004, Wang and Santure 2009, Jones and Wang 2010). Individuals 1-27 are from Black Canyon in the Methow Valley, 28-52 are from Squaw Creek in the Methow Valley, and 53-77 from Stehekin.

Table 4.1. Primer sets and PCR reactions for electrophoresis of western gray squirrel microsatellite loci analyzed by the Washington Department of Fish and Wildlife, Olympia, Washington. Primer sequences developed by Kenneth I. Warheit (Washington Department of Fish and Wildlife, unpublished data).

Primer Name	Locus Name	Primer Sequence	vector	Temp	Cycles	uM/reaction	Vector	Dye	uM/reaction
Sgr-B208 F V1	Sgr-B208	**ATCTCGCAAATAAATAAGTGCT**TCTTGGCTTCCTCATACCTG	V1	62°	40	0.27	V1	vic	0.135
Sgr-B208 R	Sgr-B208	GCAACTCAACATTACCACAGC		62°	40	0.27			
Sgr-D10 F V2	Sgr-D10	**CTTATGGTACTGTAACTGAGGA**CCCCACATTGTATGAGCC	V2	62°	40	0.22	V2	6fam	0.11
Sgr-D10 R	Sgr-D10	AGCCCACTGCCACTGTAG		62°	40	0.22			
Sgr-D237 F V3	Sgr-D237	**GTAAAACGACGGCCAGTGTCTC**TGAGCTAAGCTGACCACCTG	V3	62°	40	0.15	V3	ned	0.075
Sgr-D237 R	Sgr-D237	AGGTCCTGGGTTCAACCTC		62°	40	0.15			
Sgr-B205 F V4	Sgr-B205	**CAAAATAGGCTGTCCCATGC**ACCTCTCACTGCCTTCCTTAC	V4	65°	40	0.19	V4	pet	0.095
Sgr-B205 R	Sgr-B205	AACTTCCAGCAACACACACA		65°	40	0.19			
Sgr-A7 F V4	Sgr-A7	**CAAAATAGGCTGTCCCATGC**GCAGGTGAGCTAGACATCTG	V4	60°	37	0.25	V4	pet	0.125
Sgr-A7 R	Sgr-A7	AGTTGGGAGTGAAAGGACAG		60°	37	0.25			
Sgr-D118 F V2	Sgr-D118	**CTTATGGTACTGTAACTGAGGA**GAACTTGAACTGAGCCACATT	V2	60°	37	0.2	V2	6fam	0.1
Sgr-D118 R	Sgr-D118	TCCAGAGAGACAGAACCAATAG		60°	37	0.2			
Sgr-D216 F V1	Sgr-D216	**ATCTCGCAAATAAATAAGTGCT**GCCTCAATAACTTAGCAAGACC	V1	60°	37	0.15	V1	vic	0.075
Sgr-D216 R	Sgr-D216	AAGTGCTGTATAAGGAACGTCA		60°	37	0.15			
Sgr-D227 F V3	Sgr-D227	**GTAAAACGACGGCCAGTGTCTC**TTGGGTAGTCCAGGATAATTC	V3	60°	37	0.21	V3	ned	0.105
Sgr-D227 R	Sgr-D227	ATGTTTCAACCGAGAAGTAGAG		60°	37	0.21			
Sgr-C222 F V3	Sgr-C222	**GTAAAACGACGGCCAGTGTCTC**TGCTTTCTGCCTCTCTTTG	V3	55°	40	0.19	V3	ned	0.095
Sgr-C222 R	Sgr-C222	CCATCCAAGTGTCTTTCTCTC		55°	40	0.19			
Sgr-D119 F V2	Sgr-D119	**CTTATGGTACTGTAACTGAGGA**GGTTTCAATCCCCCACAC	V2	55°	40	0.24	V2	6fam	0.12
Sgr-D119 R	Sgr-D119	GACCTCACACACATCTCACCTA		55°	40	0.24			
Sgr-D2 F V1	Sgr-D2	**ATCTCGCAAATAAATAAGTGCT**AGAGGGGTAGATTCACTCTTTG	V1	55°	40	0.18	V1	vic	0.09
Sgr-D2 R	Sgr-D2	AATAAGACTGGCACCATGTTC		55°	40	0.18			
Sgr-B9 F V4	Sgr-B9	**CAAAATAGGCTGTCCCATGC**GCAACTTAGCAAGACCCTGTC	V4	51°	40	0.27	V4	pet	0.135
Sgr-B9 R	Sgr-B9	GTGCCAGTTCAATAATTTTTGC		51°	40	0.27			
Sgr-A101 F V3	Sgr-A101	**GTAAAACGACGGCCAGTGTCTC**GCAACCGTGCTCTCTTTAG	V3	53°	36	0.19	V3	ned	0.095
Sgr-A101 R	Sgr-A101	GCCACTTACAGTCATCCAGA		53°	36	0.19			
Sgr-A11 F V1	Sgr-A11	**ATCTCGCAAATAAATAAGTGCT**CTTTACGGCTACCCTTTCTAC	V1	53°	36	0.25	V1	vic	0.125
Sgr-A11 R	Sgr-A11	GCATTCCTGTGAGTCCATT		53°	36	0.25			
Sgr-A114 F V2	Sgr-A114	**CTTATGGTACTGTAACTGAGGA**TCATCAAATCATCAGTCAGATC	V2	53°	36	0.19	V2	6fam	0.095
Sgr-A114 R	Sgr-A114	CATTGTAACACCGGCTAAGT		53°	36	0.19			
Sgr-D211 F V4	Sgr-D211	**CAAAATAGGCTGTCCCATGC**CCAGTTCTGTTCCACTTTTG	V4	53°	36	0.25	V4	pet	0.125
Sgr-D211 R	Sgr-D211	CAGGTTCCTTTTTCTCTTCC		53°	36	0.25			
Sgr-B216 F V2	Sgr-B216	**CTTATGGTACTGTAACTGAGGA**ATACCAGATGGTTTCACTGATG	V2	58°	38	0.18	V3	ned	0.09
Sgr-B216 R	Sgr-B216	ATTTGGGTCTCTTTCTTTTCTG		58°	38	0.18			
Sgr-D108 F V2	Sgr-D108	**CTTATGGTACTGTAACTGAGGA**CTCCTTAACTCTGATGGGACTG	V2	58°	38	0.26	V2	6fam	0.13
Sgr-D108 R	Sgr-D108	TCCCTTCTGGACTGAAATAATC		58°	38	0.26			
Sgr-D7 F V1	Sgr-D7	**ATCTCGCAAATAAATAAGTGCT**GTGGAAAGGAGGGTTTAGATG	V1	58°	38	0.2	V1	vic	0.1
Sgr-D7 R	Sgr-D7	AAAGGCTCATGTGTTGAAGAC		58°	38	0.2			

Table 4.2. Descriptor variables for loci by population including number of individuals with alleles at each loci (N), observed and expected heterozygosity (Ho, He), Wright's FIS (1922), Allelic Richness (A) and probability of each locus being in Hardy-Weinberg Equilibrium (P HWE) calculated with programs GENEPOP (Raymond and Rousset 1995, Rousset 2008) and FSTAT (Goudet 2001).

		Population				
Locus		Black Canyon Creek	Stehekin	Squaw Creek	Combined Methow	Overall
Sgr-A101	N	29	26	33	62	88
	Ho	0.138	0.038	0.273	0.129	0.159
	He	0.272	0.038	0.313	0.203	0.225
	FIS	0.497	0.000	0.130	0.366	0.294
	A	2.000	1.846	2.892	3.634	2.989
	P HWE	0.005	NA	0.258	0.006	0.003
Sgr-A11	N	29	26	34	63	89
	Ho	0.448	0.577	0.235	0.270	0.404
	He	0.354	0.491	0.334	0.270	0.416
	FIS	-0.273	-0.180	0.299	0.000	0.028
	A	2.000	2.000	2.000	2.000	2.000
	P HWE	0.286	0.441	0.106	1.000	0.800
Sgr-A114	N	29	26	34	63	89
	Ho	0.586	0.308	0.471	0.492	0.461
	He	0.499	0.452	0.522	0.422	0.494
	FIS	-0.178	0.324	0.099	-0.167	0.067
	A	2.000	2.000	2.000	2.000	2.000
	P HWE	0.452	0.188	0.735	0.280	0.674
Sgr-A7	N	29	26	34	63	89
	Ho	0.793	0.577	0.735	0.683	0.726
	He	0.748	0.604	0.718	0.618	0.708
	FIS	-0.062	0.045	-0.025	-0.105	0.025
	A	4.759	3.997	4.647	4.914	4.945
	P HWE	0.547	0.009	0.089	0.221	0.452
Sgr-B205	N	25	25	32	57	82
	Ho	0.520	0.200	0.375	0.368	0.366
	He	0.532	0.254	0.445	0.388	0.427
	FIS	0.024	0.218	0.159	0.050	0.144
	A	3.879	2.999	4.281	4.900	4.971
	P HWE	0.823	0.097	0.642	0.716	0.241
Sgr-B208	N	31	25	34	65	90
	Ho	0.484	0.160	0.265	0.308	0.311
	He	0.523	0.152	0.459	0.422	0.441
	FIS	0.076	-0.051	0.427	0.272	0.296
	A	2.759	2.879	2.647	2.900	3.722
	P HWE	0.716	1.000	0.006	0.026	0.002
Sgr-B216	N	22	25	32	54	79
	Ho	0.727	0.160	0.500	0.481	0.456
	He	0.640	0.221	0.572	0.477	0.507
	FIS	-0.141	0.281	0.127	-0.009	0.102
	A	3.000	2.988	3.687	3.818	3.861
	P HWE	0.638	0.042	0.453	0.292	0.078
Sgr-C222	N	29	26	34	63	89
	Ho	0.448	0.385	0.588	0.429	0.483
	He	0.509	0.427	0.544	0.439	0.503
	FIS	0.121	0.101	-0.083	0.024	0.040
	A	2.000	2.000	2.000	2.914	2.000
	P HWE	0.714	0.662	0.011	0.018	0.011
Sgr-D10	N	28	26	33	61	87
	Ho	0.000	0.000	0.091	0.049	0.034
	He	0.000	0.000	0.089	0.049	0.034
	FIS	0.000	0.000	-0.021	-0.014	-0.008
	A	1.000	1.000	2.559	2.652	2.735
	P HWE	NA	NA	1.000	1.000	1.000

Sgr-D118	N	29	26	32	61	87
	Ho	0.379	0.308	0.281	0.246	0.322
	He	0.327	0.396	0.337	0.241	0.352
	FIS	-0.162	0.226	0.168	-0.019	0.087
	A	2.988	2.000	2.687	2.995	2.998
	P HWE	1.000	0.331	0.410	1.000	0.441
Sgr-D211	N	29	26	34	63	89
	Ho	0.724	0.462	0.618	0.571	0.607
	He	0.641	0.544	0.682	0.548	0.630
	FIS	-0.133	0.154	0.095	-0.043	0.037
	A	3.759	3.000	3.647	3.914	3.945
	P HWE	0.838	0.215	0.547	0.641	0.740
Sgr-D216	N	29	26	31	60	86
	Ho	0.655	0.654	0.484	0.467	0.593
	He	0.593	0.588	0.533	0.481	0.582
	FIS	-0.106	-0.115	0.093	0.030	-0.018
	A	3.976	3.846	2.978	3.983	3.998
	P HWE	0.116	0.830	0.883	0.147	0.216
Sgr-D237	N	22	24	22	44	68
	Ho	0.318	0.042	0.500	0.273	0.279
	He	0.439	0.042	0.470	0.343	0.341
	FIS	0.279	0.000	-0.066	0.208	0.182
	A	3.000	1.917	2.000	3.000	3.000
	P HWE	0.366	NA	1.000	0.258	0.315
Sgr-D108*	N	26	22	33	59	81
	Ho	0.577	0.227	0.485	0.525	0.444
	He	0.795	0.677	0.812	0.808	0.777
	FIS	0.278	0.670	0.406	0.352	0.430
	A	5.000	5.000	5.891	5.939	5.975
	P HWE	0.127	0.000	0.000	0.000	0.000
Sgr-D119*	N	27	26	34	61	87
	Ho	0.630	0.385	0.294	0.443	0.425
	He	0.540	0.386	0.405	0.472	0.451
	FIS	-0.169	0.004	0.276	0.062	0.058
	A	3.990	3.958	4.798	4.979	4.990
	P HWE	0.068	0.085	0.000	0.000	0.000
Sgr-D2*	N	29	26	34	63	89
	Ho	0.310	0.154	0.294	0.302	0.258
	He	0.479	0.147	0.419	0.444	0.369
	FIS	0.356	-0.049	0.301	0.322	0.302
	A	3.933	2.844	3.526	3.986	3.997
	P HWE	0.016	1.000	0.015	0.000	0.000
Sgr-D227*	N	28	25	30	58	83
	Ho	0.500	0.280	0.600	0.552	0.470
	He	0.645	0.348	0.711	0.719	0.697
	FIS	0.228	0.199	0.159	0.234	0.327
	A	3.786	3.999	3.999	4.763	4.000
	P HWE	0.236	0.065	0.008	0.002	0.000
Sgr-D7*	N	28	24	30	58	82
	Ho	0.036	0.000	0.000	0.017	0.036
	He	0.071	0.000	0.066	0.068	0.141
	FIS	0.500	0.000	1.000	0.747	0.748
	A	2.630	1.000	1.932	2.766	2.836
	P HWE	0.018	NA	0.018	0.000	0.000
Overall Loci	N	22	22	22	44	68
	Ho	0.460	0.273	0.394	0.367	0.380
	He	0.478	0.320	0.468	0.412	0.450
	FIS	0.063	0.101	0.197	0.128	0.174
	A	3.137	2.737	3.232	3.670	3.609
	P HWE	0.410	0.355	0.343	0.312	0.276

Probability of Hardy-Weinberg Equilibrum calculated with the exact HW test (Haldane 1954)
FIS calculated with Weir and Cockerham (1984) estimate
* = omitted from analyses due to null alleles and deviation from Hardy-Weinberg in multiple populations
NA = only one allele present, not possible to calculate

Table 4.3. Significant pairwise linkages (α= 0.05) among populations and loci, out of 153 total pairs for each population calculated with program GENEPOP (Raymond and Rousset 1995, Rousset 2008).

	Population			
Locus	Black Canyon	Stehekin	Squaw Creek	Total
Sgr-A101	0	4	4	8
Sgr-B208	2	3	2	7
Sgr-D216	1	3	3	7
Sgr- A7	0	3	3	6
Sgr-D118	1	2	3	6
Sgr-A11	1	0	3	4
Sgr-B216	1	1	2	4
Sgr-D10	0	0	4	4
Sgr-D211	1	2	1	4
Sgr-A114	1	1	0	2
Sgr-C222	0	1	1	2
Sgr-B205	0	0	1	1
Sgr-D237	0	0	1	1
Total Pairs	4	10	14	28

Table 4.4. F_{ST} and R_{ST} values between North Cascade western gray squirrel populations calculated with GENEPOP (Raymond and Rousset 1995, Rousset 2008) using the method of Weir and Cockerham (1984). χ^2 and P-values calculated across the 13 loci used for analysis with Fisher's method (1935).

Population Pair	FST	RST	$\chi 2$	P-Value
Black Canyon/Stehekin	0.098	0.058	∞	<0.001
Black Canyon/Squaw Creek	0.007	-0.007	23.200	0.622
Stehekin/Squaw Creek	0.065	0.268	95.443	<0.001

Table 4.5. Permutation test of difference in inbreeding between two populations of squirrels in the North Cascades: Stehekin and the Methow Valley. Inbreeding coefficients estimated with Ritland's Moment Estimator (Ritland 1996) and compared with 1000 bootstraps in program COANCESTRY (Wang 2010). Inbreeding among populations is not significantly different because the observed difference falls inside all quantiles of the expected distribution.

Data		Permutations	
Stehekin n	24	1% quantile	-0.188
Methow n	56	2.5% quantile	-0.160
Stehekin mean	0.051	5% quantile	-0.129
Methow mean	0.116	95% quantile	0.149
Stehekin variance	0.025	97.5% quantile	0.168
Methow variance	0.134	99% quantile	0.193
Mean Difference Stehekin-Methow			-0.065

Table 4.6. Effective population sizes and 95% confidence intervals calculated with program LDNe (Waples and Do 2007) which includes the bias correction method of Waples (2006) for sample size. We used a random mating model assuming neutral markers in a single, closed population. Alleles at frequency of less than 0.05 were not included in analysis.

		95 C.I.	
Population	Ne	Lower Bound	Upper Bound
Stehekin	7.2	2.8	16.5
Black Canyon	55.3	25.6	708.1
Squaw Creek	57.8	29.1	258.4
Combined Methow Valley	95.3	38.6	Infinite

Table 4.7. Permutation test of difference in relatedness between two populations of squirrels in the North Cascades: Stehekin and the Methow Valley. Pairwise relatedness estimated with the Ritland's moment estimator (Ritland 1996) and compared with 1000 bootstraps in program COANCESTRY (Wang 2010). Relatedness among populations is not significantly different because the observed difference falls inside all quantiles of the expected distribution.

Data		Permutations	
Stehekin n	276	1% quantile	-0.05
Methow n	325	2.5% quantile	-0.04
Stehekin mean	0.06	5% quantile	-0.03
Methow mean	0.02	95% quantile	0.03
Stehekin variance	0.04	97.5% quantile	0.04
Methow variance	0.07	99% quantile	0.05
Mean Difference Stehekin-Methow			0.04

CHAPTER 5: Western Gray Squirrel Diet in the North Cascades: Fungal Diversity and Composition by Location, Season, and Fire Fuel Treatment History

Abstract

Food availability affects fitness by producing energy necessary for body growth and maintenance of body condition and reproduction. An adequate diet can be especially important for threatened or endangered species such as the western gray squirrel (*Sciurus griseus*), which has declined in range and number over the past 100 years. We identified fungal spores in 136 fecal samples collected from 47 live trapped western gray squirrels at two study sites in the Washington North Cascades, the northernmost population isolate for this species. This area represents unique habitat that lacks oak trees as a food resource. Wildfire is also a common ecological disturbance in these forests and thinning and prescribed burning is used frequently to reduce wildfire risk. Fungal spores were identified to genus and categorized by relative abundance. Permutational Analysis of Variance (PERMANOVA) was used to evaluate differences in fungal community composition of squirrel diets at each study site with explanatory predictor variables: squirrel (random blocking factor), season, and wildfire/fire fuel treatment history. Fungal species richness and diversity in the diets of squirrels was higher in Spring/Summer than Fall/Winter months. Season and wildfire/fire fuel treatment history also significantly influenced fungal community composition of squirrel diets at one study site. *Rhizopogon* was the most ubiquitous and abundant genus in samples; *Geopera* was the strongest indicator species for differences between seasons. Abundance of plant material in samples drove differences between treated and untreated sites. This study is the first to examine the western gray squirrel food resource in the North Cascades and effects of forest management. We found

no evidence that recent moderate intensity wildfire and fire fuel reduction treatments have negatively affected the fungal composition of the western gray squirrel diet.

Introduction

Food availability and quality affect fitness by producing energy necessary for body growth and/or maintenance of body condition and for reproductive investment. Animals with access to higher quantity and quality of food are more likely to reproduce successfully and tend to be better competitors than animals of smaller size or lesser body mass, are better at escaping predators, and less vulnerable to parasites and disease (Cuthill and Houston 1997). Food availability had long lasting positive effects on female reproductive success of red squirrels in southwestern Yukon, Canada (Descamps et al. 2008); when mothers received supplemental food, they bred earlier on average and produced offspring with higher survival rates (Kerr et al. 2007).

An adequate diet can be especially important for threatened or endangered species such as the western gray squirrel (*Sciurus griseus*), which has already faced sharp declines in range and number due to over-hunting, automobile accidents, disease, predation, competition with non-native species, and habitat loss. Listed as a Washington state threatened species in 1993 by the Washington Department of Fish and Wildlife (WDFW) (WAC 232.12.011), the western gray squirrel is now limited to three geographically isolated areas within the state: Pierce County in the southern Puget Trough, southern Washington in Klickitat, Yakima, and Skamania counties, and the North Cascades of north-central Washington in Chelan and Okanogan counties. Throughout their range, western gray squirrels have been observed eating both epigeous and hypogeous (truffles and false truffles) fungi, pine nuts, acorns, seeds, green vegetation, fruit and insects (Cross 1969, Steinecker and Browning 1970). No previous study has specifically identified fungal genera consumed by individual squirrels, or quantified diversity and relative abundance across seasons, and geographic areas in relation to land management practices.

Stomach samples provide detailed information on an individual's last meal, however few western gray squirrel stomachs are ever available for analysis due to the state threatened status of the species, and tendency of predators to consume squirrels in entirety. Fecal pellet analysis is an alternative, non-lethal method for studying diet that allows longer term examination of temporal variation among individuals through repeated sampling. Western gray squirrels are classified as preferential mycophagists; hypogeous fungi (truffles) compose a large percentage of their diet. Truffles provide a high concentration of vitamins, minerals, fatty acids, steroids, triterpenes, amines, indoles, and phenols in addition to water (Trappe et al. 2009) and the high diversity and abundance of truffles in the Pacific Northwest (Trappe et al. 2009) make them a significant food resource. Truffles are also readily detected by squirrels and require less processing time than seeds (Cork and Kenagy 1989). Spores of both hypogeous and epigeous fungi travel through the digestive tract unaltered and are easily identified in fecal pellets. Plant material and insect parts can sometimes be recognized in fecal samples as well. We sought to identify seasonal and geographic diversity and relative abundance of fungal genera in western gray squirrel fecal pellet samples collected from two study sites within the North Cascades population: Stehekin, WA and Squaw Creek in the Methow Valley, WA.

The North Cascades is the northernmost range of the western gray squirrel and represents a unique edge habitat with potentially limited resources; it lacks oak trees (and acorns as a food source), has high average annual snowfall, and a dynamic history of wildfire and forest management practices. We began a radio-telemetry study in 2008 to evaluate resource selection of western gray squirrels in the North Cascades in relation to prescribed burning, thinning, and dwarf mistletoe removal treatments being carried out for forest fuel reduction by the National Park Service (NPS) and United States Forest Service (USFS) for fire fuel reduction. Portions of our Stehekin study area were also altered by the Flick Creek fire in 2006.

Food resources available to western gray squirrels are an important component of habitat quality that may be altered by wildfire and fire fuel treatments. Direct studies evaluating wildfire and forest management activities on truffle biomass and species richness indicate that if the organic layer of the soil remains relatively undamaged, the truffle community is not substantially altered (Jonsson et al. 1999, Korb et al. 2003). However, high-intensity, stand-replacing fires that cause total combustion of soil organic layers can severely decrease populations of ectomycorrhizal fungi (Bruns et al. 2002, Dahlberg 2002). Impacts are dependent on fire intensity and the particular species of fungi: fruiting of some truffle species is promoted by fire while others are suppressed (Trappe et al. 2006). Seasonal timing of wildfire or prescribed burning is also important: Smith et al. (2004) reported greater reductions in truffle diversity and biomass when prescribed burning was carried out in the dry season compared to the moist season. Commercial thinning has also been shown to reduce truffle production and shift species assemblages (Colgan et al. 1999). Carey et al. (2002) found lower truffle diversity in thinned versus legacy forests. However, basal area and stem density of stands are negatively associated with seed production per tree in ponderosa pine (Krannitz and Duralia 2004), so that each tree may produce more seeds in thinned areas. Over time, squirrels could potentially increase consumption of seeds and plant material in thinned areas to partially compensate for decreases in truffle abundance and diversity. Other studies also suggest that retention of a small percentage of the largest coarse woody debris at sites may help maintain fungal diversity and relative abundance through moisture retention and shading without increasing hazardous fuels (Carey et al. 2002, Lehmkuhl et al. 2004). Alterations to the truffle community due to natural disturbances and forest management practices can persist for relatively long periods of time and have the potential to negatively affect wildlife populations including the western gray squirrel. The altered tree composition and severity of winter in the North Cascades could make the food resource the key habitat requirement for western gray squirrels in this area. Squirrel density, as well as overwinter survival of Abert's squirrels (*Sciurus aberti*) in Northern Arizona, was positively correlated with fecal fungal diversity (Dodd et al. 2003).

The purpose of this study was to identify food items (particularly truffle genera) consumed by squirrels in Stehekin and the Methow Valley and compare diversity and relative abundance between seasons and fire fuel treated/burned, and untreated/unburned areas to better understand western gray squirrel diet in relation to forest management practices.

Methods

Field

Fecal sampling took place in Stehekin, WA, part of the Lake Chelan National Recreation Area of North Cascades National Park, and in the Squaw Creek drainage in the Methow Valley, Washington. Both are mixed conifer/deciduous habitats composed primarily of Douglas-fir (*Pseudotsuga menziessi*) and ponderosa pine (*Pinus ponderosa*). Summers are hot and dry; winters are cool and wet with temperatures ranging from -20 to 40°C and average rain/snowfall of 50 cm (2005-2010 NCDC NOAA Climatological Data). Additional study site information: p.4

We live trapped western gray squirrels from 2008-2011 using wire mesh 15 x 15 x 48cm Tomahawk live traps (Tomahawk Live Trap Co. Tomahawk WI, USA) baited with whole English walnuts. Traps were spaced between 50m and 80m apart and placed on the North side of the base of large diameter trees. We began trapping at sunrise and checked traps every two hours.

Captured squirrels were processed in a handling bag (Koprowski 2002) modified with an additional ventral opening and marked with uniquely numbered ear tags (model 1005-3, National Band and Tag Co., Newport, KY, USA). Three to four fecal pellets per sample were collected from captured squirrels at the trap location when possible and preserved in screw cap vials filled with 100% ethanol. Traps and the trap location were cleaned of all fecal pellets prior to re-setting to ensure that future fecal samples collected at that site were from the next squirrel captured. Effort was made to collect fecal samples every month, and to recapture and sample individual squirrels several times per year to quantify seasonal variation.

Lab

Fecal pellets were macerated and mixed with 10 drops of water in small vials to create a solution. One drop of solution was then transferred with tweezers to a microscopic slide, mixed with one drop potassium hydroxide (KOH) and covered with a 22 X 22-mm cover slip. Three slides were prepared per sample. One drop of Melzer's reagent (iodine, potassium iodide, and chloral hydrate in aqueous solution) was added to an additional slide per sample to help with spore identification when necessary. Two transects of 10 fields of view were analyzed per slide beginning 8mm from the bottom of the slide and 5mm from the left edge of the liquid moving right, and 16mm from the bottom of the slide, 5mm from the right edge of the liquid moving left. Fields of view were spaced 1mm apart and examined initially at 40X magnification. Spores were identified to genus according to Castellano et al. (1989). We were unable to reliably identify any insect parts or separate plant matter by genus or species, but did include a plant and other category. Within each field of view, relative abundance of each diet item (fungal genus, unknown spore, plant, and other) was estimated using the following categories: 3 (high): > 66%, 2 (medium): >33% and < 66%, 1 (low): >0% and < 33% aided by a 10 X 10 grid within the microscope eye piece. Categorical estimation of relative abundance decreased processing time for samples and facilitated consistency among observers. Relative abundance categorical values (1, 2, 3) were averaged across the 60 fields of view for each sample to provide an overall index of relative abundance. Oil immersion at 100X was used to re-examine fields of view and confirm genera of spores identified at 40X. Digital photos of unknown spores were taken for identification by several observers and verification by mycologist Jim Trappe (Oregon State University). Spores that could not be confidently identified were placed in an unknown spore category. Lab work was conducted by undergraduate Biology students at the University of Washington trained to identify fungal spores.

Analysis

Fecal samples were classified by study site, individual squirrel, season, and wildfire/fire fuel treatment history. Season was represented with a two-level categorical variable: Fall/Winter = September-March, Spring/Summer = April-August. These categories separated key seasonal differences in temperature and precipitation in our study areas during the sampling timeframe (2005-2011 NCDC NOAA Climatological Data); divided the two primary truffle fruiting seasons; and increased sample sizes per group. Squirrel home ranges in Squaw Creek surrounded one fire fuel treated area: the East Douglas section of the Hungry Hunter Ecosystem Management Project which was large scale prescribed burned and thinned in 2009 by the United States Forest Service (USFS); factor "treatment" had only two levels for this study site: untreated/unburned and thinning/prescribed fire. Squirrel home ranges in Stehekin surrounded one fire fuel treatment area: the Buckner Orchard/Rainbow section of the Lake Chelan National

Recreation Area Fire Fuel Reduction Area which was thinned and prescribed burned on a small scale every two years by the National Park Service (NPS) from 1996 to 2008, and one area affected by the Flick Creek wildfire in 2006; factor "treatment" had three levels for this study site: untreated/unburned, thinning/prescribed fire, and wildfire. Samples collected from squirrels with radiotelemetry information were categorized as treated or untreated depending on where the sample was collected and how much of the squirrel's home range encompassed fire fuel treated areas and/or recently burned areas compared to untreated/unburned areas. Samples from squirrels without radiotelemetry information (10/136) were classified as treated or untreated based on trap location. Eight of the 10 samples collected from squirrels without radio-telemetry information were from Squaw Creek, where the surrounding fire fuel treatment area is larger than the average squirrel home range size; the remaining two samples were collected from males in Stehekin during the non-breeding season when home range sizes are smaller and trap location is more likely to accurately represent a significant portion of the home range. Labels for seven samples from the North Cascades were unreadable and only used for analyses that did not require stratification.

The raw relative abundance scores were used to calculate species richness (sum of fungal genera detected in each sample not including plants or other material), diversity, and evenness among samples estimated with the Shannon-Weiner index:

$$H' = -\sum_{i}^{s} p_i \log\left(p_i\right)$$

which accounts for relative abundance of species, and Pielou’s J (Pielou 1966):

$$\frac{H'}{\log\left(S\right)}$$

where H' = the Shannon-Weiner diversity measure and S is the average species richness. We also generated species accumulation curves by study site to evaluate the extent of fungal diversity captured through sampling. Curves were created using the exact method which finds the expected mean species richness following the moment-based estimation method developed by Ugland et al. (2003), Colwell et al. (2004), and Kindt et al. (2006). We extrapolated species richness in a species pool to estimate how many additional genera would be found with increased sampling following the methods of Palmer (1990) and Colwell and Coddington (1994). Standard errors of the estimates were based on Chao (1987), and Smith and van Belle (1984).

We then used richness, diversity and evenness, as dependent variables for Permutational Analysis of Variance (PERMANOVA) with explanatory predictor variables: squirrel (random blocking variable nested by study site), study site, season, and fire/treatment history. PERMANOVA is a robust statistical measure with the only assumption that observations be interchangeable under the null hypothesis; multivariate normality and homogenous variances are not required, as for many other multivariate statistical tests (Anderson 2001, McArdle and Anderson 2001). The test statistic is a “Pseudo-F”, but is calculated in the same way as the traditional parametric univariate F-statistic:

$$: \frac{SSB/(t-1)}{SSW/(N-t)}$$

with *t* groups and *N* total sample size. Significance is assessed through permutation of group identities, recalculation of the test statistic, and comparison of the observed value against the permuted distribution of values. We ran PERMANOVA in the PERMANOVA + add on for PRIMER-E v6 (Clarke and Gorley 2006, Anderson et al. 2008) which calculates Pseudo-F statistics using Type III Sums of Squares. We used the Euclidean distance measure with 999 permutations of residuals under a reduced model. This method of permutation provides the best power and most accurate type I error estimation for multi-factorial designs in the widest set of circumstances (Anderson and Legendre 1999, Anderson and ter Braak 2003) and is theoretically closest to the exact test (Anderson and Robinson 2001). Pair-wise PERMANOVAs using the same data adjustments and program parameters were calculated for multiple comparisons between groups for significant terms. The PERMDISP function in the PERMANOVA + add on for PRIMER was used for analysis of homogeneity of multivariate dispersion. PERMDISP is a dissimilarity-based multivariate extension of Levene's test (1960) following the ideas of van Valen (1978), O'Brien (1992) and Manly (1994), and uses the ANOVA F statistic to compare distances from observations to their group centroid among different groups.

PERMANOVA was also used to evaluate differences in fungal community composition within squirrel diets by season and fire/treatment history at each study site. For this analysis we separated fecal samples by study site, deleted rare species that occurred in less than 5% of samples from each study site, and converted spore relative abundances to presence/absence (1,0) values. Rare species are poorly sampled and often contribute more noise than signal to predictor variable relationships. Presence/absence is a more reliable measure of the fungal community within squirrel diets because while the relative abundance of dominant food items can be assessed with some confidence, high variance around the frequency of rare items prevent estimates of proportionality and dietary importance (Colgan et al. 1997). Converting relative abundance to presence/absence retained all information on fungal community composition and diversity within squirrel diets while equalizing the importance of all dietary items within samples. We used the same parameters in PRIMER as with the diversity matrix with samples blocked by squirrel (random factor nested in fire/treatment history). Pair-wise PERMANOVAs were used for multiple comparisons between groups for significant terms, and PERMDISP for analysis of homogeneity of multivariate dispersion.

Results from PERMANOVA were visualized with Non-Metric Multi-Dimensional Scaling (NMDS), a distance based technique that uses a dissimilarity matrix to reduce the data to a smaller number of dimensions that account for as much variation as possible. NMDS also has few restrictive assumptions; it can be used with any distance measure and, again, does not require multivariate normality or homogeneous variances. Consistent with PERMANOVA parameters, we used the Euclidean distance measure. The starting configuration was the result of a Principle Coordinates Analysis (PCA) ordination in 2 dimensions; the standard fitted distance of p= 2 was used; instability was set at 0.00001 following McCune and Grace (2002, Table 16.3); the maximum number of restarts was set at 40, also as recommended by McCune and Grace (2002, Table 16.3), and interim solutions were not visualized. The adjustment of the final solution was centered around the centroid of the data cloud at its origin, rotated with PCA so that the first axis explained as much variation as possible, and the data were rescaled so that one unit along an axis corresponds to a halving of community similarity based on linear regression between distances and dissimilarities. We used two dimensions which minimized stress while allowing easier interpretability. NMDS ordinations were conducted in R version 2.7.2 (R Development Core Team 2008) with packages vegan (Oksanen et al. 2011) and labdsv (Roberts 2010).

Significant terms from study site PERMANOVAs were then analyzed with Indicator Species Analysis (ISA) to determine which diet items drove observed differences. The test statistic, or indicator value is:

$$IV_{ij} = A_{ij} \cdot B_{ij} \cdot 100$$

with

$$A_{ij} = \frac{\overline{x}_{ij}}{\sum_{j} \overline{x}_{i}} \qquad B_{ij} = \frac{n_{ij}}{n_{j}}$$

Dufrêne and Legendre (1997) classify strong indicators as those with IV scores > 25. We used the Indicator.Value function designed by Bakker (2008; Appendix S1 in Supplementary Material) in R. Version 2.7.2 (R Development Core Team 2008). This function is unique from other Indicator Species Analyses in that it assesses the significance of IV in every group j. It was more appropriate to separate Indicator Species Analyses by study site because there were several genera that occurred at only one site (Bakker 2008). All statistical analyses were evaluated at an alpha level of 0.1.

Results

We collected a total of 136 fecal samples from two study sites in the North Cascades. Sixty samples from 19 individual squirrels were collected in Stehekin; 73 from 28 individuals in Squaw Creek, with an average of 2.7 samples per squirrel (range 1-13). Within samples we identified 21 fungal genera, 17 in the truffle (hypogeous) groups Ascomycota, Basidiomycota, and Glomeromycota; the remaining four in the epigeous group Basidiomycota. Several genera were unique to one study site (Table 5.1). Some genera identified from fecal pellets in Stehekin had not been previously documented with exploratory truffle sampling conducted by Trappe and others (J.M. Trappe, Oregon State University, pers. comm.) in the Lake Chelan National Recreation Area of the North Cascades National Park Complex, including: *Leucophleps*, *Melanogaster* and *Mycoelvis*. Less truffle sampling has been completed near Squaw Creek in the Methow Valley; our study provides the first documented evidence of the presence of *Elaphomyces*, *Geopera*, *Genea*, *Gymnomyces*, *Hymenogaster*, *Hsyterangium* and *Picoa* in this area.

An average of three fungal genera were present in fecal samples from western gray squirrels. *Rhizopogon* was the most ubiquitous genus in both study areas with the highest relative abundance among samples, and highest occurrence among squirrels. *Rhizopogon* spores were present in fecal pellets of all 19 individual squirrels sampled in Stehekin and in samples from 27 of the 28 squirrels at Squaw Creek. *Geopera* had the second highest frequency among individuals from both study areas. Genera with high local frequency included *Mycolevis* in Stehekin and *Picoa* and *Hymenogaster* at Squaw Creek (Table 5.1). Species diversity was higher at Stehekin relative to Squaw Creek (*H*'= 0.659l; 0.596 respectively) despite a larger sample size at Squaw Creek (n= 60; 73). Evenness was also slightly larger at Stehekin (*J*= 0.567; 0.515 respectively), however differences between study sites were not statistically significant with PERMANOVA (*Pseudo-F*= 0.681, *P*= 0.415). Species richness, diversity, and evenness were greater among samples collected between April-August (Spring/Summer) than samples collected between September-March (Fall/Winter) in both study areas (*Pseudo-F*= 3.221, *P*= 0.071). Pairwise comparisons among seasons yielded a t-statistic of 1.795 (*P*= 0.06). There were also statistically significant differences in richness, diversity, and evenness among squirrels (*Pseudo-F*= 1.657 *P*= 0.019). There was little difference in richness, diversity and evenness between treated and untreated sites in both study sites (*Pseudo-F*= 0.366, *P*= 0.621; Tables 5.2, 5.3).

Species accumulation curves for both study sites did not reach a horizontal asymptote, indicating that additional fungal genera would likely be identified with increased sampling. However, half of the genera identified in each area were discovered within the first 10-20 samples (Figure 5.1). Extrapolated species richness from the Stehekin species pool ranged from 18.59 ± 1.5 to 22 ± 6.48 depending on the method (Chao estimate, jackknifing, or bootstrapping). Estimates were similar for Squaw Creek (range 18.47 ± 1.48 to 25 ± 10.17) depending on method.

No factors significantly influenced fungal composition of squirrel diets in Stehekin (Table 5.4). PERMDISP p-values for all factors also were non-significant. At Squaw Creek, season and treatment were significant predictor variables (*Psuedo-F*= 2.840, 1.920, *P*= 0.032, 0.078 respectively). There was also significant variation among squirrels (*Psuedo-F*= 1.595, *P*= 0.002; Table 5.5). Post-hoc Pair-Wise PERMANOVA t-statistics were 1.685 (*P*= 0.038), and 1.386 (*P*= 0.081) for season and treatment respectively. PERMDISP p-values for both factors were non-significant (*P*= 0.608, 0.563 for season and fire respectively) indicating that observed differences are due to differences in location, not dispersion. NMDS ordinations for Squaw Creek by season (Figure 5.2) and treatment history (Figure 5.3) showed separation between groups; however, relatively high dispersion among groups created some overlap of ordination distances. Final stress value for the ordination was 0.081. This level of stress indicates a "good ordination with no real risk of drawing false inferences" (Clarke 1993, p.126). The fit of the ordination, tested with a Shepard plot, was 0.989 and 0.958 for non-metric and linear correlations.

Because there were no significant differences in fungal composition of squirrel diets among seasons or treatments in Stehekin, we used results from Squaw Creek only for Indicator Species Analysis. The truffle genus *Rhizopogon* was found in most samples at relatively high abundance; thus IV scores were high for both season and fire/treatment history, however p-values were not significant (Tables 5.6, 5.7). The truffle genus *Geopera* was a strong, significant, indicator of fungal community composition within squirrel diets in the Spring/Summer season (April-August) (*IV*= 53.610, *P*= 0.010); relative abundance was 3.5 times higher in

Spring/Summer (Table 5.8). *Melanogaster* and *Hymenogaster* also had siginificant p-values for Spring/Summer (higher relative abundance), however IV scores were lower (20.101 and 13.884 respectively). Plant material had a high IV value: 40.914 for Fall/Winter (higher relative abundance), however the p-value was not significant (*P*= 0.150; Table 5.6). Plant material in fecal samples was the only strong, significant indicator of fungal community composition within squirrel diets at Squaw Creek for untreated areas (*IV*= 51.676, *P*= 0.010), indicating that there was little difference in the fungal community between treated and untreated sites. Fungal genus *Hymenogaster* had a significant p-value as an indicator of treated areas (with slightly higher relative abundance in treated areas), however the IV score was slightly less than 25 (Table 5.7, 5.8).

Discussion

All fungal genera identified in western gray squirrel fecal samples are relatively common in the Pacific Northwest. These represented approximately 1/3 of all truffle genera that have been identified in the Pacific Northwest, and were associated with the forest types of our study areas. *Rhizopogon*, the most abundant and ubiquitous genus in our samples, has an ectomycorrhizal relationship with Douglas-fir and ponderosa pine, produces truffles with high frequency at local scales over large geographic areas (Carey 1995, Molina et al. 1999, Carey et al. 2002), and has been shown to be heavily consumed by Pacific Northwest squirrel species and mycophagists in general (Maser et al. 1978, 1985, 1986; Carey, 1995; Carey et al. 1999; North et al. 1997, Lehmkuhl et al. 2004). Other potentially important genera (found at the highest frequencies) including *Geopera*, *Gautieria*, *Russula*, *Melanogaster*, *Coprinus*, and *Endoptychum*. *Geopera*, *Gautieria*, and *Melanogaster* have also been identified as important dietary items for northern flying squirrels (*Glaucomys sabrinus*) in open pine and young mixed-conifer forests in the eastern Washington Cascades (Lehmkuhl et al. 2004). The identification of previously undocumented genera in our study areas is likely due to inconsistent detectability rates between human samplers and animals that forage by smell. Flying squirrels at Fort Lewis, WA found as many genera in a two week period as human samplers found over three years, including some species never detected with human sampling (Carey et al. 2002). Additionally, some genera may be consumed with frequency disproportionate to availability due to nutritional needs met only by a diversity of species (Maser et al. 1978, Trappe et al. 2009). Also, exploratory truffle sampling by Trappe and others and fecal sampling did not occur on the same time scale or in the same sub-areas for our study. This discontinuity could be one explanation for why additional genera previously identified with truffle sampling in these areas were absent from our samples. Nineteen truffle genera were detected in fall fecal samples of northern flying squirrels in the eastern Washington Cascades compared to 12 in spring soil samples (Lehmkuhl et al. 2004). Species accumulation curves did not reach asymptotes at either study area, and extrapolated species richness indicated that additional sampling effort may have uncovered up to 10 additional genera.

It was not surprising to find season as the most significant predictor of species richness, diversity, evenness, and composition of squirrel diets at Squaw Creek because truffles fruit throughout the year, but are generally found in highest abundance in spring and fall. Species also fruit at different times leading to changing truffle community composition across seasons (Trappe et al. 2009). It was less clear why this relationship was not observed in Stehekin. The truffle community in Stehekin may include higher and more consistent diversity across seasons because this study site is a mix of east and west-side forests with a larger variety of tree species and microhabitats. We also collected nearly twice as many samples at Squaw Creek during spring and summer months compared to fall and winter which may have magnified differences in community composition at this study site. Sample sizes for Fall/Winter and Spring/Summer were more similar in Stehekin.

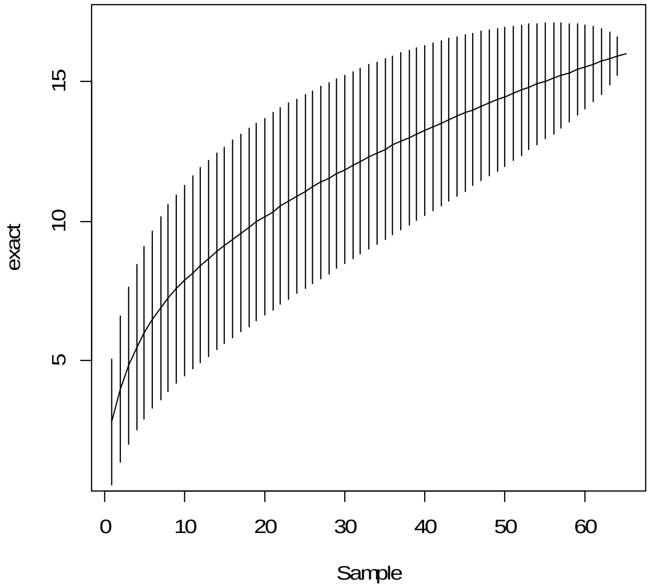
Differences in squirrel diets between treated and untreated areas may be more apparent in Squaw Creek because prescribed burning and thinning was implemented over a larger geographic area at a more intense level (compared to the step-wise, small scale fuel reduction treatments in Stehekin). Differences in Squaw Creek were driven mostly by presence of plant material in the diet, however, which explains why we did not observe significant differences in truffle species richness, diversity, and evenness between treated and untreated areas. Plant material occurred more frequently, and in higher relative abundance in samples from untreated sites, indicating that vegetative food sources may be less available after wildfire or fuel reduction treatments, increasing the importance of fungal food sources in these areas. We had a larger number of fecal samples collected from untreated areas which exhibited higher dispersion, often indicative of higher beta diversity (Anderson et al. 2006). It is possible that differences between treated and untreated areas would be minimized with increased sampling of treated areas at additional study sites.

The significant variation among squirrels for all analyses may also have influenced results; however differences among individuals are difficult to interpret due to unequal numbers of fecal samples collected per squirrel. Additional samples from each individual and from additional squirrels at both study sites could strengthen observed relationships and increase consistency among study areas. It would also be useful to sample additional sites in the North Cascades because results varied between our two sites.

A significant missing piece from our study is a measure of truffle availability within study areas. Because this analysis was part of a larger study focused on radio-telemetry and resource selection we did not have adequate time and resources to conduct habitat truffle sampling to coincide with squirrel fecal sampling. Without knowing availability, we cannot assess squirrel dietary preference. For future research we recommend following the methods of Claridge et al. (2000) for truffle sampling at study sites using paired 31.6m (1000 m^2) plots in squirrel high and low use areas, and treated and untreated areas at both study sites in fall and spring with plots systematically raked for 100 person minutes to a depth of 15cm. It would also be useful to survey the plant community in more detail so that the relationship of availability in the habitat and presence in the diet of specific plant species could be understood more clearly.

Even with the addition of site and season-specific truffle and/or vegetation sampling at study sites, there are some limitations to fecal analysis studies such as this. First, individual fecal samples only provide information on the individual's last meal, which may or may not include common and essential species. We accounted for this partially through repeated sampling of individuals. Further, only indigestible portions of food items remain in fecal samples. This is generally not a problem for truffle species with the exceptions of a few genera including *Elaphomyces*, as well as epigeous genus *Lycoperdaceae* (both identified with our sampling). These species consist of a think peridium (outer skin) which small mammals consume, and powdery gleba (spore mass) which small mammals discard. Spores are therefore only consumed accidentally and likely underrepresented in samples (Trappe and Maser 1977, Cork and Kenagy 1989). Plant material, especially from deciduous species like bigleaf maple (*Acer macrophyllum*) is also more digestible than fungal material and leaves less of a trace in fecal pellets making identification and assessment of relative dietary importance difficult.

Despite limitations, this analysis provides a first examination, and a starting point for future research, of the western gray squirrel food resource in the North Cascades and how it may be affected by forest land management activities. This study provides no evidence that recent fire fuel reduction treatments and wildfire have significantly impacted the diets of western gray squirrels in the North Cascades. Fungal composition and diversity of diets varied more by season and location than among fire fuel treatments. The plant component of western gray squirrel diets may have been reduced by the more intensive fuels management practiced in Squaw Creek compared to Stehekin, however, more research is needed to quantify the importance of plant material in the squirrel diet. The diversity of truffle genera and relative abundance of plants in squirrel diets among study sites, seasons, and treatments suggests that there is some resiliency in diet selection to changes in the food resource due to wildfire and forest management practices.



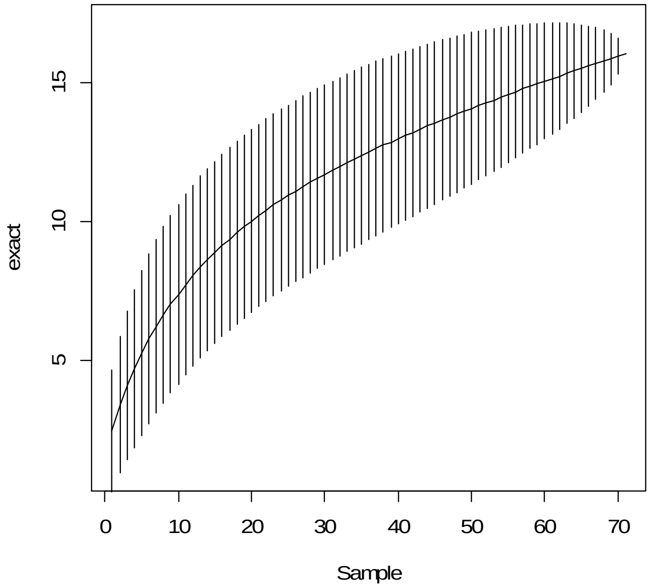


Figure 5.1. Species accumulation curves for Stehekin (top) and Squaw Creek (bottom) by sample using the exact method which finds the expected mean species richness following the moment-based estimation method developed by Ugland et al. (2003), Colwell et al. (2004), and Kindt et al. (2006). Vertical bars represent the 95% confidence intervals for mean species richness estimates. Asymptotes were not reached, and extrapolated species richness indicated that up to 10 additional genera could have been identified at each study site through increased sampling effort.

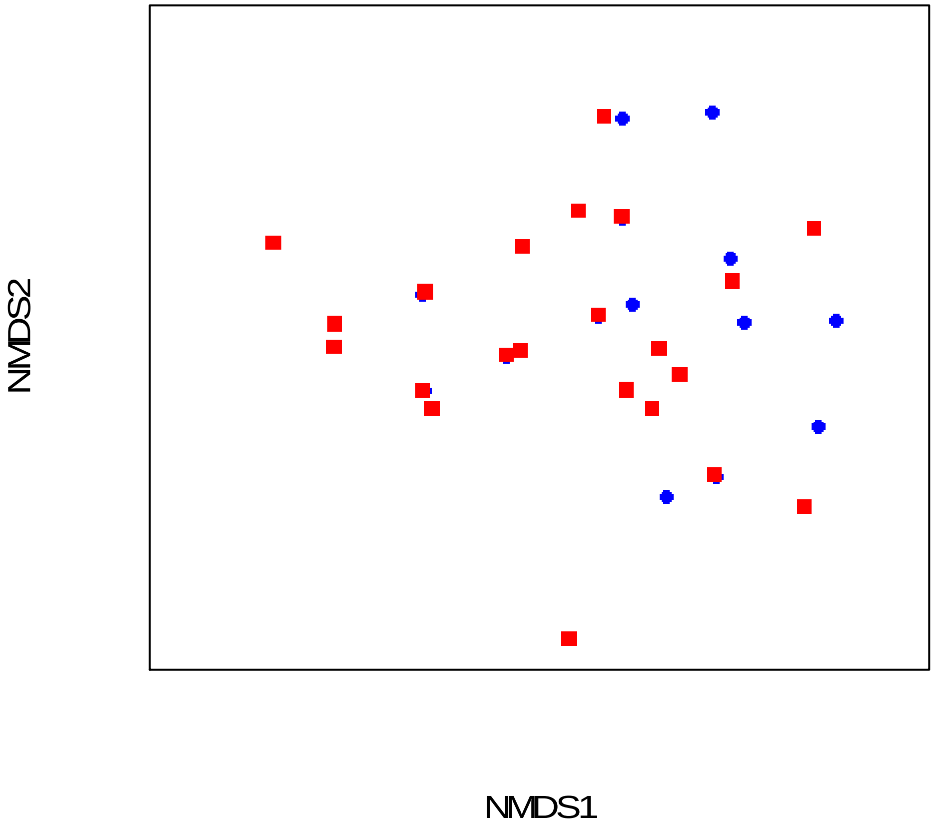


Figure 5.2. NMDS Ordination for fungal community composition at Squaw Creek by season: Fall/Winter (blue circles) and Spring/Summer (red squares). Stress = 0.081.

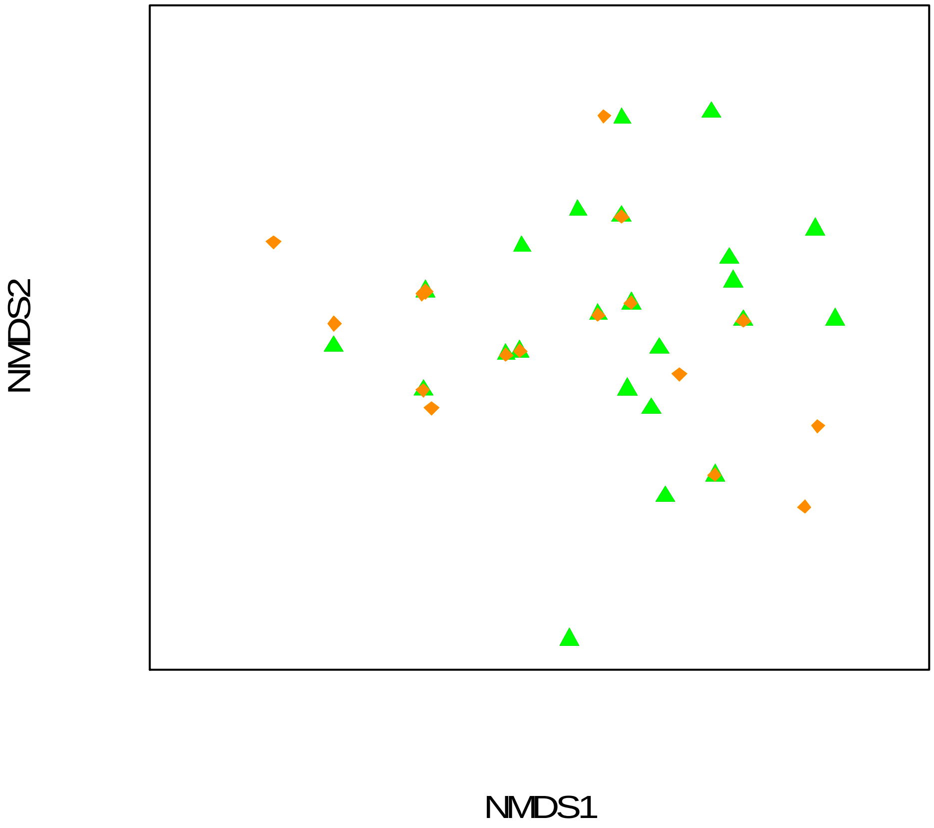


Figure 5.3. NMDS Ordination for fungal community composition at Squaw Creek by fire/treatment history: Treated (orange diamonds) and Untreated (green triangles). Stress = 0.081

Table 5.1. Relative abundance among samples (average categorical ranking between 0-3) and occurrence within squirrels (% of 19 and 28 squirrels for Stehekin and Squaw Creek respectively) of fungal genera and other dietary items at Squaw Creek by group.

		Stehekin		Squaw Creek	
Group	Genus	Relative Abundance	Occurrence	Relative Abundance	Occurrence
HYPOGEOUS					
Basidiomycota	Rhizopogon	2.133	100	2.048	96
	Gautieria	0.253	58	0.013	18
	Melanogaster	0.073	21	0.057	36
	Coprinus	0.001	5	0.052	25
	Leucophleps*	0.019	5	0.000	0
	Mycolevis*	0.001	11	0.000	0
	Hymenogaster †	0.000	0	0.057	21
	Hysterangium †	0.000	0	0.016	11
	Gymnomyces †	0.000	0	0.000	4
Ascomycota	Geopera	0.289	79	0.231	21
	Hydnotrya	0.000	5	0.000	82
	Peziza*	0.012	5	0.000	0
	Picoa †	0.000	0	0.007	7
	Sphaerosoma †	0.000	0	0.001	4
	Genea †	0.000	0	0.001	4
	Elaphomyces †	0.000	0	0.000	4
Glomeromycota	Glomus	0.001	5	0.000	4
EPIGEOUS					
Basidiomycota	Russula	0.051	58	0.011	25
	Endoptychum	0.025	16	0.013	11
	Lycoperdaceae †	0.000	0	0.016	4
	Wakefieldia †	0.000	0	0.004	4
	Unknown Spore	0.004	32	0.005	18
	Plant	0.387	95	0.410	68
	Other	0.030	42	0.006	21

* Found only in Stehekin

† Found only at Squaw Creek

Relative Abundance = average categorical ranking between 0-3

Occurrence = % within squirrels (19 for Stehekin, 28 for Squaw Creek)

Table 5.2. Species richness, diversity, and eveness by group calculated with the Shannon-Weiner Index and Pielou's J (Pielou 1966).

	Overall				Stehekin				Squaw Creek			
	Richness	Diversity	Evenness	n	Richness	Diversity	Evenness	n	Richness	Diversity	Evenness	n
Fall/Winter	2.283	0.511	0.453	60	2.412	0.578	0.503	34	2.115	0.424	0.385	26
Spring/Summer	2.781	0.727	0.596	73	2.852	0.782	0.646	26	2.766	0.696	0.566	47
Treated	2.596	0.618	0.540	57	2.433	0.700	0.622	29	2.577	0.535	0.458	28
Untreated	2.542	0.670	0.540	72	2.731	0.702	0.549	27	2.509	0.639	0.530	45
Overall	2.551	0.630	0.538	136	2.583	0.659	0.567	60	2.534	0.596	0.515	73

Table 5.3. PERMANOVA results for species richness, diversity, and evenness at both North Cascades study sites using the Euclidean distance measure with 999 permutations of residuals under a reduced model.

PERMANOVA

Factor	DF	SS	MS	Pseudo-F	P(perm)
Season	1	3.767	3.767	3.221	0.071
Location	1	1.021	1.021	0.681	0.415
Treatment	1	0.428	0.428	0.366	0.621
Squirrel(Location)	45	87.226	1.938	1.657	0.019
Season x Location	1	0.454	0.454	0.388	0.594
Season x Treatment	1	1.802	1.802	1.540	0.208
Location x Treatment	1	0.067	0.067	0.057	0.935
Residuals	77	90.056	1.170		
Total	128	192.690			

Pair-Wise Comparison PERMANOVA

Factor	t	P(perm)
Season	1.7946	0.06

Table 5.4. PERMANOVA results for fungal community composition at Stehekin using the Euclidean distance measure with 999 permutations of residuals under a reduced model.

PERMANOVA

Factor	DF	SS	MS	Pseudo-F	P(perm)
Season	1	0.772	0.772	0.599	0.601
Treatment	2	3.865	1.933	1.301	0.249
Squirrel(Treatment)	17	27.369	1.610	1.259	0.111
Season x Treatment	2	0.826	0.413	0.337	0.876
Residuals	30	38.369	1.279		
Total	55	76.446			

Table 5.5. PERMANOVA results for fungal community composition at Squaw Creek using the Euclidean distance measure with 999 permutations of residuals under a reduced model.

PERMANOVA

Factor	DF	SS	MS	Pseudo-F	P (perm)
Season	1	3.468	3.468	2.840	0.032
Treatment	1	2.340	2.340	1.920	0.078
Squirrel(Treatment)	28	40.541	1.448	1.595	0.002
Season x Treatment	1	1.274	1.274	1.043	0.445
Residuals	35	31.774	0.908		
Total	72	88.493			

Pair-wise Comparison PERMANOVA

Factor	t	P (perm)
Season	1.685	0.038
Treatment	1.386	0.081

Table 5.6. Results from Indicator Species Analysis by season for fungal community composition at Squaw Creek.

	Fall/Winter		Spring/Summer		
Genus	IV	P-Value	IV	P-Value	
Rhizopogon	44.803	0.960	50.368	0.300	
Gautieria	4.204	0.590	2.895	0.750	
Russula	6.641	0.450	3.613	0.820	
Geopera	18.504	1.000	53.610	0.010	**
Melanogaster	0.543	1.000	20.101	0.020	*
Picoa	3.652	0.770	4.470	0.590	
Coprinus	2.892	0.890	7.966	0.340	
Hymenogaster	0.709	0.990	13.884	0.040	*
UnknownSpore	4.204	0.570	2.895	0.780	
Plant	40.914	0.150	25.284	0.960	
Other	9.095	0.380	4.349	0.880	

* Significant at α = 0.05

** Significant at α = 0.05 and strong indicator (Dufrêne and Legendre 1997)

Table 5.7. Results from Indicator Species Analysis by fire fuel treatment history for fungal community composition at Squaw Creek

	Untreated			Treated		
Genus	IV	P-Value		IV	P-Value	
Rhizopogon	45.057	1.000		51.724	0.240	
Gautieria	11.111	0.120		0.000	1.000	
Russula	4.031	0.720		5.856	0.590	
Geopera	34.609	0.660		38.498	0.500	
Melanogaster	3.419	0.950		17.308	0.110	
Picoa	4.928	0.600		3.182	0.710	
Coprinus	8.682	0.300		2.492	0.890	
Hymenogaster	0.671	0.990		21.226	0.030	*
UnknownSpore	3.218	0.650		3.695	0.630	
Plant	51.676	0.010	**	13.184	1.000	
Other	4.861	0.780		8.036	0.430	

* Significant at α = 0.05

** Significant at α = 0.05 and strong indicator (Dufrêne and Legendre 1997)

Table 5.8. Relative abundance (mean ranking of percent cover across 60 fields of view per sample: 3 (high): > 66%, 2 (medium): >33% and < 66%, 1 (low): >0% and < 33%, 0) of western gray squirrel fecal diet items by season and fire fuel treatment history at Squaw Creek in the North Cascades, Washington.

	Season		Treatment	
Diet Item	Fall/Winter n= 26	Spring/Summer n= 47	Treated n= 28	Untreated n= 45
Rhizopogon	1.803	2.183	2.160	1.978
Gautieria	0.009	0.015	0.000	0.021
Russula	0.010	0.012	0.011	0.012
Geopera	0.086	0.312	0.232	0.231
Melanogaster	0.001	0.089	0.023	0.079
Picoa	0.002	0.010	0.010	0.005
Coprinus	0.037	0.060	0.021	0.071
Hymenogaster	0.006	0.086	0.115	0.021
UnknownSpore	0.003	0.004	0.003	0.004
Plant	0.621	0.293	0.208	0.536
Other	0.010	0.004	0.010	0.004

CHAPTER 6: Communicating Wildlife Research to the General Public: Application of the Reasonable Person Model

Abstract

We used the Reasonable Person Model (RPM; Kaplan and Kaplan 2005) to test the hypothesis that communicating wildlife research to the general public increases understanding and support of research. To evaluate the effectiveness of research communication tools, we administered surveys before and after three educational treatments: a presentation, website, and field trip. Field work took place in 2010 in the Washington North Cascades as part of a study on western gray squirrel (*Sciurus griseus*) habitat selection. Participant comprehension of wildlife research concepts increased significantly after all treatments, but significantly less with the website than the presentation and field trip, indicating that educational strategies may be more successful when they include personal contact with researchers and opportunities for question and answer. Ranked support for research and conservation increased similarly across all treatments; however, willingness to participate in private land management activities for western gray squirrel conservation decreased, failing to support RPM. Additional education and/or regulation may be needed to change behavior.

Introduction

Communicating current research to the general public is an obligation and opportunity for scientists. The general public funds much of research and conservation through taxes and non-profit donations. Scientists conduct research for the benefit of society and the general public; it is our responsibility to communicate findings with our donors and beneficiaries. Communicating science findings to the general public can also be advantageous to researchers: a better public understanding of current research can lead to a higher appreciation of the scientific method, validate current projects, and encourage support of future scientific endeavors. An appreciation of the scientific method, especially among young people, can help recruit new scientists and researchers. Furthermore, a better public understanding of science and current research has the potential to facilitate positive behavioral changes and informed decision making (Tressel 1981, Laetsch 1987, Irwin 1995) on issues such as environmental policy. Often a scientific understanding of a resource is needed to inspire conservation and protection.

The western gray squirrel (*Sciurus griseus*), the largest tree squirrel native to Washington, Oregon, and California, was classified as a state threatened species in 1993 by the Washington Department of Fish and Wildlife (WDFW) (WAC 232.12.011) due to a decrease in range and number. The squirrel is now limited to three geographically isolated areas in Washington: in Pierce and Thurston Counties in the southern Puget Trough, in southern Washington in Klickitat, Yakima, and Skamania counties, and in north-central Washington in Chelan and Okanogan counties. Population recovery efforts are underway, however, information on the western gray squirrel in Washington is limited, especially for the North Cascades population. We began a study in 2008 using radio-telemetry to investigate distribution and habitat selection of the western gray squirrel in the North Cascades in relation to forest management practices. This increased ecological knowledge of the western gray squirrel in the North Cascades is valuable to the scientific community and public landowners, however, ultimate recovery of the squirrel in Washington will depend on the general public, as 65% of identified western gray squirrel habitat is on private land (Linders and Stinson 2007). Key goals of the 2007 Washington State Western Gray Squirrel Recovery Plan are to work with counties, cities, and citizens to protect and enhance western gray squirrel habitat on private lands, and to develop an education and outreach strategy to gain support for western gray squirrel recovery. Many research and recovery plans have similar educational outreach components and funding agencies often require that scientific results be presented to a wide array of audiences.

Local landowners near research study sites are often curious and/or concerned and have the potential to either facilitate or impede wildlife research on both public and private lands. We conducted wildlife research in Stehekin, WA, and the Methow Valley, WA. Stehekin is a small, close-knit community of people with an appreciation of nature and the pioneering spirit. They also have a history of conflict with government authority, particularly the National Park Service (NPS), which took possession of much of the land when the Lake Chelan National Recreation Area was designated in 1969. The Methow Valley represents a larger geographic area with residents engaged in many outdoor or environmental pursuits ranging from classic farming to grassroots environmental organization. Both areas have a large constituent of retirees, many originally from the Seattle area. Residents in both study areas were expected to have an interest in wildlife research (particularly on threatened or endangered species) either because they have experience with research, science, and/or nature and want to get involved, or they are concerned about potential land management restrictions that could arise from research results.

Often what scientists think local stakeholders should know about current scientific research and what they actually want to know is different. For example, scientists often want to focus on study background and methodology when the general public may be more interested in application of results. Other times, scientists overestimate the public's prior level of knowledge, and public documents like Environmental Impact Statements (EIS), specifically designed to allow citizen input, are unreadable by the majority of the general public (Gallagher and Patrick-Riley 1989). Special challenges for science communication are the public's limited background knowledge of science and technology and fear of scientific and technical material. Even when interested, people may feel that current scientific findings are beyond their comprehension, or not relevant to their daily lives (Ross and Scanlon 1999).

Additional challenges can arise when scientific information is transferred through intermediaries, such as public information officers and journalists. It is easy for information to be lost or distorted as translators convert complex, contextualized scientific information into something that is understandable and meaningful to diverse members of the public. It is often more successful for scientists to communicate directly with the general public (Dornan 1999, Christensen 2007).

The Reasonable Person Model (Kaplan and Kaplan 2005), hereafter RPM, offers guidelines to scientists for effective educational presentations to the general public based around human informational needs: model building, being effective, and meaningful action (Figure 6.1). People and communities are more likely to be reasonable when they can relate new information to what they already know, understand and master the material, and be involved in a solution to the problem (Kaplan and Kaplan 2009). In this case we could define “reasonableness” as acting in a way that conserves the western gray squirrel on a person’s land. We can assume that people do not inherently want to harm or reduce the population of western gray squirrels near them, but if they are not aware of the squirrel’s ecology, state threatened status, and/or implications of that status, they cannot be expected to take extra caution to avoid hitting squirrels on the road during the breeding season, retain large trees on their property to preserve habitat, or allow researchers access to their land when a radio-collared squirrel crosses the public land boundary. When provided with the necessary additional information in an understandable and meaningful way – in this case that the western gray squirrel is our largest native tree squirrel and plays an important ecological role in the forest, and that a Washington state threatened status does not beget land use restrictions on private lands – we would expect most people to be willing to help participate in research and conservation efforts near their property.

Scientists often must seek the communities and audiences they wish to address, employing a variety of communication strategies rather than expecting the public to seek the information on their own. The RPM can be applied to all educational formats including press releases, newspaper articles, magazine articles, books, websites, radio, television, public lectures, exhibits at science centers and museums, and citizen science projects, which enlist volunteers for scientific data collection. Different methods will reach different members of the audience and have varied effects on understanding of and support for research projects.

Ideally a variety of educational strategies would be used to share wildlife research projects with the general public, however, because time and budget constraints may prevent wildlife managers from using more than one communication/outreach mode, we were interested in evaluating three commonly used educational strategies: a Microsoft Powerpoint (© Microsoft Corporation) presentation, a website, and a field trip. Public Powerpoint presentations are often used by wildlife managers and biologists with the NPS, Washington Department of Fish and Wildlife (WDFW), US Forest Service (USFS), and US Geological Survey (USGS; all cooperators for my study) because they address a large audience at one time and allow some interaction and question and answer. Webpages and blogs do not involve direct interaction, but allow participants to choose when to visit the website and learn the material at their own pace. The internet is currently the leading source of information on many scientific issues (NSF 2004). Citizen Science projects, in which individual volunteers or networks of volunteers, many of whom have no prior scientific training, perform or manage research-related tasks including observation, measurement, or computation, have also been increasing in popularity (Cohn 2008). Citizen science is a partnership, involving multidirectional sharing, rather than one way information transfer from scientists to the public (Brewer 2002), with a citizen science experience being similar to an interpretive experience; participants see, learn, and experience firsthand “the unencumbered delight in knowledge and experience” through a better understanding of their place in the natural world (Beck and Cable 1998). This experience can be approximated with a field trip, where scientists take participants into the field, demonstrate research methods, and allow citizen participation on a smaller scale. The objectives of this study were to survey local stakeholders of western gray squirrel research in the North Cascades to assess prior knowledge, interests, and preferences for wildlife research communication, and evaluate change in knowledge and attitude towards western gray squirrel research after communicating scientific process and findings to the general public using alternate communication strategies. We hypothesized that educational treatments would engage local stakeholders in western gray squirrel research in the North Cascades and increase understanding and support.

Methods

Field

Study sites: p.4. Treatment groups were either randomly or self selected with a goal of sampling approximately 30 people in each group. In the Methow Valley, the Powerpoint presentation took place in October 2010 at the Twisp Valley Grange as part of the Methow Conservancy’s first Tuesday natural resource lecture series. The Methow Conservancy provided advertising through an e-newsletter and posters distributed to local businesses throughout the valley. We shared this presentation with Pacific Biodiversity Institute (PBI), a non-profit organization based out of Winthrop, WA, who conducted a citizen science based distribution survey of western gray squirrels in the upper Methow Valley in 2010. We also conducted one field trip in the Methow with PBI as part of their citizen scientist training field day. An additional field trip took place at the Methow Community Center in July 2010 at Methow Coffee: a weekly, morning gathering of local residents. We solicited website respondents in the Methow Valley with fliers placed at the Sweet River Bakery in Pateros, WA, and leaflets distributed to resident acquaintances. All Stehekin winter residents (~80) were invited by letter to attend one of the three treatments, assigned through random selection of PO boxes without associated names. This sampling method can be directly compared to the United State Postal Service Delivery Sequence File random sampling method in Dillman et al. (2009). Part-time residents not in Stehekin at the time were invited to participate in the website treatment via e-mail. Non-response bias was minimized by sending the same amount of contact and follow-up information to all groups. The presentation and field trip in Stehekin took place in January 2011; the field trip took place at the Landing near the Golden West Visitor Center in the afternoon, the presentation took place in the evening at the Stehekin School.

All treatments contained the same information directly communicated from the scientist to the general public, but represented key differences in researcher interaction, time commitment, and question and answer format. For example, the field trip, which included a demonstration of wildlife research equipment and techniques including radiotelemetry, trapping, and nest surveying, was the least structured but included the most interaction and opportunity for question and answer. To meet RPM guidelines all presentations were delivered in a personable and engaging format, focused clearly on the main findings and concepts, with topics related to perceived interests and background knowledge of participants. We also used pictures whenever possible, as Kaplan and Kaplan (1989) argue that humans are especially effective at grasping information presented in pictures, relative to text, maps, or diagrams. Jargon and technical terms were avoided whenever possible, and clearly defined when necessary. Each treatment also included a section on “getting involved”, allowing participants to take part in meaningful action if desired. To avoid cross-pollination between groups (through small-town communication between participants) and ensure that all changes in comprehension and attitude were due to the specific treatment assigned to each group, all treatments in Stehekin were completed within a very short time frame and residents were told that they were part of a scientific experiment involving three educational treatments and advised to keep thoughts confidential until the completion of the study. These precautions were not needed in the Methow Valley because it is a much larger, less isolated geographic area.

We distributed pre- and post-survey questions regarding information transfer, scientific comprehension and attitude to all participants. Pre-treatment survey questions were answered prior to educational treatment; post-survey questions were distributed and collected immediately following treatments. Survey questions were selected through peer review by several social scientists and federal managers to increase the likelihood that credible and useful information would be acquired through surveys. We also collected demographic background information on survey respondents including gender, age, level of education, and residency (full-time or part-time), and how long participants have lived in the area, as potential explanatory variables for differences between subjects and to ensure the sample of respondents accurately represented the populations of interest.

Most questions were asked using a Likert scale, which includes close-ended questions with ordered choices (Salant and Dillman 1994, Dillman et al. 2009) where respondents specify their level of agreement with a statement. This gradient question design allows ranking of responses and calculation of change through simple addition or subtraction of pre- and post-survey response questions. Additional short answer questions, not used for statistical analysis, were also asked to provide further insight into scaled answers and assess general public opinion of research and science communication. The pre-survey consisted of six multiple choice and short answer information transfer questions regarding prior knowledge of the subject, preferred communication styles, and topic interests, seven true/false comprehension questions, nine 5-point Likert scale questions evaluating attitude by the level of agreement (strongly disagree, disagree, neither disagree nor agree, agree, strongly agree) with value and action statements, and five demographic questions. Post-surveys contained the same comprehension and attitude questions in addition to five new short answer and multiple choice information transfer questions for treatment evaluation. Comprehension and attitude questions were closely tied to informational content to facilitate change due to treatment. Average response time was 10 minutes per survey. Table 6.1 provides comprehension and attitude questions used for statistical analysis. The full survey is available in Appendix C.

This study was exempt from the human subject research review process at the federal and University level, as it fell under the “No Risk Research” category: “Research involving the use of educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures, or observation of public behavior” (Title 45, Code of Federal Regulations, Part 46.101.B). Office of Management and Budget (OMB) approval was not required for this survey because this branch of research was designed and funded solely by the University of Washington and NPS managers chose not to use results to guide future park management. Survey questions were approved by the University of Washington Humans Subjects Division (HSD) on

4/22/2010 under 45 CFR 46.101 (b) (2) with stipulations that respondent identities be kept confidential, and participants could skip any questions they did not wish to answer. Confidentiality was further increased by allowing respondents to mark surveys with an identifier other than their name such as a nickname, or Stehekin PO Box number. Once pre and post-surveys were matched to individuals they were given an ID number for all further analysis.

Analysis

To determine whether results could be pooled among study sites we evaluated differences in respondent demographics with Analysis of Variance. We used the same method to test for differences in demographics between treatments across and within study areas. The three sections of the survey: information transfer, scientific comprehension, and attitude were each evaluated separately. Only the comprehension and attitude sections were evaluated with statistical analysis. For the comprehension analysis correct true-false answers were assigned a value of “1” and incorrect answers “0”. The pre-survey score was then subtracted from the post-survey score for each question to calculate change by question. The change for each question was then summed across each respondent to create a total change value for comparison across treatments. A similar method was used for the attitude section; pre- and post-survey answers were already in numerical format. We used the exact Wilcoxon signed-rank test to test for increases in comprehension and attitude across pooled treatments. We then used the conservative Krukal-Wallis rank sum chi-square test to assess if a difference in increase of comprehension and attitude existed among treatments, with a multiple-comparison test after Kruskal-Wallis to evaluate treatment differences. Alpha and p-value levels were tested at 0.05. We selected non-parametric tests because the datsasets did not meet assumptions of normality and homogeneity of variance. All statistical tests were conducted in R version 2.7.2 (R Development Core Team 2008), package pgirmess (Giraudoux 2008).

Results

Demographics

One hundred and five people participated in one of the treatments at one of the two study sites (Table 6.2); 27 participants completed both pre-and post-surveys for the field trip and presentation treatments and 20 participants completed both surveys for the website treatment. Participation was higher in the Methow Valley than in Stehekin for the field trip and Powerpoint presentation treatments; Stehekin had a higher response rate for the website treatment. The Methow Valley had a larger proportion of incomplete surveys.

Participant demographics including sex, age, and level of education were statistically similar among study sites, allowing us to pool results across the entire North Cascades study area. However, there were statistically significant differences among study areas and treatments related to residence type (F= 14.73, P= 0.001,). Significantly more part-time residents in the Stehekin portion of the study participated in the website treatment (Figure 6.2). This incongruity is an artifact of the nature of the website treatment: the website was available for viewing for a longer time frame and could be accessed remotely from outside our study areas. However, we did not anticipate this to influence overall results dramatically, especially for the information transfer and comprehension sections.

The most popular careers listed by respondents were in natural resource management with the NPS or USFS (17%), and education (16%), followed by law, engineering, construction, and management (all 6%), then journalism, tourism and food services (all 5%), and biology, medical administration, and the arts (all 4%), however many respondents chose not to answer this question, therefore some careers are likely underrepresented.

Information Transfer

Most respondents had previously heard about western gray squirrel research in the North Cascades (68%); many directly from me, my field assistants, or other local wildlife researchers (35%). Printed materials ranked second (32%): Washington Department of Fish and Wildlife created a brochure on western gray squirrels in 2005, and I posted periodic updates on my research progress at the Stehekin Golden West Visitor Center and Post Office. There were also several articles written in local newspapers including the Methow Valley News, Wenatchee World, Stehekin Choice, and a radio broadcast at GoLakeChelan.com during the timeframe of my study (articles and links in Appendix C). Respondents reported hearing about western gray squirrel research in the North Cascades from friends and family (26%); from the internet (7%): WDFW provides comprehensive, reliable western gray squirrel information online, and Pacific Biodiversity Institute has a western gray squirrel research section on their website and had conducted previous citizen science projects related to the squirrel in the Methow Valley since 2009.

When asked how they generally receive information on wildlife research (not only related to the western gray squirrel in the North Cascades) respondents reported that most frequently they get information from newspapers (average score: 3.8 out of 5), followed by friends and family and the internet (both average score 3), public lectures and presentations, and exhibits and brochures in visitor centers (both average score 2.9). Respondents were also asked to rank *preference* for ways of receiving information on wildlife research (using the same categories), which was not necessarily consistent with the way they had reported receiving this information most frequently (Figure 6.3). Preference for public presentations and lectures, and brochures and exhibits scored 3.7 and 3.4 respectively, which were both higher than their scores for “general way of receiving information”.

Additional comments that help explain why people have preference for one form of communication over the other were often related to time constraints or learning style preferences. Many comments cited the ease, specificity, and low time cost of the internet, e.g., “I can read at my leisure”, “I can pick and choose what I want”, “internet is easy, speedy”. Others reported preference for newspaper articles, e.g., “I enjoy reading”, “Articles are more in-depth and usually have interesting stories”. Public presentations were preferred for the visual and personable aspects and opportunity for question and answer, e.g., “I don’t have the time or attention span to read articles on wildlife research, but people talking is great!”. Some respondents specifically preferred learning from wildlife “experts” because they are the original source and can provide in-depth explanations. Many comments addressed the accuracy, and/or trustworthiness of different sources, e.g., “pretty much all I care about is whether the information is from a trusted source or not”; however, respondents had different ideas about which types of communication are impartial and trustworthy, e.g., “it is always good to hear first hand from the experts!”, “internet/newspaper/magazines present accurate and impartial practical data”, “friends and neighbors are a trusted resource”. Some educational strategies were also more accessible than others to certain groups, e.g., “I live remote but have internet”, “time constraints”. People generally found wildlife research to be an interesting subject, and many were happy to have the information in any format, e.g., “any method would work”, “it’s an interesting subject I like reading about and talking with friends about”.

We also asked participants to rank preference for learning specific concepts about western gray squirrel research in the North Cascades, including squirrel and habitat ecology, study goals and methods, future outcomes of results, and potential for public involvement. After the treatment we asked them to evaluate how well each of these same topics were covered. People were most interested in learning about the ecological role of the western gray squirrel (average score: 4.4 out of 5), followed closely by “why the study is being done” (4.3), goals of the study (4.2), and management implications (4.1). General biology of the western gray squirrel and North Cascades habitat ranked in the middle (3.7-4), with “how to get involved” ranked lowest (2.9). Most topics covered by the treatments closely matched the amount of interest, with the exceptions of the western gray squirrel’s ecological role (interest ranked higher than topic coverage) and who was conducting the study and how to get involved (interest ranked lower than coverage) (Figure 6.4).

When asked what they liked about the treatment, participants ranked understandable, relevant, and interesting information similarly high (~4.6) for all treatments, indicating that the content and delivery style was appropriate for the audience. The field trip was ranked as slightly more fun, and the Powerpoint presentation slightly more engaging than the other treatments. Questions were answered most thoroughly with the field trip followed by the Powerpoint presentation and then website (ranked relatively low for its ability to answer questions), consistent with treatment format. Some categories unique to certain treatments, e.g., “I can learn at my own pace” for the website, and “interaction with a wildlife researcher” for the Powerpoint presentation and field trip ranked high, however, “interaction with participants”, unique to the field trip, ranked low, indicating that distinctive aspects of different educational styles can be more or less valuable.

Comprehension

There was a significant increase in comprehension (V=1515.5, *P*<.001) when data were pooled across all treatments (n=74). There also were significant differences between treatments (χ^2= 8.035, *P*=0.018): the Powerpoint presentation increased comprehension significantly more than the website (observed difference = 17.16, critical difference = 15.07) (Figure 6.5).

We also evaluated changes in comprehension by question. There were significant increases in comprehension for questions 2, 3, 5, 6, and 7 (*V* = 310.5, 511.5, 171, 230 and 300 respectively) all exhibited *P*<0.001.

Comprehension also increased for question 1 by a smaller degree *(V= 260, P=*0.002). There was no significant increase in comprehension for question 4 (*V= 38.5, P* = 0.172). Results by question were fairly consistent among study areas, however the average increase in comprehension by question was lower in Stehekin than in the Methow due to a higher level of prior knowledge: 90% of participants in Stehekin had previously heard about western gray squirrel research in the North Cascades, compared to only 57% of participants in the Methow Valley.

Attitude

There was no significant positive change in attitude due to any of the treatments (overall *V*=574, *P*=0.113; between treatments χ^2= 0.68, *P*=0.712), which on first examination failed to increase "support" for research as hypothesized. However, when responses were divided by question interesting results emerged. Questions 1, 2, 3, and 7 did change positively and significantly *(V*= 105, 228, 170.5, 85.5; *P*=0.003, 0.002, 0.005, 0.018 respectively). All changes in attitude by question were similar among treatments.

Changes in questions 4, 5, and 6 were statistically insignificant (*V*= 56.5, 123, 13.5; *P*= 0.996, 0.691, 0.856 respectively) and actually showed a decrease in level of agreement with the statements in some cases.

Most of the significant changes in attitude by question were driven by respondents from the Methow Valley (n=51). Increases in level of agreement for questions 3 and 7 were not significant in Stehekin (*V*= 14, 15; *P*= 0.242, 0.187 respectively). Of note, residents there did not want to participate in research and did not change their minds after presentations. Stehekin residents were more amenable to making land use changes on their property, however. Questions 4-6 showed slight increases in level of agreement after treatment, however changes were not significant (*V*= 21.5, 33.5, 5; *P*= 0.109, 0.099, 0.579 respectively)**.**

Discussion

Demographics

Most of the participants in our treatments were over the age of 50, highly educated, current or retired professionals. If managers and scientists are looking for volunteers for citizen science projects or want to address local stakeholders in an area, this is the group of people that is most likely to participate. It is important to note, however, that this group was difficult to reach via website. Although we had many hits on the website we received relatively few survey responses, and many partial responses: encouraging website visitors to complete a post-survey in addition to the pre-survey was a particular challenge. The social obligation of filling out surveys for the field trip and Powerpoint presentation increased response rate; however, it was still difficult to obtain both a pre-survey and post-survey for each participant. Confidentiality requirements and explanation of this social science study also lead to some non-identification in all treatments. Requirements by the Human Subjects Review Board prohibited us from requiring the name/identifier field on the website and did not allow us to request e-mail addresses for follow-up. We also received several unidentified pre-surveys from the Powerpoint presentation because we stapled a cover letter explaining the study to the front of the pre-survey directly over the line for participant name/identifier. Fortunately, six additional survey pairs were successfully matched through handwriting; verified by four separate observers. Other mismatched pairs of pre- and post-surveys from treatments were recorded but not used for analysis.

The next step in communicating wildlife research to the general public is to seek out and connect with all other groups that did not participate in the treatments for a variety of potential reasons (Table 6.3). Participation in Stehekin was very low, possibly due to timing (the presentation was on a Sunday/school night), conflicting events planned verbally in town without my knowledge, travel (many of the NPS employees that were interested in my presentations were on vacation at the beginning of the year when I was there), the fact that it was winter (although summer can be difficult too when there are many competing events to choose from), disinterest/dislike (some people in Stehekin have said to me or others that the squirrels are not worth their time and that research is a poor use of NPS/government dollars), forgetfulness, or the way it was advertised (I did not have the option of using e-mail as a way to contact participants which is the main source of communication in Stehekin). Other comments received from Stehekin residents included “If people don’t show up, it means it’s not controversial enough”, “People in Stehekin just don’t participate in anything like this very often” and “We don’t necessarily care about what happens to the squirrels, or want to know about it, but it’s nice to know that someone cares and is checking up on them”. Barriers to participation in Stehekin and in other areas could be partially overcome with a variety of solutions (Table 6.3). Additional types of educational treatments could also be used in the future to further draw interest and participation.

Information Transfer

The audience is often happy to tell you what they are interested in and how they would like to receive information; an important skill as a communicator is listening (Leshner 2006). We enjoyed the opportunity to learn what stakeholders of western gray squirrel research in the North Cascades care about and are interested in. Preference for public presentations and lectures, and brochures and exhibits ranking higher than their respective "general way of receiving information" indicate that these types of educational opportunities for learning about current wildlife research may not be as readily available to the public as they would like. One oversight on the survey was that we didn't list "directly from researchers" as a way of receiving information on wildlife research although responses indicated that it is potentially a very important type of information transfer. Comments stating preference for one form of communication over the other and penchant for unique aspects of certain educational treatments (e.g., learning at own pace with a website) indicate that a variety of formats should be used to increase opportunities for people to learn about current wildlife research in a way that is appealing and relevant to them. With regard to topic coverage, results indicate that the ecological role of the western gray squirrel could have been covered in more detail, with less time spent on who was conducting the study and how to get involved. Because educational presentations took place immediately following the pre-survey it was not possible to incorporate participant comments into treatment design and delivery. The information transfer questions from the pre-survey could be a useful tool for designing future wildlife research educational treatments if given well in advance of the presentations. This could also be incorporated by listing potential topics at the beginning of the educational presentation and allowing participants to select a subset of things they are most interested in learning about.

Comprehension

The significant increase in comprehension across all treatments supports one aspect of the original hypothesis: “actively communicating scientific findings with the general public increases *knowledge* and support for research”. The significant difference in change in comprehension between the website and the Powerpoint presentation, in addition to comments regarding preference for certain types of educational formats, indicate that educational strategies can be more successful when they include personal contact with the researcher and opportunities for question and answer. Average increase in comprehension after the field trip was very similar to that of the Powerpoint presentation; larger than the website. This suggests that website browsers tend to get information in a more cursory manner. The non-significant difference between the website and the field trip could be because the field trip was slightly shorter than the presentation with fewer participants at a time (and therefore fewer follow-up questions asked). The presentation in the Methow Valley was also shared with researchers from Pacific Biodiversity Institute who were also able to answer questions and share perspectives. The smaller, non-homogenous groups in the field trip treatment also contributed to the higher variance in response for the treatment, which masked differences between treatments. Every effort was made to keep educational presentations consistent among groups; however, this was easier to ensure for the website and Powerpoint presentation.

True/false questions 2, 3, 4, 5, 6, and 7 under the comprehension section represented the most common misconceptions about western gray squirrels and research identified through wildlife study design and fieldwork, therefore it was expected that pre-survey scores would be low. The significant increase in comprehension for these questions signifies successful educational treatment delivery. We were particularly pleased to see a significant increase in understanding on questions 6 and 7 related to western gray squirrel study methodology because this research project was often criticized for using invasive methods (trapping and radio-telemetry) to study habitat selection by squirrels and it was critical to educate managers, as well as the general public, about what types of information different research methods can and cannot provide. Question 1 was also indentified as an important concept to address through educational treatments, as federal vs. state, endangered vs. threatened status affects land use options. To further increase comprehension and support for western gray squirrel research in the future this point could be outlined more clearly in educational presentations. Comprehension did not increase for question 4: "The North Cascades are a unique habitat for the western gray squirrel" because this question was less difficult and pre-survey scores were already high.

Attitude

The non-significant overall change in attitude due to treatment is an artifact of the types of questions which fell into two basic categories: support for western gray squirrel research and conservation in general, and willingness to take action for western gray squirrel research and conservation on a personal level. The significant increase in agreement for the first two statements does support the second half of the original hypothesis: "actively communicating scientific findings with the general public increases knowledge and *support* for research". The increase in willingness to help study western gray squirrels (Question 3) after educational presentations was surprising because people had ranked learning about "how you can get involved" very low on their level of interest in the pre-survey. The response to this question was exciting because we may have recruited some future wildlife researchers and citizen scientists, and this was one way that people could take meaningful action under RPM. The positive change for question 7 was also heartening as it shows potential for friends and neighbors to share the information presented in educational treatments, expanding scope and accessibility.

Questions 4-6 further represent the “meaningful action” arm of RPM and the final outcome: behavior change, which our methods failed to achieve. In this case behavior changes were related to private land management; a controversial subject in our rural, conservative study areas. The negative and neutral responses received may have changed if we had selected more placid examples of ways to take action. However, we consider large tree and dwarf mistletoe retention as essential for preserving western gray squirrel habitat across jurisdictional boundaries and therefore wanted to address these options specifically. Prior research has shown that squirrels preferentially nest in large, mistletoe infected trees (Gregory 2005, Gregory et al. 2010); concurrent radio-telemetry research in our study areas also validates the importance of large trees and mistletoe for western gray squirrel habitat selection (K.Stuart, unpublished). Negative and neutral responses on private land management options are not surprising in these areas, but do point to a need for additional education. Significant changes in response to questions that represent behavior change can be difficult to achieve with one educational presentation and may also take more time than we allowed participants before answering the post-survey.

Discrepancy between the willingness of Stehekin and Methow Valley respondents to participate in research and conservation activities on their land is likely due to sample size and demographics. Part-time residents (represented more heavily in Stehekin) may have less of a stake in land management options because they do not spend as much time in areas that would be affected. For example, they may do less active management of trees on their property, and be more willing to allow researchers access to their lands when they are not present.

Although our educational presentations did not lead to specific behavior changes in support of western gray squirrel conservation, they did significantly increase understanding and support of wildlife research and conservation. We also contributed understanding about what interests local populations about wildlife research, constraints on participation in rural communities, advantages and disadvantages of three educational strategies, and why multiple communication methods may be needed to address all audiences.

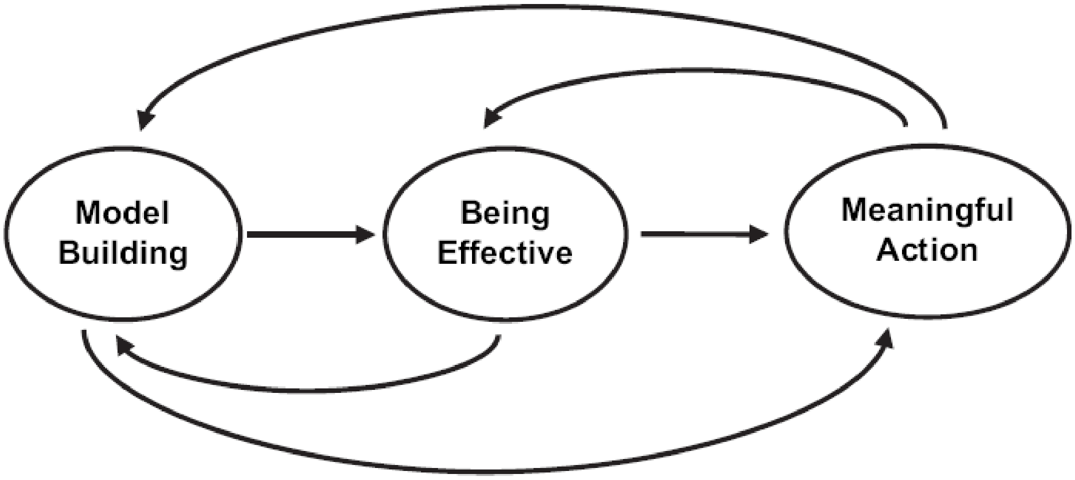


Figure 6.1. The Reasonable Person Model (Kaplan and Kaplan 2005) states that people and communities are more likely to be "reasonable" when they can relate new information to what they already know, understand and master the material, and be involved in a solution to the problem. We used this model as the basis for designing our three educational treatments and evaluating changes in support towards western gray squirrel research and conservation (our measure of "reasonableness") pre- and post-treatment.

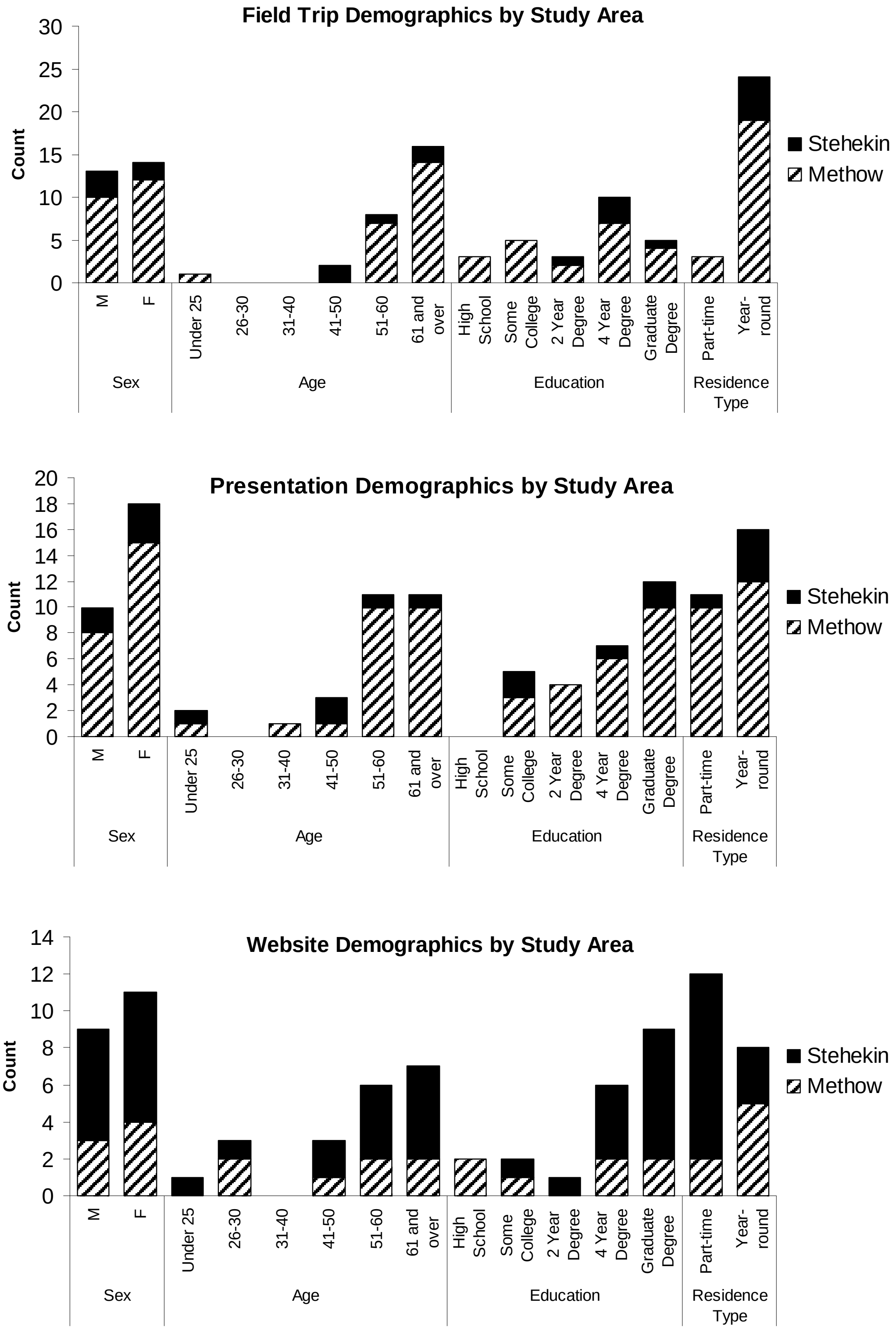


Figure 6.2. Demographic comparisons by study area and educational treatment. The only statistically significant difference between study areas and treatments was type of residence for the website treatment (*F*= 14.73, *P*= 0.001,); Stehekin had significantly more website responses from part-time residents.

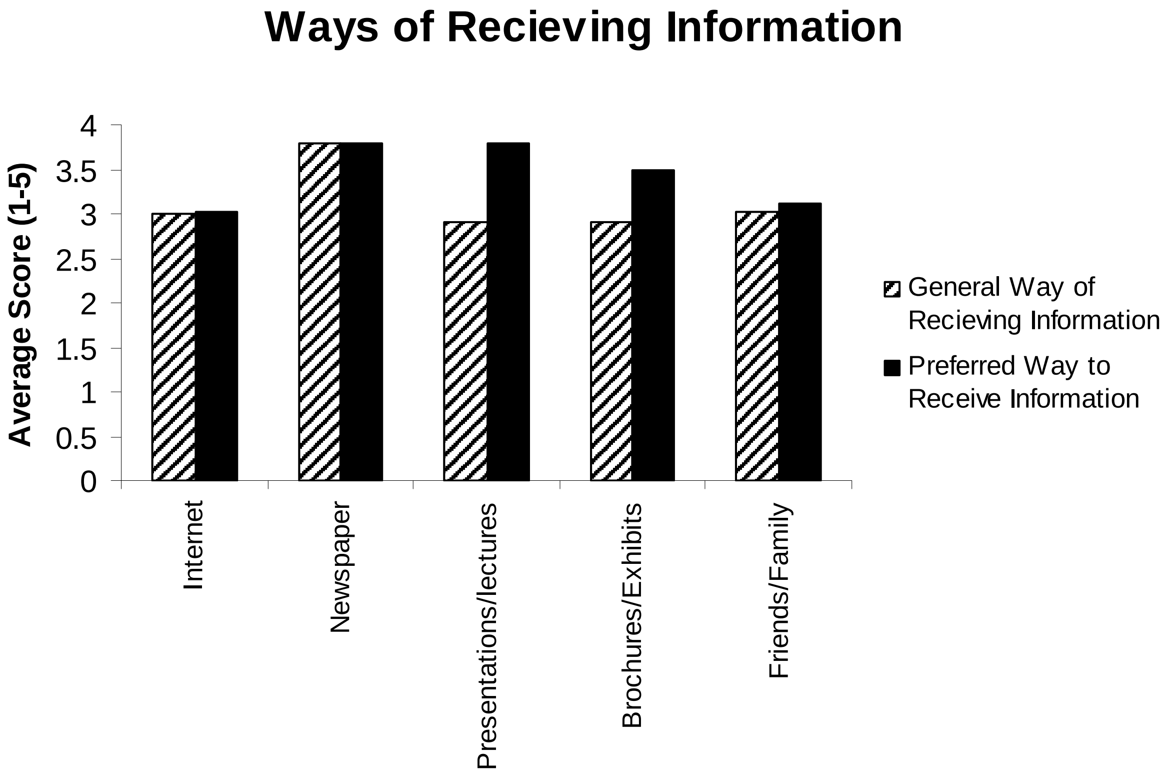


Figure 6.3. Comparison of average scores for the general ways people reported receiving information on wildlife research to ways they would prefer to receive this information.

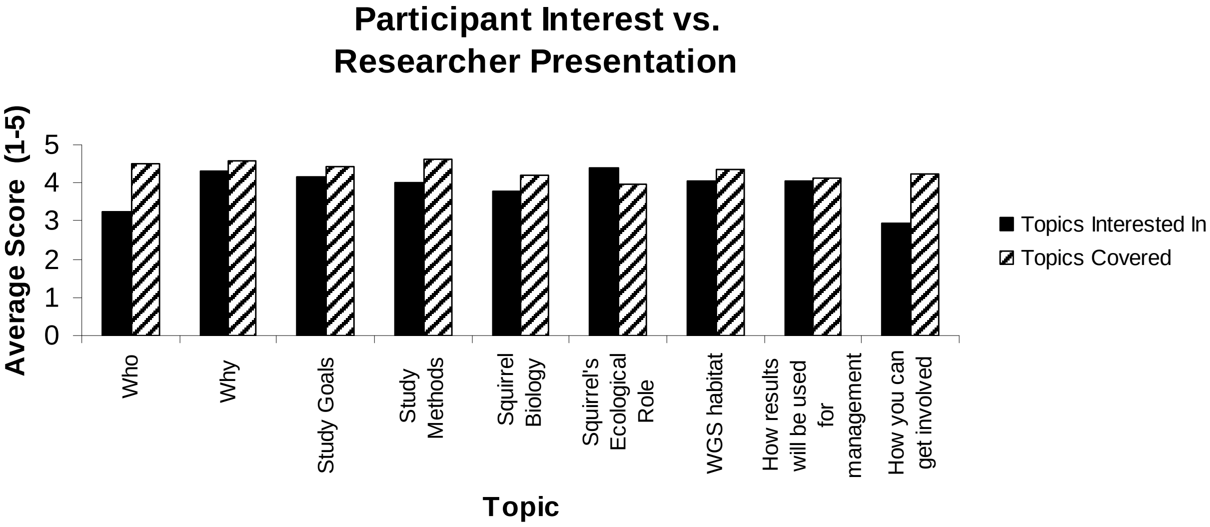


Figure 6.4. Average rankings for interest in treatment topics (pre-survey) compared to average rankings of how well each topic was covered in treatments (post-survey).

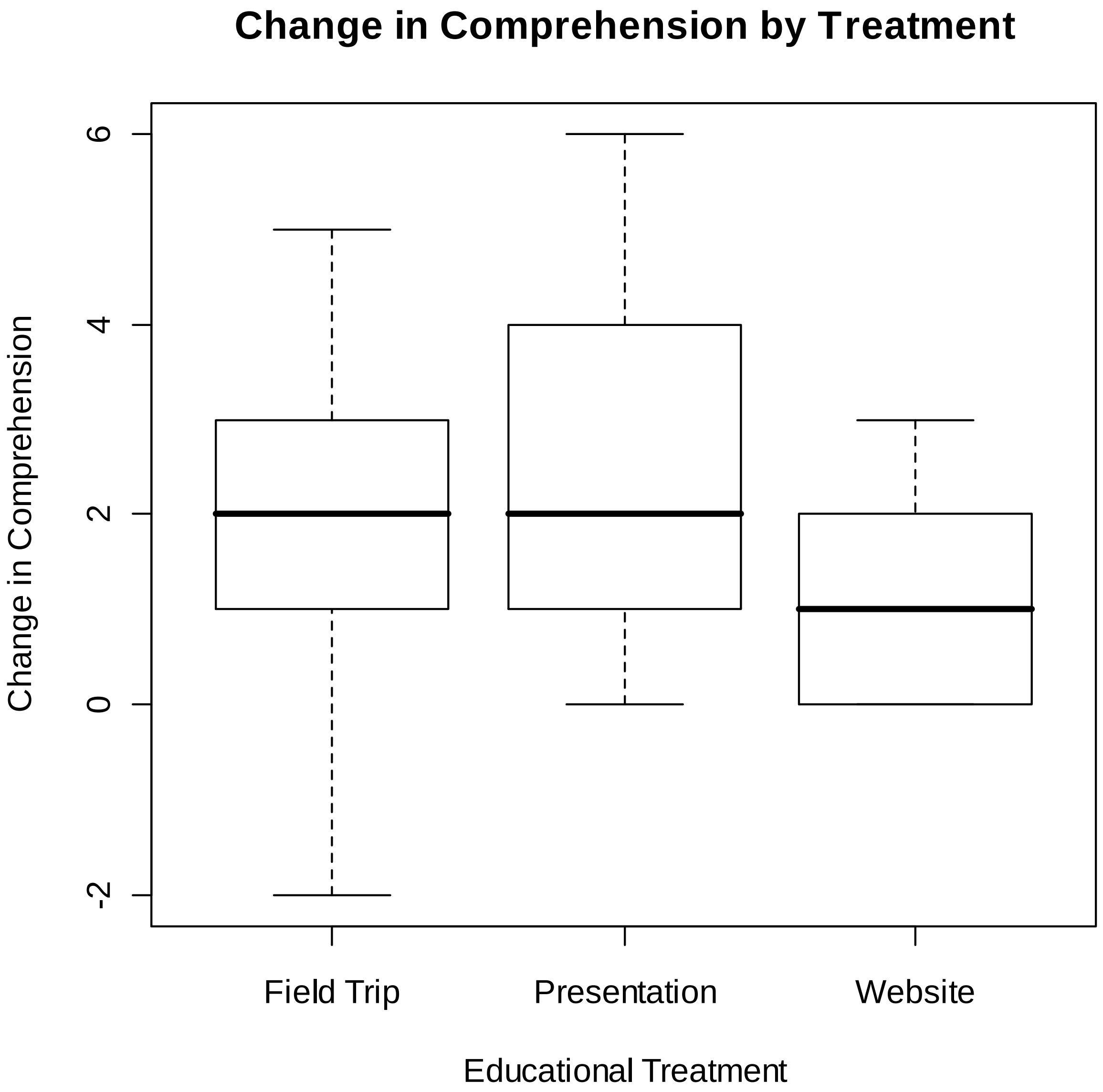


Figure 6.5. Total change in comprehension by educational treatment. Correct true/false questions were awarded a value of 1, incorrect a value of 0. Pre-survey scores were subtracted from post-survey scores then summed across each individual to create a total change value which ranged from -2 (decrease in knowledge after treatment) to 6 (increase in knowledge treatment). Significant differences between treatments (χ^2= 8.035, *P*=0.018) resulted from the Powerpoint presentation increasing comprehension significantly more than the website (observed difference = 17.16, critical difference = 15.07).

Table 6.1. Comprehension and attitude survey questions.

COMPREHENSION True/False	1. The western gray squirrel is a federally listed endangered species 2. Western gray squirrels hibernate over the winter 3. Western gray squirrels make middens (piles of food for storage) 4. The North Cascades is a unique habitat for the western gray squirrel 5. Dwarf mistletoe is an important nesting structure for western gray squirrels 6. Hair-snag tubes help us know how many squirrels live in an area 7. Radio-telemetry is the most effective way to learn about western gray squirrel habitat use
ATTITUDE 5-point Likert Scale	1. The western gray squirrel should be conserved in the North Cascades 2. Research on the western gray squirrel in the North Cascades is valuable 3. I would be interested in helping to study western gray squirrels 4. I would be willing to conserve western gray squirrel habitat by keeping or planting large trees on my land 5. I would be willing to conserve western gray squirrel habitat by keeping some dwarf mistletoe in the upper canopy of trees on my land not too near my house 6. I would be willing to conserve western gray squirrels by being extra careful not to hit squirrels on the road 7. I would be willing to share what I know about western gray squirrels, research and conservation with my friends and neighbors

Table 6.2. Participation by treatment and study site.

	Stehekin	Methow	Total	Incomplete Surveys	Grand Total
Field Trip	5	22	27	9	36
Presentation	5	22	27	6	33
Website	13	7	20	16	36
Total	23	51	74	31	105

Table 6.3. Reasons for low participation and potential solutions.

Reasons for Not Participating	Potential Solutions
- Timing	- Make extra effort to identify audiences and schedule presentations at appropriate times
- Children	- Create kid-friendly presentations to encourage families to attend
- Conflicting Events	- Be mindful of events occuring in areas of interest and schedule around
- Forgetfulness	- Have multiple events to allow more flexibility
	- Send reminders before events
- Advertising (type, lack of)	
	- Be creative about finding venues
- Dislike/Disinterest	- Advertise events with a variety of formats
	- Advertise in ways that draw many potential interests,
	- Share presentations with locals/schedule as part of outstanding community events

Management Implications

Study results from each chapter suggest the following management implications and future research for conserving western gray squirrels in the North Cascades:

The continuation of fire fuel reduction treatments is essential for preventing large-scale, high intensity wildfires, restoring natural and ecological processes, and protecting human life and property. Squirrels did not show avoidance of fire fuel treated areas indicating that previous/current fire fuel reduction treatment protocols at our study sites have retained an adequate number of suitable habitat patches for western gray squirrel nesting and foraging. We recommend future treatments focus on preserving patches of optimal habitat including large trees (our data suggest ≥50 cm) with dwarf mistletoe infection (average TBV rating ≥ 2), high canopy cover (≥ 70%), and connectivity (≥ 2 interlocking trees), with patch size and spacing following guidelines in Washington State's habitat management document for western gray squirrels (Linders et al. 2010). Identification of winter nest sites and core use areas could help prioritize land conservation efforts; because winter may be a more challenging season for squirrels, these areas may be important to retain as leave-patches.

Future research before and after fire fuel reduction treatments at additional study sites could better evaluate causes of western gray squirrel habitat selection in response to fire fuel reduction treatments. An ideal experimental design would involve randomly assigning treatments to evenly divided portions of existing squirrel home ranges.

Our analyses of squirrel space use in the North Cascades and effective population size indicated that the North Cascades habitat may be higher quality, and able to support a larger population of squirrels than previously estimated. We recommend additional research at other study sites and evaluation of additional habitat variables (for example land cover patchiness) in relation to home range size, overlap, and resource selection to enhance understanding of variability and components of habitat quality among sites. Additional monitoring of western gray squirrels over winter in the North Cascades would also enhance understanding of space use and habitat requirements across seasons.

Continuing to identify additional areas of western gray squirrel presence in the North Cascades, and conducting genetic sampling at additional sites within the Methow Valley (especially the northern range in Winthrop and Twisp, and the southern range near Manson, Washington) could enhance understanding of habitat connectivity and identify habitat corridors between populations of squirrels in the North Cascades. Additionally, comparing the genetic diversity and structure of western gray squirrels in the North Cascades to other populations of western gray squirrels in Washington, Oregon, and California, would clarify patterns and put our results into context.

We did not find any effects of fire fuel reduction treatments on diversity and relative abundance of truffles in the diets of western gray squirrels in the North Cascades. However, we recommend truffle sampling in squirrel high and low squirrel use areas and fire fuel treated and untreated areas (following the methods of Claridge et al. 2000) to compare diversity and relative abundance of truffle genera in the habitat with our results from squirrel diets. Opportunistic sampling of western gray squirrel stomachs collected from road killed squirrels in the North Cascades would also help corroborate fecal sample results. Methods for identification of plant material in squirrel fecal samples also need improvement to quantify the relative importance of plant material and effects of fire fuel reduction treatments on the overall diet of western gray squirrels in the North Cascades. It would also be informative to compare diversity and relative abundance of plant material in squirrel diets with our existing or additional vegetation sampling in squirrel high and low use and fire fuel treated and untreated areas.

My studies revealed the value of education as a tool for increasing knowledge and support for wildlife research projects, which can be enhanced by wildlife researchers directly addressing the general public through presentations and short field trips. Early and frequent communication with local landowners may help prevent misinformation and distrust of wildlife research and researchers. However, researchers should not expect immediate (if any) changes in behavior (especially related to private land management) due to one educational presentation. Several educational presentations over time, in a variety of formats, in addition to individual contact with researchers may be required to change opinion. Finally, we recommend exploring the possibility of creating citizen science projects to enlist the help of local residents who demonstrated significant interest in getting involved in wildlife research. The general public could provide a valuable resource for obtaining ongoing information on western gray squirrel population status in the North Cascades.

An increased sample size and focused study of factors influencing seasonal and annual survival rates would identify potential threats to western gray squirrel persistence in the North Cascades. Through ongoing research and continued thoughtful land management, we can maintain a viable population of western gray squirrels in the North Cascades.

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Appendix A. Notes on Relative Abundance, Mortality, and Reproduction of Western Gray Squirrels at Stehekin and Squaw Creek

The Western gray squirrel (*Sciurus griseus*) was listed as a Washington State threatened species in 1993 and is confined to three geographically isolated areas: the southern Puget Trough of Pierce and Thurston Counties, southern Washington in Klickitat, Yakima and Skamania counties, and north-central Washington in Chelan and Okanogan counties. Recovery of the species has become a priority; however, distribution and life history of the North Cascades population remains poorly understood. Land managers in the North Cascades were interested in estimates of relative abundance of local squirrel populations within forest management zones. Additionally, survival and reproductive success are fitness correlates which can be more telling of habitat quality and population status than abundance or habitat use (Van Horne 1983). We collected insufficient information for a full appraisal of demographic attributes of western gray squirrels in the North Cascades, but report here observations on relative abundance, mortality, and reproduction at two study sites: Stehekin, Washington, part of the Lake Chelan National Recreation Area of the North Cascades National Park Complex, and the Squaw Creek drainage of the southern Methow Valley, Washington.

Relative abundance was estimated from live trapping success and field observation of the minimum number of known animals alive. Mortality and its causes were documented with radiotelemetry when possible. We also attempted to monitor reproductive output of squirrels with radiotelemetry and juvenile emergence counts (Vander Haegen et al. 2005) combined with genetic analysis to estimate reproductive success.

We conducted intensive live trapping in Stehekin throughout all seasons in all known areas of western gray squirrel presence. We live trapped 12 squirrels during the 2008 field season, and an additional 11 squirrels during the 2009 field season. We continued to monitor 2 of the squirrels trapped in 2008 with radiotelemetry in 2009. We trapped an additional 2 squirrels in the 2010 field season, with a minimal amount of trapping effort, and continued to monitor 2 squirrels trapped in 2009 with radiotelemetry. Thus the minimum number of squirrels known alive in Stehekin was 12 in 2008, 13 in 2009, and 4 in 2010. With intensive live trapping at Squaw Creek we captured 28 squirrels in 2010, and an additional 9 squirrels in 2011. Twenty-two of the squirrels we captured in 2010 were re-trapped at least once during the 2011 field season. Thus the minimum number of squirrels known alive at Squaw Creek was 28 in 2010, and 44 in 2011. All estimates are smaller than the estimated census sizes from effective population sizes (N_e) using genetic samples (Chapter 4), likely because genetic sampling included multiple generations of squirrels. The relationship between census population size and N_e is also not known. There was no effort to estimate the undetected segment of the population at study sites.

Trapping success decreased in Stehekin in 2009 and 2010, but increased at Squaw Creek in 2011. These differences may have reflected high mortality observed in Stehekin compared to Squaw Creek. In Stehekin, 15 out of 22 radio-collared squirrels died before the end of the study. Most deaths were caused by predation. Avian predators were the most likely cause of death for 3 squirrels; on 1 occasion a goshawk (*Accipiter gentilis*) was observed carrying and subsequently consuming a radio-collared western gray squirrel (Figure A1.1). Mammalian predators were the most likely cause of death for an additional 4 radio-collared squirrels, with 1 squirrel tracked to the den of a pine marten (*Martes martes*) where the marten was observed (Figure A1.1). Members of the weasel family generally leave only a radio-collar and sometimes a tail, while bobcats will also leave the stomach (Gene Orth, Washington Department of Fish and Wildlife, pers. comm.). Two other mortalities were also most likely caused by predation, but we could not inspect them soon enough to discern cause of death definitively. Other likely causes of death include disease in combination with starvation and/or trauma (n = 3). Mites from the genus *Notoedres* (exact species not identified) were identified on the surface of the epidermis of 2 of these 3 squirrels by the Washington Animal Diagnostic Disease Lab at Washington State University, Pullman, Washington. This represents the first documented cases of mange outside the Klickitat County population. *Notoedres centrifera (douglasi)* mites caused outbreaks of mange in Klickitat County from 1998 to 1999 (Linders 2000, Cornish et al. 2001). Three males died for unknown reasons at the beginning of the study in 2008. Of squirrels with recorded mortality information, the average number of days survived after radio-collaring in Stehekin was 138 ± 134 [SD] (Table A1.1).

We observed fewer mortalities at Squaw Creek than Stehekin. Out of the 24 radio-collared squirrels at Squaw Creek between 2010 and 2011 we observed 5 mortalities. Again, most mortality was caused by predation. Based on evidence at the mortality site (eg: scat, squirrel remains) 2 of the mortalities were most likely caused by avian predators, 2 by mammalian predators, one of which was a pet German shepherd, the other likely a member of the weasel family. One radio-collared squirrel was killed on the road by a car during hunting season of 2010. The average number of days these squirrels survived after radio-collaring was 79 ± 85 [SD] (Table A1.2). Owners of the German shepherd reported an average of two western gray squirrel deaths caused by this dog per year. We also recorded an additional 3 road killed squirrels off Squaw Creek road (uncollared), and 8 off Highway 153 between Pateros and Methow, Washington, over our 2 field seasons.

We identified four natal nests between the two study areas, however attempts at juvenile emergence counts were unsuccessful. We observed 4 females in Stehekin and 5 at Squaw Creek with 1-3 young at least once each during routine monitoring with radiotelemetry. Co-nesting between suspected mother/offspring (juvenile young of the year) pairs was also observed 14 times in Stehekin: 4 times by a suspected mother/daughter pair from October-December 2008, and 10 times by a suspected mother/son pair from December-January 2009/2010. Co-nesting at Squaw Creek was observed 11 times between 1 mother and 3 suspected offspring (2 female, 1 male), and between another suspected mother/daughter pair 4 times from October- January 2010/2011. We captured 3 and 4 juvenile squirrels in Stehekin in 2008 and 2009 respectively, and 5, and 4 juveniles at Squaw Creek in 2010 and 2011. We were unable to assign any of the offspring to hypothesized candidate parents with confidence using genetic parentage analysis. We did not acquire enough data for comparison of reproduction at our sites to other areas in Washington.

Our estimates of relative abundance indicate that populations of western gray squirrels at both study sites are small, however, the population of western gray squirrels at Squaw Creek is approximately twice the size of the population in Stehekin. Successful reproduction was documented in both areas. The number of observed mortalities was higher in Stehekin, but mostly caused by predation which is difficult to address with management (especially in a National Park). The best strategy in this area to conserve western gray squirrels may be to maintain and/or enhance existing habitat quality through optimum placement of leave patches for nesting security within fire fuel treated areas (see Chapter 1). Conversely, at Squaw Creek, many of the recorded mortalities were human caused (domestic animals and road kills) which could be easier to mitigate with regulation or education efforts (see Chapter 5).

Figure A1.1. Top: Goshawk (*Accipiter gentilis*) with a radio-collared western gray squirrel. Bottom: Pine marten (*Martes martes*) in den with radio-collared western gray squirrel.

Table A1.1. Number of days observed and most likely cause of death for radio-collared squirrels (identified by eartag number) in Stehekin, Washington.

Squirrel	Sex	Date Radio-collared	Last Date Observed	# Days	Fate	Most Likely Cause
2600	F	4/24/2008	12/3/2009	588	M	disease/starvation
2677	M	5/14/2008	7/28/2008	75	M	unk
2679	M	5/14/2008	8/1/2008	79	M	unk
2680	M	5/15/2008	7/25/2008	71	M	unk
2681	F	8/3/2008	2/16/2009	197	M	unk predation
2684	F	8/5/2008	10/21/2008	77	U	na
2686	F	8/19/2008	2/16/2009	181	M	unk predation
2690	F	8/30/2008	10/21/2008	52	U	na
2687	F	11/9/2008	12/9/2008	30	U	na
2693	F	5/25/2009	7/30/2010	431	A	na
2694	M	5/25/2009	10/25/2009	153	M	disease
2688	M	5/27/2009	11/23/2009	180	M	disease/starvation
2695	M	6/7/2009	7/7/2009	30	M	avian
2696	M	8/15/2009	11/18/2009	95	A	na
2697	F	8/25/2009	11/23/2009	90	M	mammalian
2698	F	9/11/2009	7/30/2010	322	A	na
2699	F	9/29/2009	12/30/2009	92	M	mammalian
2663	F	10/16/2009	12/12/2009	57	M	avian
2700	F	10/16/2009	1/4/2010	80	M	mammalian
2664	M	10/16/2009	1/25/2010	101	M	mammalian
2666	M	3/7/2010	6/8/2010	93	M	avian
2668	M	3/8/2010	8/21/2010	166	A	na

Fate M = Observed mortality
Fate A = Alive at end of study
Fate U = Unknown fate
Most likely cause na = not applicable (mortality not observed)

Table A1.2. Number of days observed and most likely cause of death for radio-collared squirrels (identified by eartag number) in the Squaw Creek drainage of the southern Methow Valley, Washington.

Squirrel	Sex	Date Radio-collared	Last Date Observed	# Days	Fate	Most Likely Cause
2669	M	3/22/2010	12/15/2010	268	A	na
2670	F	3/23/2010	10/28/2010	219	M	avian
2828	M	4/15/2010	12/4/2010	233	A	na
2829	M	4/29/2010	11/9/2010	194	A	na
2827	M	5/9/2010	12/18/2010	223	A	na
2830	M	5/9/2010	10/25/2010	169	A	na
2831	M	5/9/2010	5/9/2010	0	U	na
2674	F	5/23/2010	12/30/2010	221	A	na
2835	M	5/25/2010	11/3/2010	162	A	na
3080	M	7/19/2010	12/5/2010	139	A	na
3100	F	7/30/2010	11/4/2010	97	M	domestic dog
3081	F	8/2/2010	8/23/2011	386	A	na
3086	M	8/5/2010	8/23/2010	18	M	avian
3099	F	8/24/2010	12/30/2010	128	U	na
3087	F	8/28/2010	10/15/2010	48	M	road kill
3088	F	10/11/2010	10/22/2010	11	M	mammalian
3091	F	10/15/2010	12/17/2010	63	A	na
3084	M	10/28/2010	4/21/2011	175	A	na
3092	F	11/9/2010	12/31/2010	52	A	na
3090	F	4/29/2011	8/19/2011	112	A	na
3093	F	4/29/2011	8/22/2011	115	A	na
3076	M	5/18/2011	9/16/2011	121	A	na
2672	M	6/22/2011	9/7/2011	77	A	na
3082	F	7/14/2011	9/15/2011	63	A	na

Fate M = Observed mortality
Fate A = Alive at end of study
Fate U = Unknown fate
Most likely cause na = not applicable (mortality not observed)

Appendix B. Squirrel and Habitat Data Collection Forms

Western Gray Squirrel Trap Check Form

Date:

Set Time: **Close time:** **Initials:**

Condition: B = Bait in trap, G = Bait gone WGS = western gray squirrel
DS = Dougals Squirrel, RS = Red Squirrel, S = Sprung (no animal captured), CL = closed by operator

Trap Location:

	Check 1		**Check 2**		**Check 3**		**Check 4**	
	Start:	Finish:	Start:	Finish:	Start:	Finish:	Start:	Finish:
Trap #	Condition	Animal #	Condition	Animal #	Condition	Animal #	Condition	Animal #

Trap Location:

	Check 1		**Check 2**		**Check 3**		**Check 4**	
	Start:	Finish:	Start:	Finish:	Start:	Finish:	Start:	Finish:
Trap #	Condition	Animal #	Condition	Animal #	Condition	Animal #	Condition	Animal #

Stehekin Western Gray Squirrel Capture Form **Date**__________________ **Observer(s)**____________________

Katy Stuart kshipe@u.washington.edu (206) 953-2716

Site	Trap #	**Species**	Sex	Age	Ear Tag#	Radio freq.	New radio attached?	Magnet removed?

Restraint time	Time begin: Time released:
Reason for capture	a) routine grid trapping b) attach radio collar c) replace radio collar d) collect biosample e) assess condition f) other (describe)

Biometrics (length in mm)			
Body length			
Tail length			
Hind foot			
Ear			
Neck			
Gross Weight (g)		Bag weight:	
Net Weight (gross wt. – bag wt.)			
Reproductive Data			
♀		♂	
Vulvar swelling?		Scrotal?	
Nuzzle marks?		Testes length	
Ventral color	a) white b) yellow c)white and yellow		
Teat length			
Teat width			

Comments: (include old radio freq. if collar removed)

Teat color		Teeth √		Mange (% of body involved)		Tissue sample?		Fecal sample?

Western Gray Squirrel Telemetry Form

Study Area: Observer(s):

mm/dd/yy			
Time: 24 hr			
Eartag (ID) #			
Collar frequency			
Subarea: eg: Orchard, Landing			
GPS Easting			
GPS Northing			
GPS Accuracy			
Location Accuracy: 1m = visual/nest 5m=tree, no visual, 10m=b/w trees			
Substrate (tree type or ground)			
Active signa?l Y/N			
Visual? Y/N			
Animal condition			
Activity/behavior			
Nest? Y/N nest # (if new nest fill out nest form)			
Mistletoe? y/n			
Dominant tree spp.			
Subdominant tree spp.			
DBH of substrate			
Canopy closure:% in 10's			
Woody shrub cover: % in 10's			
Avg. shrub height			
Cloud cover: % in 10's			
Precipitation; none, rain, snow			
Wind: none, slight, moderate, severe			
Comments			

North Cascades Western Gray Squirrel Nest Form

Study Site:

Date: mm/dd/yyyy				
GPS Northing				
GPS Easting				
GPS Accuracy				
Nest Number				
Squirrel Observed Here? Ear tag ID/radio frequency				
Nest Type				
Nest Condition				
Nest Color				
Nest Height				
Tree Height				
Tree DBH				
Tree Species				
Mistletoe? TBV				
Picture Taken?				
Comments				

Nest Type:
P= Platform; flat nest usually made of conifer boughts (can include other materials such as oak, lichen, grass etc.)
S= Shelter; spherical nest usually made of conifer boughs (can include other materials)
C= Cavity

Nest Condition:
A= Fully constructed or partially constructed nest that contains some fresh material
B= Nest is substantial, but may have lost material or be partially falling from tree
C= Most material is gone, but material indicates western gray squirrel

Nest Material Color:
G= Green (any amount)
R= Red or Rusty (any amount, but no green)
B= Brown/Black

Date: PLOT #: Observers:

NEST/FOCAL TREE . UTME__________UTMN__________Species_________ Nest Aspect_____DBH_______
Canopy connectivity_____ Relative Tree Height_____ Mistletoe TBV__/__/__Tot.___Live Canopy____Tree condition____ Height______Maximum Scorch Height_____LLC_____
Nearest water source w/in 25.25m plot_____

8 RANDOM TREES ----- **25.25m radius plot, DBH ≥ 20cm**

1. Bearing_______Distance_______Species _________ DBH _____ Rel. Ht._____ Canopy connectivity_____
Mistletoe TBV__/__/___Tot.__Live Canopy_____ Tree condition_____Ht._____ Max. Scorch_____ LLC ____

2. Bearing_______Distance_______Species _________ DBH _____ Rel. Ht._____ Canopy connectivity_____
Mistletoe TBV__/__/___Tot.__Live Canopy_____ Tree condition_____Ht._____ Max. Scorch_____ LLC ____

3. Bearing_______Distance_______Species _________ DBH _____ Rel. Ht._____ Canopy connectivity_____
Mistletoe TBV__/__/___Tot.__Live Canopy_____ Tree condition_____Ht._____ Max. Scorch_____ LLC ____

4. Bearing_______Distance_______Species _________ DBH _____ Rel. Ht._____ Canopy connectivity_____
Mistletoe TBV__/__/___Tot.__Live Canopy_____ Tree condition_____Ht._____ Max. Scorch_____ LLC ____

5. Bearing_______Distance_______Species _________ DBH _____ Rel. Ht._____ Canopy connectivity_____
Mistletoe TBV__/__/___Tot.__Live Canopy_____ Tree condition_____Ht._____ Max. Scorch_____ LLC ____

6. Bearing_______Distance_______Species _________ DBH _____ Rel. Ht._____ Canopy connectivity_____
Mistletoe TBV__/__/___Tot.__Live Canopy_____ Tree condition_____Ht._____ Max. Scorch_____ LLC ____

7. Bearing_______Distance_______Species _________ DBH _____ Rel. Ht._____ Canopy connectivity_____
Mistletoe TBV__/__/___Tot.__Live Canopy_____ Tree condition_____Ht._____ Max. Scorch_____ LLC ____

8. Bearing_______Distance_______Species _________ DBH _____ Rel. Ht._____ Canopy connectivity_____
Mistletoe TBV__/__/___Tot.__Live Canopy_____ Tree condition_____Ht._____ Max. Scorch_____ LLC ____

10.6m radius plot

Canopy Cover: Densitometer Y/N

N1		E1		S1		W1	
N2		E2		S2		W2	
N3		E3		S3		W3	
N4		E4		S4		W4	
NE1		SE1		SW1		NW1	
NE2		SE2		SW2		NW2	
NE3		SE3		SW3		NW3	

Coarse woody debris	Decay Class 1	Total	Decay Class 2	Total	Decay Class 3	Total
DBH 10-25cm						
DBH 25-50cm						
DBH >50cm						

5.6m radius plot

Understory Cover: Shrubs/Saplings/etc. ≤ 5cm DBH

Low: 0.5-2m			**High: 2-8m**		
Species	**Cover Class**	**Modal Height**	**Species**	**Cover Class**	**Modal Height**
Total Deciduous			Total Deciduous		
Total Evergreen			Total Evergreen		

Ground Cover

Category	**Cover Class**
Total Vegetation	
Cheat Grass	
Total Grasses	
Forbs	
Moss	
Litter	
Bare Ground	
Rock	
CWD	
Water	
Road	
Building	
Other:	

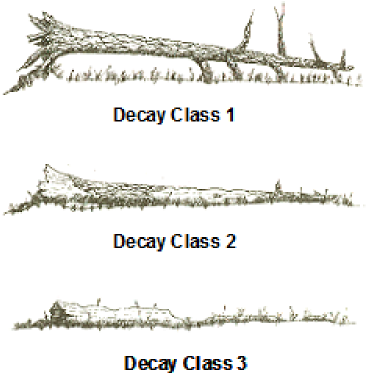

Figure 1. Decay classes

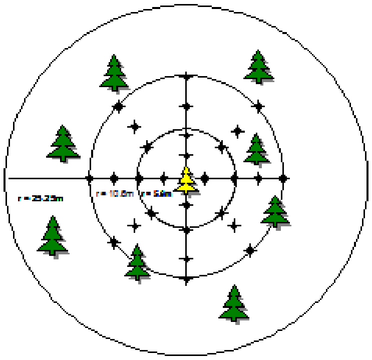

Figure 2. Plot Set up

Reference Section

Decay classes: 1) Trees that just fell over, retaining bark and branches, little decay in wood and resting largely above ground. 2) Logs in contact with the ground, lost some bark and branches, some decay. 3) Logs that have begun decomposing into the forest floor, are not intact, and lack both bark and limbs (Fig. 1).

Mistletoe TBV: For each third of the tree ground level to top: 0) no brooms 1) 1-33% volume of tree occupied by brooms 2) 34-66% volume occupied 3) >67% occupied by brooms. Maximum rating for tree = 9.

Relative Height: 1) Dominant 2) Co-dominant 3) Subdominant (overtopped)

Tree Condition: 1) Alive 2) Declining 3) Dead with branches and needles 4) Dead with branches 5) Dead no branches/needles

Tree and shrub species codes: Use first two letters of genus and species. Eg: Douglas-fir = PSME. If overlap between species use additional letters or codes and explain in comments section. Stay consistent between plots.

Shrub/ground cover classes: 1) 0-1% 2) 2-5% 3) 6-25% 4) 26-50% 5) 51-75% 6) >76%

COMMENTS:

Appendix C. Communicating Wildlife Research Surveys

Name: ____________________

1. Have you heard about western gray squirrel research in the North Cascades before? (check **one**)

   ☐**Yes** ☐**No**

2. If you have, where did you get your information?
   (check all that apply)

   ☐ Newspaper Article(s)
   ☐ North Cascades Visitor Center(s)
   ☐ Friends/family
   ☐ North Cascades Squirrel brochure
   ☐ Directly from researcher(s)
   ☐Other________________________________

3. In what format do you ***generally*** receive information on wildlife research and conservation?
   (Rate each)

	Never		**Occasionally**		**Most Often**
The Internet	1	2	3	4	5
Newspaper/Magazine Articles	1	2	3	4	5
Public Presentations/Lectures	1	2	3	4	5
Brochures/Exhibits at Visitor Centers	1	2	3	4	5
Friends/Family	1	2	3	4	5
Other _________________________	1	2	3	4	5

4. In what format do you ***prefer*** to receive information on wildlife research and conservation?
   (Rate each)

	Least Preferred		**Somewhat Preferred**		**Most Preferred**
The Internet	1	2	3	4	5
Newspaper/Magazine Articles	1	2	3	4	5
Public Presentations/Lectures	1	2	3	4	5
Brochures/Exhibits at Visitor Centers	1	2	3	4	5
Friends/Family	1	2	3	4	5
Other__________________________	1	2	3	4	5

   Why?

5. What are you interested in learning about this project?
   (Rate each)

	Least Interested		**Somewhat Interested**		**Most Interested**
Who is working on this project	1	2	3	4	5
Why this project is being done	1	2	3	4	5
Project goals	1	2	3	4	5
Methods used to study squirrels	1	2	3	4	5
General western gray squirrel biology	1	2	3	4	5
The ecological role of the western gray squirrel (why is it important?)	1	2	3	4	5
North Cascades habitat (where to find squirrels)	1	2	3	4	5
How study results will be used	1	2	3	4	5
How you can get involved in the project	1	2	3	4	5
How this project relates to other, current wildlife research	1	2	3	4	5
Other _____________________________	1	2	3	4	5

True/False (circle)

6. The western gray squirrel is a federally listed endangered species. **T** **F**

7. Western gray squirrels hibernate over the winter **T** **F**

8. Western gray squirrels make middens (piles of food for storage) **T** **F**

9. The North Cascades is a unique habitat for the western gray squirrel. **T** **F**

10. Dwarf mistletoe is an important nesting structure for western gray squirrels **T** **F**

11. Hair-snag tubes help us know how many squirrels live in an area **T** **F**

12. Radio-telemetry is the most effective way to learn about western gray squirrel habitat use **T** **F**

Please rate your level of agreement with the following statements (circle one):

		Strongly Disagree	**Somewhat Disagree**	**Neither Agree Nor Disagree**	**Somewhat Agree**	**Strongly Agree**
13.	The western gray squirrel should be conserved in Stehekin	**1**	**2**	**3**	**4**	**5**
14.	Research on the western gray squirrel in Stehekin is valuable	**1**	**2**	**3**	**4**	**5**
15.	I would be interested in helping to study western gray squirrels (eg: sharing sightings and observations with researchers, volunteering field help)	**1**	**2**	**3**	**4**	**5**
16.	I would be interested in helping to study western gray squirrels *on my land* (eg: distributing and checking hair snag tubes on my property)	**1**	**2**	**3**	**4**	**5**
17.	I would be willing to conserve western gray squirrel habitat by keeping or planting large trees on my land	**1**	**2**	**3**	**4**	**5**
18.	I would be willing to conserve western gray squirrel habitat by keeping some dwarf mistletoe in the upper canopy of trees on my land, not too near my house	**1**	**2**	**3**	**4**	**5**
19.	I would be willing to conserve western gray squirrels by being extra careful not to hit squirrels on the road	**1**	**2**	**3**	**4**	**5**
20.	I would be willing to share what I know about western gray squirrels, research and conservation with my friends and neighbors	**1**	**2**	**3**	**4**	**5**

Gender:☐Male ☐Female Age: ☐ under 25 ☐ 26-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ 61 and over

Level of Education:☐ High School/GED ☐ Some College ☐ 2-year degree ☐ 4 year degree ☐ Graduate degree (Master's +)

Years of Residence in Stehekin/Methow Valley: _______ ☐Year-round ☐Part-time

Occupation (or former) ______________________

Name: ____________________

1. What did you like about the field trip? (Rate each on a 1-5 scale)

	Poor	**Fair**	**Neutral**	**Good**	**Excellent**
The interaction with a wildlife researcher	**1**	**2**	**3**	**4**	**5**
The interaction with other participants	**1**	**2**	**3**	**4**	**5**
The information was interesting	**1**	**2**	**3**	**4**	**5**
The information was relevant	**1**	**2**	**3**	**4**	**5**
The information was understandable	**1**	**2**	**3**	**4**	**5**
My questions were answered	**1**	**2**	**3**	**4**	**5**
The field trip was engaging	**1**	**2**	**3**	**4**	**5**
The field trip was fun	**1**	**2**	**3**	**4**	**5**
It was a short time commitment/easy to get to	**1**	**2**	**3**	**4**	**5**
Other _______________________________					

2. Please provide additional comments on what you particularly liked.

3. What could have been improved?

4. Were these topics adequately covered? (Rate each on a 1-5 scale)

	Poor	**Fair**	**Neutral**	**Good**	**Excellent**
Who is working on this project	**1**	**2**	**3**	**4**	**5**
Why this project is being done	**1**	**2**	**3**	**4**	**5**
Project goals	**1**	**2**	**3**	**4**	**5**
Methods used to study squirrels	**1**	**2**	**3**	**4**	**5**
General western gray squirrel biology	**1**	**2**	**3**	**4**	**5**
The ecological role of the western gray squirrel (why is it important?)	**1**	**2**	**3**	**4**	**5**
North Cascades habitat (where to find squirrels)	**1**	**2**	**3**	**4**	**5**
How study results will be used for management	**1**	**2**	**3**	**4**	**5**
How you can get involved in the project	**1**	**2**	**3**	**4**	**5**
How this project relates to other, current wildlife research	**1**	**2**	**3**	**4**	**5**

5. Do you have any other questions? Please be specific

Name: ____________________

1. What did you like about the website? (Rate each on a 1-5 scale)

	Poor	Fair	Neutral	Good	Excellent
The information was interesting	1	2	3	4	5
The information was relevant	1	2	3	4	5
The information was understandable	1	2	3	4	5
My questions were answered	1	2	3	4	5
The website was engaging	1	2	3	4	5
The website was fun	1	2	3	4	5
It was a short time commitment/easy to get to	1	2	3	4	5
I could learn at my own pace	1	2	3	4	5
Other _______________________________					

2. Please provide additional comments on what you particularly liked.

3. What could have been improved?

4. Were these topics adequately covered? (Rate each on a 1-5 scale)

	Poor	Fair	Neutral	Good	Excellent
Who is working on this project	1	2	3	4	5
Why this project is being done	1	2	3	4	5
Project goals	1	2	3	4	5
Methods used to study squirrels	1	2	3	4	5
General western gray squirrel biology	1	2	3	4	5
The ecological role of the western gray squirrel (why is it important?)	1	2	3	4	5
North Cascades habitat (where to find squirrels)	1	2	3	4	5
How study results will be used for management	1	2	3	4	5
How you can get involved in the project	1	2	3	4	5
How this project relates to other, current wildlife research	1	2	3	4	5

5. Do you have any other questions? Please be specific

Name: ____________________

1. What did you like about the presentation? (Rate each on a 1-5 scale)

	Poor	**Fair**	**Neutral**	**Good**	**Excellent**
The interaction with a wildlife researcher	**1**	**2**	**3**	**4**	**5**
The information was interesting	**1**	**2**	**3**	**4**	**5**
The information was relevant	**1**	**2**	**3**	**4**	**5**
The information was understandable	**1**	**2**	**3**	**4**	**5**
My questions were answered	**1**	**2**	**3**	**4**	**5**
The presentation was engaging	**1**	**2**	**3**	**4**	**5**
The presentation was fun	**1**	**2**	**3**	**4**	**5**
It was a short time commitment/easy to get to	**1**	**2**	**3**	**4**	**5**
Other _______________________________	**1**	**2**	**3**	**4**	**5**

2. Please provide additional comments on what you particularly liked.

3. What could have been improved?

4. Were these topics adequately covered? (Rate each on a 1-5 scale)

	Poor	**Fair**	**Neutral**	**Good**	**Excellent**
Who is working on this project	**1**	**2**	**3**	**4**	**5**
Why this project is being done	**1**	**2**	**3**	**4**	**5**
Project goals	**1**	**2**	**3**	**4**	**5**
Methods used to study squirrels	**1**	**2**	**3**	**4**	**5**
General western gray squirrel biology	**1**	**2**	**3**	**4**	**5**
The ecological role of the western gray squirrel (why is it important?)	**1**	**2**	**3**	**4**	**5**
North Cascades habitat (where to find squirrels)	**1**	**2**	**3**	**4**	**5**
How study results will be used for management	**1**	**2**	**3**	**4**	**5**
How you can get involved in the project	**1**	**2**	**3**	**4**	**5**
How this project relates to other, current wildlife research	**1**	**2**	**3**	**4**	**5**

5. Do you have any other questions? Please be specific

True/False (circle)

6.	The western gray squirrel is a federally listed endangered species.	**T**	**F**
7.	Western gray squirrels hibernate over the winter.	**T**	**F**
8.	Western gray squirrels make middens (piles of food for storage).	**T**	**F**
9.	The North Cascades is a unique habitat for the western gray squirrel.	**T**	**F**
10.	Dwarf mistletoe is an important nesting structure for western gray squirrels.	**T**	**F**
11.	Hair-snag tubes help us know how many squirrels live in an area.	**T**	**F**
12.	Radio-telemetry is the most effective way to learn about western gray squirrel habitat use.	**T**	**F**

Please rate your level of agreement with the following statements (circle one):

		Strongly Disagree	**Somewhat Disagree**	**Neither Agree Nor Disagree**	**Somewhat Agree**	**Strongly Agree**
13.	The western gray squirrel should be conserved in Stehekin	**1**	**2**	**3**	**4**	**5**
14.	Research on the western gray squirrel in Stehekin is valuable	**1**	**2**	**3**	**4**	**5**
15.	I would be interested in helping to study western gray squirrels (eg: sharing sightings and observations with researchers, volunteering field help)	**1**	**2**	**3**	**4**	**5**
16.	I would be interested in helping to study western gray squirrels *on my land* (eg: distributing and checking hair snag tubes on my property)	**1**	**2**	**3**	**4**	**5**
17.	I would be willing to conserve western gray squirrel habitat by keeping or planting large trees on my land	**1**	**2**	**3**	**4**	**5**
18.	I would be willing to conserve western gray squirrel habitat by keeping some dwarf mistletoe in the upper canopy of trees on my land, not too near my house	**1**	**2**	**3**	**4**	**5**
19.	I would be willing to conserve western gray squirrels by being extra careful not to hit squirrels on the road	**1**	**2**	**3**	**4**	**5**
20.	I would be willing to share what I know about western gray squirrels, research and conservation with my friends and neighbors	**1**	**2**	**3**	**4**	**5**

21. Would you be interested in participating in future wildlife research and conservation projects in the North Cascades?

☐YES ☐NO

THANK YOU!! ☺

Vita

Kathryn Stuart was born in Moses Lake, Washington and has lived in the greater Seattle area since. She earned her Bachelor's of Science in Conservation of Wildland Resources and Wildlife Science with minors in Aquatic and Fisheries Sciences and Quantitative Science from the University of Washington College of Forest Resources in 2006. In 2012 she earned a Doctor of Philosophy at the University of Washington with the school of Environmental and Forest Sciences, College of the Environment.