

**Determining Habitat Associations of Virginia and Carolina Northern Flying Squirrels in
the Appalachian Mountains from Bioacoustic and Telemetry Surveys**

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Abstract

The Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*) and the Carolina northern flying squirrel (*G. s. coloratus*) are geographically isolated subspecies of the northern flying squirrel found in montane conifer-northern hardwood forests the Appalachian Mountains of the eastern United States. Both subspecies were listed under the Endangered Species Act in 1985 as endangered, and accordingly, the Virginia northern flying squirrel and the Carolina northern flying squirrel are considered high conservation priorities by state and federal agencies. Although the listing prompted work to determine the broad distribution and habitat associations of both subspecies, numerous data gaps remain, particularly with regard to habitat management and development of efficient monitoring techniques. Regional interest in restoration of red spruce (*Picea rubens*) forests in the central and southern Appalachian Mountains, considered to be the flying squirrels' primary habitat, increases the importance of understanding habitat selection and managers' ability to detect squirrels at multiple spatial and temporal scales.

I compared two novel survey techniques (ultrasonic acoustics and camera trapping) to a traditional technique (live trapping) to determine which method had higher probability of detection (POD) and lower latency to detection (LTD, number of survey nights to initial detection) of northern flying squirrels in the region. Both novel techniques performed better than the traditional techniques with higher POD and lower LTD. I found that ultrasonic acoustics and camera trapping had similar POD, whereas LTD was significantly lower with ultrasonic acoustics versus camera

trapping. Additionally, the ability to distinguish between northern flying squirrels and the parapatric southern flying squirrel (*G. volans*) also is possible with ultrasonic acoustics, but not with camera trapping. This ultimately makes ultrasonic acoustics the most effective and efficient method to obtain detection/non-detection data. To better inform management decisions and activities (i.e., red spruce restoration), this method should be used in conjunction with existing traditional monitoring techniques that provide demographic data such as nest boxes.

I assessed habitat selection of radio-collared Virginia and Carolina northern flying squirrels at multiple spatial scales with use-availability techniques. I analyzed field data from paired telemetry and random points and determined Virginia northern flying squirrels microhabitat (within-stand habitat) selection showed preference for conifer-dominant stands with deep organic horizons, a factor that might be directly linked to food (hypogean fungi) availability.

Similar to previous studies on the Virginia northern flying squirrel on the landscape- and stand-level using Euclidean distance based analysis, Carolina northern flying squirrels also selectively preferred montane conifer forests in greater proportion than their availability on the landscape. Additionally, Carolina northern flying squirrels did not select for or against northern hardwood forests regardless of availability on the landscape. Habitat preference of both subspecies indicates that red spruce restoration activities may be important for the persistence of Appalachian northern flying squirrels into an uncertain future, as anthropogenic climate change may cause further reduction of the quality and extent of high-elevation montane conifer forests in the region.

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Preface

Several chapters of this dissertation have been accepted or are in review in peer-review journals, with co-authors.

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Chapter 1: Introduction

Northern flying squirrels (*Glaucomys sabrinus*) are nocturnal, arboreal small mammals found in northern North America and are typically associated with coniferous and mixed coniferous-deciduous forests (Wells-Gosling and Heaney 1984). The majority of this species' range is contiguous throughout Canada and northern portions of the United States (e.g., Pacific Northwest, Great Lake states, New England), however disjunct populations occur in the southern Rocky Mountains, the Black Hills, and the Appalachian Mountains (Wells-Gosling and Heaney 1984, Ford and Rodrigue 2007). Although northern flying squirrels were known from the northeastern United States (Howell 1918), they were not known to occur south of Pennsylvania until the late 1930s. A distinct subspecies (Virginia northern flying squirrel, *G. s. fuscus*) was discovered in the Allegheny Mountains of the central Appalachians in West Virginia in 1936 and extended the known range of this species approximately 600 km southwest (Miller 1936, Figure 1.1). Another distinct subspecies (Carolina northern flying squirrel; *G. s. coloratus*) was discovered in the Blue Ridge Mountains of the southern Appalachians in 1951 (Handley 1953). The Carolina northern flying squirrel is geographically isolated from the Virginia northern flying squirrel by an additional 300 km southwest along the Appalachian Mountain chain (Figure 1.1) and both subspecies are considered genetically distinct from each other and the contiguous northern flying squirrel populations in New England and Canada (Arbogast et al. 2005).

Habitat Associations

The Virginia northern flying squirrel (*G. s. fuscus*) is found in high-elevation montane conifer (i.e., red spruce, *Picea rubens*; balsam fir, *Abies balsamea*; eastern hemlock, *Tsuga*

canadensis) forests of the Allegheny Mountains of eastern West Virginia and western Virginia (Stihler et al. 1995, Menzel et al. 2006, Ford et al. 2007). Similar to the Virginia northern flying squirrel, the Carolina northern flying squirrel is associated with high-elevation montane conifers (i.e., red spruce; eastern hemlock; Fraser fir, *A. fraserii*; Weigl et al 1999, Hughes 2006; Ford et al. 2014, 2015). Both subspecies (hereafter “Appalachian northern flying squirrels”) and their primary habitat, red spruce forests, are Pleistocene relicts, remaining from the last glaciation event 18,000 years ago (Delcourt and Delcourt 1984, Odum et al. 2001). Both subspecies and their habitat occur on the highest peaks and ridges in the region (Figure 1.1; Weigl et al. 1999, Menzel et al. 2005, Ford et al 2015). Due to the broad topography of the Allegheny Mountains, red spruce forests of the central Appalachians are considered more contiguous than the red spruce forests found in the southern Appalachians, where the more dramatic topography and lower latitude have fragmented this forest type into highly disjunct “sky islands” (Eager and Adams 1992).

Threats to Appalachian Northern Flying Squirrels

When both Appalachian northern flying squirrels were listed as Endangered under the U.S. Endangered Species Act in 1985, the two major threats to the subspecies were 1) degradation and reduction of suitable habitat and 2) interspecific competition and parasite-mediation from southern flying squirrels (*G. volans*; USFWS 1985).

Habitat Loss and Degradation

At the turn of the 20th century, industrial logging resulted in widespread clearcutting of red spruce stands and destructive fires (Korstian 1937, Minckler 1940, Clarkson 1960), causing a 37-60% and 88-90% reduction in the extent of this forest type in the southern and central Appalachians, respectively (Boyce and Martin 1993), further negatively influencing the

connectivity of this naturally fragmented forest type. The reduction of these forest types was driven by loss of the red spruce seed bank from fire and erosion (Davis 1930, Korstian 1937, Krustchinsky 2007). Additionally, the rapid establishment of northern hardwood species over slow-growing red spruce regeneration caused a shift away from red spruce-dominant stands, especially at lower elevations (Ayres and Ashe 1905, Korstian 1937, Pielke 1981, Hayes et al. 2006), effectively “shrinking” the extent of this forest type to higher elevations (Busing et al. 1993, Hayes et al. 2006, Nowacki et al. 2010).

After industrial logging ceased in the early 20th century, high-elevation montane conifer stands, especially red spruce, Fraser fir, and balsam fir, were further degraded by anthropogenic activities such as atmospheric acid deposition and introduction of the balsam woolly adelgid (*Adelgid balsamea*; Hay et al. 1978, Bruck and Robarge 1988, Dull et al. 1988, Eager and Adams 1992). However, the largest current and future threat to this system is anthropogenic climate change, as warming temperatures and changes in precipitation may influence habitat suitability for montane conifers throughout the region (Beane and Rentch 2015, Koo et al 2015). Species shifts to higher elevations to compensate for a changing climate may be impossible if a species currently occurs at the highest elevations, therefore increasing the risk of extinction (McDonald and Brown 1992, Parmesan 2006, Issac 2009). Impacts from acid deposition have lessened due to the Clean Air Act of 1990 (Bintz and Butcher 2007, Banks 2013), however, extant air pollution may interact with climate change to produce increased negative effects on forest health (Koo et al 2015). Since the Appalachian northern flying squirrel’s habitat was previously reduced in extent and remaining habitat degraded, there is higher potential for relatively modern climate to have a greater impact than in undisturbed systems (Issac 2009).

Interspecific Threats

Southern flying squirrels occur throughout most of the eastern United States, from southern Quebec and the Great Lake states south to the Gulf of Mexico, east to the Atlantic Ocean, and west to eastern Texas, with disjunct populations in Mexico and Central America (Dolan and Carter 1977). Within the central and southern Appalachian Mountains, this species typically occurs at lower elevations than northern flying squirrels, although both species are sympatric to parapatric along the montane conifer – northern hardwood ecotone (Stihler et al. 1995, Reynolds et al. 1999, Weigl et al. 1999). Southern flying squirrels occupy more austral habitats regionally due to two factors: 1) physiological constraints and 2) dietary constraints. Southern flying squirrels cannot tolerate colder temperatures found at higher elevations, especially during the winter months (Weigl et al. 1999, Wetzel and Weigl 1994). However, southern flying squirrels may seasonally occupy montane conifer habitat and reproduce at these sites during the summer months (Urban 1988, Reynolds et al. 2009; C. Diggins, personal observation). Southern flying squirrels have a smaller body size than northern flying squirrels, which require high quality food and nesting aggregations with conspecifics for efficient energy and thermoregulation to survive colder temperatures at higher elevations (Stapp et al. 1991, Weigl et al. 1999, Thorington and Weigl 2011). Habitat occupancy by southern flying squirrels also is limited at higher elevation because of their reliance on hard mast species (i.e., oak *Quercus* spp., hickory *Carya* spp., American beech *Fagus grandifolia*; Fridell and Litvatis 1991, Weigl et al. 1999, Holloway and Malcolm 2007), which are important for caching to survive the winter months (Weigl 1978, Weigl et al. 1999). However climate change may facilitate southern flying squirrel invasion of northern flying squirrel habitat due to warming temperatures and expected altitudinal increase of local oak and American beech distribution and in high elevation stands (Bowman et al. 2005, Myers et al. 2009, Wood et al.

2016). Expansion and persistence of southern flying squirrels within Appalachian northern flying squirrel habitat may increase potential hybridization between the subspecies, which has been recorded in northern Pennsylvania and southeastern Canada (Garroway et al. 2010).

Both southern flying squirrels and northern flying squirrels are secondary cavity nesters and southern flying squirrels may compete for denning sites with northern flying squirrels, in areas of sympatry (Weigl 1978). Potential use of den sites by both species may help facilitate the transmission of *Strongyloides robustus*, a parasitic nematode which reduces survival and productivity in northern flying squirrels (Weigl 1978). Transmission occurs from free-living stages found in feces, which can infect nest material or soil substrates of nest sites shared by both species (Wetzel and Weigl 1994, Weigl et al. 1999, Weigl 2007). This parasite has detrimental effects (i.e., emaciation, susceptibility to pneumonia) on northern flying squirrels, but southern flying squirrels act as reservoirs and seemly are unaffected (Wetzel and Weigl 1994, Weigl et al. 1999, Pauli et al. 2004), which could be due to year-round probability of infection at warmer, low-elevation sites. Colder, high-elevation sites occupied by Appalachian northern flying squirrels intermittently supported *S. robustus*, but the nematode cold intolerant and cannot persist at higher elevations throughout the year due to freezing temperatures (Wetzel and Weigl 1994). Within Appalachian northern flying squirrel habitat, *S. robustus* only occurs at sites where southern flying squirrels are sympatric with northern flying squirrels (Krichbaum et al. 2010). Therefore, even limited interactions may have negative effects for the already vulnerable Appalachian northern flying squirrels (Weigl et al. 1999). Potential range expansion of southern flying squirrels from climate change (Bowman et al. 2005, Myers et al. 2009, Wood et al. 2016) and warmer winter temperatures may increase *S. robustus* infection in Appalachian northern flying squirrels (Weigl et al. 1999, Krichbaum et al. 2010).

Management and Conservation Status

Since the listing of the Virginia and Carolina northern flying squirrel in 1985 (USFWS 1985), state and federal agencies in the central and southern Appalachians have been monitoring both subspecies. The West Virginia Department of Natural Resources (WVDNR) and the Virginia Department of Game and Inland Fisheries (VDGIF) established long-term nest box monitoring sites in suitable habitat (i.e., montane conifer) in 1985 (Stihler 1987, 1995; Reynolds et al. 1999). The North Carolina Wildlife Resources Commission (NCWRC) and Wake Forest University supported an exploratory study from 1986-1991 to determine the distribution and ecology of Carolina northern flying squirrels in western North Carolina (Weigl et al. 1999). After the conclusion of Weigl et al (1999)'s study, NCWRC established a long-term nest box monitoring program in 1996 (C. Kelly, NCWRC, personal communication). Both WVDNR and NCWRC continue their nest box monitoring programs to date, however, VDGIF ceased their nest box monitoring program in 1996 due to extremely low capture rates (R. Reynolds, VDGIF, personal communication). Nest box monitoring has been the primary technique used to monitoring both subspecies, as capture rates in live traps are extremely low and require more time, personnel, and resources (Ford et al. 2015).

Currently, the majority of the range for both subspecies occurs on federal and state lands (63% for Virginia northern flying squirrel and ~90% for the Carolina northern flying squirrel), thereby conceivably providing long-term protection of their habitat. Work by Menzel et al. (2004, 2005, 2006) and Ford et al. (2004, 2007, 2010), demonstrated that the Virginia northern flying squirrel was more widespread than originally thought and occupancy on the landscape was stable. Because of its stable status and the protection of the majority of its habitat, the Virginia northern flying squirrel was recently delisted as federally endangered in 2013 (USFWS 2013) and is

currently going through required post-delisting monitoring for approximately 10 years. However, this subspecies is still listed as state endangered in Virginia. The Carolina northern flying squirrel is currently listed as both federally endangered and state endangered in North Carolina, Virginia, and Tennessee. Conservation efforts to increase the extent, connectivity, and quality of habitat through activities, such as red spruce restoration, may help persistence of both subspecies into the future.

Red Spruce Restoration

A current goal of land management agencies within the central and southern Appalachians is to restore high-elevation red spruce forests to a larger portion of their former extent than current to increase resiliency to climate change. Red spruce is regaining its former range by establishing regeneration in hardwood dominant forests (Rollins et al. 2010), but the natural shift in these stands from hardwood dominant to red spruce dominant forests could take centuries. Through the collaborative efforts of the Central Appalachian Spruce Restoration Initiative (CASRI) and the Southern Appalachian Spruce Restoration Initiative (SASRI), preliminary research on restoration of red spruce has shown the most efficient way to restore red spruce is through single-tree canopy gap creation (Schuler et al. 2002; Rentch et al. 2007, 2010, 2016). Red spruce is shade-tolerant and can exist in the understory for decades waiting to capture a canopy gap (White et al. 1985, Smith and Nicholas 1999, Rentch et al. 2010). Red spruce has exhibited natural recovery by way of reestablishment as understory regeneration in areas where it was previously the dominant overstory species prior to industrial logging (Mayfield and Hicks 2010, Nowacki et al. 2010, Rollins et al. 2010). Overstory canopy gaps in a gap-phase regeneration dynamic are the main disturbance type in these forests (White et al. 1985, Rentch et al. 2010) and restoration activities

aim to mimic this, allowing suppressed red spruce to reach the overstory to become a steady-state, self-replacement driver in the ecosystem (Rentch et al. 2010, 2016). Additionally, red spruce restoration may retard invasion of southern flying squirrels into Appalachian northern flying squirrel habitat, thereby decreasing the risk of parasite mediation and hybridization.

Nonetheless, multiple studies in the western United States have indicated negative effects of several silvicultural practices on the abundance of northern flying squirrels (Waters and Zabel 1995, Carey et al. 2000, Holloway and Malcolm 2006, Holloway and Smith 2011, Holloway et al. 2012), although these studies tended to focus on harvesting techniques such as clearcuts, shelterwood cuts, and green-tree retention rather than restoration-based activities. Within the Pacific Northwest, Holloway et al. (2012) found that varying levels of basal area removal and spatial arrangement of harvest treatments influenced northern flying squirrel populations, with more intensive harvests causing negative effects on squirrels. Silvicultural treatments can reduce den sites and abundance of food resources, as well as increase predation (Holloway and Smith 2011). However, scale and heterogeneity of treatments may influence the impacts of silvicultural activities on flying squirrels and red spruce restoration canopy-gap treatments would be patchier and on a smaller scale than harvesting efforts in the western United States. Therefore, understanding habitat selection and developing better survey protocol for both subspecies is critical to understanding how management of their primary habitat might impact the Appalachian flying squirrels.

Research Needs

For each subspecies, there are specific knowledge gaps which may influence further management, especially in relation to red spruce restoration efforts. Work on landscape scale and

stand-level habitat selection was conducted for the Virginia northern flying squirrel using telemetry data (Menzel 2004, 2005, 2006; Ford et al. 2007), whereas some work on habitat selection for Carolina northern flying squirrels was conducted, but inferences were limited due to small sample sizes and single study sites (Hackett 2002, Ford et al. 2014) or were based on nest box data (Ford et al. 2015). Additionally, Appalachian northern flying squirrels are difficult to capture using traditional techniques, such as live trapping and nest boxes (Stihler 1995, Reynolds et al. 1999, Terry 2004, Menzel et al. 2006, Ford et al. 2010), requiring more effective and efficient techniques to detect Appalachian northern flying squirrel. To establish better monitoring and management techniques for both Appalachian subspecies, my objectives for my dissertation were:

1. Determine which survey techniques are most effective for the Appalachian northern flying squirrels by comparing live trapping, camera trapping, and ultrasonic acoustics on detection rates of the Carolina northern flying squirrel.
2. Understand microhabitat selection by evaluating where radio-collared Virginia northern flying squirrels are selecting habitat vs. available habitat.
3. Assess home range and landscape- and stand-level habitat selection of radio-collared Carolina northern flying squirrels.

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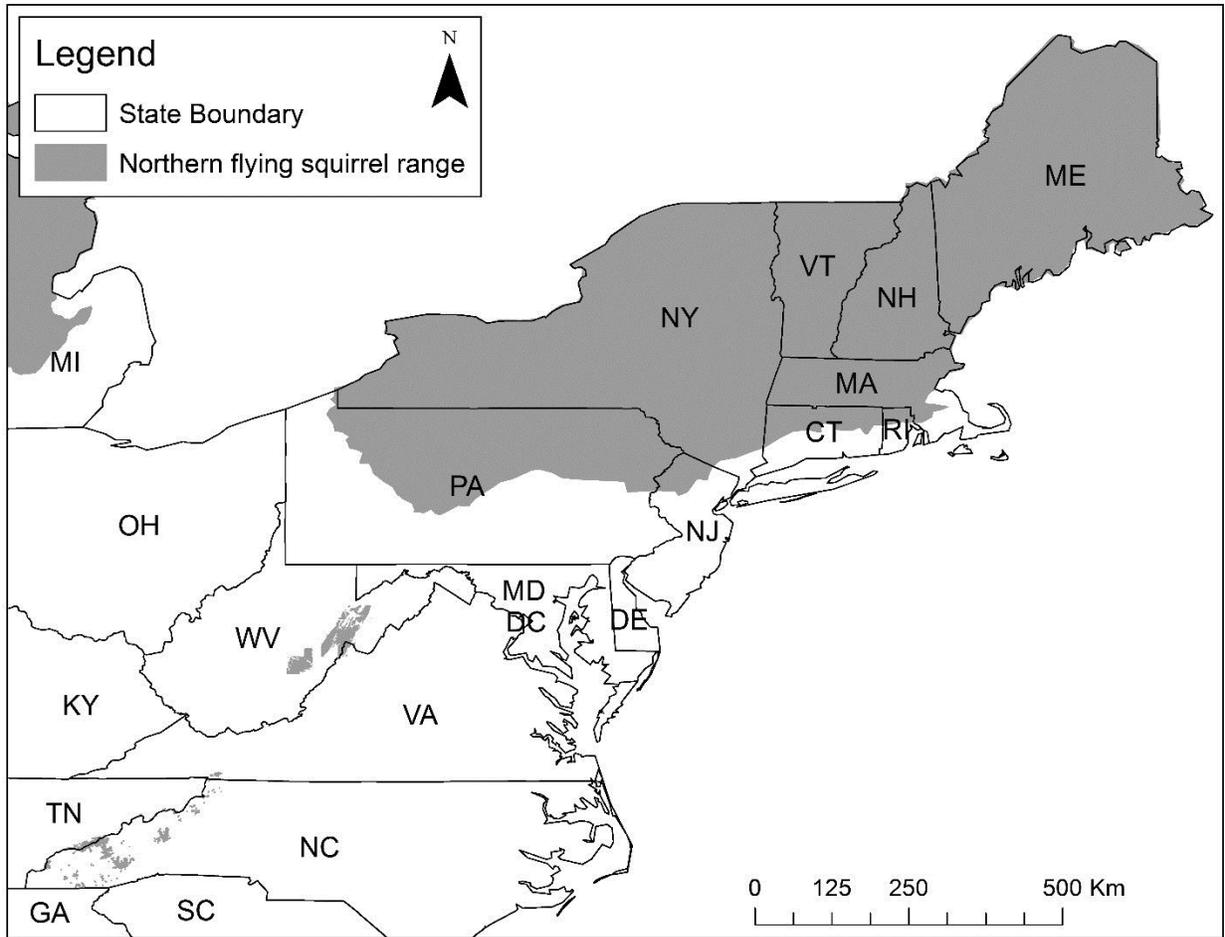


Figure 1.1. Geographic range of the northern flying squirrel (*Glaucomys sabrinus*) in the eastern United States.

Chapter 2: Comparison of survey techniques on detection of Northern Flying Squirrels: Live traps, camera traps, and ultrasonic acoustics

Abstract

The ability to detect a species is central to the success of monitoring for conservation and management purposes, especially if the species is rare or endangered. Traditional methods such as live capture can be labor intensive, invasive, and produce low detection rates. Technological advances and new approaches provide opportunities to more effectively survey for a species both in terms of accuracy and efficiency than previous methods. I conducted a pilot comparison study of one traditional technique (live trapping) and two novel non-invasive techniques (camera trapping and ultrasonic acoustic surveys) on detection rates of the federally endangered Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) in occupied habitat within the Roan Mountain Highlands of North Carolina. In 2015, I established 3 5x5 live trapping grids (6.5 ha) with 4 camera traps and 4 acoustic detectors systematically embedded in each grid. All 3 techniques were used simultaneously over 2 4-day survey periods. I compared techniques by assessing probability of detection (POD), latency to detection (LTD; i.e., number of survey nights until initial detection), and survey effort. Acoustics had the highest POD (0.37 ± 0.06 SE), followed by camera traps (0.30 ± 0.06), and live traps (0.01 ± 0.005). Acoustics had a significantly lower LTD than camera traps ($p=0.017$), where average LTD was 1.5 nights for acoustics and 3.25 nights for camera traps. Total field effort was highest with live traps (111.9 hrs) followed by acoustics (8.4 hrs) and camera traps (9.6 hrs), although processing and examination for data of non-invasive techniques made overall effort similar among the 3 methods. This pilot study demonstrates that both non-invasive methods are a better rapid-assessment detection technique for Carolina northern

flying squirrels than live traps. However, determining seasonal effects between survey techniques and further development of protocols for both non-invasive techniques is necessary prior to widespread application in the region.

Introduction

The ability to estimate the occupancy of a species is critical to effective conservation and management of wildlife. Understanding population trends, dynamics and habitat associations, including changes in species occupancy rates, allows managers to determine the effects of habitat condition, habitat management and other conservation measures (e.g., harvest restrictions, ecological restoration, invasive species management) on species distribution and viability. However, to effectively estimate occupancy, reliable techniques to detect target species are required (MacKenzie et al. 2002, Pollock et al. 2002, Silveira et al. 2003), as inefficient techniques may increase sampling error due to low probability of detection (Gu and Swihart 2004). Various techniques can produce differing estimates of detection (e.g., Gompper et al. 2006, Hackett et al. 2007, Vine et al. 2009, Coleman et al. 2014a), that in turn may influence how a species or its associated habitat is managed. This may especially be true for rare or cryptic species, where underestimation of occupancy resulting from the use of certain sampling techniques might fail to detect a species when it is present (Gu and Swihart 2004), potentially providing false negative data on distribution, population status or trends (Thompson 2004). Techniques that improve estimates of detection probability allow for more effective monitoring and conservation of rare species (McDonald 2004).

For small mammals, traditional methods typically include capture-recapture or direct take (i.e., kill traps), to determine demography and population density (Carey et al. 1991, Manley et al.

2006, Sikes and Gannon 2011). However, traditional methods can be labor intensive (Villette et al. 2016) and can result in no or low capture rates for sensitive, threatened or endangered species, especially when those species are cryptic in habit (de Bondi et al. 2010, McDonald et al. 2013). Additionally, these methods are invasive, directly affecting animal welfare, inducing stress, or directly affecting population via trap-induced mortality (O'Farrell et al. 1994, Place and Kenagy 2000, Fletcher and Boonstra 2006, Sullivan and Sullivan 2013). Technological advances have made non-invasive options available to survey small mammal occupancy in the wild, including camera trapping (Di Cerbo and Biancardi 2013, Villette et al. 2016), and ultrasonic acoustics (Kalcounis-Rueppell et al. 2006).

Over the past 2 decades, camera trapping has been used widely on large and medium carnivores, ungulates and raptors (Karanth and Nichols 1998, Silver et al. 2004, Roberts et al. 2006, Kelly et al. 2008, Jachowski et al. 2016), but has not received widespread use for monitoring small mammals (de Bondi et al. 2010, Mills et al. 2016). However, camera traps were successfully employed to monitor arboreal mammal use of road crossing structures (Goldingay et al. 2011, Kelly et al. 2013*a*) as well as surveys for cryptic and rare species (e.g., Taylor et al. 2014; McDonald et al. 2015*a, b*).

Small mammals (Rodentia, Soricomorpha) are known to emit ultrasonic vocalizations (Sales and Pye 1974, Wilson and Hare 2004, Kalcounis-Rueppell et al 2006, Soltis et al. 2012, Gilley 2013, Zsebök et al. 2015). Commercial ultrasonic detectors developed to monitor bat activity also are able to record ultrasonic vocalizations produced by small mammals (Kalcounis-Rueppell et al. 2006, Gilley 2013). Bat researchers have used ultrasonic acoustics for decades (Griffin 2004), and have developed methodology to account for how habitat type, location and orientation of detectors, and weatherproofing influence species detection (Weller and Zabel 2002,

Patriquin et al. 2003, Limpens and McCracken 2004, Pettersson 2004, Britzke et al. 2010, Coleman et al. 2014b). Researchers also have developed methods and software to identify recorded bat echolocation pulses to the species level (Parsons 2004, Britzke et al. 2011). The limitations and assumptions of data collected from ultrasonic acoustic surveys are well established for bat studies (Hayes 1997, 2000; Broders 2003; Gannon et al. 2003; Britzke et al. 2013; Coleman et al. 2014a), and can be adapted to other species. The development of call libraries from captive and wild populations of small mammal species allows for utilization of this method as a small mammal monitoring technique in the field. However, to date there has been limited use of small mammal monitoring via ultrasonic acoustics and few assessments of how these types of monitoring compare to more traditional techniques.

The recent discovery and characterization of ultrasonic calls of North American flying squirrels (*Glaucomys spp.*, Gilley 2013) has increased the potential for use of ultrasonic acoustics to monitor these species in the wild. The Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*), a federally endangered subspecies, is a secretive, nocturnal gliding mammal found in disjunct, high-elevation “sky islands” of red spruce (*Picea rubens*) – Fraser fir (*Abies faseri*), eastern hemlock (*Tsuga canadensis*), and adjacent northern hardwood forests with a boreomontane conifer component in the southern Appalachian Mountains (USFWS 1985, Pyane et al. 1989, Weigl et al. 1999, Hackett and Pagels 2003, Kelly et al. 2013a; Ford et al. 2014, 2015). Similar to northern flying squirrels in boreal forest habitats of Canada and the northern United States, the southern Appalachian subspecies is traditionally surveyed using live-trapping and artificial nest box monitoring (Carey et al. 1991, Loeb et al. 1999, Ford et al. 2015). However, traditional methods are labor intensive and result in extremely low capture rates for this subspecies (USFWS 1990, Reynolds et al. 1999, Weigl et al. 1999, Hughes 2006, Ford et al. 2015). In multiple areas

of North Carolina, Tennessee, and Virginia, efforts to document Carolina northern flying squirrel presence via trapping or nest box monitoring often have been unsuccessful or have taken years of surveys, even if habitat quality and quantity was similar to known occupied areas (Weigl et al. 1999; C. Kelly, unpublished data; R. Reynolds, Virginia Department of Game and Inland Fisheries, personal communication). Capture rates for these long-term nest box monitoring programs are low: 5.8% and 8% of box checks produced captures in North Carolina and Virginia, respectively (Reynolds et al. 1999; C. Kelly, unpublished data). Past nest box surveys have been focused on lower-elevation or easily accessible sites as efforts to survey higher elevation sites or more remote sites were logistically difficult (Ford et al. 2015; C. Kelly, unpublished data). In addition to being labor-intensive during installation and subsequent monitoring, nest boxes typically have lag-time of several years before utilization by flying squirrels occurs and produce low-recapture rates, making population estimates difficult (Ford et al. 2010). Moreover, capture rates from live-trapping tend to be lower than nest boxes, even in high quality habitat (Weigl et al. 1999; C. Kelly, unpublished data; C. Diggins, unpublished data). To effectively manage for this rare subspecies, a more efficient and reliable method of monitoring than traditional techniques needs to be established.

Within designated recovery areas in western North Carolina (USFWS 1990), a preliminary field study using ultrasonic acoustic surveys provided data to confirm the Carolina northern flying squirrel can be differentiated from the common and more widespread southern flying squirrel (*G. volans*) in the region (Gilley 2013). Ultrasonic acoustic surveys determined the presence of 4 new populations of Carolina northern flying squirrels in areas that were unsuccessfully surveyed using traditional methods over the previous three decades (Kelly et al. 2013b). Of the 4 new populations, two were confirmed with subsequent camera trap surveys (Kelly et al. 2013b), highlighting the

potential use of this technique to monitor Carolina northern flying squirrels. Although preliminary data shows that monitoring flying squirrels with ultrasonic acoustics is a promising technique, no research has been done to compare this novel technique directly to other survey techniques. The purpose of our study was to determine how detection probability of two non-invasive techniques (ultrasonic acoustics and camera traps) compared to traditional live-trapping techniques for Carolina northern flying squirrels and to assess the advantages and disadvantages of each method and applicability for wildlife managers.

Study Area

My study took place in the Blue Ridge subdivision of the southern Appalachian Mountains physiographic province on Roan High Bluff (latitude 36°5.65'N, longitude 82°8.49' W) in the Roan Mountain Highlands, Pisgah National Forest, Mitchell County, North Carolina. I conducted my surveys in pure red spruce – Fraser fir forests above 1,800 m. The study site is considered high quality Carolina northern flying squirrel habitat (Ford et al. 2007, 2015) and recent and concurrent capture records of this subspecies were obtained at the site from live trapping and telemetry data (C. Diggins, unpublished data). The elevation of my study site excluded potential captures of southern flying squirrels, which cease to be sympatric with Carolina northern flying squirrels at the spruce-northern hardwood ecotone at approximately 1,370 m and above. Although the two species can be differentiated with high degree of certainty using ultrasonic acoustics (Gilley 2013), in my experience, the species can be still be relatively difficult to differentiate using camera traps unless photographs are of very high quality (C. Kelly, unpublished data). Therefore, I focused my study in an area where southern flying squirrels were excluded by high elevation and unsuitable habitat (Weigl et al. 1999).

Methods

To survey Carolina northern flying squirrels, I placed 3 survey grids at elevations between 1,815 m – 1,900 m on Roan High Bluff (Figure 2.1). We established 5 x 5 live-trapping grids (N = 25 traps/grid) with 50 m between each trap (6.25 ha). Trapping grids were modelled after Carey (2000) and are similar to other trapping efforts for this subspecies in the southern Appalachians (C. Kelly, unpublished data). I systematically imbedded 4 camera traps and 4 ultrasonic acoustics detectors within each grid (Figure 2.1), so camera traps and acoustics were proportionally surveying similar areas of each grid, reducing spatial variation on detection rates. The number of survey grids was constrained to the number that could be completed by a 2-person crew and was similar to past trapping efforts in the region. I placed grids ≥ 100 m apart from each other. Prior to the start of my survey, I pre-baited live traps and camera traps for a 4-day period. During the survey, grids were sampled for two 4-day periods, using all techniques simultaneously to reduce temporal variation on detection rates, with 3 days of no surveys between trapping periods. I conducted surveys between late May and early June 2015. My methods for live trapping, camera trapping, and acoustic surveys were approved by the Virginia Tech Institutional Animal Care and Use Committee (permit #11-120-FIW).

For live trapping (hereafter “live traps”), I used Tomahawk 201 traps (14 x 14 x 41 cm, Tomahawk Live Trap Co., Hazelhurst, Wisconsin). I set traps 1.5 m up on the bole of trees to increase captures of flying squirrels (Loeb et al. 1999). I covered traps with plastic sheeting and duct tape and placed polyfill batting into each trap to reduce potential trap stress and hypothermia. Each trap was baited with a slice of apple covered in a peanut butter-molasses-bacon grease-oatmeal mixture. Fresh bait was placed in all traps when I opened traps for each survey night. I opened traps at dusk and checked them at dawn. I closed all traps between sunrise and sunset to

reduce the capture of diurnal species such as red squirrels (*Tamiasciurus hudsonicus*). I identified Carolina northern flying squirrels to species by gray base color of the ventral pelage and hind foot measurements >34mm (Wells-Gosling and Heaney 1984). I determined sex, age, and weight of all captured individuals. All captures were marked with a uniquely numbered ear tag (No. 1005-1, National Band and Tag Company, Newport, Massachusetts) to aid in the identification of recaptured individuals. I released all individuals at the site of capture.

For camera trapping (hereafter “camera traps”), I used Bushnell 6MP Trophy Trail Cameras (Bushnell Corporation, Overland Park, Kansas). Cameras were set 1.5 m up on the bole of a tree. Each camera was baited with 332 g peanut butter suet cake placed in a metal suet basket (12.7 x 14 x 4.4 cm; C & S Products Co., Fort Dodge, Iowa). I used peanut butter suet for camera traps instead of the peanut butter-molasses-bacon grease-oatmeal mixture. Preliminary camera trap trials using this technique on southern flying squirrels showed that peanut butter suet was more persistent over multiple survey nights and attracted less non-target species (i.e., black bears, *Ursus americanus*, and raccoon, *Procyon lotor*), which are show to have effects on squirrel detection rates (C. Diggins, unpublished data). Each metal suet basket was nailed 1.5 m up on the bole of a tree directly opposite of the camera trap. To ensure proper identification of squirrels, I placed bait between 1-2 m from the camera trap tree. Due to the flying squirrels’ quick movements, I used a 3-photo capture every 30 sec to ensure identifiable pictures of squirrels. I placed masking tape over the flash of the camera to reduce excessive glare, making species easier to identify. All cameras were re-baited between the two sampling periods.

For ultrasonic acoustics (hereafter “acoustics”), I used Pettersson D500x ultrasonic detectors (Pettersson Eleckrontik AB, Uppsala, Sweden). The D500x is a full-spectrum detector with a directional microphone that records in real-time and stores call files onto CF cards allowing

for multi-day passive sampling. Each detector was placed in a steel ammunition box (30.5 x 15.6 x 19 cm; model MA21; BLACKHAWK!, Overland Park, Kansas) modified with a 35° elbow polyvinyl chloride tube to provide weatherproofing for the detector (Britzke et al. 2010) and to protect the detector from black bear damage. I attached detectors 1.5 m up on the bole of a tree using bungee cords and pointed the detector in the direction with the least clutter (i.e., vegetation), allowing better call recording quality (Weller and Zabel 2002). Detectors were oriented in the same direction for both sampling periods. I did not place bait for acoustic detectors. I programmed detectors to start recording at civil twilight and cease recording 30 min after sunrise.

Survey Effort

I conducted an effort analysis of all three techniques in the field over the course of my 8 day survey, as well as data entry and analysis of each technique. Additionally, I calculated cost of nightly detection effort for each technique by determining field effort costs for nightly detection rates per unit. For live traps, I determined the number of technician hours to establish and remove trap grids, open and bait traps at dusk, check traps the following morning, and enter trapping data. For camera traps, I calculated the number of technician hours to prepare camera traps for deployment, deployment and removal of camera traps from the field, and formatting of SD cards for data downloading. All camera trap images obtained during the survey were analyzed visually. For acoustics, I calculated number of technician hours to prepare detectors and acoustic equipment for deployment, acoustics deployment in and removal from the field, and configuration of CF cards for data downloading. Prior to data analysis, all acoustic data were filtered through SonoBat Batch Scrubber 5.4 (DND Design, Arcata, California) to remove the majority of files that did not contain acoustic calls (i.e., noise files from wind or rain). Maintained files included calls from flying

squirrels as well as bats, insects, *Peromyscus* spp., and other animals. I then sorted all maintained call files visually for identification following Gilley (2013).

One technician hour was calculated at \$15 US (Coleman et al. 2014a). For both camera traps and acoustics, I did not count download time of data towards my cost or effort analysis because data downloading did not require full attention of technicians (e.g., data download may take 45 min, but configuration took the technician ≤ 5 min). Additionally, I did not account for the time it took to filter acoustic data towards cost or effort analysis because it did not require technician attention during the process (i.e., it could take up to 5 hrs for data from one acoustic unit to filter through SonoBat Batch Scrubber, but only took the technician ≤ 2 min to initially run the data in the program). Data entry for live trap data was calculated in total hours, whereas data analysis for camera traps and acoustics was determined as average number of hours it took to analyze a single gigabyte of data, as entry rates varied between individual observers. For acoustic data, Carolina northern flying squirrel call files were visually and aurally identified in SonoBat 2.9.8 (DND Design, Arcata, California) using captive and wild flying squirrel call libraries and a double observer technique, where calls were identified by trained observers and confirmed by an experienced observer (C. Diggins) to assure bat call files were not misidentified as squirrels. I included these analysis hours for acoustics in my overall effort calculation.

Data Analysis

To compare detection rates of each method, I assessed probability of detection (POD) and latency to initial detection (LTD; the number of survey nights until an initial detection of a species occurred at a survey site; Gompfer et al. 2006). I determined POD by analyzing data using an occupancy modeling framework, where ψ is the probability of a species occupying a defined area and p is the probability of detecting that species given it is present (MacKenzie et al. 2005b).

Occupancy modeling uses a maximum likelihood approach to estimate the probability of detecting a species at least once given that it is present at the site, whereby assuming the likelihood of detecting that species does not change over the course of the survey (MacKenzie et al. 2002). To determine how the different techniques might influence flying squirrel detection, I used type of detection (i.e., live traps, camera traps, or acoustics) to determine differences in detection probability among the different methods, while keeping ψ constant. I conducted the analysis in program PRESENCE 8.3 using a single-season model (Hines 2014).

To determine which technique was more efficient in determining presence of Carolina northern flying squirrels, I assessed LTD (Gompper et al. 2006) for each technique. Because live traps were checked daily and both camera and acoustic data had time stamps, I was able to calculate comparable LTD rates for all techniques. Additionally, I calculated a catch per unit effort of each method by estimating the percentage of survey points of each live trap, camera trap, or acoustic detector that had at least one detection over the course of the study, as well as the average number for nights each unit detected flying squirrels over the course of the survey (unit capture nights/survey). Accordingly, the most effective survey technique should result in higher POD and lower LTD (Gompper et al. 2006), with a higher number of survey points detecting flying squirrels at least once over the course of the study. I analyzed differences in LTD and catch per unit effort using non-parametric Kruskal-Wallis (K-W) test and Wilcoxon Rank-Sum (WR-S) test in Program R 3.1 (R Development Core Team 2015).

Results

I accumulated a total of 572 live trap nights (corrected for sprung traps), 96 acoustic survey nights, and 96 camera trap nights. I captured 3 individual Carolina northern flying squirrels and 2

re-captures with live-trapping ($N = 0.008$ captures/trap night). I only captured flying squirrels in 1 of the 3 trapping grids and never captured more than 2 squirrels in a single trap night. Camera traps collected a total of 27GB (32,944 images) of data with 318 images of Carolina northern flying squirrels ($N = 0.219$ captures/trap night). I recorded a total of 148.1GB (53,111 sound files) of data from acoustic surveys. After I removed noise files using software filtering, I analyzed 17.2GB (6,172 sound files) of data and identified 438 Carolina northern flying squirrel call files ($N = 0.313$ captures/trap night). I classified the majority of call files as trills (71.6%) or whistles (38.1%; Gilley 2013). Other call type classifications included ‘barks/yelps’ and ‘crows’ (L.M. Gilley, Mars Hill University, unpublished data).

Comparison Surveys

From occupancy modeling, I found that POD varied with survey technique. Overall, the average POD for acoustics was higher than camera traps (0.37 ± 0.06 SE and 0.30 ± 0.06 , respectively), however detection rates for these two methods are comparable (Figure 2.2). Both methods had substantially higher POD than live traps (0.01 ± 0.005 , Figure 2.2). Although at least one acoustic and one camera trap unit detected flying squirrels on the first survey night, LTD for acoustics was significantly lower than LTD for camera traps (WR-S test: $W = 111.5$, $P = 0.018$). The average LTD for acoustics was 1.5 survey nights (range = 1-6) and for cameras was 3.25 survey nights (range = 1-5).

Additionally, 75% of acoustic survey points detected flying squirrels the first survey night compared to only 8.3% of camera traps survey points. It took a minimum of 5 survey nights before 75% of camera trap survey points detected a flying squirrel. Acoustics detected flying squirrels at 83.3% of survey points, whereas camera traps and live traps detected flying squirrels at 75% and 6.7% of survey points, respectively. Over the 8 days of surveying, the average capture nights per

individual unit were: live traps at 0.07 ± 0.03 unit capture nights/survey (range = 0-1), camera traps at 1.75 ± 0.49 unit capture nights/survey (range = 0-5), and acoustics at 2.5 ± 0.48 unit capture nights/survey (range = 0-5). Both camera traps and acoustics had significantly higher unit capture nights/survey versus live traps (K-W test: $\chi^2 = 53.22$, $df = 2$, $P = 0.000$), whereas unit capture nights/survey for acoustics and camera traps did not significantly differ (WR-S test: $W = 90.5$, $P = 0.289$).

Survey Effort

The three survey techniques varied in field collection and laboratory processing effort. For combined effort, acoustics was the least expensive technique (\$1,522.50), followed by live traps (\$1,698), and camera trapping (\$1,872), although all three methods were comparable in price. Cost of field effort was higher for live traps and cost of lab effort was higher for camera traps and acoustics (Figure 2.3). Laboratory effort for average data analysis effort for acoustics and camera traps was 93.1 hrs (4.2 ± 0.9 hrs/GB) and 115.2 hrs (5.4 ± 0.4 hrs/GB), respectively, whereas data entry for live traps was 1.3 hrs. Total field effort for acoustics and camera traps was 8.4 hrs and 9.6 hrs, respectively, compared to 111.9 hrs of field effort for live traps.

For the amount of field effort exerted for live traps, an additional 13.3 acoustic sites or 11.6 camera trap sites could have been surveyed. Field effort for capture/trap night for each technique was as follows: acoustics = 0.28 hr/capture effort (\$4.20 technician cost/capture effort), camera traps = 0.46 hr/capture effort (\$6.90 technician cost/capture effort), and live traps = 22.38 hr/capture effort (\$335.70 technician cost/capture effort).

Discussion

Survey techniques used to monitor rare and elusive species require balancing survey effectiveness and cost efficiency (Silveira et al. 2003). Rare and cryptic small mammals, such as the Carolina northern flying squirrel, are inherently difficult to monitor and more effective survey methods may be available with new technologies versus traditional techniques. My preliminary study highlights the potential of two novel non-invasive techniques, acoustics and camera traps, compared to live traps. Although POD are comparable between the two methods, I found that acoustics has a lower LTD than camera traps, making this technique more conducive for rapid assessment surveys. Additionally, the majority of acoustic units detected flying squirrels the first survey night versus the several nights that camera traps required to reach the same level of effectiveness. A possible explanation may be a lag time between bait placement at camera traps and detection of bait by flying squirrels, although pre-baiting camera trap sites should have alleviated this lag time. I do not think baiting for camera traps and live traps influenced detection rates for acoustics. Because flying squirrels are a gregarious species (Wells-Gosling and Heaney 1984), individuals may frequently produce ultrasonic calls to communicate with conspecifics, potentially making calls easier to detect. A regional pilot study for Carolina northern flying squirrels in high-quality habitat used acoustic in areas that were not pre-baited or had other baited techniques deployed simultaneously. During this study, acoustics had a POD of 0.36 over 8-10 survey nights (W.M. Ford, unpublished data), similar to the POD and survey length of my study.

Live traps had the highest LTD and lowest POD compared to the other two techniques, highlighting the inefficiency of traditional techniques versus the effectiveness of non-invasive techniques to obtain detection/non-detection data, as has been observed with numerous other wildlife species (e.g., Hackett et al. 2007, Coleman et al. 2014a, McDonald et al. 2015a). Although

my study was of a shorter duration than most live trapping studies with in the southern Appalachians, captures/trap night were similar to other regional live trap efforts (Hackett 2002, Menzel et al. 2006; C. Diggins, unpublished data). Additionally, due to limited funding and personnel, my study was only conducted during the spring season. Seasonality is known to influence live trap capture rates of flying squirrels (Vernes 2004) and may have influenced my ability to capture squirrels in live traps since populations are assumed to be lower in the spring vs. later in the year, although this may not be true for the southern Appalachians (Reynolds et al. 1999). Additionally, cost per capture effort for live traps was 79.9 and 46.6 times more expensive than acoustics and camera traps, respectively. Although capture rates were low and field effort was greater than either of the non-invasive methods, live traps may allow biologists to determine population estimates from capture-recapture data and demographic information from individual captures (i.e., sex, age, reproductive condition, etc.). Because it is not currently possible to differentiate individuals using acoustics or camera traps, abundance and density estimates are not obtainable with these techniques (Hayes 2000). However, for rare species, monitoring for spatial and temporal changes in occupancy may be more effective for understanding long-term trends as species detection data may be a surrogate for abundance or population size (MacKenzie et al. 2005a). Both camera traps and acoustics also require less field effort than live traps and have higher POD and LTD, increasing the feasibility and efficiency of large-scale occupancy surveys. Large-scale efforts could show regional trends across habitat patches, as occupancy-based monitoring programs can identify habitat patches with high-levels of persistence (MacKenzie 2005).

Nonetheless, a serious drawback to both acoustics and camera traps is the large amount of data that potentially can be generated, increasing data management and requiring data to be

analyzed visually and aurally, which can be extremely time-consuming. However, automated methods for both techniques may potentially reduce data analysis to a fraction of the time. Acoustic data from flying squirrel vocalizations could be analyzed using similar automated systems as have been developed for bats (Parsons and Jones 2000, Adams et al. 2010, Britzke et al. 2011), making the potential to analyze large datasets less time-consuming. Automated systems for analysis of flying squirrel calls using a machine learning technique are in the initial testing stage (A. O'Connor, Georgia Institute of Technology, unpublished data), but development is in its infancy and validation is needed prior to widespread use within the southern Appalachians and beyond. Within the region occupied by Carolina northern flying squirrels, a developed discriminant function analysis separates known northern flying squirrel from known southern flying squirrel calls with >96% accuracy (Gilley 2013), highlighting the potential ability of quantitative methods to discriminate between species in the wild. From a visual examination, some field recorded Carolina northern flying squirrel calls, particularly the whistle vocalization, can be similar to echolocation pulses of hoary bats (*Lasiurus cinereus*) in call shape, call lengths, and frequency (typically between 20-30 kHz; Thomas et al. 1987). When shape, call length, and frequency made call identification via spectrogram visually difficult, I was able to qualitatively differentiate the calls by ear, as sound quality of bat and squirrel calls differ and are easy to distinguish.

Automated software for identification of mammalian species in camera trap pictures are being developed (Yu et al. 2013), but are not currently commercially available. Due to similarities in their size, distinguishing between Carolina northern flying squirrels and southern flying squirrels with visual analysis is difficult (C. Kelly, unpublished data) and overall misclassification rates and observer bias have not yet been evaluated for flying squirrels. Because of their morphological similarities, automated systems' ability to differentiate between the two species

may be limited (R. Kays, North Carolina Museum of Natural History, personal communication) as conclusive differentiation requires examination of ventral body hair coloration and hind foot length (Wells-Gosling and Heaney 1984). Because the two species' ranges only overlap in southeastern Canada, New England, Great Lake states, and the southern Appalachians along the deciduous forest-boreal forest latitudinal or elevation tension zone, camera traps may be suitable for determining occupancy of either species in parts of the range where the two squirrels are not sympatric. Currently, I do not recommend the use of camera traps for monitoring in areas where the species are sympatric until better methodology for differentiating species can be developed, as misidentification of the Carolina northern flying squirrel could influence forest management regionally, leading to inappropriate actions where the subspecies is actually present or cessation of certain management in areas with false positive identifications.

Combined field and lab effort for all 3 methods were comparable over the course of my study. However, my study was on a small spatial and temporal scale and should be regarded as a pilot effort. The geographic separation of the sky islands inhabited by the Carolina northern flying squirrel, including lack of roads, steep terrain, and presence of designated wilderness areas, would make field work for large-scale live trapping efforts more costly and difficult, whereas large-scale efforts would increase the amount of data analysis for the other 2 methods. The amount of data analyzed for acoustics was reduced by 88.3% by using bat call analysis software to remove noise files. The validation of recently developed automated software for analyzing flying squirrel calls would drastically reduce lab effort for this technique, making it the most efficient and effective technique compared to camera traps and live traps. Additionally, more work is required relative to assessing differences among camera and detector type. For example, the capabilities of different brands of ultrasonic detectors and microphones as well the type of ultrasound transformation used

(e.g., frequency division, time-expansion or full spectrum) vary (Adams et al. 2012). Similarly, camera type, photo capture rate, photo quality, camera placement, and bait type may influence detection rates and species identification (Kelly and Holub 2008, Paull et al. 2011, Glen et al. 2013).

In regions with large numbers of rare, threatened or endangered species such as the biologically rich southern Appalachians, the ability of state and federal agencies and NGOs to devote large amounts of time and effort conducting field surveys for single species is limited – particularly if the species has a low detection rate with traditional techniques. Improvement of rapid assessment techniques could provide detection/non-detection data to assist in discovering unknown or small populations and the ability to monitor long-term trends in species occupancy. I believe this study shows promise for ultrasonic acoustics and camera traps as rapid assessment techniques for surveying the Carolina northern flying squirrel, as well as the recently delisted Virginia northern flying squirrel (*G. s. fuscus*) in the central Appalachians and the petitioned San Bernardino flying squirrel (*G. s. californicus*) in the Pacific Southwest. However, my study was on a small spatial and temporal scale and should be regarded as a pilot effort with inference to the southern Appalachians. Seasonality and geographic location (i.e., Pacific Northwest vs. Black Hills vs. southern Appalachians) may influence the effectiveness of the new techniques vs. traditional techniques and should be further examined. For example, densities of Carolina northern flying squirrels may be lower than in other parts of the northern flying squirrel's range (i.e., Pacific Northwest, Canada), where live trapping may be more effective than in the southern Appalachians. Also, minimal sampling intensity to determine occupancy efficiently using each technique needs to be further explored and will most likely vary seasonally and geographically. Additionally, protocol for both novel techniques needs further development prior to widespread use in the field.

These non-invasive techniques in conjunction with traditional nest box monitoring or live trapping may provide a better understanding of spatial and temporal dynamics of flying squirrel populations across the landscape.

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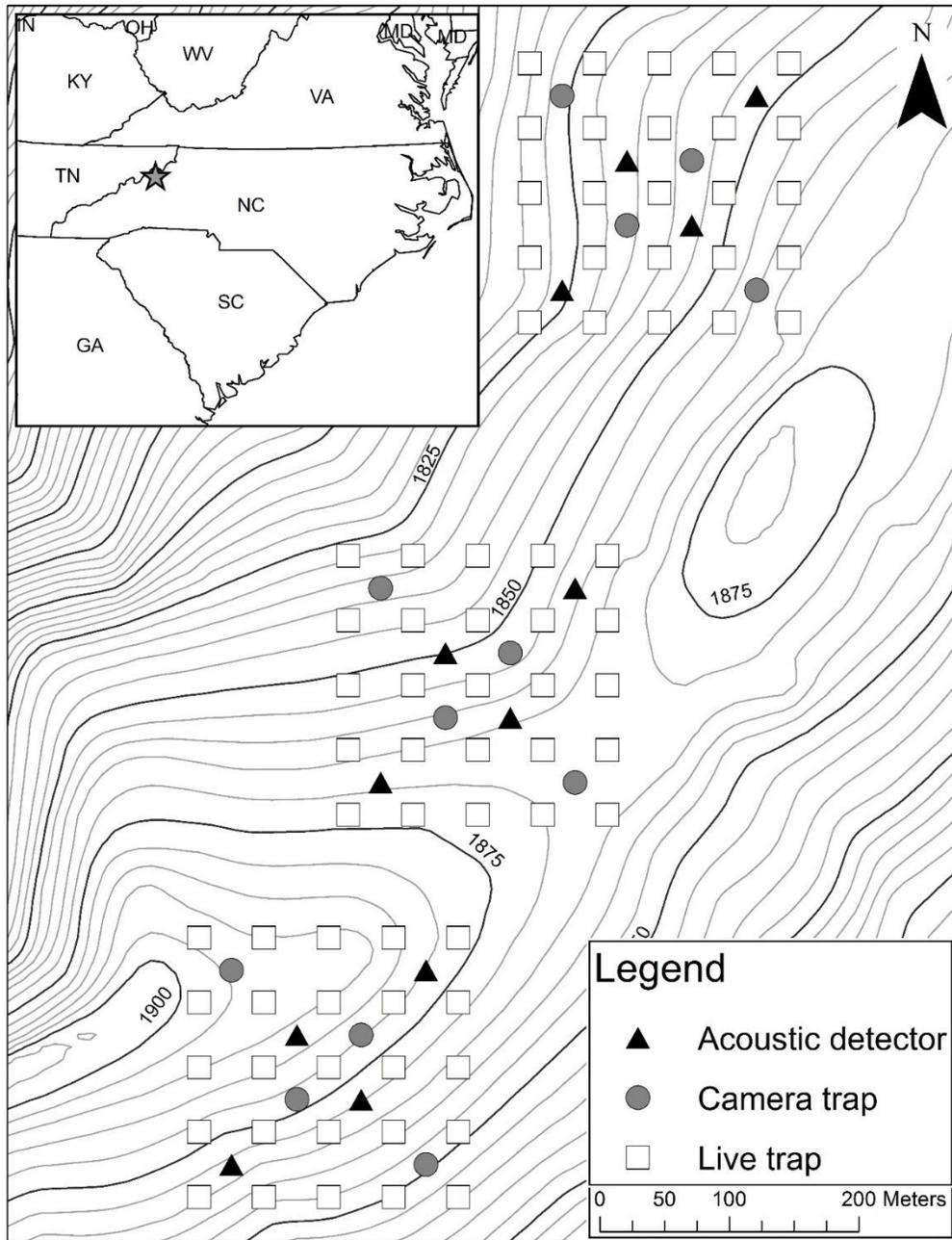


Figure 2.1 Study site for survey technique comparison grids of Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) in a red spruce (*Picea rubens*)-Fraser fir (*Abies fraseri*) stand at Roan High Bluff, Roan Mountain Highlands, Mitchell County, North Carolina. Surveys occurred during late May – early June 2016. Elevation is shown in meters.

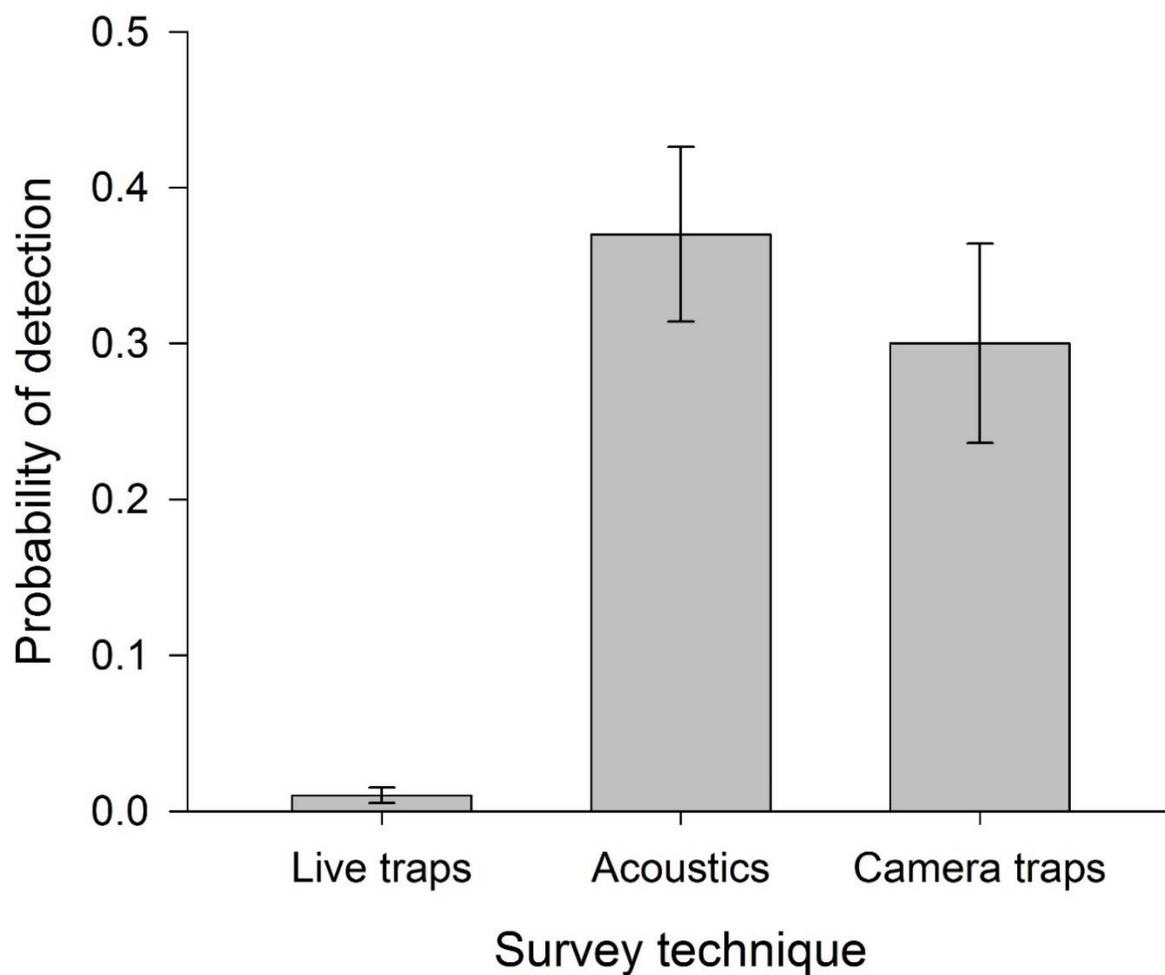


Figure 2.2. Average probability of detection (POD) \pm SE of three survey techniques used to determine presence of Carolina Northern Flying Squirrels (*Glaucomys sabrinus coloratus*) in a red spruce (*Picea rubens*) – Fraser fir (*Abies fraseri*) stand at Roan High Bluff, Roan Mountain Highlands, Mitchell County, North Carolina during late May – early June 2016.

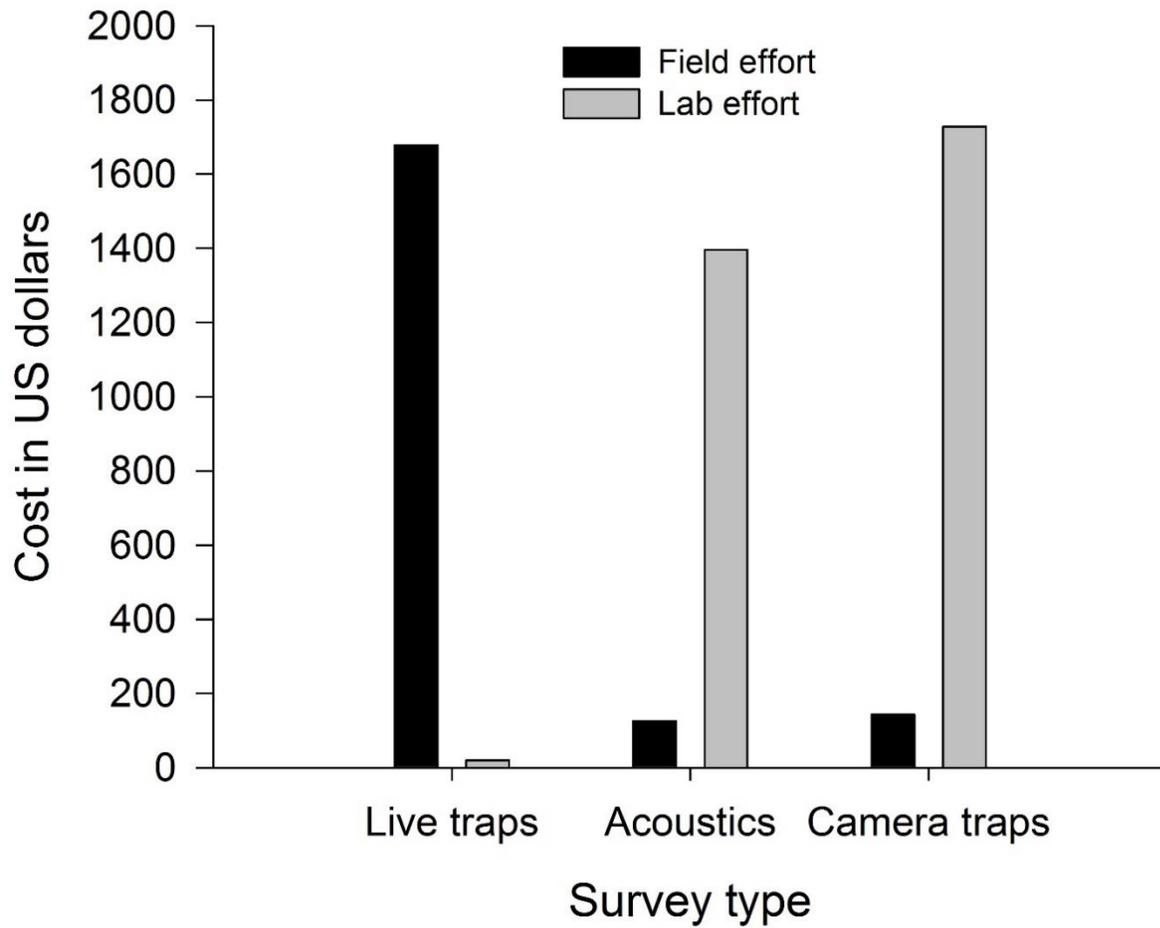


Figure 2.3. Field and lab effort costs for three survey techniques used to determine presence of Carolina Northern Flying Squirrels (*Glaucomys sabrinus coloratus*) in a red spruce (*Picea rubens*)-Fraser fir (*Abies fraseri*) stand at Roan High Bluff, Roan Mountain Highlands, Mitchell County, North Carolina during surveys in late May – early June 2015.

Chapter 3: Microhabitat selection of the Virginia Northern Flying Squirrel (*Glaucomys sabrinus fuscus*) in the central Appalachians

Abstract

The Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*) is a rare Sciurid occurring in the Allegheny Mountains of eastern West Virginia and northwest Virginia. Previous work on this subspecies has confirmed close associations with red spruce (*Picea rubens*) at the landscape- and stand-level in the region. However, ongoing red spruce restoration actions using canopy-gap creation to release single or small groups of trees requires a better understanding of within-stand habitat selection of Virginia northern flying squirrels to assess potential short- and medium-term impacts. To address these questions, I conducted a microhabitat study using radio-collared squirrels in montane conifer and mixed-conifer hardwood stands. I used points obtained from telemetry surveys and randomly generated points within each squirrel's home range to compare microhabitat variables for 13 individuals. I found that Virginia northern flying squirrels preferentially selected plots with conifer dominant overstories and deep organic soil horizons. Squirrels avoided plots with dense red spruce regeneration in the understory in stands with hardwood-dominated overstories – the types of areas targeted for red spruce restoration. I also opportunistically searched for hypogean fungi at Telemetry Points and found three species of *Elaphomyces* during my surveys. My results indicate that microhabitat selection is associated with red spruce dominant forests. Efforts to restore red spruce where hardwoods dominate in the central Appalachians may improve the connectivity and extent of habitat of the Virginia northern flying squirrel.

Introduction

The Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*) is a rare subspecies of northern flying squirrel found in high-elevation montane conifer and conifer-northern hardwood forests in the central Appalachian Mountains of eastern West Virginia and western Virginia (Stihler et al. 1995, Reynolds et al. 1999). The preferred habitat of the Virginia northern flying squirrel is red spruce (*Picea rubens*)-dominated forests (Stihler et al. 1995, Menzel et al. 2006, Ford et al. 2007). This forest type was reduced from estimated >200,000 ha in the late 1800s to less than 20,000 ha at present (Newins 1931, Korstian, 1937, Schuler et al. 2002, Byers et al. 2013) due to industrial clear-cut logging and subsequent fires during the early to mid-20th century (Clarkson 1964). Since that time, habitat has been further degraded by acid precipitation (Edgar and Adams 1992) and introduced exotic pests (i.e., balsam woolly adelgid, *Adelges picea*) and is presumed to be highly vulnerable to future anthropogenic climate change (Beane and Rentch 2015). Because of reduced extent and degradation of this forest type, Noss et al. (1995) listed the red spruce-dominated forests of the region as one of the most critically endangered forested ecosystems in the United States.

Multiple studies indicated a positive relationship between the Virginia northern flying squirrel and red spruce forest at the landscape- (Odom et al. 2001, Menzel et al. 2005, Ford et al. 2010) and stand-level in the region (Menzel et al. 2004, 2006; Ford et al. 2007). In the Appalachians, past research indicated that denning habitat often has been associated with the northern hardwood-conifer ecotone (Weigl et al. 1999, Hackett and Pagels 2003). However, radio-collared Virginia northern flying squirrels in the central Appalachians and endangered Carolina northern flying squirrels (CNFS; *Glaucomys sabrinus coloratus*) in the southern Appalachians use red spruce as foraging habitat more than expected based on their availability (Menzel et al. 2006,

Ford et al. 2007, Ford et al. 2014) as important food items for the Virginia northern flying squirrel are less abundant in northern hardwood forests (Loeb et al. 2000). Moreover, recent research suggests these subspecies readily den in red spruce-dominated stands (Ford et al. 2010, 2014; Diggins et al. 2015). Habitat use of this subspecies has been studied on larger scales, but within-stand habitat associations are not well-defined. Ford et al. (2004) assessed habitat variables in Virginia northern flying squirrel occupied and unoccupied sites as determined by nest boxes and trapping. However, for the Virginia northern flying squirrel, many nest box locations are biased by placement in the montane conifer-northern hardwood ecotone where denning was thought to exclusively occur as a function of regulatory survey needs on public lands (Ford et al. 2010). Additionally, due to low capture rates of this subspecies (Terry 2004, Menzel et al. 2006, Ford et al. 2010), non-presence at nest box and trapping sites does not necessarily specify unoccupied habitat, which may have limited the ability of Ford et al. (2004) to distinguish which within-stand habitat variables influence the Virginia northern flying squirrel occupancy.

All subspecies of northern flying squirrels are preferential mycophagists throughout their distribution (Maser et al. 1985, Maser et al. 2008). The fruiting bodies of hypogeous fungi (i.e., truffles) are subterranean and rely on small mammals, such as northern flying squirrels, for dispersal throughout the forest (Fogel 1976, Fogel and Trappe 1978, Maser et al. 1978, Johnson 1996). Hypogeous fungi form an obligatory symbiotic relationship with the roots of certain tree species, aiding host trees in absorption of nutrients and water from the soil (Harley and Smith 1983), making this relationship critical to tree and forest health (Amaranthus 1998). Home ranges of northern flying squirrels are larger than other small mammals that consume hypogeous fungi (e.g., southern red-backed vole, *Myodes gapperi*; Orrock and Pagels 2002), and therefore, they may have a greater ecological role in dispersing fungi across the landscape (Loeb et al. 2000). In

West Virginia, the diet of Virginia northern flying squirrel includes the fruiting bodies of hypogean fungi, however the subspecies has a more generalist diet, including lichens and tree buds (Mitchell 2001). Many hypogean fungi species are associated with roots of specific tree species (Castellano et al. 1999, Trappe et al. 2007) and typically fruit within 2 m of host trees (Fogel 1976). Therefore, location of hypogean fungi host trees may influence the Virginia northern flying squirrel's microhabitat selection.

Although the Virginia northern flying squirrel was listed as endangered under the Endangered Species Act (ESA; USFWS 1985), this subspecies was delisted in 2013 (USFWS 2013) after the determination that the distribution is largely on protected (63% of suitable habitat) on public land (Menzel et al. 2005) and also that occupancy rates were indicative of long-term persistence (as determined from long-term nest box monitoring data; Ford et al. 2010). Paradoxically, the delisting of this subspecies has allowed more flexible forest habitat management strategies to affect habitat improvement than when it was listed, including ecological restoration of red spruce forests using canopy-gap creation (Schuler et al. 2002, Rentch et al. 2007, Rentch et al. 2010, Rentch et al. 2016). Presently, the Virginia northern flying squirrel is considered a focal rationale for red spruce restoration in the central Appalachians, as these activities are designed to increase the extent and connectivity of the squirrel's habitat. However, biologists and land managers are still debating what constitutes the preferred habitat of this subspecies. This discussion centers on determining what habitat type is more important to the Virginia northern flying squirrel: montane conifer forests (i.e., red spruce, and eastern hemlock, *Tsuga canadensis*) or high-elevation northern hardwood forests dominated by yellow birch (*Betula alleghaniensis*), American beech (*Fagus grandifolia*) and other species (i.e., adjacent forest types to red spruce stands that might be designated for restoration treatments).

As a shade-tolerant species, red spruce has been increasing in extent as understory regeneration in some areas where northern hardwoods became dominant following logging and burning in the past 100 years (Mayfield and Hicks 2010, Rollins et al. 2010). This process may reflect signs of natural recovery of red spruce forests (Nowacki et al. 2010), although natural recovery is slow due to the slow establishment characteristics of red spruce (White et al. 1985, Smith and Nicholas 1999, Rentch et al. 2010). Spodic soils with deep organic horizons are correlated with red spruce forests and evidence of these soil types may still be present under forests that regenerated as hardwoods after industrial logging (Nauman et al. 2015). Restoration using single canopy-gap to small group selection cuts permit suppressed mid- and understory red spruce to be released for eventual accession to the overstory at a faster rate than natural recovery through successional trajectories (Rentch et al. 2016). Accelerating the process allows red spruce to regain their status as a foundation species, driving the structure and processes within the ecosystem (Ellison et al. 2005). Prioritization of red spruce restoration sites is being determined from comparing of current red spruce cover to the historic extent (i.e., Korstian 1937, Byers et al. 2013, Nauman et al. 2015), as well as important habitat patches and corridors for other red spruce-associated wildlife (Rentch et al. 2007). Currently, little research beyond anecdotal observations have examined microhabitat selection of the Virginia northern flying squirrel, thereby limiting effective prioritization of future forest restoration actions for this subspecies (Odom et al. 2001, Ford et al. 2004), especially with within-stand restoration treatments, such as single-tree canopy gap creation.

My objective was to determine variables that influence microhabitat selection by the Virginia northern flying squirrel in the central Appalachians. I used squirrel locations obtained from telemetry data of radio-collared individuals and compared microhabitat characteristics for

selected sites versus available sites within a squirrel home ranges. I also sought to determine whether radio-collared individuals are associated with characteristics of montane conifer forests by determining if selected sites occur in areas with deep organic soil horizons, which are growth media for hypogean fungi (Meyer et al. 2005) and also may represent areas where red spruce dominated before logging (Nauman et al. 2015). I hypothesized that flying squirrels would preferentially select microhabitats associated with montane conifer forests (i.e., deep organic soil horizons, and conifer dominance in overstory and/or understory).

Study Area

I conducted my study in Pocahontas and Randolph counties, West Virginia in the Allegheny Mountains and Plateau sub-physiographic region of the central Appalachians. The region is characterized by broad plateau-like ridges, steep slopes, and narrow valleys (Fenneman 1938, Byers et al. 2010). The mountains of the region are capped with Pennsylvanian siltstones and sandstones characterized by frigid silt or sandy loam soils that are rocky, highly acidic and low in productivity (Allard and Leonard 1952, Pyle et al. 1982, Flegal 1999). My study sites included: Kumbrabow State Forest (KSF; 38°37' N, 80°6' W), Snowshoe Ski Resort (SSR; 38°24' N, 79°59' W) and the Greenbrier Ranger District (GRD; 38°39' N, 79°47' W) on the Monongahela Nation Forest (Figure 3.1). The site on SSR was dominated by red spruce, whereas sites on GRD were dominated by red spruce, red spruce-Eastern hemlock, and northern hardwood forests composed of black cherry (*Prunus serotina*), yellow birch, and red maple (*Acer rubrum*). Sites on KSF were dominated by planted Norway spruce (*Picea abies*)-eastern hemlock and northern hardwood forests. All sites were secondary growth stands, as all areas had been cutover at least once in the preceding 100 years and many had experienced multiple reentry to harvest

merchantable, high value timber such as black cherry. Elevations at the study sites ranged from 930 – 1450 m. Climatic conditions were cool and moist with frequent fog, high annual precipitation (120-150 cm distributed throughout the year, with >350 cm of average annual snowfall in the winter months), and possibility of freezing temperatures year round (Stephenson 1993, Rentch et al. 2007).

Methods

I obtained Virginia northern flying squirrels to radio-tag using a combination of nest box and live trap surveys. I coordinated nest box surveys with the annual spring and fall checks conducted by the West Virginia Division of Natural Resources and the GRD staff (C. Stihler, WVDNR, unpublished data). Nest boxes (33.0 x 12.7 x 12.7 cm with a 4.4 – 5.7 cm opening) were placed in single transects per site ranging from 15-30 nest boxes spaced at 30-50 m intervals (Stihler et al. 1987; S. Jones, U.S. Forest Service, personal communication). Nest boxes were mounted 4-5 m on the trunks of trees and checked using a ladder. I supplemented nest box checks with live trapping using Tomahawk 201 live traps (14 x 14 X 41 cm, Tomahawk Live Trap Co., Hazelhurst, Wisconsin) baited with a slice of apple rolled in a peanut butter-molasses-bacon grease-oatmeal mixture. To reduce trap stress and potential hypothermic effects of trapping, I placed polyfill batting in each of the traps and partially covered traps with plastic and duct tape. Traps were set on the ground as well as on the bole of trees to increase captures of flying squirrels (Loeb et al. 1999). I opened traps at dusk and checked them at dawn. All traps were closed between sunrise and sunset to reduce capture of diurnal species.

For captured flying squirrels, I distinguished Virginia northern flying squirrels from the sympatric southern flying squirrels (*Glaucomys volans*) by coloration of ventral fur and hind foot

measurement (Wells-Gosling and Heaney 1984). For all captured squirrels, I recorded sex, age, reproductive condition, and mass. Individuals were tagged with uniquely number ear tag (No. 1005-1, National Band and Tag Company, Newport, MA). All adult captures were radio-collared using 3.4 and 4.0 g PD-2C radio-collars (Holohil Systems Ltd., Carp, Ontario, Canada), consisting of $\leq 5\%$ of their total body weight. My methods for capture and tagging were approved by the Virginia Tech Institutional Animal Care and Use Committee (permit #11-120-FIW).

I radio-tracked individuals and surveyed squirrel locations in May-July 2013, October 2013, and May-June 2014. To determine home-range, I tracked radio-collared squirrels for a minimum of 5 nights or until the radio-collar signal was lost. I used Wildlife Materials TR4-2000S receivers (Wildlife Materials, Carbondale, Illinois) and 2-element folding yagi antennas to determine the location of squirrel location points. Before nighttime telemetry, I located each squirrel's diurnal den. I obtained telemetry points using triangulation by manning two fixed stations taking telemetry points simultaneously to minimize temporal error (Schmutz and White 1990). Telemetry stations were located >50 m apart to minimize bearings taken at less than 90° angles (White 1985). I tracked each squirrel for at least 2 hrs after civil twilight and typically ended surveys before or at midnight when squirrel activity drastically decreased (Menzel et al. 2006, Ford et al. 2007). I obtained bearings on individual squirrels every 5 to 10 min, to determine frequent movements associated with nocturnal activity. Because I used simultaneous bearings, I assumed animal movements would not influence telemetry error rates (Schmutz and White 1990). All personnel were tested for telemetry bias by using known locations of transmitters hidden in the field and actual bearings from telemetry stations to the location of the transmitter (White and Garrott 1990). I calculated our telemetry bearing error to be $3^\circ \pm 1^\circ$, although I did not estimate polygon error because I used the triangulation technique (Reding et al. 2013).

Microhabitat Points and Home Range

I entered locations of stations and triangulation bearings into the software program LOCATE II (Pacer Co., Truro, Nova Scotia, Canada) to obtain UTM coordinates of locations of individual squirrels. Using all locations for each squirrel, I estimated home ranges using 100% Minimum Convex Polygon (MCP) function in Biotas 2.0 (Ecological Software Solutions LLC, www.ecostats.com/web/Biotas; Figure 3.2). I selected MCP because it does not explicitly depict high and low use areas (Getz and Wilmer 2004), as this estimator assumes use across the home range is continuous (Mitchell and Powell 2008). My primary use of MCP was simply to define an area of potential habitat available to a squirrel in proximity to known squirrel locations. Since high-use areas as denoted by other home range estimators may include higher quality habitat, random points may not fall in lower quality habitat, thereby biasing microhabitat comparison data. Home range shapefiles obtained from Biotas were imported into ArcMap 10.0 Geographic Information Systems (Environmental Systems Research Institute, Inc., Redlands, California, USA). For VNFS with ≥ 30 points, I randomly generated 20 points within each home range using ArcMap to serve as Random Points during microhabitat surveys. I also randomly selected 20 locations obtained from telemetry surveys (hereafter “Telemetry Points”) for each individual, allowing us to compare points squirrels were selecting for versus random points within the squirrel’s home range (Figure 3.2).

Microhabitat Surveys

For each individual squirrel tracked, I surveyed microhabitat variables at Random Points and Telemetry Points. At each point, I set up a circular plot with a 10 m radius. Within each plot, I placed a 1 x 1 m subplot on the plot center. I followed the microhabitat measurement protocol provided by Castleberry et al. (2002) for Allegheny woodrats (*Neotoma magister*) in the Allegheny

Mountains as modified based on previous habitat association studies on the Virginia and Carolina northern flying squirrels (Pyane et al. 1989, Ford et al 2004, Menzel et al. 2006, Ford et al. 2014, 2015). At each Random Point and Telemetry Point, I qualitatively assessed dominant and co-dominant tree species within the circular plot. I measured the distance of the nearest tree within 10 meters from the center of the plot and recorded its species and diameter at breast height. I measured percent canopy cover at plot center using a spherical densitometer. I assessed percent ground cover within the 1 x 1 m subplot using the following variables: herbaceous vegetation, bare soil/rock, woody debris/roots, moss/lichen, and duff (i.e., leave/needle litter). Additionally, I sampled the depth of the forest floor (i.e., organic horizon) by measuring four soil plugs within the subplot and averaging them for each subplot. For Telemetry Points, I assessed if there are any signs of small mammal digs in the subplot to determine active foraging for the fruiting bodies of hypogeous fungi. I indicated two methods of small mammal fungal foraging types: digs and scratches. I determined a dig as fresh if the dig did not have debris (i.e., leaf fragments, needles) or spider webs covering the dig (Castellano et al. 1999, Castellano et al. 2003). I opportunistically excavated digs within Telemetry Points, searching for hypogeous fungi for ≤ 5 min within 0.5 m² area of the dig. All hypogeous fungi found were destructively sampled, placed in wax bags, and saved for later identification to species.

Data Analysis

Before comparison, I assessed if any of my variables were highly correlated using a Pearson's correlation in *R* statistical program version 3.1 (R Development Core Team 2015). No variables were significantly correlated, so all were retained for subsequent analysis. I compared differences between variables at Telemetry and Random Points averaged across all individual squirrels. Because my data was not normally distributed, I analyzed data using a permuted

multivariate analysis of variance (perMANOVA; Anderson 2001). Assumptions of analysis of variance are not generally met by most biological data that tend to be highly skewed or aggregated. The PerMANOVAs use a permutation procedure to assess significance without requiring data be normally distributed (Anderson 2001). I ran PerMANOVAs based on a Euclidean distance matrix with 9999 permutations per run in the *R* statistical software package ‘vegan’ (Dixon 2003, Oksanen et al. 2009). Closest overstory tree was assessed by comparing distance from plot center to the tree and tree type (i.e., conifer versus hardwood species). For dominant overstory tree, I assessed % conifer presence (i.e., red spruce, Norway spruce, eastern hemlock) averaged across all sites. For understory conifer, I determined if pole or seedling red spruce or eastern hemlock trees were present in the understory, including regeneration thickets, and averaged presence across all sites. Additionally, I determined soil depth associated with Telemetry Points with truffles versus Telemetry Points without truffles using a perMANOVA.

Results

I captured 28 Virginia northern flying squirrels (21 adults, 7 juveniles) in 746 nest box checks and approximately 1,300 trap nights. I fitted 19 adult squirrels with radio-collars. Of these squirrels, I was able to conduct microhabitat surveys on 13 individuals (7 males, 6 females). For these 13 squirrels, I gathered a total of 802 telemetry locations (average per squirrel = 62 ± 5). Mean MCP home ranges were 4.2 ± 1.0 ha (mean male MCP = 5.8 ± 1.6 ha, mean female = 2.3 ± 0.5 ha).

The three most important explanatory habitat variables were organic soil horizon depth, dominant overstory tree, and presence of understory red spruce. The mean organic soil horizon depth was greater at Telemetry Points versus Random Points (Table 2.1). Montane conifers were the dominant overstory tree at 73.5% of Telemetry Points versus 57.7% Random Points. Of the

Telemetry Points with a dominant hardwood overstory, 69.6% had some conifer component in the overstory and 4% had red spruce regeneration present in the understory. Understory red spruce was greater in Random Points versus Telemetry Points (Table 2.1), with red spruce regeneration in Random Points occurring in both conifer and hardwood dominant plots (48.5% and 9.2%, respectively). Other microhabitat variables (i.e., variables quantifying ground cover and canopy cover) were not significantly different between Telemetry and Random Points (Table 2.1).

Species of the closest overstory tree included red spruce (25.8% and 20% of Telemetry and Random Points, respectively), red maple (25.0% and 29.2%), yellow birch (17.3% and 22.7%), Norway spruce (11.9% and 6.5%), black cherry (7.3% and 8.5%), American beech (3.5% and 4.2%), Eastern hemlock (3.1% and 2.7%), and sweet birch (*Betula lenta*; 2.7% and 0.4%). Other species found at a small number of plots included American basswood (*Tilia americana*), white pine (*Pinus strobus*), striped maple (*Acer pensylvanicum*), sugar maple (*A. saccharum*), cucumber magnolia (*Magnolia acuminata*), and Fraser magnolia (*M. fraseri*). The closest overstory trees at Telemetry Points were 59.2% hardwood species and 40.8% conifer species. The closest overstory tree at Random Points were 70.0% hardwood species and 29.2% conifer species, whereas 0.8% of plots had no tree species within 10 meters of the plot center. The average distance of closest tree was 2.2 m in Telemetry Points and 2.8 m in Random Points, although this was not significantly different (Table 2.1).

Of the 260 Telemetry Points surveyed, hypogean fungi digs and scratches were found on 25.4% of the subplots and approximately half of those subplots contained hypogean fungi. Three species of hypogean fungi were found: *Elaphomyces americanum*, *E. verruculosus*, and *E. macroporous*. The majority of digs I observed were found during the spring seasons (86.4%), as leaf deposition in mixed forests during the fall season made locating digs difficult. Montane

conifers were the closest overstory tree at 61.8% of plots where hypogean fungi were present, although all plots with hypogean fungi had either red spruce, Norway spruce, or Eastern hemlock as a dominant overstory tree (88.2%) or present in the midstory (11.8%). Soil depth was significantly deeper at Telemetry Points with truffles (12.5 ± 0.5) than telemetry points with truffles (8.6 ± 1.0 ; $F_{259} = 8.888$, $p = 0.003$).

Discussion

Typically small mammal microhabitat studies obtain data from capture-no capture live-trapping grids (e.g., Rossell and Rossell 1999, Pyare and Longland 2002, Jorgensen 2004, Meyer et al. 2007), but this can be misleading because baited capture sites do not necessarily indicate microhabitat selection (Trainor et al. 2005), especially of foraging habitat. Although there are error rates associated with VHF telemetry (White and Garrott 1990), I measured our error rate and corrected for potential bias by using a larger plot size. The majority of the radio-collared individuals were obtained from nest boxes, which were placed along the conifer-northern hardwood ecotone. Trapping and placing nest boxes along the ecotone is associated with higher capture rates of northern flying squirrels in the Appalachians (Weigl et al. 1992, Hackett and Pagel 2003), however ecotonal habitat is associated more with nesting habitat versus foraging habitat (Loeb et al. 2000, Menzel et al. 2006, Ford et al. 2007, C. Diggins, unpublished data). Using telemetry data reduced the biased effects of baited trap sites or selectively placed nest boxes on potential foraging habitat.

Macrohabitat conditions are typically considered better predictors of small mammal habitat selection than microhabitat (e.g., Morris 1987, Orrock et al. 2000, Coppeto et al. 2006). This is because microhabitat partitioning is usually constrained by macrohabitat (Jorgensen and Demarais

1999), although this may be dependent on average home range size of the species in question (e.g., Castleberry et al. 2002). Although Virginia northern flying squirrels are highly mobile and can have home ranges > 20 ha (Menzel et al. 2006, Ford et al. 2007), I found the majority of within-stand habitat variables measured did not differ between selected and random sites. Canopy cover and herbaceous cover are known to influence northern flying squirrel microhabitat selection (Pyare and Longland 2002, Smith et al. 2004, Meyers et al. 2005), as northern flying squirrels may be more vulnerable to predation in open stands (Carey et al. 1992). Urban (1988) found that fern cover was positively correlated within Virginia northern flying squirrel core-activity areas versus low-activity areas, whereas canopy cover was negatively correlated. However, neither variable influenced microhabitat selection in my study. I posit this finding to be explained by the fact that herbaceous cover was generally low at most sites I surveyed (Table 2.1) and largely absent from plots with hypogean fungi. Canopy cover was similar between Random and Telemetry Points, regardless if plots were dominated by hardwoods ($75.1\% \pm 1.2$ canopy cover) or montane conifers ($78.8\% \pm 0.9$ canopy cover). Accordingly, this probably reduced my ability to detect a difference in overstory cover between Telemetry and Random Points.

Three habitat variables were significantly different between Random and Telemetry Plots: organic soil horizon depth, dominant overstory tree, and presence of understory red spruce. Virginia northern flying squirrels selected for deep organic soil horizons that are typically associated with conifer stands and may be linked to selection of hypogean fungi at foraging sites. Deeper organic soil horizons also may be associated with higher abundances of hypogean fungi (Meyer et al. 2007), although I found truffles at Telemetry Points with shallower soils than Telemetry Points without truffles. Because my exploration of truffles was opportunistic and I had a limited time search, searches for truffles were incomplete at most Telemetry Points and therefore

further assessment needs to be made in regards to soil depth and truffle presence. *Elaphomyces spp.* are known to occur in high proportions in Virginia northern flying squirrel diets (Mitchell 2001). All three species of hypogean fungi I found are associated with red spruce or other conifer surrogates, such as Norway spruce, and eastern hemlock (Castellano et al. 2012; M. Castellano, personal communication). In a study of the Carolina northern flying squirrel's habitats in the southern Appalachians, red spruce was considered the most important tree species to plots with hypogean fungi (Loeb et al. 2000). In my study, the majority of digs (62%) were located within 2 m of a conifer, the zone near the host tree where most hypogean fungi occur (Fogel 1976). Presence of downed coarse woody debris is considered an important indicator to flying squirrels for hypogean fungi detection (Pyare and Longland 2001), but coarse woody debris itself does not influence squirrel occurrence (Pyare and Longland 2002, Meyer et al. 2007). I did not measure coarse woody debris due to the low abundance observed throughout my study sites. Although I only opportunistically sampled for hypogean fungi, my success in obtaining hypogean fungi was much higher than a previous study on the Virginia northern flying squirrel in the central Appalachians (Ford et al. 2004) in part because that study was based on survey plots approximate to nest box sites, which were not necessarily related to foraging sites.

Similar to studies at the landscape and stand-level for northern flying squirrels in the central and southern Appalachians, I found that the Virginia northern flying squirrel's microhabitat selection was linked to forests dominated by red spruce or other conifer surrogates (Pyane et al. 1989, Odom et al. 2001, Ford et al. 2004, Menzel et al. 2004, Ford et al. 2005, Menzel et al. 2005, 2006; Ford et al. 2007, 2014). Pyare and Longland (2002) suggested that flying squirrel microhabitat selection may be hierarchical, but my results indicate that presence of red spruce or a conifer surrogate is more important in predicting microhabitat habitat selection of Virginia

northern flying squirrels than other habitat features, such as herbaceous cover. Northern flying squirrel home ranges are smaller in pure montane conifer stands versus stands on the conifer-northern hardwoods ecotone (Menzel et al. 2006, Ford et al. 2014; C. Diggins, unpublished data), suggesting conifer stands have higher quality habitat than ecotonal stands. Menzel et al. (2006) found that radio-collared Virginia northern flying squirrels selectively chose red spruce dominant stands and mixed stands, while avoiding pure northern hardwood stands. Ford et al. (2014) and Diggins (Chapter 4) found that radio-collared Carolina northern flying squirrels selected red spruce more than expected based on availability at both the stand-level and within stand-level in the southern Appalachians. Variation in microhabitat within red spruce stands such as stand maturity and tree condition may influence the frequency of use at a site, but I did not specifically address this within my study.

Understory red spruce was significantly lower in Telemetry Points versus Random Points. Red spruce regeneration, not herbaceous understory, was associated with higher amounts of absolute understory cover, although I did not empirically quantify density of regeneration over the whole of my study sites. In southeast Alaska, Smith et al. (2004) found that conifer regeneration was higher at trap sites that produced no captures of flying squirrels versus capture sites. However, other research has indicated increased understory vegetation might be important for cover from predators, especially when foraging and handling food items (Longland and Price 1991, Pyare and Longland 2002). Increased understory cover may also hinder mobility on the ground (Carey 2000), which is considered a less efficient form of locomotion for flying squirrels (Scheibe et al. 2006). In my study, I found that red spruce regeneration was significantly higher in Random Plots and hardwood dominated forests. Therefore, microhabitat selection was not associated with hardwood dominant stands, regardless of presence of red spruce regeneration in the understory.

My findings link the Virginia northern flying squirrel's microhabitat selection to deep organic soil horizons and overstories comprised of montane conifer dominated stands. Although adjacent hardwood stands may provide denning opportunities (Hackett and Pagels 2003, Menzel et al. 2004), my data suggests that conifer stands were more important to foraging habitat. If the expansion of red spruce regeneration into adjacent hardwood stands is indicative of ecological recovery of this forest type (Mayfield and Hicks 2010, Nowacki et al. 2010, Rollins et al. 2010), these areas seemingly should be high priority for restoration to increase the extent and connectivity between habitat patches. Girdling, stem-injection herbiciding, or felling of hardwoods to mimic natural canopy gaps (Rentch et al. 2010) would release single or small groups of suppressed spruce to the overstory and potentially provide a near-term increase in potential den sites in newly created snags (Rentch et al. 2016). Although northern flying squirrels are known to be sensitive to silvicultural treatments (Carey 2000, Holloway and Smith 2011, Holloway et al. 2012), red spruce restoration occurs on a relatively small scale within the forest compared to more common forest harvest methods (i.e., clearcutting, green tree retention). Red spruce restoration is proposed to occur in northern hardwood dominant stands with established red spruce in the understory or midstory, which may not strongly impact Virginia northern flying squirrels because they tend to avoid these habitats. Currently, no study on the Virginia northern flying squirrel's response to spruce restoration has occurred, but part of this is due to the current small-scale application of red spruce treatments versus implementation of a landscape-scale study, which would allow land managers to measure wildlife responses (Covington et al. 1997, Block et al. 2001), especially of a highly mobile arboreal mammal. Therefore, a better understanding of response to both commercial and non-commercial treatments still needs to occur to understand short-term impacts of red spruce restoration in the central Appalachians on the Virginia northern flying squirrel.

This is the first microhabitat selection study of northern flying squirrels in the Appalachian Mountains and may provide pertinent information to habitat management of this subspecies. Denning habits of northern flying squirrels in the Appalachians may be plastic (Hackett and Pagels 2003, Diggins et al. 2015) and foraging habitat could be a more important indicator of habitat occupancy of Virginia northern flying squirrels. Red spruce is predicted to decline in extent due to anthropogenic climate change, potentially leading to the extirpation of this species from the central Appalachians (Beane and Rentch 2015). Links to red spruce and other conifer surrogates on the landscape-, stand-, and microhabitat-level should help managers prioritize areas for habitat management and red spruce restoration to be able to increase or retain as much of this threatened habitat type as possible in the future. Restoration will help increase the extent, connectivity, and quality of the Virginia northern flying squirrel's habitat, increasing the potential for population persistence into an uncertain future climate within the central Appalachians.

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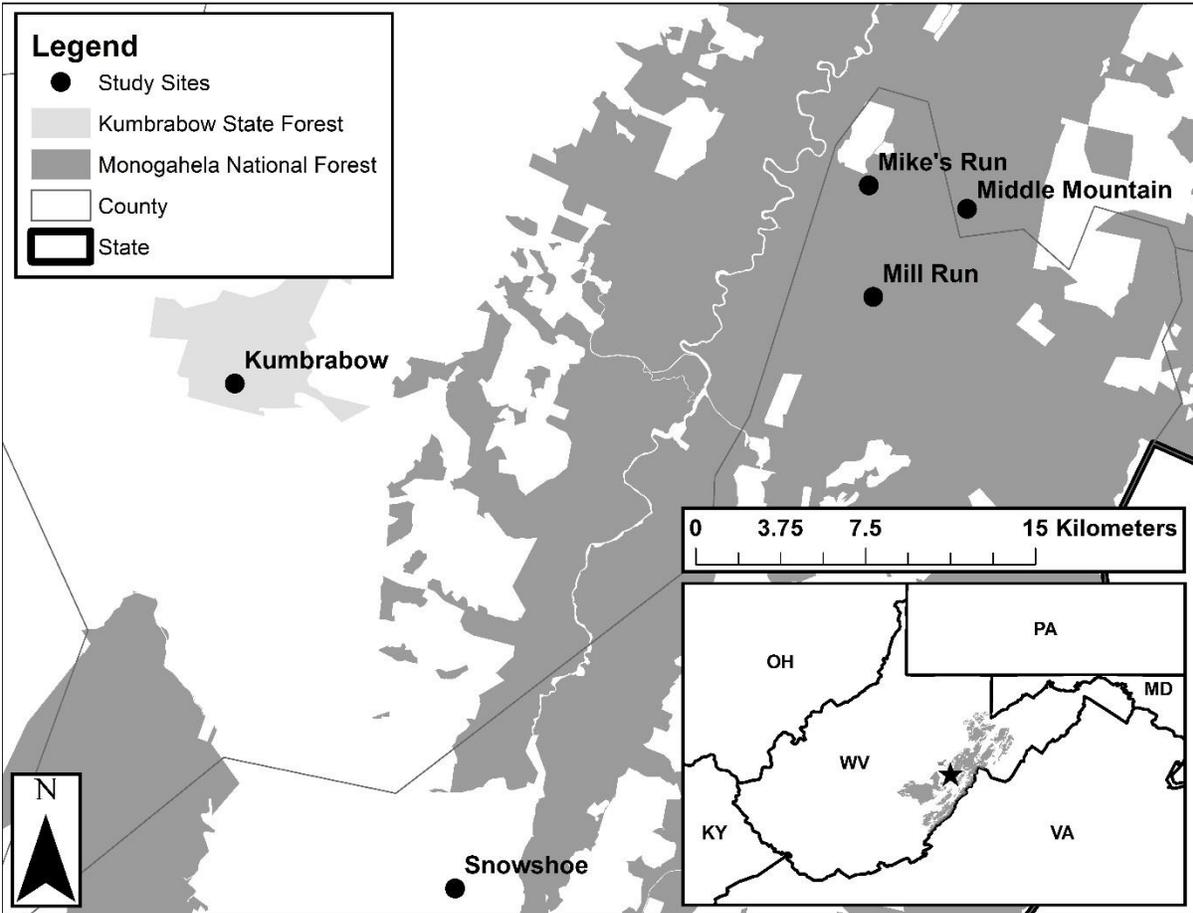


Figure 3.1. Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*) microhabitat survey sites during 2013 and 2014 in the central Appalachian Mountains of West Virginia.

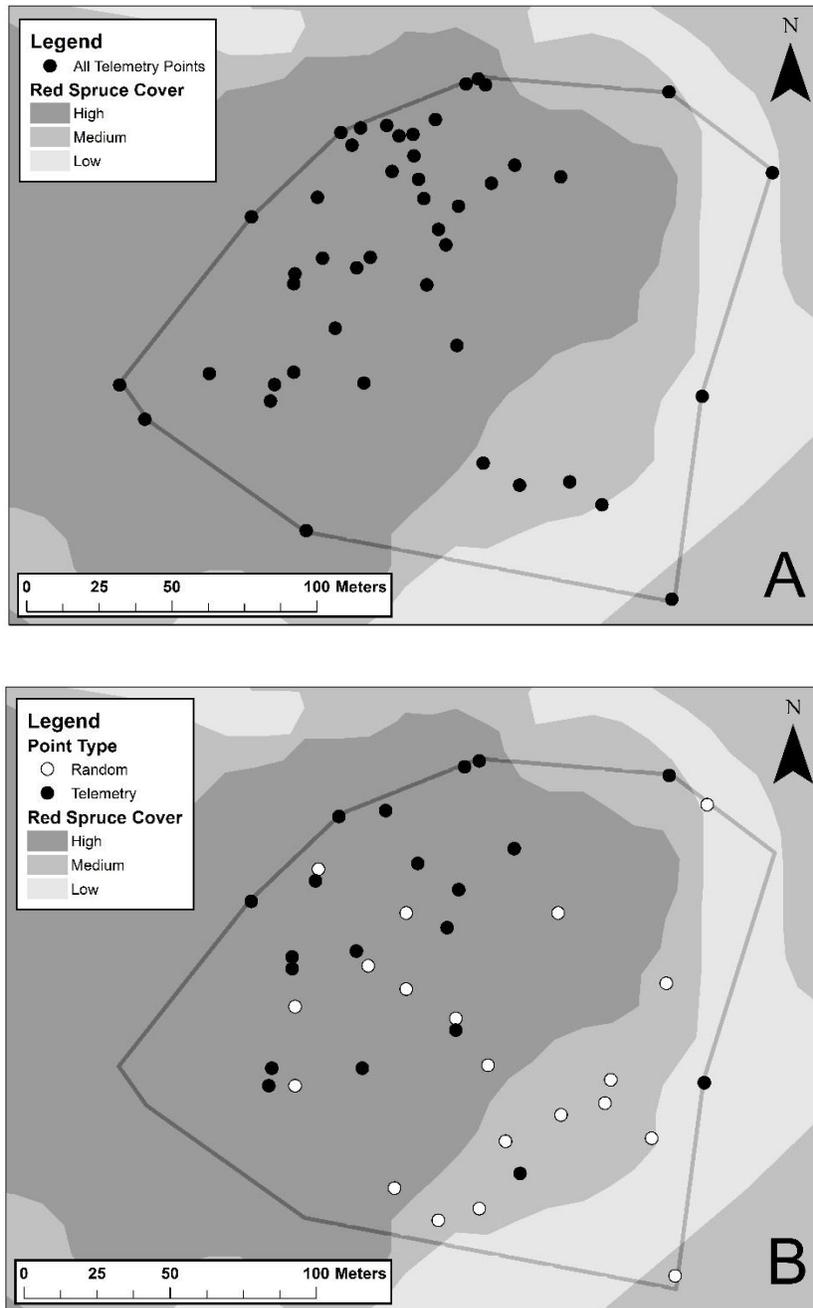


Figure 3.2. 100% Maximum Convex Polygon home range of male Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*) at Greenbrier Ranger District in summer 2014 on the Monongahela National Forest, West Virginia: A) The original telemetry point locations; B) Randomly selected Telemetry Plots and randomly generated Random Plots where microhabitat surveys were conducted. Red spruce cover from Byers et al. 2013.

Table 3.1. Mean (\pm SE) and PerMANOVA results of microhabitat variables average across all individual Virginia Northern Flying Squirrels (*Glaucomys sabrinus fuscus*) from 2013-2014, Allegheny Mountains, West Virginia.

Habitat Variable	Telemetry Plots	Random Plots	F-statistic (df =1, 24)	P-value
Organic Matter Depth (cm)	12.0 \pm 1.3	8.6 \pm 0.8	4.686	0.043
% Bare Ground	0.5 \pm 0.4	0.7 \pm 0.3	0.190	0.705
% Moss Cover	5.6 \pm 1.4	5.2 \pm 1.1	0.049	0.850
% Duff Cover	77.5 \pm 2.6	74.9 \pm 2.8	0.490	0.483
% Woody Debris	5.5 \pm 0.5	4.7 \pm 0.6	0.908	0.097
% Herbaceous Cover	11.0 \pm 1.6	14.3 \pm 2.4	1.362	0.350
% Canopy Cover	76.4 \pm 3.9	77.4 \pm 2.3	0.050	0.838
Distance of Closest Overstory Tree	2.2 \pm 0.2	2.8 \pm 0.3	3.302	0.080
Closest Overstory Tree (% Plots with Conifer)	40.8 \pm 0.04	30.4 \pm 0.04	3.388	0.066
Dominant Overstory Conifer (%)	73.5 \pm 0.05	57.7 \pm 0.06	4.105	0.048
Understory Spruce (% Plots with Regeneration)	22.3 \pm 0.03	83.1 \pm 0.07	59.040	0.000

Chapter 4: Home Range and Habitat Use of Carolina Northern Flying Squirrels (*Glaucomys sabrinus coloratus*)

Abstract

Understanding habitat selection is important for determining conservation and management strategies, such as ecological restoration designed to benefit threatened, endangered or sensitive species. The Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) is an endangered subspecies found in the high-elevation montane forests of the southern Appalachians. Previous habitat associations have been obtained over the past three decades largely from live traps and nest box surveys, however, actual habitat selection from free-ranging animals is not well understood. I conducted a radio-telemetry study on Carolina northern flying squirrels ($N = 20$) in western North Carolina in 2012 and 2014-2015. I estimated home range using the minimum convex polygon (MCP) and biased random bridge (BRB) methods. The MCP at the 95% and 50% level for males were 5.2 ± 1.2 ha and 1.3 ± 0.4 , respectively, and for females were 4.0 ± 0.7 and 0.6 ± 0.1 , respectively. The BRB at the 95% and 50% level for males were 10.8 ± 3.8 ha and 2.2 ± 0.8 , respectively, and for females were 8.3 ± 2.1 and 1.6 ± 0.4 , respectively. In contrast to other studies, I found no difference in home range size for either home range estimator between the sexes at the 95% (BRB: $t_{14} = -0.57$, $P = 0.58$; MCP: $t_{14} = -0.81$, $P = 0.43$) or 50% level (BRB: $t_{12} = -0.66$, $P = 0.52$; MCP: $t_{11} = -1.57$, $P = 0.14$). Den site ($N = 82$) selection occurred more frequently in conifer dominate forests (81.1%) versus northern hardwood forests (19.9%; $X^2 = 31.1$, $df = 5$, $P = 0.000$). I assessed habitat selection using Euclidean distance based analysis at the 2nd order (home range selection within the landscape) and 3rd order scale (within home range selection). I found that squirrels were non-randomly selecting for habitat at both 2nd ($F = 54.83_{3,17}$, $P < 0.0001$) and

3rd order scales ($F = 363.95_{3,17}$, $P < 0.0001$). At both scales, squirrels preferentially selected for montane conifer forests more than expected based on availability on the landscape, followed by selection of grassy/shrub habitat, although use of these habitats were along the edges with closed-canopy forest. Squirrels neither selected for or against northern hardwood forests, regardless of availability on the landscape. As such, my results highlight the importance of montane conifer to Carolina northern flying squirrels in the southern Appalachians. Management and restoration activities that increase the quality and extent of this naturally rare forest type may be important for long-term conservation of this subspecies in face of anthropogenic climate change.

Introduction

Conservation and management of wildlife is dependent on understanding selection and use of resources by a species (Gaston 2003), habitat associations (Manly et al. 2002, Morrison et al. 2006), and response to changes in habitat availability via natural or anthropogenic disturbance (Block et al. 2001, Martin 2001, Heller and Zaveleta 2009). Species management efforts can be limited when resource selection is not understood, particularly for rare or endangered species (Thompson 2004). Moreover, for montane species that are vulnerable to climate change impacts, understanding current habitat selection is needed on multiple spatial scales to determine where habitat management and conservation efforts should be focused.

Within the southern Appalachians, the Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) is a Pleistocene relict that occurs in disjunct “sky islands” of montane conifer (red spruce, *Picea rubens*; Fraser fir, *Abies fraseri*; Eastern hemlock, *Tsuga canadensis*) and montane conifer-northern hardwood ecotone above 1,400 m in western North Carolina, eastern Tennessee and southwestern Virginia, USA (USFWS 1985, Pyane et al. 1989, Brown et al. 1999,

Weigl et al. 1999, Hughes 2006, Ford et al. 2015). This subspecies was listed as federally Endangered in 1985 due to the drastic decrease in the condition and extent of spruce-fir forests, the squirrel's primary habitat (USFWS 1985, Pyane et al. 1989, Ford et al. 2014, 2015). Following the industrial logging and associated fires at the turn of the 20th Century, loss of this forest community type was estimated to be 35-57% and was mainly due to replacement by faster-growing northern hardwoods, especially at the lower elevations where red spruce formerly had occurred (Korstian 1937, Pyle and Schafale 1988, Busing et al. 1993, Boyce and Martin 1993, Hayes et al. 2006). These forests were further degraded by acid precipitation and the introduced balsam woolly adelgid (*Adeges balsamea*; Hay et al. 1978, Dull et al. 1988, Eager and Adams 1992). Together, these factors make southern Appalachian spruce-fir forests one of the most endangered forested ecosystems in the United States (Noss et al. 1995).

Additionally, since spruce-fir forests occupy the highest peaks and ridges in the region, they are under extreme threat from anthropogenic climate change (Iverson et al. 2008, Beane and Rentch 2015, Koo et al. 2015). Under a climate change scenario of doubled carbon emissions, Carolina northern flying squirrels are predicted to become extirpated from the Great Smoky Mountains National Park (Burns et al. 2003), where the largest contiguous spruce-fir forest in the southern Appalachians occurs, including the only substantial old-growth spruce-fir in the region (Dull et al. 1988, Ford et al. 2015). This subspecies is also threatened by interspecific den-site and parasite-mediated competition from the parapatric southern flying squirrel (*G. Volans*; USFWS 1985, Weigl 1978, Wetzel and Weigl 1994, Weigl et al. 1999), the effects of which may be exacerbated by climate change as southern flying squirrels occupy forests formerly only occupied by Carolina northern flying squirrels (Bowman et al. 2005, Wood et al. 2016).

Under the Endangered Species Recovery Plan for this subspecies, understanding habitat use of the Carolina northern flying squirrel is important for long-term management and recovery (USFWS 1990, 2013). This subspecies is assumed to be highly associated with montane conifer forests (Payne et al. 1989, Ford et al. 2015), although there has been speculation that montane conifer forests may not be critical as the Carolina northern flying squirrel also uses northern hardwood forests and are captured more readily along the montane conifer-northern hardwood ecotone (Weigl 2007). Past studies on habitat associations assessed using nest boxes, live traps, and limited telemetry data (Weigl et al. 1999, Hackett and Pagels 2003, Hughes 2006, Ford et al. 2014, Ford et al. 2015). However, many live traps and nest boxes typically were placed on the montane conifer-northern hardwood ecotone (Weigl et al. 1999, Reynolds et al. 1999, Ford et al. 2015; C. Kelly, North Carolina Wildlife Resources Commission, unpublished data), potentially biasing interpretation of habitat use as information was not from tracked individuals.

To date, the majority of limited telemetry work on the Carolina northern flying squirrel also has been conducted on the montane conifer-northern hardwood ecotone (Weigl et al. 1999, Hughes 2006, Ford et al. 2014). However, previous telemetry studies on another Appalachian subspecies, the Virginia northern flying squirrel (*G. s. fuscus*), in the Allegheny Mountains in the central Appalachians of West Virginia, USA showed that although denning occurs along the conifer-northern hardwood ecotone or in small hardwood inclusions (Ford et al. 2007, Menzel et al. 2004), foraging occurs in conifer stands (Ford et al. 2007, Menzel et al. 2006, Diggins Chapter 2). Moreover, telemetry studies to date did not spatially analyze nightly habitat use by Carolina northern flying squirrels (Weigl et al. 1999, Hughes 2006), with exception of Hackett (2002) and Ford et al. (2014). However, these two studies had limited sample sizes (≤ 6 squirrels) and only

occurred at one study site, therefore have a limited range of inference (Hackett 2002, Ford et al. 2014).

In the southern Appalachians, there is an increasing interest in the restoration of red spruce forests along the spruce-northern hardwood ecotone using the canopy-gap creation method that mimics gap-phase regeneration that is believed to be the dominant disturbance/reinitiation dynamic in these systems (Rentch et al. 2007, 2016). Since the Carolina northern flying squirrel is the main regulatory driver for restoring red spruce in the southern Appalachians, a better understanding of how this subspecies selects habitat is required to determine the best restoration techniques. Because previous surveys of this subspecies have been focused along the montane conifer-northern hardwood ecotone, little survey effort and no telemetry studies have been conducted in pure montane conifer stands (Ford et al. 2015). The objective of my study was to 1) determine home range estimates of Carolina northern flying squirrels and 2) build on work previously conducted by Ford et al. (2014) and understand habitat selection of radio-collared Carolina northern flying squirrels on multiple spatial scales, including within habitat types previously under-surveyed, i.e., spruce-fir forests at the highest elevations regionally. Therefore, I included telemetry data from squirrels tracked in both pure montane conifer stands and montane conifer-northern hardwood ecotone to better understand comprehensive habitat use across the Carolina northern flying squirrel's geographic range. I hypothesized that 1) home range estimates will be similar to other isolated populations of northern flying squirrels and 2) Carolina northern flying squirrels will preferentially select for spruce-fir forests over other habitat types.

Study Area

I conducted my study in montane conifer and spruce–northern hardwood forests (hereafter “montane conifer-northern hardwood forests”) in the Blue Ridge sub-physiographic province of the southern Appalachian Mountains in western North Carolina and southwestern Virginia. Montane conifer forests occur in disjunct high-elevation “sky islands” across the region, with red spruce occurring between approximately 1,400 – 1,830 m and northern hardwood forests occurring <1,400 m throughout, whereas pure Fraser fir stands occur > 1,675 m on some sky islands. My study sites were located at Mount Rogers National Recreation Area in Virginia on Whitetop Mountain in the Balsam Mountains, Grayson County, and Pisgah National Forest in North Carolina at Carver’s Gap and Roan High Bluff in the Roan Mountain Highlands, Mitchell County, and Buckeye Gap and Little Sam’s Knob in the Great Balsam Mountains, Haywood County, North Carolina (Figure 4.1). The sites at Whitetop Mountain, Buckeye Gap, Little Sam’s Knob, and Carver’s Gap were red spruce-northern hardwood forests. Roan High Bluff was a red spruce-Fraser fir dominant forest. All stands had been clearcut at the turn of the 20th century during industrial logging era, with exception of a relict old-growth stand on Whitetop Mountain (T. Blevins, USDA Forest Service, pers. comm.). Within the Blue Ridge Province at these elevations, the growing season ranges between 150-180 days, and precipitation is ≤ 200 cm equally distributed throughout the year (McNab and Avers 1994).

Methods

Capture and Telemetry Surveys

I conducted my study in March-June 2012, February-May 2014, and May-June 2015. I obtained Carolina northern flying squirrels from a combination of live trap and nest box surveys.

I conducted live trapping using Tomahawk 201 live traps (14 x 14 x 41 cm and 14 x 14 x 48 cm; Tomahawk Live Trap Co., Hazelhurst, WI, USA). I baited traps with a slice of apple rolled in a peanut butter-molasses-bacon grease-oatmeal mixture. Traps were set 1.5 m up on the boles of trees (Loeb et al. 1999) and on the ground at the base of trees and along log runs. Traps were opened at dusk, checked at dawn, and closed during the day to reduced captures of diurnal species, such as red squirrels (*Tamiasciurus hudsonicus*). I checked nest boxes established by the North Carolina Wildlife Resources Commission's long-term nest box monitoring program (McGrath and Patch 2003). Nest boxes were constructed of plywood with dimensions of 30 cm x 18 cm x 15 cm and a 5 cm x 5 cm entrance (Kelly et al. 2013). Nest boxes typically were placed 3.6 m up on the trunks of trees in single transects of 10-15 nest boxes per site (Ford et al. 2014).

I distinguished the Carolina northern flying squirrel from the sympatric southern flying squirrel by coloration of ventral fur and hind foot measurement. Adult Carolina northern flying squirrels have underbelly hairs that are lead grey at the base and hind feet >34 mm, whereas southern flying squirrels have underbelly hairs that are white at the base and hind feet <33 mm (Dolan and Carter 1977, Wells-Gosling and Heaney 1984). I recorded sex, age, reproductive condition, and mass for all captured squirrels. I tagged adult squirrels with a uniquely numbered ear tag (No. 1005-1, National Band and Tag Company, Newport, MA, U.S.A.) and radio-collared with 3.4 or 4.0 g PD-2C radio-collars (Holohil Systems Ltd., Carp, Ontario, Canada). My methods for capture and tagging followed the American Society of Mammalogists guidelines (Sikes and Gannon 2011) and were approved by the Virginia Tech Institutional Animal Care and Use Committee (protocol #14-120-FIW).

I tracked Carolina northern flying squirrels during February-May 2012, February-April 2014, and May-June 2015. I used Wildlife Material TR4-2000S receivers (Wildlife Materials,

Carbondale, Illinois) and 3-element folding yagi antennas to determine the location of individual squirrels to diurnal den sites and during nighttime activity. To determine home range, I tracked collared squirrels for a minimum of four nights or until the radio-collar signal was lost. Tracking began at civil twilight and was continued until activity ceased, which was typically 2 hours after civil twilight (Weigl and Osgood 1974, Menzel et al. 2006, Ford et al. 2014). To obtain telemetry points, I used triangulation by manning 2 fixed stations and simultaneously taking telemetry points to minimize temporal error (Schmutz and White 1990). This method also reduces alteration of squirrel behavior that occurs when following the squirrel to exact locations on foot (Witt 1992). To minimize bearing taken at less than 90° angles, I located telemetry stations > 50 m apart (White 1985). Since this subspecies can be highly mobile during periods of activity (Weigl et al. 1999) and decreased time intervals between successive observations takes into account the behavior of the animal (de Solla et al. 1999), I took points on squirrels every 5-20 min (Menzel et al. 2006, Ford et al. 2010, 2014; Diggins Chapter 2). All personnel were tested for telemetry bias prior to the initiation of tracking to determine potential location error rates. I used known locations of transmitters hidden in the field and personnel took bearings from a predetermined telemetry station to the location of the transmitter (White and Garrot 1990). I calculated our telemetry bearing error to be $2^\circ \pm 0.5^\circ$. However, I did not estimate polygon error because I used the triangulation technique (Reding et al. 2013, Ford et al. 2014).

Home Range Analysis

I obtained UTM coordinates of telemetry data for individual squirrels using location of stations and triangulation bearings entered into LOCATE II (Pacer Co., Truro, Nova Scotia, Canada). I calculated home ranges for telemetry and den locations using the biased random bridge (BRB) method of Benhamou (2011) and minimum convex polygons (MCP) with package

adehabitat (Calenge 2006) in *R* version 3.1.2 (*R* Development Core Team 2014). Calculation of MCP allowed us to compare home ranges with previous studies on Carolina northern flying squirrel and other subspecies (e.g., Virginia northern flying squirrel). The BRB method utilizes an advective-diffusive movement process that links sequential points to estimate local space use density, thereby incorporating abilities of animals to preferentially select for more attractive areas within home ranges (Benhamou 2011). For serially correlated relocation data, this approach yields a reliable way to estimate home range utilization distributions. Therefore, use of the BRB method allowed the use of serial autocorrelation telemetry locations to more accurately represent the movement process of CNFS within their home range.

I estimated the diffusion parameter used in calculating BRB home ranges with the maximum likelihood method, using a maximum time of 30 min between successive relocations (Benhamou 2011). I specified a minimum distance of 1 m between successive relocations used in calculating BRB home ranges. I estimated that localization errors were less than 1 m, with a standard deviation of less than 0.5 m. Therefore, I set minimum diffusion parameter at 3 m, which also accounts for uncertainty in habitat configuration. I specified that relocation variance have constant weight. I allowed individual home ranges to be calculated on independent 1000x1000 pixel grids. *A priori*, I elected to use 50 and 95% home ranges, as defined by probability contours, as core and whole home ranges. I compared differences between male and female home ranges for both MCP and BRB at both the 50 and 95% levels using a *t*-test in program *R*.

Den Tree Analysis

I tracked squirrels to diurnal dens. Initially, squirrels were tracked every 1-3 days for the first 1 – 2 weeks, and afterwards every 2-5 days. I flagged den trees to determine den tree frequency of use and took GPS coordinates to spatially analyze den tree locations in ArcGIS 10.2

(Environmental Systems Research Institute, Inc., Redlands, California). I identified den types as dreys (i.e., external leaf nests), cavities, or subterranean. For drey and cavity trees, we determined tree species, status (i.e., live or dead), and diameter at breast height. I determined distance in meters of each den tree from the nearest spruce dominate stand using Spatial Analyst in ArcGIS. I also determined what habitat type den trees fell in using reclassified habitat shapefiles in ArcGIS (see *Habitat Use Analysis* section). I determine the difference between the sexes for number of dens used, duration of frequency of use, and diameter at breast height using a t-test. I determined sex difference in den type selection using a chi-squared equitable distribution test, as well as determined the location of den trees in conifer dominant and northern hardwood dominant forests using a chi-squared equitable distribution test. All den tree analysis were performed in Program R.

Habitat Use Analysis

To assess habitat use versus availability, I used Euclidean distance based analysis. This method assesses habitat use by comparing distances from telemetry locations and random points to the edge of each habitat type using a linear approach (Conner and Plowman 2001). The use of Euclidean distance incorporates edges between habitat types (i.e., dominant vegetation community) as an important habitat feature and reduces the effects of telemetry error associated with other methods of habitat analysis (Conner et al. 2003, Bingham and Brennan 2004). Additionally, this method can be applied to multiple spatial scales (Conner and Plowman 2001). I used this method to assess habitat selection at the 2nd order (home range selection within the landscape) and 3rd order (within home range selection) habitat scale with the 95% BRB home range for each individual squirrel. To assess habitat selection on the 2nd order scale, I assessed habitat availability within a 1000 m buffer from the combined area of home ranges for each site (Ford et al 2014). I generated random points within each individual squirrel's home range (3rd order scale

analysis) and random points within the 1000 m buffer (2nd order scale analysis) in ArcGIS. I reclassified habitat shapefiles from Southeastern Gap Analysis data (www.basic.ncsu.edu/segap/) as spruce-fir forests and grass/shrub habitat (i.e., grassy balds, heath balds, old fields). Habitat shapefiles for northern hardwood forests were obtained from Evans et al. (2014). I confirmed habitat reclassification using aerial photography for montane conifer and grass/shrub habitat, whereas Evans et al. (2014) verified predicted northern hardwood habitat from ground-truthed points. I created ratios of distances of random points and telemetry points to the closest representative habitat feature for both scales. Random habitat use is indicated by ratios equal to 1.0 (Conner and Plowman 2001). Non-random use is indicated when habitat types were used more than expected (telemetry/random distance ratios <1.0) or habitats were used less than expected (telemetry/random distance ratios >1.0; Conner and Plowman 2001). To determine if selection was occurring between habitat types, I ran a multivariate analysis of variances (MANOVA) to determine if distance ratios were different from 1.0. I then used a *t*-test to determine which habitat types were used more or less by squirrels based on their availability for both spatial scales. Lastly, I used a series of *t*-tests to rank habitat preferences between the different habitat types at both spatial scales. I determine statistical significance using $\alpha \geq 0.05$. All habitat selection analyses were conducted in Program R.

Results

Home Range

I captured 24 squirrels (13 males, 11 females) over 4,340 live-trap nights and 36 nest box checks. I fitted 22 squirrels with radio-collars. Of these squirrels, I obtained > 30 telemetry points on 20 squirrels (10 males, 10 females), for a total of 1,317 telemetry points (average points per

squirrel 65.8 ± 19.9 SE, range 31–107). Individual squirrels were tracked for an average of 5.1 track nights/squirrel (range = 3 – 9) over the course of 0.25-4 months. All individuals with ≥ 30 points I considered appropriate for the calculation of home ranges (Seaman et al. 1999; A. Silvis, unpublished data). Mean time between nightly locations was 21.7 ± 3.2 min. Inter-location movement distances were non-normally distributed, with strong positive skew; average median distance moved between consecutive points was 50.4 ± 6.2 m.

MCP home ranges were non-normally distributed at both the 50% and 95% levels, also with strong positive skew. Mean MCP home range for males were 1.3 ± 0.4 ha (range 0.2–3.7 ha) and 5.1 ± 1.2 ha (range 1.0–12.2) at the 50 and 95% levels, respectively. Mean MCP home range for females were 0.6 ± 0.1 ha (range 0.02–1.4 ha) and 4.0 ± 0.7 ha (range 1.4–9.6) at the 50 and 95% levels, respectively. The MCP home range size was not significantly different between male and female squirrels at the 50% ($t_{11} = -1.57$, $P = 0.14$) or 95% levels ($t_{14} = -0.81$, $P = 0.43$).

Median diffusion parameter used in the BRB home range estimator was 213.4 m²/min (± 129.6 SE). Home ranges were non-normally distributed and displayed strong positive skew at both the 50% and 95% levels. Mean BRB home range size for males were 2.2 ± 0.8 ha (range 0.2-8.6 ha) and 10.8 ± 3.8 ha (range 0.8-38.6 ha) at the 50 and 95% levels, respectively (Figure 4.2). For females, mean BRB home range size were 1.6 ± 0.4 ha (range 0.6-4.5 ha) and 8.3 ± 2.1 ha (range 4.0-24.0 ha) at the 50 and 95% levels, respectively (Figure 4.2). BRB home range size was not significantly different between male and female squirrels at the 50% ($t_{12} = -0.66$, $P = 0.52$) or 95% levels ($t_{14} = -0.57$, $P = 0.58$). There was no significant difference between home range size in pure montane conifer (N = 5) and montane conifer/northern hardwood (N = 15) at the 50 and 95% BRB home range (Wilcoxon Rank Sum Test: $W = 30$, $p = 0.55$; $W = 35$, $p = 0.87$, respectively).

Den Tree Results

I tracked 21 Carolina northern flying squirrels (10 males, 11 females) to 82 dens sites on 171 occasions. Individual squirrels had an average of 4.2 ± 0.4 dens sites over the course of being subject to radio-tracking. The number of den sites did not vary between males and females ($t_{17} = -0.847, P = 0.48$). The average duration a squirrel remained at a den site was 3.3 ± 0.8 days (range: 1-14) and did not vary between males and females ($t_{10} = 1.068, P = 0.31$). Of the den surveyed, 50% were dreys, 47.6% were cavities, and 2.4% were subterranean. The type of den used did not significantly differ between males and females ($X^2 = -0.847, df = 2, P = 0.07$), although only males utilized subterranean dens. Dreys only occurred in living trees, whereas 56% of cavity trees occurred in snags. The following proportion of species were used as cavity trees: 35.9% Fraser fir, 28.2% yellow birch (*Betula alleghaniensis*), 17.9% unidentifiable conifer snags, 12.8% red spruce, 2.6% red maple (*Acer rubrum*), and 2.6% black cherry (*Prunus serrotina*). The follow proportion of species were used as drey trees: 80.5% red spruce, 14.6% Fraser fir, 2.4% yellow birch, and 2.4% rosebay rhododendron (*Rhodendron maximum*). Trees with dreys had significantly larger diameter at breast height than cavity trees (average: 26.7 ± 1.6 cm and 34.1 ± 2.1 cm, respectively). Males and females did not differ in the size of den trees they were selecting for ($t_{71} = 1.332, P = 0.19$). The majority of den sites were in conifer dominant stands (81.1%), whereas 19.9% were in northern hardwood dominant stands ($X^2 = 31.1, df = 5, P = 0.000$). The average distance of dens from conifer dominant stands was 38.9 ± 11.4 m.

Habitat Use

The average distance between a Carolina northern flying squirrel telemetry location and the nearest spruce-fir, northern hardwood, and grass/shrub habitat was 5.6, 121.1, and 284.4 m,

respectively. The average distance between random points and the nearest spruce-fir, northern hardwood, and grass/shrub on the 3rd order scale was 8.9, 113.8, and 295.8 m, respectively, and on the 2nd order scale was 117.23, 81.28, and 548.01, respectively. A comparison of these distances indicated that non-random habitat selection was occurring on the 2nd order ($F = 54.83_{3,17}$, $P = 0.000$) and 3rd order scale ($F = 363.95_{3,17}$, $P = 0.000$) and did not differ between male and female squirrels ($F = 0.12_{3,16}$, $P = 0.9485$). The squirrels I tracked were located significantly closer to spruce-fir habitat than expected based on availability at both the 2nd ($t_{19} = -35.35$, $P = 0.000$) and 3rd order ($t_{19} = -5.35$, $P = 0.000$) scales. Squirrels also occurred significantly closer to grass/shrub than expected based on availability at the 2nd ($t_{19} = -5.56$, $P = 0.000$) and 3rd ($t_{19} = -2.8$, $P = 0.01$) order scales. Squirrels neither avoided nor selected northern hardwood habitat at the 2nd order ($t_{19} = 0.72$, $P = 0.48$) or 3rd order scale ($t_{19} = 1.87$, $P = 0.07$).

Pairwise comparison of distances between squirrel locations and each habitat type indicate that tracked squirrels occurred significantly closer to spruce-fir forests versus other habitat types (Table 4.1). Squirrels occurred significantly closer to grass/shrub habitat versus northern hardwood forests. Based on the proportion of each habitat type on the landscape, squirrels preferred spruce-fir, followed by grass/shrub habitats, and northern hardwood forests.

Discussion

Home range estimates reported for this study were comparable to, albeit slightly smaller than values reported in studies throughout the geographic range of the northern flying squirrel (Table 4.2). Unlike the majority studies conducted on this species (Table 4.2), I did not find a significant difference in the size of home ranges between males and females at the 95% or 50% levels, although my home range estimates were slightly larger for males versus females. The

average MCP home ranges observed for male Carolina northern flying squirrels is the smallest reported average home range size for males of all northern flying squirrel subspecies (Table 4.2). My MCP home range estimates were similar to Virginia northern flying squirrel home ranges reported by Diggins (Chapter 2) in the central Appalachians, but much still smaller than the home ranges reported by Menzel et al. (2006) and Ford et al. (2007) for the same subspecies. The longer tracking duration (~ 12 weeks) of Menzel et al. (2006) may have been reflected in larger home range estimates for the Virginia northern flying squirrel, as Diggins' (Chapter 2) tracking duration for the same subspecies was 2-4 weeks and had much smaller home ranges for both sexes (Table 4.2). However, Menzel et al. (2006) tracked squirrels in an industrial forest and areas with lower quality mixed habitat than in my study areas. Ford et al. (2007) conducted their study during winter, potentially indicating larger home range sizes during the time of year that incorporates the breeding season. Although I tracked squirrels from February to June, I did not assess potential seasonal variation due to small sample sizes among seasons. Seasonal differences in home range size of Carolina northern flying squirrels were reported by Hughes (2006) and Weigl et al. (1999), which found that average winter home ranges (12.5 ha and 11.5 ha, respectively) were larger than average summer home ranges (8.3 ha and 6.2 ha, respectively). However, due to a small sample sizes, neither Hughes (2006) nor Weigl et al. (1999) statistically determined if season influenced home range size.

Carolina northern flying squirrels are considered montane conifer forest associates within the southern Appalachian Mountains (Pyane et al. 1989, Weigl et al. 1999, Hughes 2006, Ford et al. 2015), although the majority of research along the montane conifer-northern hardwood ecotone has made the relationship between this subspecies and montane conifer stands unclear (Weigl 2007). Previously, the ecotone between conifer dominant stands and northern hardwood stands

was assumed to be important for denning (Hackett and Pagels 2003, Menzel et al. 2004, Weigl 2007), however, my results indicate that conifer dominant stands are more frequently used for denning than northern hardwood dominant stands. The majority of observed cavity trees and drey trees used by radio-collared squirrels were conifer species (66.6% and 95.1%, respectively). Weigl et al. (1999) found dreys used by Carolina northern flying squirrels were exclusively in red spruce trees. I found similar results, although I also observed a male denning in a drey nest in a rosebay rhododendron (*Rhododendron maximum*) canopy (Diggins et al. 2015). Within the Appalachians, northern flying squirrels are thought to be plastic in their denning habits (Hackett and Pagels 2003, Weigl et al. 2007, Diggins et al. 2015), therefore denning habitat may not be the most important predictor of habitat use of the Carolina northern flying squirrel.

My study is the first to assess habitat selection on multiple spatial scales using telemetry data of Carolina northern flying squirrels in pure montane conifer and montane conifer-northern hardwood forests. Although the ecotone between montane conifer forests and northern hardwood forests are believed to be the optimal habitat for this subspecies (Weigl et al. 1999), I found squirrels selectively preferred spruce-fir forests, whereas they did not select for northern hardwood forests regardless of availability at the 2nd or 3rd order scale. My results are similar to work on Virginia northern flying squirrel, where red spruce or red spruce-northern hardwood forests were preferentially selected by squirrels over northern hardwood forests (Menzel et al. 2006). Isolated subspecies of northern flying squirrels may utilize northern hardwood forests as diurnal denning sites and then spend the nighttime foraging in coniferous forests (Menzel et al. 2006, Hough and Dieter 2009, Ford et al. 2014, Diggins Chapter 2). The increased activity within conifer forests during nighttime foraging activity may be associated with preferred food items, such as hypogean fungi (Hackett and Pagels 2003, Holloway and Malcolm 2007, Hough and Dieter). Within the

southern Appalachians, hypogean fungi abundance is higher in montane conifer stands versus northern hardwood stands (Loeb et al. 2000), which may be reflected in preferential selection of this forest type.

The associations with montane-conifer forests to Carolina northern flying squirrels may also be important for decreasing interactions with the southern flying squirrel in areas of sympatry (Weigl et al. 1999). In high elevation and high latitude parts of its distribution, southern flying squirrel habitat occupancy is limited by availability of hard mast-producing trees (e.g., oak *Quercus* spp.) or American beech (*Fagus grandifolia*; Fridell and Litvatis 1991, Weigl et al. 1999, Holloway and Malcolm 2007), that are important to their survival during colder temperatures at higher elevations or during the winter months when they rely on cached food (Weigl 1978, Weigl et al. 1999, Odum et al. 2001). Virginia northern flying squirrels in the central Appalachians avoided mixed stands with a significant red oak (*Quercus rubra*) component (Menzel et al. 2006), which may reflect avoidance of southern flying squirrels due to increases in mast trees within those stands or avoidance due to unsuitable foraging habitat for preferred food items, such as hypogean fungi (Loeb et al. 2000, Diggins Chapter 2). In my study, northern red oak-dominated stands occurred at lower elevations (< 1,400 m) outside my study areas, so I could not empirically determine Carolina northern flying squirrel preference or avoidance of this forest type. However, none of the squirrels I tracked over the course of this study were located in or near this habitat type.

Additionally, I found Carolina northern flying squirrels selected for grass/shrub habitat at the 2nd and 3rd order scales. The majority of grass/shrub habitat were heath balds dominated by *Rhododendron* spp., grassy balds dominated by grass species (e.g., mountain oat grass *Danthonia compressa*), or small open wet areas dominated by blueberry (*Vaccinium* spp.) or sphagnum moss

(*Sphagnum* spp) and comprised a relatively small proportion of the landscape, with the exception of the grassy bald at White Mountain and the grassy bald on Round Bald at Carver's Gap. When the size of the grass/shrub areas were smaller (i.e., \leq the average glide distance of a northern flying squirrel; Vernes 2001), squirrels travelled across these habitat types to access other foraging areas (Menzel et al. 2006). Radio-collared squirrels found adjacent to larger grass/shrub habitats (i.e., grassy balds at Round Bald and Whitetop) were either denning near these habitats or had nighttime movements located along the edges of these habitats in shrubby areas, but did not occur within the middle of these habitats where herbaceous vegetation was dominant. Although I found squirrels denning in trees along the edges of grass/shrub habitats, there is a record of this subspecies denning in large stems of rosebay rhododendron (Diggins et al. 2015), which may be reflective of the flexibility in denning selection of this subspecies (Hackett and Pagel 2003, Weigl 2007). Edges of open habitats may also provide additional food items for Carolina northern flying squirrels, such as soft mast (i.e., blueberries; Mitchell 2001), although it is not known if squirrels are foraging in these areas. Lack of movements across larger open areas may be due to limited gliding capabilities, lack of traversable canopy, and the squirrel's perceptual range across areas of non-forest cover (Scheibe et al. 2006, Flaherty et al. 2008, Kelly et al. 2013, Smith et al. 2013).

Similar to Virginia Northern Flying Squirrel, the degradation and reduction of montane conifer habitat for Carolina northern flying squirrel make actions to restore these habitats important for long-term conservation of these subspecies (Schuler et al. 2002, Menzel et al. 2006). Climate change may further reduce habitat suitability and extent of spruce-fir forests in the southern Appalachians (Koo et al. 2015), which could lead to the eventual extirpation of Carolina northern flying squirrel (Burns et al. 2003). Ecological restoration of red spruce through canopy-gap creation may help increase resiliency of this forest type to climate change (Rentch et al. 2016) and

may reduce habitat invasion by southern flying squirrels into Carolina northern flying squirrel habitat. Restoration treatments to release red spruce should focus on girdling mast-producing species, such as northern red oak, while maintaining species, such as yellow birch, which are important denning sites and whose bark provides denning substrate for Carolina northern flying squirrels. High-elevation spruce-fir restoration within the southern Appalachians would also benefit other species of conservation concern, such as the northern saw-whet owl (*Aegolius acadicus*) and the federally endangered spruce-fir moss spider (*Microhexura montivagans*). Additionally, little work has been done to understand Carolina northern flying squirrel habitat selection within the Great Smoky Mountains National Park, the only sky island with a substantial amount of old-growth habitat remaining (Dull et al. 1988, Ford et al. 2015). Although I tracked 3 squirrels in old growth habitat at Whitetop Mountain in Virginia, there is a need to better determine habitat selection between old growth and second growth stands to better inform ecological restoration trajectories within southern Appalachian spruce-fir forests.

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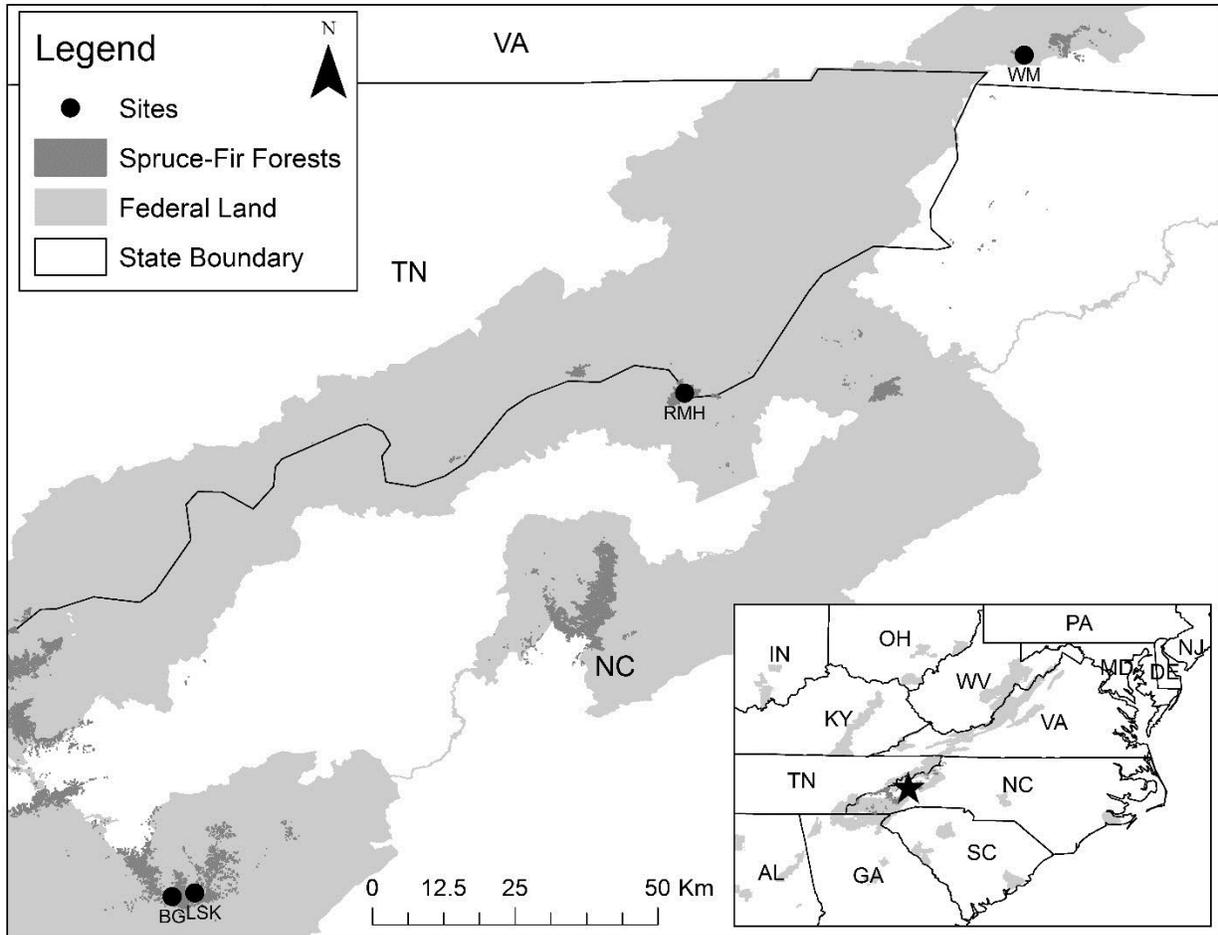


Figure 4.1. Survey sites for Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) home range and habitat selection study in western North Carolina, during 2012 and 2014-2015. BG = Buckeye Gap, LSK = Little Sams Knob, RMH = Roan Mountain Highlands, and WM = Whitetop Mountain.

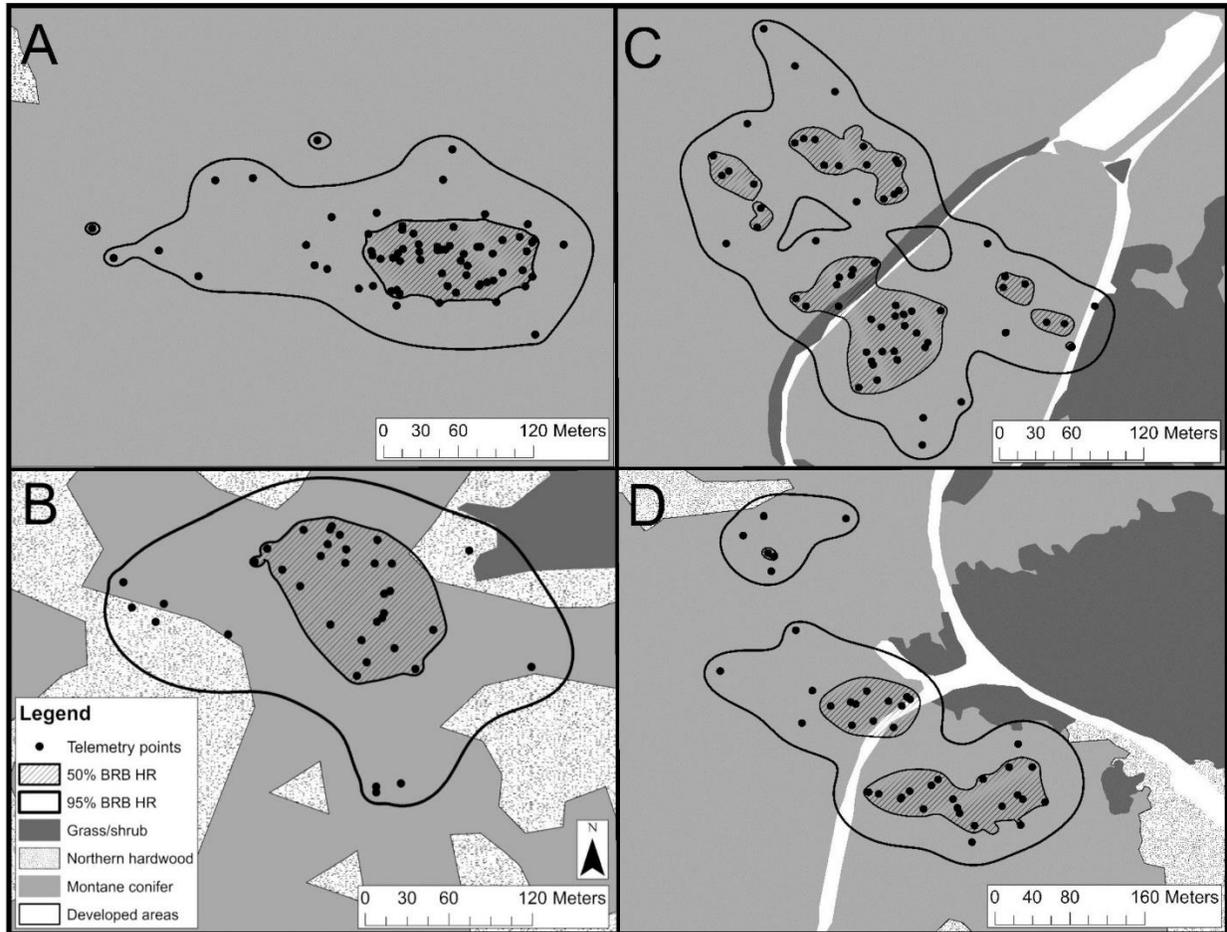


Figure 4.2. Examples of 50% and 95% biased random bridge home ranges of Carolina northern flying squirrels (*Glaucomys sabrinus coloratus*) in western North Carolina during 2012 and 2014-2015. A) Female home range in montane conifer forest at Roan High Knob, Mitchell County, B) female home range on montane conifer-northern hardwood ecotone at Buckeye Gap, Haywood County, C) male home in montane conifer forests at Roan High Knob, Mitchell County, and D) male home range on montane conifer-northern hardwood ecotone at Carver's Gap, Mitchell County.

Table 4.1. Ranking matrix of Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) habitat selection in western North Carolina during 2012 and 2014-2015. Results are t-statistics (P-values) of pairwise comparisons of habitat type telemetry/random distance ratios.

2 nd Order Scale			
	Spruce-fir	Northern hardwood	Grass/Shrub
Spruce-fir	---	-5.97(0.000)	-5.11(0.000)
Northern Hardwood	5.97(0.000)	---	2.98(0.008)
Grass/Shrub	5.11(0.000)	-2.98(0.008)	---
3 rd Order Scale			
	Spruce-fir	Northern hardwood	Grass/Shrub
Spruce-fir	---	-3.12 (0.005)	-6.17 (0.000)
Northern Hardwood	3.12 (0.005)	---	1.16(0.259)
Grass/Shrub	6.17 (0.000)	-1.16 (0.259)	---

Table 4.2. Comparison of average Minimum Convex Polygon home ranges of northern flying squirrels (*Glaucomys sabrinus*) from previous studies in North America.

Source	Subspecies	Location	Habitat Type ^D	Season	Male MCP (ha)	Female MCP (ha)	Average MCP (ha)
This study	<i>G. s. coloratus</i>	North Carolina/Virginia	Red spruce-Fraser fir; red spruce-northern hardwood	Feb. - June	5.1 (10) ^B	4.0 (10)	4.5 (20)
Hackett 2002	<i>G. s. coloratus</i>	Virginia	Red spruce – northern hardwood	Year Round	8.9 (3) ^B	8.0 (3)	9.4 (6)
Hughes 2006	<i>G. s. coloratus</i>	North Carolina	Eastern hemlock – northern hardwood	Year Round	17.5 (5) ^C	4.9 (2)	13.9 (7)
Weigl et al. 1999	<i>G. s. coloratus</i>	North Carolina/Tennessee	Red spruce – northern hardwood	Feb. – Sept.	10.1 (6) ^C	6.9 (4)	8.9 (10)
Urban 1988	<i>G. s. fuscus</i>	West Virginia	Red spruce – northern hardwood	Unknown	---	---	5.2 (3)
Menzel et al. 2006	<i>G. s. fuscus</i>	West Virginia	Red spruce – northern hardwood	May – Sept.	59.8 (4) ^A	15.9 (8)	29.7 (12)
Ford et al. 2007	<i>G. s. fuscus</i>	West Virginia	Red spruce – northern hardwood	Winter	72.2 (2) ^C	8.4 (1)	50.9 (3)
Diggins Chapter 2	<i>G. s. fuscus</i>	West Virginia	Red spruce/eastern hemlock – northern hardwood	May – June, Oct.	5.8 (7) ^B	2.3 (6)	4.2 (13)
Gerrow 1996	<i>G. s. gouldi</i>	New Brunswick	Red spruce – birch spp.	Unknown	12.5 (7) ^A	2.8 (8)	3.1 (15)
Holloway and Malcolm 2007	<i>G. s. macrotis</i>	Ontario	Sugar maple – yellow birch	Summer	11.2 (7) ^B	3.8 (7)	---
Hough and Dieter 2009	<i>G. s. bangsi</i>	South Dakota	Ponderosa pine	May – Aug.	11.2 (30) ^A	6.9 (19)	9.6 (49)
Wilson et al. 2008	<i>G. s. lascivus</i>	California	Red fir; mixed conifer forests	May – Oct.	23.5 (9) ^B	23.8 (4)	23.6 (13)
Witt 1992	<i>G. s. oregonensis</i>	Oregon	Douglas-fir – western hemlock – western redcedar	Dec., June – Sept.	---	---	3.7 (4)
Martin and Anthony 1999	<i>G. s. fuliginosus</i>	Oregon	Douglas-fir – western hemlock	June	5.9 (20) ^A	3.9 (19)	---

^A Found a significant difference in home range size between males and females

^B Found no significant difference in home range size between males and females

^C Study did not test for differences in home range size between males and females.

^D Red spruce = *Picea rubens*, Fraser fir = *Abies fraserii*, Eastern hemlock = *Tsuga canadensis*, birch spp. = *Betula* spp., sugar maple = *Acer saccharum*, yellow birch = *Betula alleghaniensis*, ponderosa pine = *Pinus ponderosa*, red fir = *Abies magnifica*, Douglas fir = *Pseudotsuga menziesii*, Western hemlock = *Tsuga heterophylla*, Western redcedar = *Thuja plicata*

Chapter 5: Conclusion

The Virginia and Carolina northern flying squirrels (*Glaucomys sabrinus fuscus* and *G. s. coloratus*, respectively) are high priority species on both the state and federal level. The ability to monitor for both subspecies and conduct management activities that improve the extent, quality, and connectivity of their habitat is important for long-term persistence and occupancy within the central and southern Appalachian Mountains. The squirrels' nocturnal and arboreal nature, in addition to their rarity on the landscape, make these subspecies inherently difficult to monitor and manage. My research sought to 1) determine the most effective monitoring technique for northern flying squirrels in Appalachia, and 2) fill data gaps on multi-scale habitat use for both subspecies.

Major Findings

1. Novel techniques (i.e., ultrasonic acoustics and camera traps) were more effective at detecting Carolina northern flying squirrels than traditional live trapping. Probability of detection (POD) was similar between ultrasonic acoustics and camera traps. However, ultrasonic acoustics had a lower Latency to Detection (LTD) than camera traps, making it the most effective method. Additionally, ultrasonic acoustics can differentiate between northern flying squirrels and southern flying squirrels, which are sympatric in some areas of the Appalachian Mountains.
2. Radio-collared Virginia northern flying squirrel selected microhabitat where conifers were dominant, the understory was open, and organic soils were deeper than random points. Squirrels did not select areas where northern hardwoods were dominant in the overstory. Microhabitat selection was similar to macrohabitat (i.e., home range, landscape) selection,

where presence of conifer species in the overstory is preferred (Stihler et al. 1995, Odom et al. 2001, Menzel et al. 2006, Ford et al. 2007, 2010).

3. Radio-collared Carolina northern flying squirrels preferentially selected montane conifer forests on multiple spatial scales. Squirrels also preferentially selected denning habitat in conifer dominant stands, even when the squirrel's home range was located on the montane conifer – northern hardwood ecotone. There is no evidence of selection for or against northern hardwood forests by this subspecies, highlighting the importance of montane conifer forests noted by other antidotal and observational studies of non-radio-collared individuals (Payne et al. 1989, Weigl et al. 1999, Hackett 2002, Hackett and Pagels 2003, Ford et al. 2014, 2015).

Management Implications

Conservation and management of the Virginia and Carolina northern flying squirrel is dependent on the ability of managers to assess changes in populations of both subspecies. However, due to low capture rates via traditional methods (i.e., live traps and nest boxes) exhibited in this study and other studies (Stihler et al. 1995, Reynold et al. 1999, Weigl et al. 2002, Terry 2004, Hughes 2006, Menzel et al. 2006), habitat has traditionally been used as a surrogate to manage for the Appalachian northern flying squirrels (Pyane et al. 1989, Ford et al. 2004). Ultrasonic acoustics presents a promising method to rapidly assess habitat occupancy by both subspecies of northern flying squirrel, while having the ability to differentiate between northern and southern flying squirrels, which are sympatric at the montane conifer – northern hardwood ecotone. Ultrasonic acoustics can provide information on probability of occupancy across the landscape and allow for managers to understand what landscape (i.e., elevation, habitat type,

landform index) and local effects (i.e., presence of southern flying squirrels, varying levels of invasive insect infestation, within-stand structural characteristics) are driving habitat occupancy. Ultrasonic acoustics can also determine how habitat management or ecological restoration influence site occupancy over time, allowing for managers to assess how the application of treatments and structural changes induced by treatments influence the persistence of occupancy of both subspecies.

Anthropogenic climate change is one of the most concerning factors that will impact Appalachian northern flying squirrels and their preferred high-elevation montane conifer habitat, especially red spruce (*Picea rubens*) forests. Climate change can have indirect or direct influences on the expansion or contraction of a species' range (Gaston 2003, Sexton et al. 2009), potentially causing extinction in species with range restrictions (Parmesan 2006). In mountainous regions, such as the Appalachian Mountains, shifts to higher elevations may be impossible if a species' habitat presently occurs at the highest elevations, making mountain top species vulnerable to extinction due to severe range contractions (McDonald and Brown 1992, Parmesan 2006, Issac 2009), especially when species are located in mountainous regions at more southern latitudes (Guralnick 2006) or at the southern extent of their range (McCain and King 2014). The position of montane conifer forests on the highest peaks and ridgelines within the central and southern Appalachians make them extremely vulnerable to climate change (Beane and Rentch 2015, Koo et al. 2015). Additionally, red spruce forests and northern flying squirrels reach their most southern extent within the central and southern Appalachian Mountains (Miller 1936, Handley 1953, Edgar and Adams 1992). Reduction in the extent of red spruce forests after industrial logging has shifted the elevational range of this species to higher elevations (Pielke 1981, Busing et al. 2003, Hayes et al. 2006).

My results indicate that both Virginia and Carolina northern flying squirrels preferentially select for montane conifer habitat, such as red spruce. Both subspecies are currently flagship species for red spruce restoration efforts in the central and southern Appalachians. Red spruce restoration aims to mimic fine-scale natural disturbances (i.e., tree fall gaps, White et al. 1985, Rentch et al. 2010) to release suppressed understory trees into the overstory (Rentch et al. 2016). Goals of restoration are to increase the extent and connectivity of red spruce forests similar to what existed prior to industrial logging. The increase in the extent and shifting from hardwood dominant to spruce dominant forests may increase the resilience of this forest type to anthropogenic climate change, therefore improving and protecting Virginia and Carolina northern flying squirrel habitat into the future.

Future Research Directions

Although I filled several data gaps with my research on Virginia and Carolina northern flying squirrels, new questions need to be address to further improve management and conservation of these subspecies. The two main issues that need to be focused on are 1) development of protocol for ultrasonic acoustic surveys, and 2) determine how natural and anthropogenic disturbance influences Appalachian northern flying squirrel habitat occupancy.

Standardized protocol for conducting ultrasonic acoustic surveys on Appalachian northern flying squirrels is important to ensure the technique is being used in an effective and meaningful way. Due to studies on the methodology of ultrasonic acoustics for survey bats, methods for effective detector placement and weatherproofing have already been determined (Weller and Zabel 2002, Brtizke et al. 2010) and have been adapted for flying squirrel surveys. However, other technique-related issues (i.e., detector type, seasonality, survey length, detector configuration)

have not yet been assessed to any appreciable extent and therefore are important for proper utilization of this technique.

The ability to assess how each subspecies is impacted by multiple, interacting factors (e.g., climate change, competition, habitat degradation) is important in determining how they may persist on the landscape, which has profound conservation implications at mid- and long-term scales. Using a resource utilization analysis, data from radio-collared squirrels from my research and past research (i.e., Menzel et al. 2006, Ford et al. 2007) can help improve range-wide distribution maps and may be paired with climate change models to predict future changes to suitable habitat in the future. These maps may also help highlight areas for red spruce restoration by assessing potential corridors between isolated habitat patches. Ultrasonic acoustics could be used to determine the influence of habitat quality, habitat management, and disturbances on long-term habitat occupancy by Virginia and Carolina northern flying squirrels. Because ultrasonic acoustics can also be used to survey for southern flying squirrels, this method can be employed to assess the seasonal invasion of southern flying squirrels into Virginia and Carolina northern flying squirrel habitat. Ultrasonic acoustics can be used to determine how balsam woolly adelgid (*Adelgid balsamea*) infestation influences flying squirrel occupancy, as infested areas have lower amounts of canopy cover, which may negatively affect flying squirrels (Smith 2007). Lastly, this method can also be used to determine how ecological restoration treatments via canopy-gap creation affect Virginia and Carolina northern flying squirrel habitat occupancy, helping to assess the initial impact of restoration treatments and long-term effects on both subspecies.

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