Movement patterns, food availability, and fungal diets of sympatric flying squirrels in the Kawartha Highlands

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Abstract

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Northern flying squirrels (NFS) are mycophagous specialists (fungi-dominated diet) that may be displaced with southern flying squirrel (SFS) range expansion, thereby limiting fungal dispersal in forest communities. To understand the implications of squirrel species turnover on mycophagy, we investigated the home ranges of both flying squirrel species who are living in stable sympatry. We found no significant difference in home range sizes and identified spatial overlap between the two species. Through habitat selection ratios we found SFS were strongly selecting for deciduous-dominated habitats more than NFS. Lastly, we conducted microscopy on flying squirrel scat and found NFS were eating more fungi than SFS. We conclude that the squirrels are sharing the same habitat landscape but are finding ways to partition the habitat accordingly to allow for sympatry. SFS may contribute to the spore-dispersal cycle similarly to their northern counterpart through moderate fungus consumption and large home range sizes.

Keywords: mycophagy; Glaucomys; flying squirrels; diet; home range; sympatry; fungi

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Chapter 1

General Introduction

This thesis is about the role of northern flying squirrels (*Glaucomys sabrinus*) as mycophagists in forest ecosystems. Northern flying squirrels provide an ecosystem service by dispersing fungal spores that help to establish mycorrhizal networks. Under a warming climate, southern flying squirrels (*Glaucomys volans*) are expanding their range northwards from southern latitudes, and this may lead to a species turnover to southern flying squirrels. Northern flying squirrels are likely to contract their range in response. This thesis is about the implications on mycophagy and animal dispersal of fungal spores should turnover of flying squirrel species occur.

Note: Throughout this thesis I may address northern flying squirrels as “NFS” and southern flying squirrels as “SFS”.

Dispersal

Dispersal is a natural ecological process that allows the establishment of an organism into a new community (Clobert et al. 2012). Dispersal is important in the dynamic nature of ecosystems and determines how populations are balanced over time. There are many factors that contribute to the dispersal process. The dispersal of seeds, for example, is highly facilitated by animal-based vectors (Nathan et al., 2008). In some instances, animals have the capacity to disperse more seeds and are able to germinate them more easily, than from wind conditions (Vander Wall, 2008).

The dispersal process is also important for fungus, the spores of which are sometimes dispersed by animals with fungi-dominated diets. Animals that persist on a diet of fungi are called mycophagists, and they are dominant vectors for fungus spore dispersal. Some fungi rely on animals to spread their spores because these fungal fruiting bodies, such as truffles, spend their entire life cycle underground (Fogel & Trappe, 1978) and hence require excavation by animal vectors for the spores to be released to the air. This benefits the truffle spores, as the spores can be easily germinated into the soil after passing through the gut of animals (Colgan & Claridge, 2002; Kotter & Farentinos, 1984). The dependence on animals to carry out spore dispersal is how these underground truffles are able to continue to spread in forest ecosystems (Johnson, 1996). It is difficult to track how far animals can disperse fungal spores, but records on small scale studies revealed small mammals have the capacity to spread truffle spores up to 35 m from the original fruiting body (Frank et al., 2009) and in some cases up to 900 m (D.Luoma, personal communication, April 2023), although this is likely an underestimate given known dispersal distances of small mammals (Carey 1995). In some regions, rodents and large mammals like deer are important vectors for establishing mycorrhizal networks in newly exposed soils after a volcanic eruption (Allen 1987) or retreating glaciers (Cázares & Trappe, 1994). This is most interesting when comparing this process to air-borne fungal spores independently, as wind has a relatively short dispersal distance (Li, 2005).

In areas where there is some habitat patchiness and landscape disturbance, small mammals offer a stronger effect for fungal dispersal than relying on airborne vectors (Jacobs & Luoma, 2008; Luoma et al., 2004). Similar to seed dispersal, animals play a large role through mycophagy to contribute to long distance spore dispersal and can create inoculums in new soil environments (Ashkannejhad & Horton, 2006; Cázares & Trappe, 1994).

Animals eat fungi because they are able to locate and detect fungi easily through olfactory senses and morphological characteristics of fungi (Elliott et al., 2022), and animals do not need to exert energy into processing the food item, like removing shells from acorns and pine cones (Trappe et al., 2009). Fungi also supply water for hydration, minerals, vitamins, fats, and proteins to their herbivores; all nutrients that are especially more concentrated in truffles than mushrooms (Trappe et al., 2009). Overall, there are more vitamins and minerals in fleshy macro-fungi than fruits and nuts, and leaves of plants (Claridge et al., 1999). However, many of the nutrients in truffles are locked inside the indigestible parts of the fungi (e.g., cellulose), so eating one type of fungus genus may be limited in nutrients for the animal (Trappe et al., 2009). For example, mycophagists need to eat diverse fungi to obtain a proper nutrient balance (Claridge et al., 1999). This may be why there are few obligate mycophagists (i.e., individuals that are entirely dependent on a fungus diet). Species that are solely mycophagists are often associated with boreal habitats where fungus is plentiful because fungi taxa are highly associated with conifer trees and the surrounding habitat within the boreal (i.e., snag and coarse woody debris). In North America, examples of obligate mycophagists include the southern red-backed vole (*Clethrionomys gapperi*) and the northern flying squirrel (*Glaucomys sabrinus*) (Orrock & Pagels, 2002; Trappe et al., 2009).

Flying Squirrels

There are three species of North American flying squirrels (*Glaucomys* spp.). Humboldt's flying squirrel (*Glaucomys oregonensis*) (Arbogast et al., 2017) is a newly identified species in the Pacific Northwest, from British Columbia in Canada to California in the US. There is one subspecies of this population that is critically imperilled; the San Bernardino flying squirrel (*Glaucomys oregonensis californicus* (Arbogast et al., 2017; Rhoads, 1897). Northern flying squirrels (*Glaucomys sabrinus*) (Shaw, 1801) have the largest geographic range from as far north as Alaska at the extent of the treeline; covering west from Vancouver to the east coast of Newfoundland; and south to the mountains in the United States, such as the Appalachians and Sierra Nevada (Weigl, 2007). Most northern flying squirrels are a group of low population concern, however the conservation status of some of the subspecies differs. The Virginia northern flying squirrels (*Glaucomys sabrinus fuscus*) (Miller, 1936) subspecies once was listed and recently delisted due to a population recovery (U.S Fish and Wildlife, 2013). The Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) (Handley, 1953) subspecies is considered endangered and at risk due to anthropogenic disturbances (Arbogast, 2007). Southern flying squirrels (*Glaucomys volans*) (Linnaeus, 1758) have the second largest range, occupying northern

Mexico, much of the southeastern US and some provinces in Canada; as far south as the Gulf of Mexico and as far north as central Ontario with one of the northern ranges extending in Temagami, Ontario (47^{o} deg N) (Bowman et al., 2005; COSEWIC, 2006; Stabb, 1988).

Southern flying squirrels have expanded their range northwards and come into increasing secondary contact with northern flying squirrels (Bowman et al., 2005). There is an expectation that increased contact between these species could lead to the extirpation of northern flying squirrels through competition (Weigl, 1978). Parasite-mediated competition is possible as southern flying squirrels are reservoir hosts of *Strongyloides robustus* which is thought to be deleterious to the northern flying squirrel (O'Brien et al., 2022; Weigl, 1978). Further, southern flying squirrels appear more aggressive for nesting sites than northern flying squirrels (i.e., Muul, 1968; Weigl, 1978). Although southern flying squirrel aggression may be true in studies of captive animals, it has also been observed that the southern flying squirrels are social nesters, in that they were more often found nesting in groups, whereas northern flying squirrels were found more in solitude (Olson et al., 2018; Weigl, 1974), and these patterns might confound inferences about nest competition. These differences in nesting strategies can be attributed to their reproductive successes and thermal ecology. Southern flying squirrels have their litters in early spring, April and May, and have larger litters in spring than northern flying squirrels (Dolan & Carter, 1977; Wells-Gosling & Heaney, 1984), which offers more opportunities for social nesting. Southern flying squirrels are also more reliant on social thermoregulation than their northern counterparts (Olson et al., 2018). However, flying squirrels are limited in nest types because both species prefer tree cavities for nests and they gravitate towards nesting in similar-sized trees based on the ambient temperature; both were found to select larger trees in the winter (Holloway & Malcolm, 2007; Minns et al. (in review)). Competition for nests remains a testable hypothesis for understanding the displacement of northern flying squirrels in light of southern expansion (Weigl, 2007).

Mycorrhizal networks

Mycorrhizae is a term used to describe the mutual relationship that exists between plant roots and fungus networks underground (Smith & Reed, 1997). It has been said that nearly all plants in forested ecosystems are connected through mycorrhizal networks in which fungal hyphae attach to plant roots and through symbioses, they both benefit through this process (Cairney, 2000; Smith & Reed, 1997). Mycorrhizal relationships are known to be one of the first colonizers of land plants and played a large role in how terrestrial plants were able to establish (Cairney, 2000). Fungal hyphae existing in the soil can mediate the plant nutrient, water, and mineral uptake. In exchange, the plant roots provide the fungus hyphae with carbohydrates and fats, since fungi are sugar limited. This relationship is unique because it promotes efficient energy transport and the thin-web like networks are able to reach in narrow areas of the soil where plant roots are too thick to navigate (Smith & Read, 2008). Mycorrhizal networks have a role in facilitating plant growth, depending on the nutrient availability in its proximity (Allen et al., 2003). The colonization of mycorrhizae has the potential to increase plant growth rates and survivorship depending on the plant host and fungal inoculum (Allen et al., 2003; Smith & Read, 2008). These networks help to provision nitrogen to trees, and are responsible for adding to the forest natural biomass and facilitating nutrient cycling underground (Fogel, 1980). Without these symbioses in a given ecosystem, plant health may be affected and altered.

With rapid climate warming, mycorrhizal networks face a variety of effects. One review identified that warming temperatures may increase the nutrient exchange between plant hosts and fungal hyphae; accelerating the symbioses (Duarte & Maherali, 2022). Another research paper found that mycorrhizal fungi has higher colonization power to plant roots when soil nutrient levels (i.e., nitrogen availability) are high and environmental factors are favourable for temperate environments, meaning higher precipitation and warmer temperatures (Soudzilovskaia et al., 2015). But overall, the general consensus is that direct impacts of climate change on mycorrhizal networks are unknown as the effects are variable in different regions and they are difficult to measure (Bennett & Classen, 2020). However there is concern that climate warming can affect the phenology of plant emergence, thus altering the interactions between plants and their relationship with fungi (Bennett & Classen, 2020).

Warming temperatures from climate change has shifted the ranges of many animals. This impact makes climate warming especially vulnerable for those located at the edge of their population distribution (Myers et al., 2009). Climate warming, coupled with human landscape alterations and human-induced habitat loss, have also predicted to shift the balance of carbon and nitrogen ratios in soils, due to elevated nitrogen levels from human input in the atmosphere and fertilizers (Nadelhoffer et al., 1999). These changes all pose critical problems for fungal and mycorrhizal communities, affecting how they are able to grow, in what density, and the ability to colonize and spread in forested areas (Soudzilovskaia et al., 2015). Some mycorrhizal networks depend on mycophagists to spread their reproductive fruiting bodies and in turn dispersing the mycorrhizal networks and connecting to more plant hosts (Fogel & Trappe, 1978).

Mammalian mycophagy has likely existed since the last interglacial period (Trappe et al., 2009) and the ecological significance was first documented by Reess & Fisch (1887). This fundamental system will be altered with climate warming affects (i.e., Myers et al., 2009), because plant, fungus, and animal distributions are shifting, potentially decoupling or altering symbiotic relationships. My thesis will assess the potential implications of such range shifts to the relationship between mycophagous northern flying squirrels and animal-dispersed fungus.

The main objective of this thesis is to understand the mycophagic role of both flying squirrel species and the implications of the ecosystem services if species turnover were to occur, with southern flying squirrel range expansion and subsequent northern flying squirrel displacement. I investigated the home range size, habitats used, and mycophagy of two flying squirrel species that are currently existing at a location with stabilized sympatry. My first object is to understand the home ranges of both species to determine space and area used on the landscape. This relates to the second objective, in which I explored the habitats selected for within individual home ranges. The third objective identified the fungal diet of both squirrel species to understand the fungal taxa and abundance consumed. Lastly the fourth objective identified the availability of common macrofungi present at the site that may contribute to habitat selection patterns and fungal diet preferences. These objectives would explore how these two squirrels can continue to exist in sympatry on the landscape for 21 years as identified with recent incidents of hybridization at this location (Garroway et al., 2010). If I identify differences among home range, habitat selection and diet, I can attribute this to resource partitioning, which allows the species to live in sympatry. This research will aid our understanding of the ecosystem service role of both flying squirrels, and the implications on this role should southern flying squirrels displace northern flying squirrels from range expansion.

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Chapter 2

Home range size, habitat selection, and mycophagy of sympatric North American flying squirrels

Author: Rebekah Persad[1]

Abstract

Northern flying squirrels (NFS) are a keystone species that specialize on a fungus diet and facilitate the dispersal of fungi spores, thereby supporting the growth of underground mycorrhizal networks. Given the range expansion of the closely related southern flying squirrels (SFS), a dietary generalist, we wished to understand the implications of squirrel species turnover on fungi dispersal. At a sympatric site where food availability is similar between squirrel species, we investigated the home ranges, habitat selection, and fungal diets of flying squirrels to test if northern flying squirrels have larger home ranges attributed to their time spent foraging in coniferous habitats, and that they favour fungi taxa more than their southern counterparts. We live-trapped flying squirrels for one year (2020-2021), deployed 31 radio collars (NFS n = 18, SFS n = 13), and radio-tracked individuals to investigate home range size using 95% minimum convex polygons (MCP). We identified no significant differences in home range sizes between species ($P > 0.1$), and subsequently, we found both squirrel species had home range overlap. We conducted a habitat selection analysis using binomial GLM of mean ratios of selectivity and identified southern flying squirrels selected deciduous-dominated habitats more than northern flying squirrels within their home ranges. Finally, we identified that northern flying squirrels had significantly higher spore loads

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[1] The authors of the paper that will originate from this thesis will be: Rebekah Persad, Samantha M. Stead, Ryan B. Stephens, Jeff Bowman.

and spore richness in their scat compared to their southern counterpart. This study identifies stable sympatry between northern and southern flying squirrels, due to their ability to partition habitats and diets in slightly different ways while sharing the landscape. Our research suggests that if northern flying squirrels were to be replaced by their southern counterparts, southern flying squirrels have the potential to provide a similar ecosystem service to disperse fungal spores.

Keywords: mycophagy; Glaucomys; squirrels; diet; range; sympatry

Introduction

Mycophagy

Small mammal mycophagy is often poorly examined and underappreciated in wildlife sciences (Stephens & Rowe, 2020). Mycophagy can serve a similar function to seed dispersal or pollination, in which animals act as a vector to spread a substrate in new habitats, or in the surrounding environment. Small mammals with a predominantly fungal diet act as vectors that disperse fungal spores in forest stands, directly and indirectly enhancing the forest community diversity (Johnson, 1996). The fecal pellets of small mammal mycophagists (i.e., individuals who eat fungi) act as “pills” of symbiosis containing viable fungal spores that can be readily germinated in the soil and establish new mycorrhizal communities; thereby supporting symbiotic relationships to exchange nutrients with vascular plants and facilitating the growth of trees (Caldwell et al., 2005; Maser & Maser, 1988; Smith & Reed, 2008). These fungal-packed feces not only contain spores, but also contain yeast and nitrogen-fixing bacteria that help facilitate decomposition in the soil, providing available nitrogen and nutrients to plant roots (Li et al., 1986).

The fruiting bodies of hypogeous fungus (i.e., fungi growing below ground) rely on mycophagous animals to disperse their spores (Maser et al., 1978), making mycophagy by mammals especially important for dispersal. Hypogeous fungi, also known as truffles, have their spores are locked into their fruiting bodies. Truffles emit a strong odour that attracts small mammals and helps them locate these fruiting bodies (Maser et al., 1978; Maser & Maser, 1988), encourages them to bite into the peridium (outer casing), and ultimately facilitate the dispersal process (Maser et al., 1978). Mycophagists only consume the peridium, not the entire truffle. They scrape the peridium that contains the most nutrients, and incidentally consume spores during this process (Cork & Kenagy, 1989; Fogel & Trappe, 1978). This natural spore dispersal process can be thought of as the forest's natural fertilizer, inoculating soil throughout the forest with viable fungal spores, with higher inoculation success nearby tree roots, facilitating forest regeneration and uninhibited growth of seedlings and plants (Maser et al., 1978; Weigl, 2007).

Flying squirrels

Flying squirrels are highly mobile creatures and need to cover large distances to find food. Male squirrels travel farther distances than females, thus expanding home range in search of females to mate, and notably they travel far in search for food (Taulman & Smith, 2004). Females have smaller home ranges because they forage nearby nests to tend to young, (Fridell & Litvaitis, 1991; Taulman & Smith, 2004), at least during the maternal period. Both southern and northern flying squirrels exhibit similar sex-related differences in home range size for these reasons. However, there has not been a study to document home range sizes of both flying squirrel species existing in local sympatry. A study in central Ontario that trapped allopatric flying squirrels in different locations found northern flying squirrels have larger home range sizes than southern flying squirrels (Holloway & Malcolm, 2007b).

Home range size differences in past research seem to vary with the tree types present in flying squirrel home ranges (Jacques et al., 2017); landscape features such as elevation and mountains which shape food availability (Diggins et al., 2017; Menzel et al., 2006); and seasonal effects (Weigl, 2007). Moreover, home range sizes also may depend on conditions of the woodlot or forest. For example, if fragmentation and logging has occurred, squirrel species are likely to have larger ranges to incorporate sufficient habitat (Holloway & Malcolm, 2007b; Menzel et al., 2006; Taulman & Smith, 2004). Northern flying squirrels may travel farther within woodlots due to their stronger reliance on fungal fruiting bodies, which are patchily distributed (Weigl, 1978). Northern flying squirrels are thought to be important mycophagists, since they have co-evolved their fungal diet in their boreal forest range where conifer trees have supplied this diet item (Maser et al., 1985). In comparison, southern flying squirrels have smaller home range estimates because they depend on a diet of hard mast seeds that are more often in abundance and diverse in seed type in hardwood habitats. Specifically, southern flying squirrels are found to be strongly tied to hardwood habitats such as oak trees (*Quercus* spp.) (Sonenshine & Levy, 1981) and are known to prefer a diet of acorns and other tree seeds (Helmick et al., 2014; Weigl, 1978). However, they are generalist enough that they will also eat fungi, fruits and plant parts depending on availability (Fogel & Trappe, 1978; Harlow & Doyle, 1990). Southern flying squirrels are known to be habitat specialists in deciduous-dominated habitats (Taulman & Smith, 2004; Weigl, 1978), but more of a generalist in terms of their diet. In contrast northern flying squirrels are identified to be habitat generalists in that they adapt to various nest and tree types, and forest types year round (O'Brien et al., 2021; Rosenberg & Anthony, 1992; Weigl, 1978), but are known to be fungal specialists for their diet. These divergent characteristics appear to allow both species to live in sympatry in some locations.

Coniferous forest stands without the presence of the obligate mycophagist, the northern flying squirrel, may suffer detrimental ecological effects. Northern flying squirrels play a significant role with fungus spore dispersal in forests and therefore facilitate the growth of fungi and mycorrhizal networks within the ecosystem (Fogel & Trappe, 1978). If northern flying squirrels were absent from the forest, it is predicted spore dispersal rates will be reduced because some fungi may rely on heavy mammalian consumption, likely from fungi specialists, to effectively spread (Nuske et al., 2017). In conjunction, there may be lower fungal diversity in their associated forest habitats and vascular plants would not sufficiently grow because plants heavily depend on mycorrhizal networks for nutrient cycling and facilitating biomass production (Fogel, 1980; Vernes, 2007). Therefore northern flying squirrels are considered a keystone species because of their integral role in forest ecosystem functioning and their strong associations with old-growth forests (North et al., 1997). This is what makes flying squirrels very interesting animals to study as mycophagists; their quick mobile nature to glide far distances can spread spores to adjacent woodlots and beyond (Frank et al., 2006). There are currently no studies conducted to identify the fungal diet of both northern and southern flying squirrels while existing in sympatry, and ours is the first study to compare the diet of both species at the same location.

Range shifts

In the last 20 years, southern flying squirrels have expanded their range northwards (Bowman et al., 2005; Myers et al., 2009). During several years preceding 2003, southern squirrels moved 200 km northwards past their historic range limit (Bowman et al., 2005). This range expansion was attributed to a series of warm winters from climate warming. This change in distribution provided an opportunity for increased secondary contact and sympatry with northern flying squirrels (Bowman et al., 2005; Garroway et al., 2010). Sympatry occurs when two species are able to coexist through niche partitioning and when habitat supports both animals (Weigl, 1978). Localized sympatry is dependent on the ability of these squirrel species to eat different diets, since this would limit competition for food (Maser & Maser, 1988). However increased sympatry can promote hybridization between the two species or create competition for nesting cavities or other resources (Garroway et al., 2010; Weigl, 1978).

The shift in range limits of southern flying squirrels as a result of climate change and mast fluctuations (i.e., Bowman et al., 2005), has unknown implications for long-term population trends of northern flying squirrels, in the face of expanding southern flying squirrel populations. There is evidence for parasite-mediated competition between northern and southern flying squirrels. Northern flying squirrels have been shown to be limited by the presence of an intestinal parasite, *Strongyloides robustus*, whereas southern flying squirrels are a reservoir host (Wetzel & Weigl, 1994). Therefore, the presence of southern flying squirrels might limit northern flying squirrels due to the introduction of this parasite. In addition, there is some evidence that northern flying squirrels are outcompeted by southern flying squirrels for tree cavity nests where cavities are limiting (Weigl 1978). Southern flying squirrels are social nesters compared to northern flying squirrels, which tend to nest in smaller numbers (Olson et al., 2018). Therefore, northern flying squirrels might face competition for suitable nests and may be displaced from forest territories as a result of southern expansion (Weigl, 1978). As southern flying squirrels continue to expand their range, populations of northern flying squirrels face extirpation at their rear edge and will contract their ranges in response (Hampe & Petit, 2005).

Objectives

Our overall objective was to assess the potential implications on the ecosystem service of mycophagous animals should climate change lead to a turnover from northern flying squirrels to southern flying squirrels. We conducted this study at a long-term research site that has been monitoring flying squirrels for 21 years. The location has both flying squirrel species and they have been present at the site for the full 21 years. This provides an interesting study area to investigate questions about range boundary dynamics of both flying squirrel species. Our first objective was to measure the home range sizes of both flying squirrels. Northern flying squirrels have a larger body size and travel long distances in northern forests to search for widely dispersed food such as truffles. In contrast, southern flying squirrels are smaller and forage on the relatively rich food source of tree seeds. Therefore, we predicted that northern flying squirrels would have larger home range sizes to account for time spent and space use to locate fungi, which are often patchily distributed. Our second objective was to identify differences in habitat selection between flying squirrel species. We hypothesized that coniferous habitats support the growth of rich fungal communities which should attract northern flying squirrels, and separately southern flying squirrels will be most active in deciduous habitats that supply their diet of mast seeds. Therefore, we predicted that northern flying squirrels should select coniferous habitats within our study area, whereas southern flying squirrels should select deciduous habitats. Third, we investigated the fungal diet of each flying squirrel species to compare mycophagous behaviour present at this location. We hypothesized that at a sympatric site where food availability is consistent between the species, northern flying squirrels will favour fungi taxa. If northern flying squirrels favour fungi taxa, we will see a higher spore richness and abundance (load) in their scat. If northern flying squirrels favour fungi taxa more than southern flying squirrels, we should also see that northern flying squirrels strongly select for a particular fungal taxon, such as identifying strong associations with *Elaphomyces* (truffle species), in that truffle spores were found more often in the scats of northern flying squirrels than those of southern flying squirrels (Carey et al., 2002; Fogel & Trappe, 1978; Mitchell, 2001). Finally, we investigated the macro-fungi present at the site to determine fungal food availability and abundance present within squirrel home ranges. Our study will disentangle dietary niches of both flying squirrel species living in sympatry within a forest ecosystem that has sustained healthy populations of both species for 21 years. By understanding the habitats and space used, as well as fungal diets and food availability of the squirrels, we can better understand the role of northern flying squirrels in the ecosystem, and the implications on mycophagy should they be replaced by southern flying squirrels.

Methods

Study Site

This study took place near the Kawartha Highlands Provincial Park within Harvey Township, Ontario (44.685°N, 78.335°W). The total area of the field site is approximately 100 ha in size, surrounded by marsh and Mississauga Lake. Squirrels have been extensively monitored at this site for more than 21 years (Bowman et al., 2005). This site is also located just at the southern latitudes of the Canadian shield and is located on crown land. The 100-ha mature forests are relatively undisturbed and dominated by mixed-wood habitats. This site has been found to have abundant populations of both northern and southern flying squirrels and is known as a sympatric location. There has been hybridization documented at this long-term research site (Brown et al., 2021; Wolf et al., 2022).

The abundance of mast seeds at this location was quantified by annual acorn surveys. During the duration of this study, 2020 was a mast year, whereas both 2021 and 2022 were mast bust years (J.Bowman, personal communication, Oct 2022). Mast seeding events are difficult to predict because seed production is highly variable between years (Sork et al., 1993) and becomes more complex to understand interannual variation with ecological interactions with rodent seed eaters and spongey moth outbreaks (Ostfeld et al., 1996). Though this study didn't directly study mast seed variability as we were focused on fungi diet and availability, we can infer dietary selection based on the range of food available to the squirrels, and the lack thereof due to the outbreak of the spongey moth and subsequent mast failure (see discussion). We focused our efforts to understand the fungi diet and the implications of mammal mycophagy.

An array of 70 traps covering 6 ha was centrally placed in the study area. We used stratified random sampling techniques to conduct habitat surveys to identify dominant forest types. We delineated habitat types using ground truthing and SCOOP satellite imagery (Land Information Ontario, 2018) to identify the variety of forest stand types and calculate the area of each different habitat present on the site. This involved classifying habitats based on dominated tree type from satellite imagery and using knowledge from ground-truthed habitat classifications. The dominant forest types were classified in the following categorizes: pine-oak-barren, pine mixed-wood, eastern hemlock, deciduous-dominated, and birch (tree species within forest types are described in the results). Based on the area of each type we distributed random points within each of these forest types which then became the location for the fungal plots and tree surveys. In addition to these forest types, we identified road, water, marsh, and development (i.e., cottage properties) as other landcover types. Together with the 5 forest types, this resulted in 9 distinct landcover types that together produced a seamless map of the study area (see Figure 1).

<u>Live-trapping</u>

All fieldwork for this study was conducted from November 2020 until early December 2021. Squirrels were captured using Tomahawk model 102 live traps (14 cm x 14 cm x 41 cm, Tomahawk Live Trap Co., Hazelhurst, WI) placed approximately 2-m from the ground secured on wooden platforms. For the study period, trapping generally occurred twice a week, with exceptions due to adverse weather or local SARS-CoV-2 restrictions (no trapping occurred January and February 2021). Each trap was baited with sunflower seeds, peanuts, or peanut butter. We were not concerned with bait bias as we trapped both species in relative equal proportions in different forest types throughout the 21 years of live-trapping. Traps were set at dusk for approximately four hours each trapping night, checked every hour, and closed at the end of the evening session. Upon capturing squirrels, new individuals were tagged using a passive integrated transponder (PIT) (model TX1411SST, 12.50 mm × 2.07 mm, 134.2 kHz ISO, 0.1020 g; Biomark Inc. Boise, Idaho, USA), and identified to species and sex. An ear clipping was taken for DNA and we conducted morphological measurements to collect body condition information such as weight, skull measurements, right hind foot, and tail length. We collected fecal pellets when handling squirrels. Pellets were also opportunistically collected on the trap platforms when a fresh pellet could be confidently linked to the captured squirrel. Fecal pellets were stored in microcentrifuge vials and frozen at -18 ° C until they needed to be processed. We radio-collared 31 squirrels using 1.8-g BD-2C radio collars (Holohil Systems Ltd., Carp, Ontario, Canada), with 13 collars on northern and 18 on southern flying squirrels.

Radio-telemetry

We tracked individuals with collars, until collars were no longer sending signals, or collars were found on the ground (see results for tracking times). Whenever possible, we recaptured squirrels and removed radio collars when batteries were no longer functioning. We used location estimates from telemetry to calculate home range size using diurnal roost locations (Witt, 1992), nocturnal activity patterns, and trapping locations. Tracking individuals to diurnal roosts occurred 2-3 times a week, usually by homing the signal directly to the roost. Nocturnal locations were tracked through triangulation at least twice a week. Triangulation methods used at least two bearings and at least two GPS locations. Triangulated locations were estimated with maximum likelihood techniques using the Location of a Signal (LOAS 4.0) software.

Fungi and tree surveys

From 16 May 2021 until 31 October 2021 we conducted 12 weeks of fungal surveys to survey the availability of fungal fruiting bodies. We alternated between one week of truffle surveys and one week of mushroom surveys, conducting a total of 12 surveys of each type. Fungal survey plots were selected using a stratified random sample through ArcGIS Pro 2.7.0 (Environmental Systems Research Institute, Redlands, CA). We chose to set up 50 permanent plots through stratified random sampling for 12 weeks to survey for mushrooms, since they are known to be patchily distributed. Each mushroom plot was surveyed within a 2-m radius from the plot center. Any form of macrofungi (fungi with visible fruiting bodies) found within the boundaries of the plot was counted and unique individuals were collected for later identification. We also conducted tree surveys to record the tree species and tree DBH within a 5-m radius from the center of each mushroom plot. We only selected trees within the plot that were at least 8 cm in diameter.

We selected a different design for truffle surveys. We chose 30 temporary plots through the same process of stratified random sampling for 12 weeks. Due to the destructive nature of truffle sampling which involves raking the top layer of soil, only 30 plots were used and a new set of 30 plots were randomly chosen for each sampling week. Moreover, we set a time constraint of 5 minutes to rake the topsoil to avoid extensive digging. Truffles that had unique morphological characteristics were collected during the survey period, taken back to the lab to be dried for biomass and further identification.

Fungal microscopy from pellet samples

The following seasons were grouped across both years (2020 and 2021): winter = November to February, spring = March to June, summer and fall = July to October. Seasons were binned in four-month intervals to allow for proportional comparison. We followed established protocols (R.Stephens, personal communication, March 2021) to process pellet samples for fungal spore identification. Pellets were freeze dried for approximately 12 hours after taken out of the freezer. The dry mass was weighed, and 15-20 mg was the standard mass extracted from the matter. Pellets were ground with water and approximately 1 ml of 5% KOH was added to the vial to rehydrate the spores. The vials were shaken and then left for 10 minutes to settle. Vials were sieved into 10 ml centrifuge tubes, using 100-micron paint filters to filter out coarse fractions. The 10 ml tubes were centrifuged at 3000 RCF for 10 minutes, and the supernatant layer was pipetted out. The remaining sediment layer was then put into 1.5 ml microcentrifuge tube topped off with 70% ETOH. This vial was centrifuged again for 10 minutes at the default speed 14.5 RPM. Intermixing with ETOH further broke down fungal spores and separated the supernatant layer. The supernatant was pipetted off again and the final pellet remaining was a thick solution between 100-600 µl or half a milliliter. We left a bit of ETOH in the vial to pipette 25 µl of this solution into a new tube with 100 µl of distilled water. This new mixture was pumped a few times with the pipette and 25 µl of this diluted solution was then placed on a microscope slid and stained with 15 µl of Lugol's iodine. We put a 100x100 grid sticker on the slide to identify field of view squares. 20 squares were randomly selected, and spores were grouped by morphotype and counted for abundance estimates.

Data Analysis

Home range

Home range analysis was conducted using 95% Minimum Convex Polygons (MCP) using location estimates from diurnal roosts, nocturnal triangulation estimates, and trap locations. We selected MCP methods as it is frequently used for home range analyses, and we wanted to see coarse extents of squirrel range used on the landscape, and the associated habitat use. We calculated spatial error by occasionally triangulating individuals at a known roost tree during the day when they were stationary. We calculated average distances between paired locations. We noticed live-trap locations were in the center of the majority of squirrel roosts and nocturnal locations. Because flying squirrels have relatively small home ranges (i.e., see Holloway & Malcolm, 2007b), we assumed trap locations were already part of their home range, thus they were included in the MCP. Due to low spatial error in location estimates (see results), we incorporated all location estimates for 31 squirrels that fit into 95% MCP. We computed 90% MCP for one individual (SFS, male) because the 95% MCP included what appeared to be large movements from the core home range, and we considered those location estimates to be unlikely. A two-way ANOVA was performed to assess the effect of flying squirrel species and sex on home range size. We also tested for interactions between species and sex. We used R and R studio for all statistical analyses using Rstudio version 2022.12.0+353 (R Core Team, 2022). For home range analysis we used packages adehabitatLT and adehabitatHR (Calenge, 2006).

Habitat selection indices

Resource selection models were created to determine the proportions of habitat used over the available habitat. We defined habitat used as the area (in hectares) of habitat used among each habitat type within an individual's MCP home range. Habitat availability is defined as the area that is available for each individual to access across the landscape, and can be within or extending outside of the home ranges. Selection indices used a ratio equation using methods from Mcloughlin et al., (2002). We calculated 9 categorical habitat layers. The following calculation was used.

w_i = proportion of habitat used / total area of individual home range

h_i = proportion of habitat available / total area of all habitats

$b_i = w_i / h_i * 100$

The resulting standardized index (b_i) is a resource selection ratio of habitat selection where an individual is more or less likely to select that habitat given its equal proportion to availability. If the habitat selection index (b_i) is equal to the ratio value of 1, the individual uses the habitat in equal proportion to its availability. If the index b_i exceeds the ratio value of 1, the individual is more frequently selecting for this habitat than its availability. If the index b_i is near the ratio value of 0, the individual is less likely selecting the habitat, or avoiding the habitat.

The following models were fit under binomial generalized linear models to determine selection of habitat types by both species: 1) habitat use values (w_i), 2) mean ratio values (b_i) using 95% confidence intervals. Logistic binomial generalized models are commonly used to assess likelihood of habitat use in resource selection functions (Muff et al., 2020). Our response variable was species as a binary variable (SFS or NFS), and the predictor variables were the mean ratios (b_i) of habitats used by squirrels for each habitat type. We did not test between sex because it was not relevant to understand habitat selection between both flying squirrel species. We conducted linear models in R studio using the package stats (R Core Team, 2022).

Fungal availability

From the set of macrofungi that we surveyed, we selected four families of known macro-fungi that matched the known types of fungal spores identified from squirrel pellets. Three of these were common mushrooms observed in the field from the families Boletaceae, Russulaceae, and Suillaceae, and the fourth was the single most abundant family of truffles observed, Elaphomycetacea. Boletaceae was comprised of mushrooms in the following genera; *Chalciporus, Leccinum, Tylopilus*, and the family Russulaceae; *Lactarius* and *Russula*, family Suillaceae; *Suillus,* and Elaphomycetaceae; *Elaphomyces*. We focused on family level to match the family-level analysis from the spores identified in the squirrel pellets. We plotted their distribution in each habitat and calculated the mean abundance per plot, by summing the total number of each fungi taxon within each habitat type, divided by the total number of plots over the season. We removed road, development, and marsh habitat types from this analysis as we did not conduct fungal surveys on these landcover types.

Spore richness and spore load

We calculated both spore richness and spore load index using the spore morphotypes identified in the scat. Spore load index was used as an estimate of spore abundance. Richness values tallied number of unique spore taxa within each individual pellet. To calculate spore load index within each sample, the frequency of spores in each spore taxon was square-root transformed to reduce dynamic variation and extreme values in the numbers. This number was rescaled across each individual fungi taxon and standardized between 0 and 1, and then all spore taxa were totaled within each sample.

We used Welch's two sample t-test to determine significant differences between northern and southern flying squirrel scats within each seasonal bin, binned in the corresponding dates: Spring (March – June 2021), Summer/Fall (July – October 2021), Winter (November – February).

NMDS

We used the package vegan in R Studio (Oksanen et al., 2022) to conduct a two-dimensional non-metric multidimensional scaling (NMDS) analysis using Bray Curtis distance metrics. A NMDS was computed to understand the abundance of fungal communities (spore morphotypes) consumed by each squirrel species, and if there are patterns of similarity among spore taxa consumed between species. We also computed the NMDS across the spring, summer and fall, and winter to see patterns of spore morphotypes consumed and their prevalence in seasons when the samples were collected. We also used vegan to conduct a PERMANOVA to test for significant variations in spore taxa consumed between squirrel species. We also ran the PERMANOVA to test for significant variations in spore taxa consumed among seasons. We conducted a species indicator analysis, package indicspecies (De Caceres & Legendre, 2009) to see if one squirrel species was strongly associated with any kind of fungi taxa compared to the other squirrel species.

Results

Location estimates

We radio-tracked 13 northern flying squirrels (4 female and 9 male) and 18 southern flying squirrels (9 female and 9 male) from May to December 2021. On average, the radio collars for northern flying squirrels were active for 10.15 ± 3.32 weeks and southern flying squirrel tags were active for 5.33 ± 4.12 weeks. Northern flying squirrels had a mean of 32.92 ± 15.09 location estimates and southern flying squirrels had a mean of 26.22 ± 9.66 location estimates (Figure A.1). The two species seemed to occupy the same areas when looking at the location estimates, apart from a few areas dominated by conspecifics. Northern flying squirrels seemed to occupy areas northwest of the study location where southern flying squirrels were absent (Figure A.1, A.2, A.3). Southern flying squirrels were mostly found in the core use areas, however some southern species deviated towards cottage owner properties (Figure A.1, A.3). Spatial error for the radio transmitters on northern flying squirrel locations (n = 10) had a mean distance of 56.48 ± 43.95 m and southern flying squirrel locations (n = 12) had a mean error distance of 61.10 ± 79.64 m.

Home range analysis

There was considerable overlap between the home ranges of southern and northern flying squirrels (Figure 1; Figure A.2 and A.3). Moreover, both species of flying squirrel had similar home range sizes (species effect: $F_{1, 28} = 0.153$, $p = 0.698$) (Table 1). We identified no significant interaction between species and sex on home range size (interaction: $F_{1, 28} = 0.448$, $p = 0.509$). However we identified that on average, male southern flying squirrels and male northern flying squirrels had larger home ranges compared to their sex counterpart (Figure 2).

Table 1. Mean home range size (SE) of southern flying squirrels (SFS) and northern flying squirrels (NFS) near the Kawartha Highlands Provincial Park (2021) (n=31).

	Southern flying squirrels (SFS)		Northern flying squirrels (NFS)	
95% MCP*	*Female (n=9)*	*Male (n=9)*	*Female (n=4)*	*Male (n=9)*
Mean Area (Ha)	4.71 (1.52)	9.46 (2.85)	2.58 (0.67)	10.3 (1.46)

**Note: With the exception for one male SFS at 90% MCP*

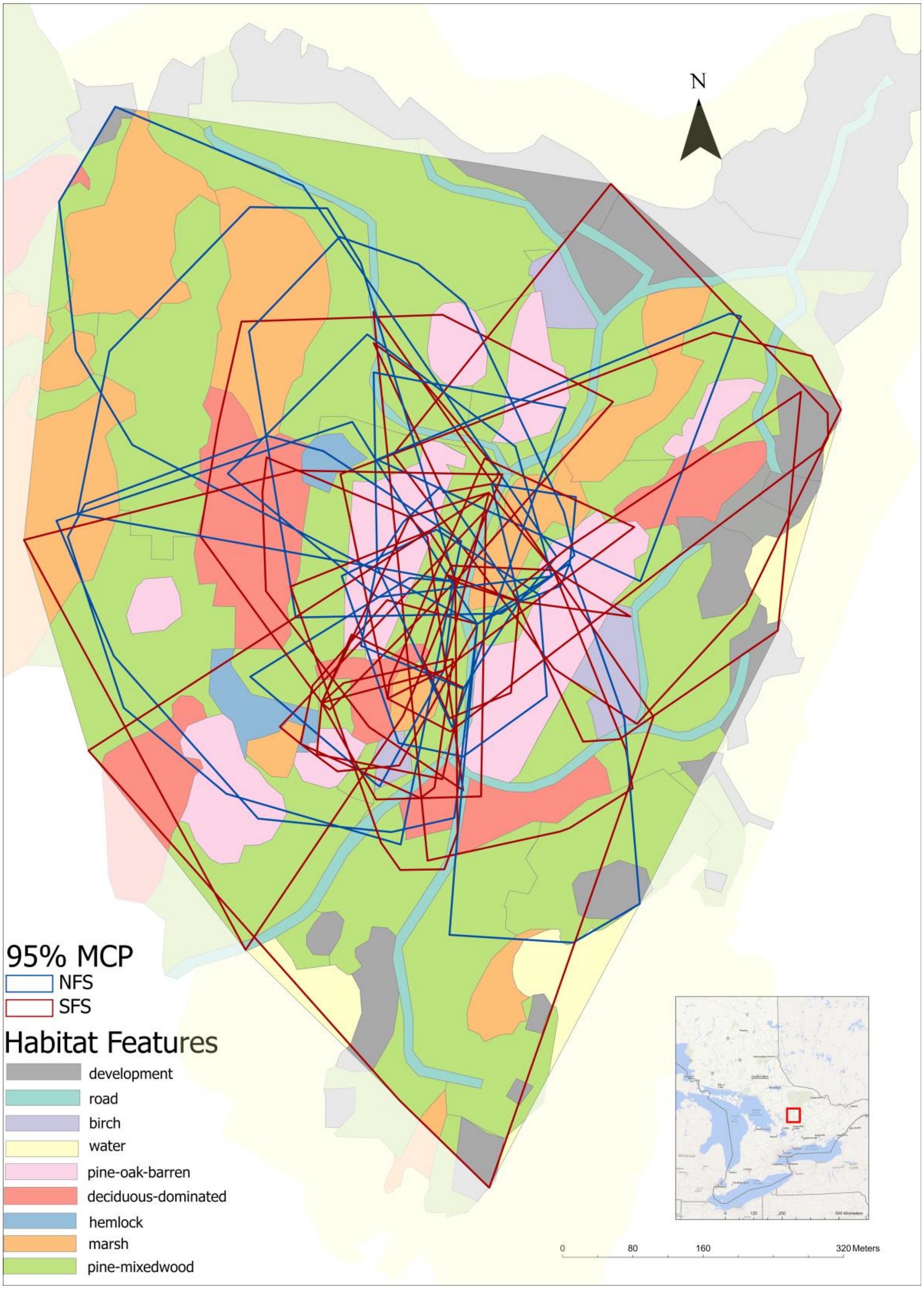

Figure 1. 95% MCP home ranges of northern (NFS) and southern (SFS) flying squirrels on habitat features near the Kawartha Highlands Provincial Park (n=31).

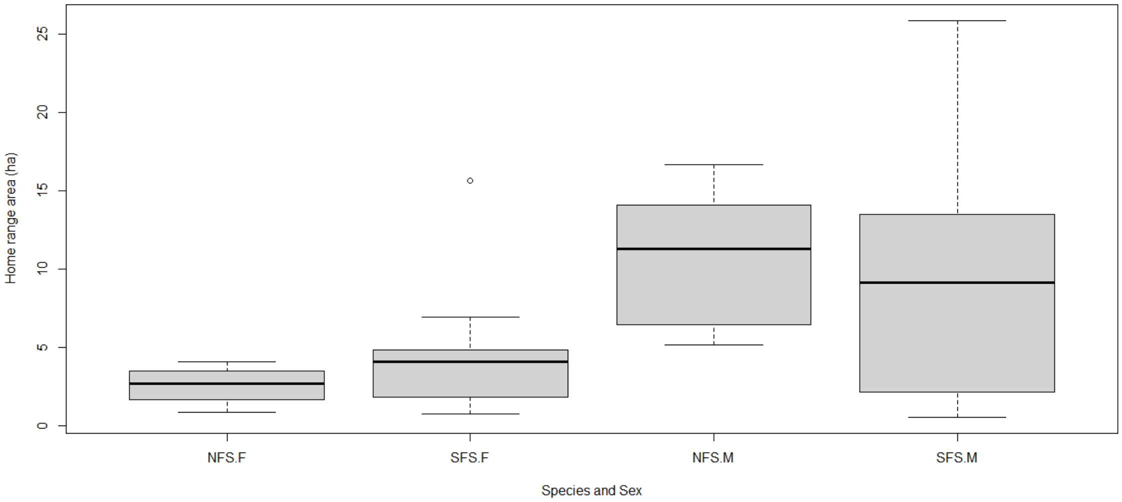

Figure 2. Boxplot of mean home range sizes between flying squirrel species and sex; northern (NFS) and southern (SFS) flying squirrels, where F = female and M = male.

Site characteristics

Pine-mixedwood habitats made up the most habitat within the two species' home ranges and represented 47.8% of the total landcover (Table A.1). The site dominated by pine-mixedwoods made up largely of eastern white pine (*Pinus strobus*) and red oak (*Quercus rubra)* tree species, but there is also intermixed white oak (*Quercus alba*), balsam poplar (*Populus balsamifera*), and ironwood (*Ostrya virginiana*). The second most abundant habitat type were pine-oak barren. This habitat made up 11.3% of the total habitat available (Table A.1). The tree species in pine-oak barrens were primarily eastern white pine and red oak. The third most common forest type on the landscape were deciduous-dominated land covers, which made up 8.7% of the total habitat available (Table A.1). Deciduous-dominated habitats were made up of white oak, red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), ironwood and balsam poplar, bigtooth aspen (*Populus grandidentata*), white ash (*Fraxinus americana*); and sparsely bitternut hickory (*Carya cordiformis*). Birch habitats were dominated by sugar maple, yellow birch (*Betula alleghaniensis*) and white birch (*Betula papyrifera*), and were nearby or located within marsh-treed habitats, and had one of the smallest areas making up 1.8% of total available habitat (Table A.1). Similarly, hemlock habitats were the smallest land class at this site making up 1.2% of the total available habitat (Table A.1). Conifer species in hemlock habitats were made up of balsam fir (*Abies balsamea*), eastern hemlock (*Tsuga canadensis*), eastern white cedar (*Thuja occidentalis*).

Habitat selection indices

To estimate habitat selection, we first identified the proportions of habitat used (w_i) within individual home ranges across each habitat type (n=9). Using habitat use proportions (w_i), we identified both species of flying squirrel mostly used pine-mixed-wood and pine-oak-barren habitats within their home range (Table 2). We computed a Pearson's product-moment correlation test (Pearson's r) to assess the linear relationship between each habitat category used within the habitat selection index. We established a correlation coefficient threshold of $r = 0.70$ (see Dormann et al., 2013) to determine the variables used for the binomial generalized linear model (Table 2). We identified no significant correlations between the habitat variables and proceeded using all variables for the analysis ($r < 0.70$). However, we removed water and marsh habitat features from our analysis because they were not ecologically relevant for understanding squirrel habitat use and selection. Flying squirrels are known to forage and have nests nearby water sources, such as riparian zones and marshes as they provide ideal habitat for fungi growth (Hackett & Pagels, 2003; Meyer et al., 2005). Our water habitat feature is Mississauga Lake, surrounding the study area, which does not meet flying squirrel selection preferences for water sources. Further, distance to water is not considered a requirement in flying squirrel habitat selection (Timossi et al., 1995). This infers the squirrels are to be attracted to the habitat near it and adjacent, but not directly selecting within water sources.

Seven habitat features were measured for n=31 squirrels and average ratios of habitat selection indices (b_i) were identified using 95% confidence intervals (Figure 3). Binomial generalized linear models (GLM) were generated for proportions of habitat used within each individual home range (w_i) and with habitat selection indices from mean ratios of habitat used over available habitat (b_i) (Table 2). To analyze differences in habitat selection, we used a binomial GLM for the habitat selection indices (b_i). The only statistically significant difference that we observed in habitat selection between species was that southern flying squirrels were more likely to select deciduous-dominated habitats, compared to northern flying squirrels (Table 2). On average southern flying squirrels significantly selected deciduous-dominated habitat 2.08 times more than it was available (SFS mean = 2.08, se = 0.34) (Figure 3). We also found that both species used pine-oak barren habitats the most compared to the other habitat types, in a similar way at 2.49 times as much as it was available (SFS mean = 2.49, se = 0.24; NFS mean = 2.49, se = 0.27) (Table 2). Southern flying squirrels also selected birch habitat averaging nearly 2.47 times more than it was available, however there was low statistical power (SFS mean = 2.47, se = 0.70) (Table 2). Conversely, northern flying squirrels were found using hemlock habitats twice as much as it was available, however the difference between the two species is also not statistically significant (NFS mean = 2.15, se = 0.69) (Table 2). There was avoidance of development from both species (SFS mean = 0.47, se = 0.25; NFS mean = 0.09, se = 0.08) (Table 2). Using a Spearman's rank correlation test, mean ratios of habitat selection indices (b_i) for northern and southern flying squirrels were moderately correlated suggesting the species were using habitats somewhat similarly (*r*(34) = 0.39, *p* = 0.396).

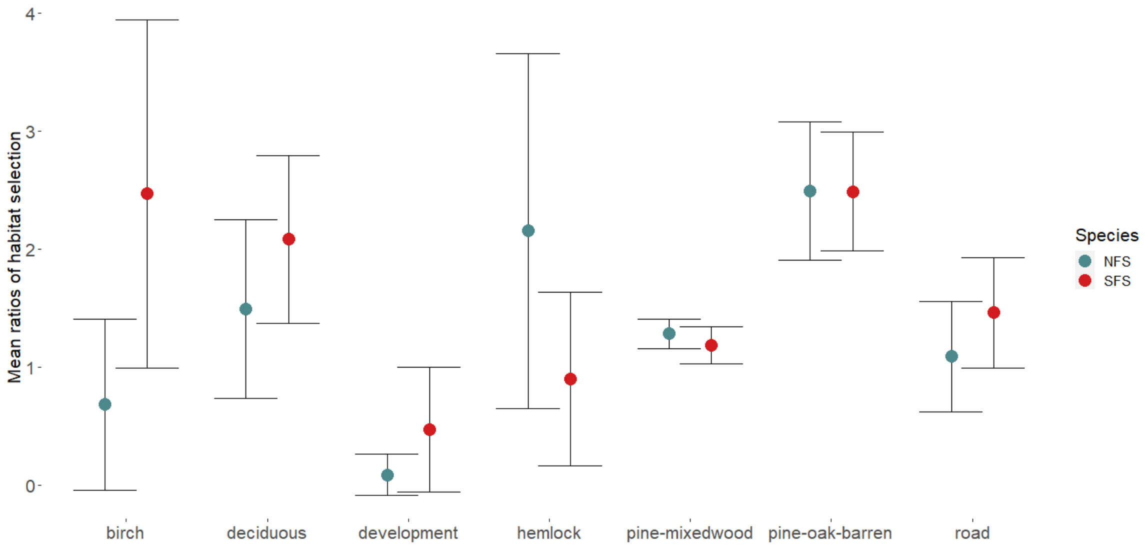

Figure 3. Mean ratios of habitat selection indices ($\boldsymbol{b_i}$) for northern (NFS) and southern (SFS) flying squirrels (n=31) at 95% confidence intervals.

Table 2. Binomial generalized linear models (GLMs) describing differences in habitats used **(w_i)** and habitat selection indices **(b_i)** between northern flying squirrels (NFS) and southern flying squirrels (SFS). Species was coded as a binary response where NFS was 0 and SFS was 1.

	Habitat Use (w_i)			Habitat selection (b_i)		
	Estimate	SE	P	Estimate	SE	P
Intercept	-3.181	4.173	0.445	-3.18	4.172	0.445
Birch	-10.407	32.096	0.745	-0.139	0.430	0.745
Deciduous dominated	**25.664**	**12.660**	**0.042**	**1.659**	**0.818**	**0.042**
Pine-mixedwood	-1.969	6.332	0.755	-0.698	2.245	0.755
Hemlock	-60.451	38.086	0.112	-0.549	0.346	0.112
Pine-oak-barren	1.520	6.978	0.827	0.127	0.584	0.827
Development	12.883	18.271	0.480	0.708	1.004	0.480
Roads	46.095	27.678	0.09	1.579	0.948	0.09

Spore richness and spore load

We collected and processed 119 fecal samples from squirrels. We sampled a relatively equal proportion of northern and southern flying squirrel pellets throughout each season from 2020-2021. Three of the fecal samples did not have any spores and were removed from the analysis. Using 116 samples, we grouped and tallied 13 different spore morphotypes. We compared standard deviations to see the variations within the mean of each species (Figure 4). We found in the summer and fall and winter months, northern flying squirrel pellets had significantly higher spore richness levels in their fecal pellets than southern flying squirrel pellets (summer and fall: t(37) = 2.83, p = 0.008, n = 39; winter: t(32) = 2.64, p = 0.015, n = 34). Similarly, northern flying squirrel pellets had significantly higher spore load indices during the summer and fall, and winter months compared to southern flying squirrel pellets (summer and fall: t(37) = 2.34, p = 0.025, n = 39; winter: t(32) = 2.73, p = 0.012, n = 34). By contrast, during the spring months there was no difference in either spore richness or spore loads between northern and southern flying squirrel pellets (spore richness (t(44) = -1.36, p = 0.182, n = 46; spore loads (t(44) = 2.73, p = 0.615, n = 46). See Table A.2 for mean spore richness and spore load indices in squirrel pellets for both species across each season.

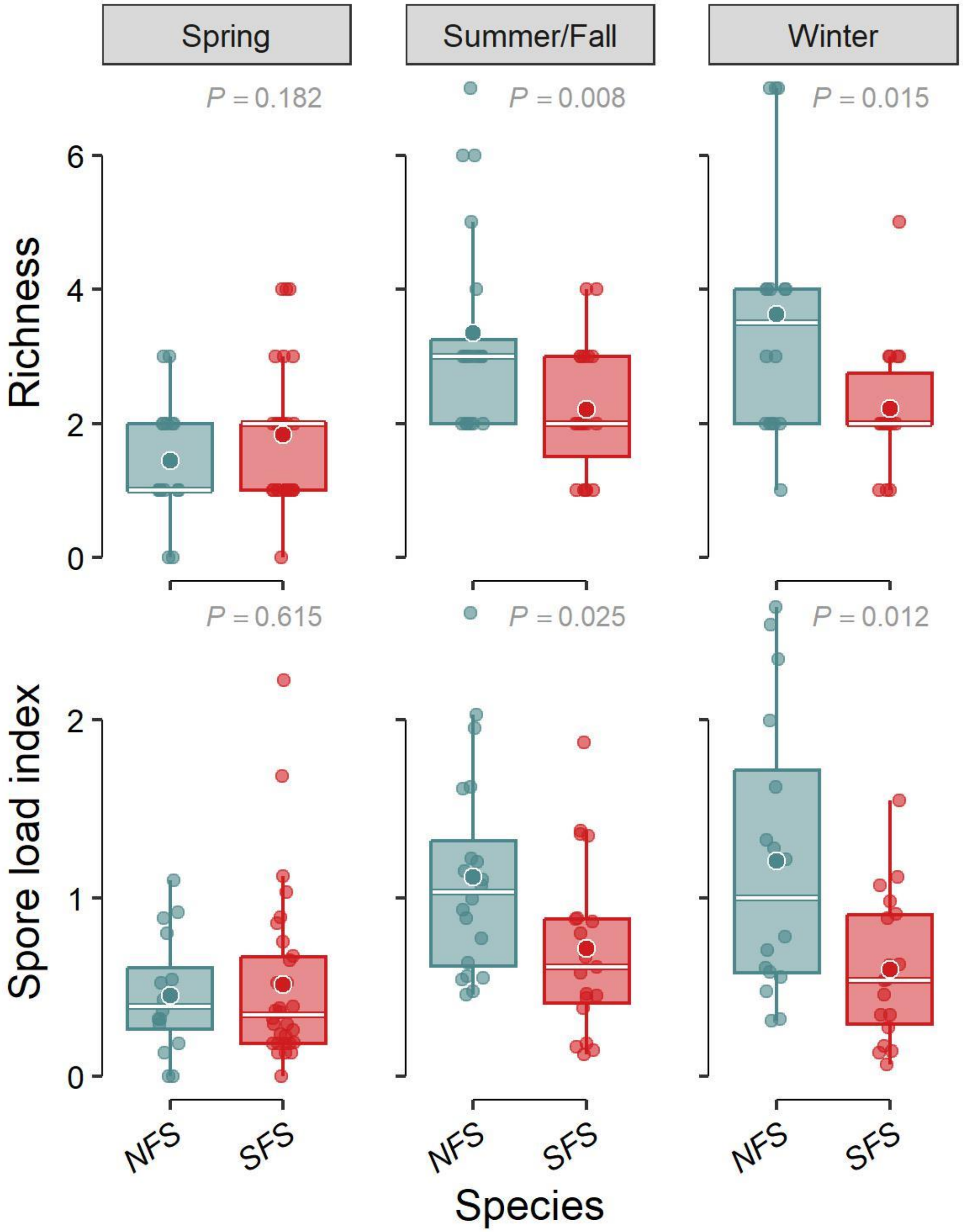

Figure 4. Fungal spore load richness and spore load index within each sampling season across 116 flying squirrel scats (northern flying squirrel (NFS) n = 50, southern flying squirrel (SFS) n = 66).

NMDS

We found that northern and southern flying squirrels were eating similar fungal communities, as indicated by substantial overlap between the fungal types; and we conducted a PERMANOVA that indicated little variation in the communities was explained by squirrel species ($F_{1,115} = 2.52$, $R^2 = 0.02$, $P = 0.01$) (Figure 5 a). In contrast, we found more variation between fungal communities was explained by the seasons ($F_{2,115} = 9.02$, $R^2 = 0.14$, $P = 0.001$) (Figure 5 b). We also found no significant interactions occurring between species and seasons; therefore, northern and southern squirrels behaved similarly across seasons. Although there was overlap of fungi taxa consumed by both squirrel species, we were able to determine fungi taxa associations. To understand what fungi communities were found more often by one squirrel species than the other, we conducted an indicator species analysis. We found both northern and southern flying squirrels similarly favoured two fungi taxa on average; one type from the Suillaceae family, "Suillus_type1" (NFS $\mu = 0.30 \pm 0.03$; SFS $\mu = 0.30 + 0.03$), and one unknown type "Unknown 8" (NFS $\mu = 0.20 \pm 0.03$; SFS $\mu = 0.16 + 0.03$), however these fungi taxa did not have statistically significant associations with either of the squirrel species. Therefore, using an indicator species analysis, we found northern flying squirrels were significantly associated with fungi taxa from Boletaceae and Suillaceae families: "Boletaceae_type2" (IndVal = 0.238, $P < 0.001$), "Boletaceae_type1" (IndVal = 0.199, $P = 0.01$), "Suillus_type2" (IndVal = 0.170, $P < 0.05$). In contrast, southern flying squirrels did not have any significant associations with fungi taxa.

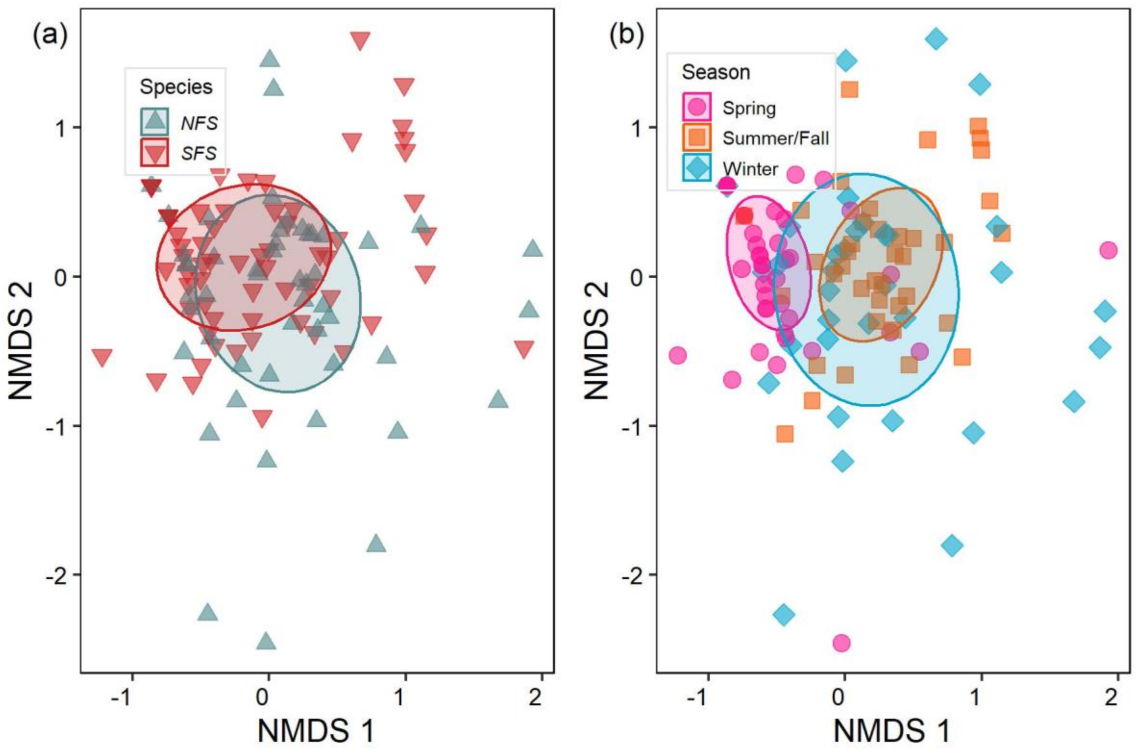

Figure 5. A biplot of two-dimensional non-metric multidimensional scaling (NMDS) of the spore abundances rescaled of the fungal morphotypes (n=13) and the relationship a) among squirrel species and b) across seasons (n=116).

<u>Fungal distribution</u>

There were 29 families of macrofungi surveyed and a total sum of 1193 individual fungal fruiting bodies identified from the 12 weeks of surveys. Pine-oak barren and pine-mixedwood habitats both had the most fungi compared to other habitats sampled. Pine-mixedwood habitats had the most diverse macrofungal communities, with all four families found within these habitats (Figure 6). Within pine-mixedwoods, macrofungi in the Russulaceae family had the second most abundant number of mushrooms across habitat types, however it had the highest mean abundance per plot (n = 109, µ = 0.70 ± 0.14) (Figure 6). Similarly, macrofungi in the Russulaceae family in pine oak barren habitats were the most abundant and had a high mushroom mean abundance per plot (n = 223, µ = 0.64 ± 0.10) (Figure 6). Overall, mushrooms in the Russulaceae family had the highest frequency of mushrooms compared to other families that we surveyed during the 12 weeks (n = 349). Notably, we found the greatest abundance of Elaphomycetacea (truffles) in pine-oak barren habitats compared to the other habitat types, with the highest truffle mean abundance per plot (n = 36, µ = 0.18 ± 0.07).

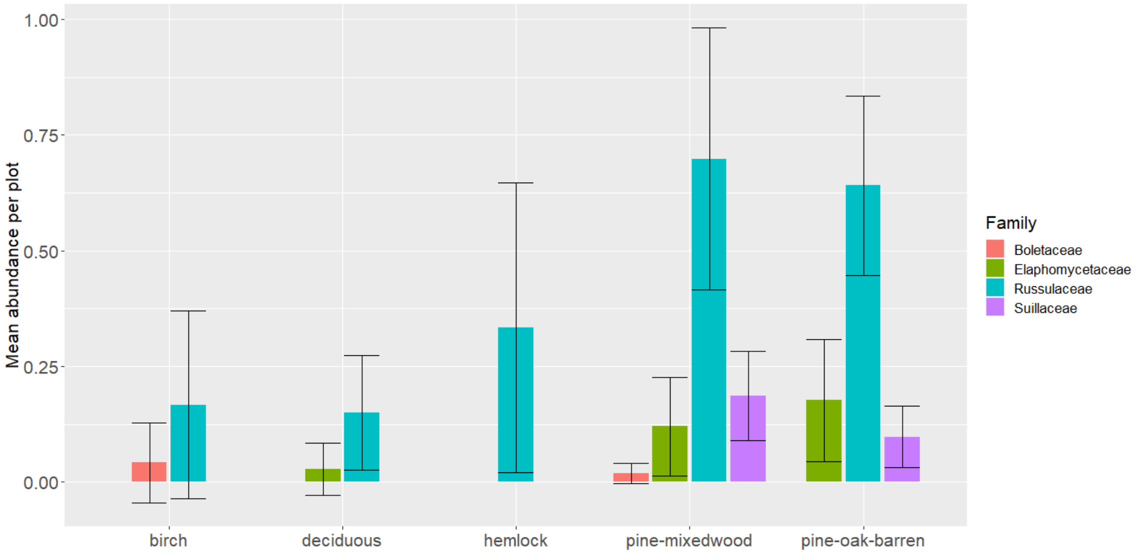

Figure 6. Mean abundance per plot of four common macrofungi families surveyed in each habitat type at the Kawartha Highlands Provincial Park.

Discussion

Our objective of this study was to identify mycophagy of northern and southern flying squirrels in central Ontario and identify the implications for the ecosystem if species turnover displaces northern flying squirrels. We identified no significant differences between northern and southern flying squirrels home range size, and identified a spatial overlap in the home ranges. To tease apart this result, we conducted a habitat selection analysis and found southern flying squirrels selected deciduous-dominated habitats more often than northern flying squirrels. Northern flying squirrels did not have strong selectivity to any type. Thirdly, we found northern flying squirrels had more fungi spore richness and higher spore loads in their scat in the summer/fall and winter seasons than southern flying squirrels. Both species consumed fungi similarly in the spring. We also investigated if a species was skewed towards a particular fungi taxon, and we found that northern flying squirrels were strongly associated with Boletaceae and Suillaceae mushrooms, but that southern flying squirrels did not have any strong fungi associations. We also found that the habitats that squirrels shared and selected for the most were pine-mixedwood and pine-oak-barren, which also had the most diverse and abundant fungi taxa. Our study provided evidence that these flying squirrels are behaving similarly but could be partitioning resources in different ways that allows both species to live in sympatry. Southern flying squirrels are consuming fungi taxa and contributing to the spore dispersal cycle similarly to their northern counterpart. Should northern flying squirrels get displaced in forest ecosystems in light of southern species expansion, southern flying squirrels will have the potential to replace at least some of the ecosystem service role of northern flying squirrels in their absence.

Location estimates

Three different types of location estimates (diurnal roosts, nocturnal and trap locations) were incorporated into the MCP to maximize location estimates for each squirrel species. We aimed to radio-track both species of flying squirrel simultaneously, but southern flying squirrels were tracked for less time likely because squirrels dispersed from the woodlot in the fall likely because there were no acorns identified on the annual acorn survey, during the mast year at this study location (J.Bowman, personal communication, July 21 2023). Further, some radio collars on southern flying squirrels were removed early from collar size constraints, or for other on-going projects. Therefore, northern flying squirrels seemed to have longer radio-tracking time.

We also identified a spatial overlap of the total location estimates between both flying squirrel species (Figure A.1). However, we can see more locations of northern flying squirrels at the edge of their home range, as they expanded throughout the northwest corner of the woodlot. Holloway & Malcolm (2007b) identified northern flying squirrels nesting at the edge of their home range, which offers a plausible reason for this pattern. Northern flying squirrels could also expand their range to cover more land area to forage for fungi, or in search for coniferous trees and the habitat associated with it (Holloway & Malcolm, 2007b). We can also see that locations of southern flying squirrels were concentrated in or around deciduous-dominated habitats, nearby roads, and in cottage properties (Figure A.1). This may be due to the abundance of mast seeds from deciduous trees in these habitats; the southern flying squirrels preferred food (Weigl, 1968). Southern flying squirrels could also gravitate towards feeders in homeowner's backyard which often attract the presence of flying squirrels, especially when feeders are surrounded by forest (Stephenson & Calcarone, 1999).

Home range

We found no significant difference between our estimates of northern and southern flying squirrel home range size. We also found substantial spatial overlap in the estimated home ranges of the two squirrel species. They seemed to occupy similar habitat features as well on the shared landscape. This contrasts with past research, as others have found a difference in home range size between the two species, whereas we found that southern flying squirrels at our study site have larger home ranges than previous estimates. For example, an earlier study in central Ontario allopatric flying squirrels found that southern flying squirrel home range size was considerably smaller than northern flying squirrels (Holloway & Malcolm, 2007b). Using a 95% MCP they estimated on average male southern flying squirrels had home range sizes of 3.07 ± 0.40 (ha), female southern flying squirrel home ranges were 2.99 ± 0.54 (ha), male northern flying squirrel home ranges of 11.22 ± 3.40 (ha), and female northern flying squirrel home range sizes were 3.76 ± 0.57 (ha). Similarly, one of the first few radio-telemetry studies for southern flying squirrels found a similar result. Bendel & Gates (1987) found southern flying squirrels had small home range sizes with males at 2.45 ± 0.88 (ha) and females at 1.95 ± 0.76 (ha).

Southern flying squirrels at our field site may have larger home ranges than previous studies due to the dominance of pine-mixedwood habitats present on the landscape, with eastern white pine (*Pinus strobus*) comprising 46% of trees in the core areas, and red oak (*Quercus rubra*) making up 19% of the trees. The mixedwood nature of the forest may require animals to travel farther to obtain resources available in either coniferous or deciduous habitats. The existence of large proportions of both pine and oak species encourages shared space use by both species since these habitats offer favorable nest sites for both species and promotes the growth of available food for both squirrel species across the landscape. Pine-mixedwood and pine-oak forests have abundant snags that also provide cavities, and are composed of many dead trees or those declining in health condition, that act as den sites for both species of squirrels (Holloway & Malcolm, 2007a; O'Brien et al., 2021; Minns et al. (in review)). Additionally, another study identified a large mean home range size for male southern flying squirrels of 16 ± 4.36 ha, which the authors attributed to using pine-hardwood stands as corridors to reach mast hardwood stands with abundant seeds (Taulman & Smith, 2004). Similarly, in the Central Appalachian Mountains, Menzel et al. (2006) also identified a large mean home range size for male northern flying squirrels, with a range of 59.8 ± 23.5 ha, which the authors inferred was because animals used many different habitat zones to supply their diet due to fragmentation. This may explain why squirrels at our study site were not significantly selecting pine-mixedwoods, as they used this common forest type to cross to their selected destination: deciduous-dominated habitats for southern flying squirrels and coniferous-based habitats for northern flying squirrels.

We identified that squirrel movements at night are within range of the trap locations and diurnal roosts, so we are able to make conclusions on habitat use within home ranges. We identified little spatial error between day triangulations and known roosts (see results), therefore we can assume there is little spatial error between nocturnal locations and diurnal roosts. Previous studies have combined telemetry location estimates from each squirrel to generate MCP's, such as nocturnal triangulations, diurnal roosts, and nest sites (Bendel & Gates, 1987; Lehmkuhl et al., 2006; Holloway & Malcolm, 2007b). Gerrow (1996) suggested incorporating known squirrel locations into the home range analysis is more accurate rather than relying on triangulation data alone. Those that relied on nocturnal locations using triangulation alone seemed to have larger spatial error (i.e., Menzel et al. 2006; Fridell & Litvaitis, 1991). Holloway & Malcolm (2007b) identified that southern flying squirrel nests were located in proximity to their nocturnal core use areas, and some northern flying squirrels did as well. Therefore, our method of combining all 3 types of location estimates is appropriate and we can draw conclusions of habitat use with these location estimates.

Habitat Selection

Our findings for selection are congruent with past research on habitat selection. When both species are in sympatry, we see strong significant selection by southern flying squirrels for deciduous-dominated habitats and strong selection by both species for pine-oak barren habitats. Both species in North America are often found in pine-hardwoods that are composed of oak and pine tree species that promote ideal habitat for both species (Arbogast, 2007; Dolan & Carter, 1977). This is aligned with past research that has identified that southern flying squirrels strongly select deciduous habitat in their home range (Taulman & Smith, 2004; Weigl, 1978). Furthermore, our research on the fungal distribution shows that pine-mixedwood and pine oak barren habitats have the most diversity of fungi taxa that squirrels were found to consume, using data on our fungal diet study. This promotes habitat use in these zones within their home range, providing the possibility to consume more fungi in their diet.

In 2021, there was a mast defoliation of many of the tree species in our study area due to the outbreak of the spongy moth (*Lymantria dispar*), an invasive moth species, which consumed all foliage of needles and leaves from conifers and hardwoods respectively (Ontario Ministry of Natural Resources and Forestry, 2023). As a result, we identified no acorns at this field location during autumn 2021. According to Taulman et al. (1998) if there is a substantial disturbance that affects food resources, then squirrels will adjust their home range accordingly. Therefore, it is possible that southern flying squirrel home range size and habitat selection in deciduous-dominated habitats could have been affected by the lack of hard masts during this sampling year. The effects of low mast production may have been an amplified by the moth outbreak, but could also be impacted from a high density of seed-eating squirrels the year prior and resulting in a cyclical low mast production the following year, as this was previously identified in white-footed mice (*Peromyscus leucopus*) (Ostfeld et al., 1996). The ability to disperse and travel far can be advantageous when foods and habitats are unstable (Roff, 1975). If acorns and seeds are abundant, we might expect that southern flying squirrels will have smaller home ranges and will stay locally without the need to travel far for food (Roff, 1975; Taulman et al., 1998). As a result, periodic food competition may be minimized for northern flying squirrels. However competition may remain for nest sites if southern flying squirrels are dispersing less.

The study by Holloway & Malcolm (2007b) investigated home range of both flying squirrel species in Algonquin Park, located 150 km north from our study site. The Park is dominated by conifer species such as white spruce (*Picea glauca*) and eastern hemlock (*Tsuga canadensis)*. It is known that truffles are highly associated with conifer groups like spruce (*Picea* spp.), fir (*Abies* spp.) and an assortment of pines (*Pinus* spp.) (Zhang & Minter, 1989). Compared to research at our location, our forest matrix has a lower diversity of conifers, with the dominant conifer being white pine (*Pinus strobus*) and make up 46% of the tree species at this location. Moreover, hemlock habitats made up

1.2% of the total habitat available (Table A.1). Perhaps only one truffle genus was found because of the smaller area of pure coniferous stands and the lack of conifer richness present at the study site. Additionally, the pine-oak dominance promotes habitat that is suitable for both species' occupancy, also promoting sympatry between squirrel species. These factors may have influenced the home ranges between the two species, in which the species overlapped in ranges, but had some slight differences in how habitats were used on the landscape.

We found males from both species had larger home ranges than females. Mammalian males generally have larger home ranges than females usually to seek out females (Fridell & Litvaitis, 1991; Lindstedt et al., 1986). While we did not collar pregnant or lactating females, we can assume females had smaller home ranges so they could conserve energy and forage close to nests, tend to their young, and avoid nest predation (Fridell & Litvaitis, 1991; Taulman & Smith, 2004).

Analysis of fungal diets

We identified that northern flying squirrels had significantly higher spore richness and spore loads in their fecal pellets compared to southern flying squirrels in the summer/fall and winter seasons. However, in the spring, both squirrel species had similar richness and spore load amounts in their fecal pellets. One reason can be explained by the dietary generalist role of southern flying squirrels, which may have been magnified by the low mast year. Recent research has identified that small mammals that acts as generalists also contribute an unexpected portion to the spore dispersal system (Stephens & Rowe, 2020). Moreover, during a low mast year in 1999, Vernes et al. (2004) identified northern flying squirrels and red squirrels (*Tamiasciurus hudsonicus*) consumed more fungi than normal, and speculated their diet was related to the lack of available mast crops.

There are few studies that examine the fungal diet of southern flying squirrels (i.e., Fogel & Trappe, 1978; Harlow & Doyle, 1990), and if identified, they describe that fungi play a partial role in their diet. We also found little research that discussed fungal diets of both flying squirrel species in sympatry. We identified that both flying squirrel species are eating fungi, however northern flying squirrels are just eating significantly more. This is consistent with past research that identifies northern flying squirrels as obligate mycophagists when using microscopy to identify spores (Maser et al., 1986). Summer and fall months were the most rich in spores likely because these months facilitated the growth of the most productive fungal communities, especially truffles (Fogel & Hunt, 1979). Winter seasons yielded high spore taxa and spore loads because of the ability for squirrels to cache the truffles from the summer harvest (Currah et al., 2000). There may be no difference in richness and loads in spring because both species may be more focused on eating new plant emergences. A study by Mitchell (2001) examined spring and fall diets of West Virginia northern flying squirrels and found during the same spring months as our study (March – June) they found plant buds comprised majority of the squirrel’s diet. And similarly to our study, the squirrels consumed more fungi and more fungi taxa during the fall months (Sept – November) (Mitchell, 2001). Furthermore, availability of fungi taxa may play a role in this result of seasonal differences in fungal diets. In our study, we identified fungi taxa became more abundant towards late summer in August and were mostly absent before this month. This may have left squirrels to switch their diet to be more plant-based in the spring. These reasons can partially explain why there are seasonal differences in the fungi taxa consumed by both squirrel species.

We identified a strong overlap of fungi taxa consumed by both squirrel species. However, there are slight differences in the northern flying squirrel diet to show that they may be eating some different taxa that southern flying squirrels are not eating, or not eating as much. When we looked at these differences, we found northern flying squirrels were strongly associated with a type of Boletaceae fungi and Suillaceae fungi, relative to all other fungi taxa in the scat. This skew is interesting considering *Elaphomyces* spores appeared in many northern flying squirrel scat samples and is known to be a strong preference for this species (Loeb et al., 2000; Pyare & Longland, 2001; Vernes et al., 2004). However research identifies this could be region dependent, as one study in the Alberta boreal found northern flying squirrels consumed more mushrooms such as Boletaceae than truffles (like *Elaphomyces*) (Currah et al., 2000). Nonetheless, Boletaceae and Suillacae are known common fungi families consumed by these squirrels (Carey, 2000; Currah et al., 2000; Mitchell, 2001). Perhaps they were selected because they offer more nutritional value than *Elaphomyces* truffles, which are limited in nitrogen and other vital nutrients (Cork & Kenagy, 1989). Squirrels often must consume multiple food sources to satisfy their full nutritional needs (Cork & Kenagy, 1989; Dubay et al., 2008). Moreover, as mentioned above, our pine-oak dominated site influenced the prevalence of *Elaphomyces*, but if there were more spruce-or conifer-rich habitat, we might see more truffle genera found in the scat, as identified by Holloway & Malcolm (2007b) in Algonquin Park. Perhaps there was a skew towards a mushroom diet at this site because of the lack of available truffle genera at this study location (R.Persad, personal communication, Oct 2021). Interestingly, the focused fungi taxa groups we used for this study; Russulaceae, Boletaceae, Suillaceae, *Elaphomyces*, all have mycorrhizal symbiosis with plant hosts (Smith & Read, 2008). Fungi taxa fruiting bodies of *Elaphomyces*, *Suillus* and *Russula* have long expansive mycorrhizal connections that have strong associations with pine trees (Bonello et al., 1998; Smith & Read, 2008), which also happened to be the most dominant trees in our study.

Implications

Although these squirrels are found to live in stable sympatry, there is a risk of species turnover when ecological gradients and species distributions are shifting. Species turnover is most influenced by changing environmental conditions and the species level of tolerance or adaptation (Shurin, 2007). Turnover is also expected to occur when there is high interspecific competition between species, in which a species is displaced, and the competitor can dominate the region (Martin & Ghalambor, 2023). There is concern that northern flying squirrels are subject to displacement from southern flying squirrels when they come into secondary contact (Weigl, 1978). Secondary contact and hybridization have been identified at our study site, and thus at risk of species turnover from northern to southern flying squirrels because of the rapid range shift of southern flying squirrels (Bowman et al., 2005).

Rapid climate warming has affected the range distributions of animals towards a poleward shift (Walther et al., 2002). Populations on the marginal edges are most vulnerable to extirpation from this shift, particularly those on the rear edge (Hampe & Petit, 2005). In response, populations of northern flying squirrels in the southern edge are subject to a range contraction due to southern flying squirrel expansion (Bowman et al., 2005). By contrast, southern flying squirrels in the northern edge are expanding poleward. The transition zone between the two populations range edge is where we should expect species turnover to occur, which overlaps within our study location.

Flying squirrel ranges distributions are hypothesized to be controlled by several ideas discussed earlier such sociality and parasite-mediation (O'Brien et al., 2022; Olson et al., 2018). Flying squirrel populations are also vulnerable to forest fragmentation and rely on large woodlots for their survival (Walpole & Bowman, 2011). However, it is unknown what mechanism is truly responsible for poleward animal movements. Our study provides a possible reason for what we should expect to occur from climatic shifts in southern flying squirrel range expansion and northern flying squirrel range contraction. We suspected a species turnover from northern to southern flying squirrel to occur, and we predicted the effect this will have on the ecosystem functions.

With the rising threat of climate warming affecting southern flying squirrel range expansion, we had previously thought species turnover would reduce the functionality of forest ecosystems with reduced spore dispersal from northern flying squirrels. However, this research suggests species turnover may not provide detrimental effects to the forested environments as expected. If southern flying squirrels were to replace northern flying squirrels in forest patches in central Ontario, the role of fungal dispersal may not be profoundly affected because we identified that southern flying squirrels consume fungi in moderate proportions. Because of their larger than expected home range size, southern flying squirrels also have similar opportunities to spread fungal spores in larger areas, covering more habitat which contributes to the higher spread of mycorrhizal inoculum. Our findings suggest that if northern flying squirrels were to be replaced in our study site, southern flying squirrels can also replace at least some of the ecosystem services of northern flying squirrels.

Dietary generalists like southern flying squirrels may not replace the entire role of fungal specialists in forested environments since specialists contribute to a higher proportion of fungal dispersal than non-specialists (Nuske et al., 2017). However when there is food scarcity or when fungal specialists are absent from habitats, fungal generalists can contribute to a substantial amount of fungal dispersal in replacement, thereby increasing the opportunity for fungal growth and interactions with the underground mycorrhizal networks (Nuske et al., 2017; Stephens and Row, 2020). However, more research and monitoring on the ecological roles and interspecific interactions between fungal specialists and fungal generalists is needed as species turnover could lead to a “functional redundancy” in the ecosystem, which could inversely strengthen the structure of the ecosystem (Walker, 1995) (see Chapter 3 General Discussion). This means if turnover from northern to southern flying squirrels were to occur, the role of fungi dispersal would be maintained because southern flying squirrels could possibly fill in this role as replacement for their northern counterpart.

The similarity in ecological behaviour between the species can be attributed to the habitat and the consistency of the food it provides to both squirrels. This study location in the Kawartha Highlands is in a high dense pine-oak mixed forest where fungi taxa are diverse and plentiful. Because these flying squirrel species are in sympatry, this enables the two species to act in similar ways, yet they appear to have minor ways to partition resources possibly to avoid competition. This was found when northern flying squirrels had higher spore richness and spore loads in their scat than their southern counterpart, and northern flying squirrels were strongly associated with two mushroom families, and southern flying squirrels did not have strong associations with any fungi type. Another factor that probably influences this dietary decision is the low mast year, in which southern flying squirrels selected for fungi more than usual.

Conclusions

We have evidence to show that northern and southern flying squirrels are using habitats and resources similarly, but are finding ways to partition resources accordingly to be in sympatry. This is the first study in central Ontario to identify the diets of the two species in a hybrid zone and at a location with long-term sympatry. We identified that southern flying squirrels had larger than average home ranges in central Ontario (in comparison to Holloway & Malcolm (2007b). Moreover, with the low hard mast year, squirrels appeared reluctant to use or cross over multiple habitat types in search for food resources. Along with home range overlap, we found that both flying squirrel species were sharing pine-oak habitats similarly, likely to supply a fungus diet. While there is overlap, our research highlights some resource and habitat partitioning respectively, in that that northern flying squirrels relied on fungi a bit more than southern flying squirrels, and through the significant use of deciduous-dominated habitats for southern flying squirrels.

Overall, the squirrels are sharing the landscape together which has implications towards future conservation and management of our forest woodlots. This is pertinent for forest patches with old-growth and mature trees that face logging. If these habitats continue to be lost through substantial cutting (i.e., blocks of large hectares), squirrels will not have enough space to forage, nest, and use resources, as we've identified the minimum habitats and space used within their home ranges. This research highlights the importance that both flying squirrel species provide an ecosystem service in the forest; facilitating the dispersal of fungal spores. Special attention should focus on both species of squirrels for future conservation and management decisions.

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Chapter 3

General Discussion

Synthesis

The goal of this study was to understand the mycophagous role of both *Glaucomys* flying squirrel species, should there be turnover from northern flying squirrels to southern squirrels. I sought to understand the implications on the ecosystem roles if species replacement were to occur from rising threats of climate warming. Through a home range analysis in Chapter 2, I identified both species of flying squirrel had similar home range sizes and that there was significant spatial overlap between their ranges. Contrary to my first hypothesis, there was no difference in home range size between species, and I further explored why the two species might be behaving in similar ways by investigating each species habitat selection and seasonal fungal diet. My second hypothesis was partially supported in that I identified southern flying squirrels were significantly selecting for deciduous-dominated habitats in their home range, more so than northern flying squirrels. Although northern flying squirrels did not have strong relationships with a particular habitat type, I identified the two species behaved similarly as they both selected for pine-oak barren habitats in similar ratios of selectivity. My third hypothesis was also supported in that I found northern flying squirrels had significantly higher spore richness and spore loads in fecal samples compared to southern flying squirrels. Moreover, northern flying squirrels were consuming more diverse fungi in higher loads in the summer/fall and winter seasons compared to their southern counterpart, and both species consumed fungi in similar ways in the spring. I also found northern flying squirrels were strongly associated with Boletaceae and Suillaceae mushroom families, and that southern flying squirrels did not have strong fungi taxa preferences. Lastly, in the fourth objective, I found that both squirrel species used habitats with the highest fungal availability; in pine-oak barrens and pine-mixedwood habitats. I conclude that both species are ecologically similar at this long-term research site where sympatry has been observed for 21 years, yet there are slight differences in habitat choices and diet that allow both to adapt and co-exist together. Therefore, these species appear to partition their habitats and resources while sharing the same space and behaving similarly on the landscape. I can assume that if northern flying squirrels were to be replaced by southern species in light of climate warming, there is potential that the fungal-dispersal role in the ecosystem will be preserved by the presence of southern flying squirrels at this location.

Climate change and shifting ecosystem services

Although my research exploring the implications of species turnover of flying squirrels in central Ontario provided a different outcome than expected, research has suggested potential general negative consequences from this phenomenon. It has been said that climate warming can influence a shift in the species functional role in an ecosystem (Grimm et al., 2013). Climate change has also been found to heavily impact range shifts of species sensitive to temperature changes, such as ectotherms in the tropics (Gibson-Reinemer et al., 2015). These climatic shifts have variable effects across taxa and overall promote a phenological mismatch, ultimately affecting the time of food availability and the trophic cascade (Donnelly et al., 2011). Global predictions on bird communities infer that the ecosystem shifts are region dependent; the largest effect being the seed dispersal process in tropical areas with a decline in these specialists, leading to reduced dispersal opportunities (Stewart et al., 2022). Unpredictable and extreme weather events from climate warming can alter the timing of mast seed production (i.e., warmer temperatures can promote earlier fruiting) and result in intermittent seed germination, thereby affecting the future frequency of mast seed events and substantial seed production (Bogdziewicz et al., 2021).

Functional redundancy

Climate warming can also increase the potential for functional redundancy of various species; if there is increased species loss, range shifts, or an alteration of species interactions (Fetzer et al., 2015; Rosenfeld, 2002). However, this concept does not necessarily have a negative connotation. If one species is lost due to ecosystem shifts, other species in the area that function in similar ways could replace the species functional role. Thus the balance will be restored and the ecosystem services will be maintained (Rosenfeld, 2002). Temperature increases might strain the functional roles of all species in ecosystems however, thus more species diversity and richness is needed to continue ecosystem functions (Fetzer et al., 2015; García et al., 2018). Moreover where sympatry exists, there must be some niche partitioning, or some differences in species ecological functions in order for a balance to be maintained throughout the ecosystem

(Pigot et al., 2018). This is evident in my study location since both squirrels partition their diets and habitats in slightly different ways, which allows both species to live in sympatry. Yet I can draw conclusions that their functional roles are ecologically similar, and therefore if both species act as mycophagists in the ecosystem, I can assume resource dispersion will be maintained and fungi and mycorrhizal networks will continue to spread regardless if there is a population deficit in one flying squirrel species.

Recommendations

This study provides evidence to support the importance of flying squirrels to the ecosystem service of fungus spore dispersal as both species consumed fungi. Small mammals like southern and northern flying squirrels both have an important role to disperse fungal spores in forested ecosystems as shown in our study. Though this fungal-dispersal role is more strongly aligned with obligate mycophagists or fungal specialists like northern flying squirrels, this still provides the opportunity for dietary generalists like southern flying squirrels to facilitate this ecological role. I found that the southern flying squirrels consumed fungi in higher proportions than previously expected. Therefore, I recommend more research attention towards generalist fungal consumers, and to understand if climate warming plays a role in altering these ecological functions and dietary decisions. As identified by Stephens & Rowe (2020), rodent generalists can have ecologically meaningful effects in fungal dispersal, especially when fungal specialists are absent, and are still considered reliable dispersers to facilitate mycorrhizal networks.

To get a full measure of the dietary decisions of these squirrels, I recommend conducting a full scat analysis to estimate the proportions of the total diet of each flying squirrel. With this information, I would be able to understand the proportions of mast seeds and other food items that were consumed but not identified with fungal spore microscopy. Full diet analysis can be done using DNA metabarcoding or metagenomics and is a recently used method to understand the mycophagy diet of small mammals (i.e., Cloutier et al., 2019). This should be coupled with microscopy methods to identify the spores that are intentionally consumed, while still accounting for the other food items that are not fungi. However, microscopy remains a useful tool in mycophagy studies since users are able to visually count spores from individuals that are purposely consumed in one digestive period, whereas DNA-based methods can provide a matrix of all spores consumed, so some spores may be eaten unintentionally or indirectly from their prey (Cloutier et al., 2019).

A 95% MCP home range was created to identify the minimum area and space used by each individual squirrel species. I used the MCPs to calculate the proportions of habitats used over the availability through a resource selection ratio. Using a MCP is commonly used for home range research (Calenge, 2006), however it provides a coarse estimation of areas used within home ranges. There are alternative methods that can be used to refine home ranges to accurately judge individual habitat selection. Kernel density estimations could be used to generate home ranges by investigating density of telemetry locations on the landscape; to identify intensity of use in a particular habitat (Calenge, 2006) which would refine both home range estimations and habitat selection. I incorporated trap locations into home ranges to increase the number of location estimates for each individual squirrel, which may bias kernel estimates. If I had a sufficient number of telemetry locations within each individual squirrel, I would not need to use trap locations when calculating home range size, and thus would conduct kernel density analyses instead. I recommend future research can consider using kernel functions for calculating home range estimations.

I also recommend sampling with flying squirrel populations occupying different and larger habitat types. My sampling site is located in a heavy pine-oak matrix. The area of the hemlock plots I used were smaller than the pine-oak-barren and pine-mixedwood habitats, which lacks a proper representation for the hemlock forest type due to the ecological nature of the study site. It was identified that diverse truffle taxa favours growth in coniferous trees and the associated environmental conditions of the habitat (Fogel & Trappe, 1978). Truffles are most associated with trees such as spruce and pine and hemlock (Zhang & Minter, 1989; Stephens et al., 2017). If I used a larger variation of sampling sites such as using several dense coniferous forests with spruce and hemlock, and comparing these to dense deciduous-dominated habitats, I may find different patterns of squirrel space use and habitat selectivity, compared to a pine-oak dominated study site. If I continued to sample across a different cyclical time period (i.e., during a spongy moth bust or during mast seeding events), then I may see a shift in the fungal diets of these squirrels and perhaps not as much overlap in diet and home range.

Flying squirrels are sensitive to forest fragmentation because they rely on mature woodlots for travelling through corridors and providing ideal nesting and foraging habitat within their home range (Taulman & Smith, 2004). Old-growth trees provide snags for cavities that flying squirrels use for roosting and nesting (Weigl, 1968). Moreover, old-growth forests provide the habitat structure (i.e., coarse wood debris), habitat heterogeneity and suitable conditions for truffles to flourish (Waters et al. 1997). Mature and old-growth forest are increasingly at risk from logging practices and habitat loss from development (DellaSala et al., 2022). There are a few management recommendations to address the loss of our mature woodlots to retain ideal habitat for flying squirrels and other wildlife that rely on these habitats for survival. One recommendation is to promote green-tree retention, which provides an alternative to clear-cutting. Green-tree retention would provide sustainable logging and would leave behind live trees and other structural properties that are commonly found in old-growth forest (Sullivan et al., 2001). It was found flying squirrels would benefit from this approach if a high retention percentage were maintained (Holloway et al., 2012). Ultimately, the goal of forest management provides alternatives to clear-cutting by preserving the structural integrity and microhabitat features from old-growth forests, and retaining these features in second-growth forests, which are more common and widespread today (Carey & Johnson., 1995). I recommend following retention practices as I identified that both flying squirrel species were found to consume fungi and select pine-oak habitats in their home range, which are all inclusive characteristics of mature woodlots. Southern flying squirrels depended on large and live deciduous trees for nesting and providing acorns (Minns et al. (in review)). Therefore, forest managers should consider these alternatives and characteristics of mature woodlots before logging.

I recommend continued monitoring of these flying squirrels to investigate long-term effects of these populations, and to further understand the implications of turnover over longer periods. After 21 years of long-term sympatry at this study location, there is still concern of species turnover with accelerating threat of climate warming. Southern flying squirrels expanded their range 200 km northwards past their historic range limit in the last two decades (Bowman et al., 2005), from a dramatic change in temperature and mast crop failure. Mammalian ranges are rapidly shifting and the changes in species distributions has cascading effects on the communities they become in contact with (Myers et al., 2009). A species turnover is likely to occur from an extirpation of trailing edge populations, such as northern flying squirrels in their southern edge, and when leading edge populations dominate through expansion, such as southern flying squirrels in their northern edge (Hampe & Petit, 2005). Although the proximate cause of limitation at the southern range boundary of northern flying squirrels remains uncertain, it is quite possible that a poleward range contraction of this species will occur, and that southern flying squirrels will occupy habitats formerly occupied by northern flying squirrels and can continue the role of fungi dispersal.

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Appendix A.

Table A.1. Total habitat available of nine landcover classifications using ground-truth data and SCOOP 2018 satellite imagery.

TOTAL AVAILABLE (*Hi*)	**HECTARES**	**(RATIO) % OF SUM**
Birch	1.339843	**1.80409**
Deciduous-dominated	6.467103	**8.707908**
Development	5.496138	**7.400511**
Hemlock	0.90862	**1.22345**
Marsh	10.6663	**14.36209**
Pine-mixedwood	35.46719	**47.75632**
Pine-oak-barren	8.378145	**11.28111**
Road	3.427457	**4.615047**
Water	2.116214	**2.849467**
SUM	**74.26701**	**100**

Table A.2. Mean ± SD of spore richness and spore load index of 116 scat samples of northern flying squirrels (NFS) and southern flying squirrels (SFS) across each sampling season.

Species	**Number of samples**	**Season**	**Metric**	**Mean**	**SD**
NFS	16	Spring	Richness	1.44	0.892
NFS	16	Spring	Spore load index	0.453	0.328
NFS	20	Summer/Fall	Richness	3.35	1.5
NFS	20	Summer/Fall	Spore load index	1.12	0.584
NFS	16	Winter	Richness	3.62	1.93
NFS	16	Winter	Spore load index	1.21	0.799
SFS	30	Spring	Richness	1.83	1.02

SFS	30	Spring	Spore load index	0.514	0.49
SFS	19	Summer/Fall	Richness	2.21	0.976
SFS	19	Summer/Fall	Spore load index	0.717	0.488
SFS	18	Winter	Richness	2.22	0.943
SFS	18	Winter	Spore load index	0.6	0.411

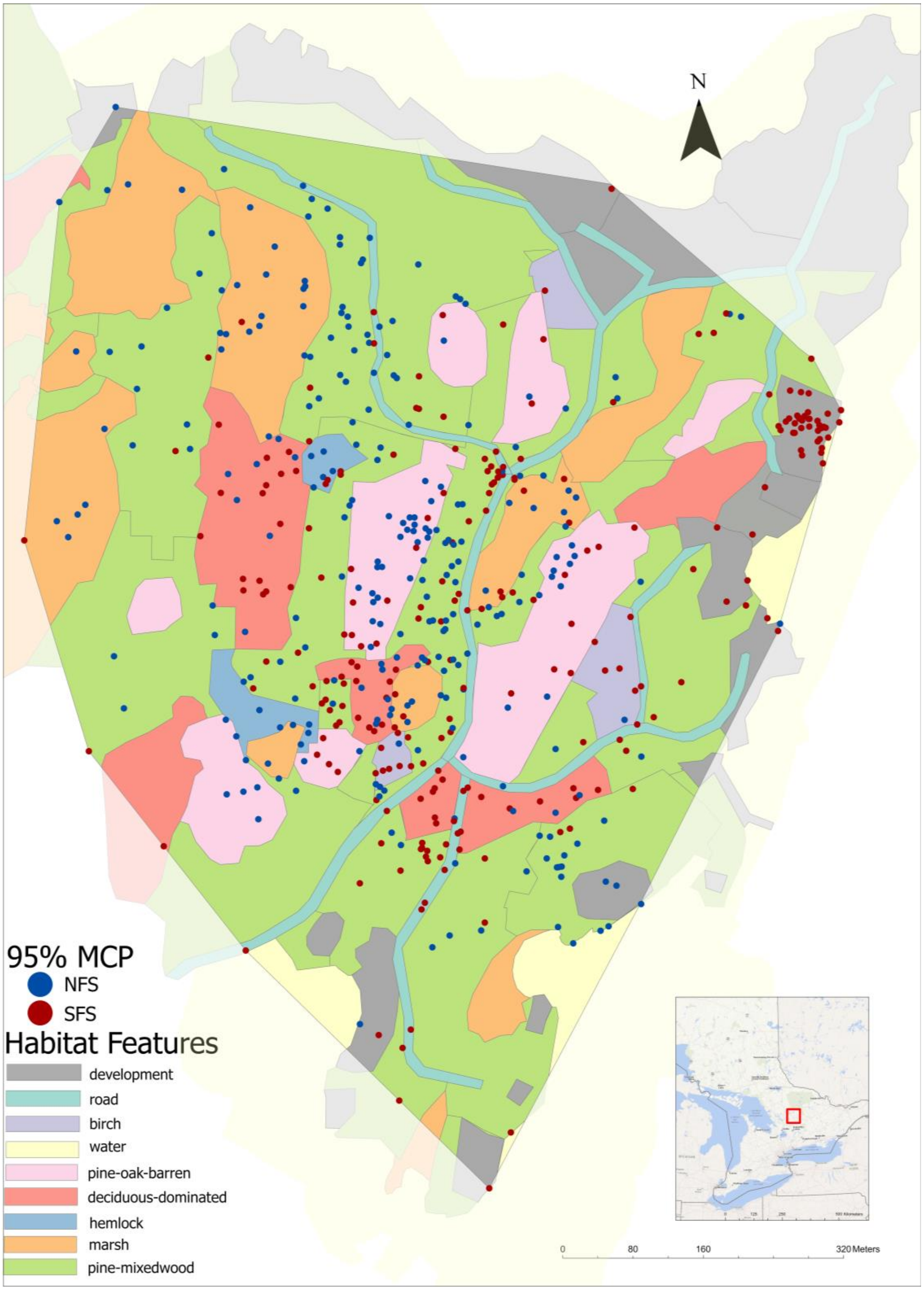

Figure A.1. All location estimates using diurnal roosts, nocturnal triangulations, and trap locations of northern (NFS) and southern (SFS) flying squirrels on habitat features near the Kawartha Highlands Provincial Park (n=883).

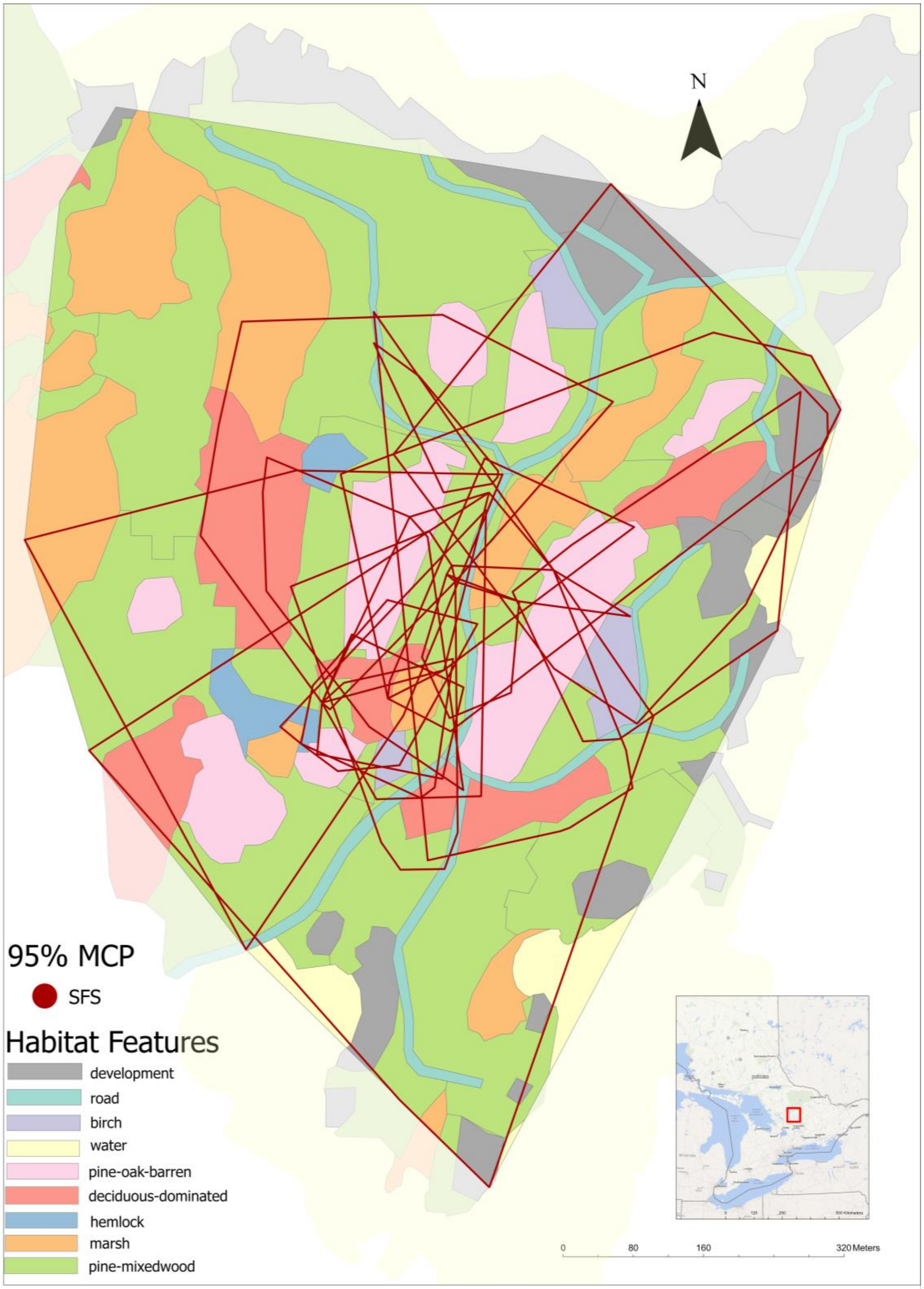

Figure A.2. 95% MCP home ranges of southern flying squirrels (SFS) on habitat features near the Kawartha Highlands Provincial Park (n=18).

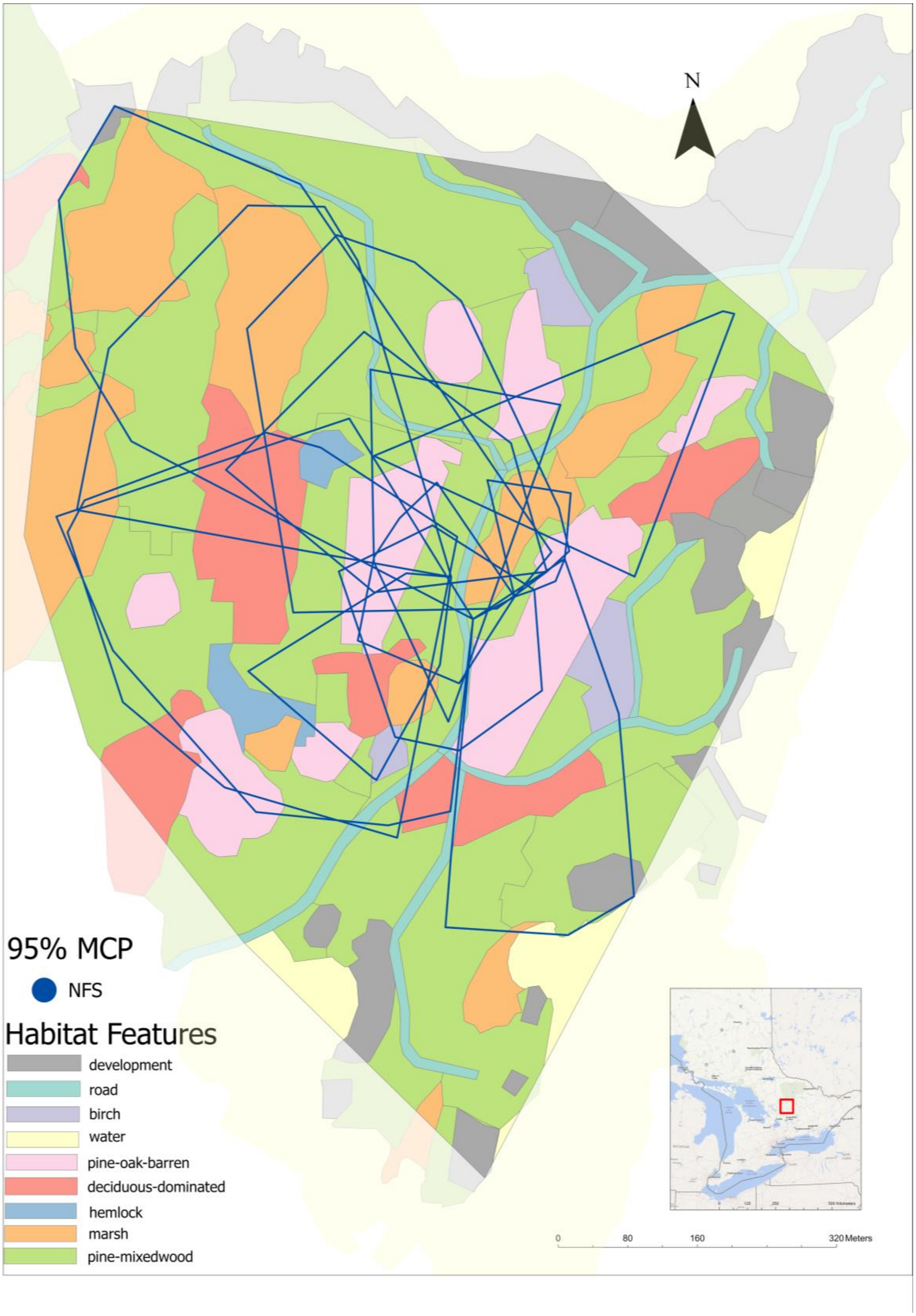

Figure A.3. 95% MCP home ranges of northern flying squirrels (NFS) on habitat features near the Kawartha Highlands Provincial Park (n=13).