Behavioural and dietary responses of red squirrels to environmental constraints at their northern range boundary

By

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Abstract

The geographical limits of a species' distribution are shaped by interacting biotic and abiotic factors, with northern range boundaries typically constrained by abiotic factors and southern boundaries by biotic factors. Species exhibiting behavioural and physiological plasticity can persist at range boundaries despite sub-optimal environmental conditions. This dissertation examines how American red squirrels (*Tamiasciurus hudsonicus*) respond to food availability, low tree density, extreme climates, and predation pressure at their northern range boundary, the sub-Arctic treeline. Annual cone production of white spruce (*Picea glauca*) was substantially lower than in interior forests, with mast year cone production reduced by 70-80%. This limitation was compounded by consistently low filled-seed production. In response, squirrels relied primarily on fungi as a dietary resource during both mast and non-mast years, a marked departure from the seed-based diets typical of interior populations. Nevertheless, spring body mass, overwinter mass loss, and survival remained strongly linked to annual seed and cone availability. Thermal benefits drove squirrels to primarily use underground burrows for nesting, which reduced exposure to larger predators like red foxes (*Vulpes vulpes*) and American martens (*Martes americana*), but increased vulnerability to smaller, burrowing predators such as short-tailed weasels (*Mustela erminea*). Larger predator activity peaked in summer and autumn when squirrels were more active, while burrowing predator activity peaked in winter, when squirrels were sheltering in burrows. Thus, burrows may trade protection from weather and larger predators for increased vulnerability to small, subterranean hunters. American martens, a primary squirrel predator, exhibited a diet dominated by snowshoe hares (*Lepus americanus*) regardless of annual fluctuations in abundance of typical primary prey (voles and squirrels). These results suggest a preference for larger, high-caloric prey in low-productivity environments, with opportunistic shifts to smaller prey when more accessible. This research highlights the importance of behavioural and physiological plasticity in enabling populations to persist at ecological margins and enhances our understanding of how species navigate constraints of climate, habitat structure, and resource limitation at range boundaries. As climate change accelerates environmental shifts, insights into such dynamics are critical for predicting range dynamics and guiding conservation in rapidly changing ecosystems.

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Contributions of Authors

Chapter 2

A.E. Windsor led the Conceptualization, Methodology, Investigation, Formal analysis, and Writing – original draft. J.H. Markham contributed to Conceptualization and Methodology, provided Resources (seed data), and contributed to Writing – review & editing. J.D. Roth contributed to Conceptualization and Methodology, and provided Writing – review & editing. J.D. Roth additionally provided Supervision, Project administration, and Funding acquisition for this chapter. The manuscript is currently in review with *Oecologia*.

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Chapter 3

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Chapter 4

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Chapter 5

A.E. Windsor contributed to Conceptualization, Methodology, Investigation, Formal analysis, and Writing – original draft. J.D. Roth contributed to Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, and Writing – review & editing. C. Warret-Rodrigues contributed to Conceptualization, Methodology, Resources (prey stable isotope data), and Writing – review & editing.

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Chapter 1: Introduction

Species distributions and range boundaries

The geographical range of a species, and the subsequent distribution of individuals and populations within that range, are shaped by a combination of biotic and abiotic factors (Kirkpatrick & Barton 1997, Holt 2003, Kubisch et al. 2014). At a local scale, interactions such as competition and predation influence how individuals are distributed across landscapes (Grayson & Johnson 2018). At a broader scale, climate and resource availability are often the primary drivers of species distributions (Gaston 2003, Sexton et al. 2009). Together these factors constrain populations along latitudinal and environmental gradients, defining the species' northern and southern range boundaries (Emlen et al. 1986), locations where a species transitions from present to absent (Louthan et al. 2015).

Range boundaries are not universally static. While some are fixed by physical features such as coastlines or mountain ranges, others are dynamic, subject to ongoing shifts in response to changing environmental conditions, biotic interactions, or disturbance regimes (Brown et al. 1995). This raises a fundamental ecological question: what prevents species from expanding beyond their geographical range? The edges of a species' range are influenced by a complex interplay of ecological factors, including biotic elements like competition and predation, as well as abiotic elements like climate, habitat structure, and availability of shelters and nesting sites.

Generalizing the processes that define range boundaries can be challenging each species occupies a distinct ecological niche governed by a unique set of limiting environmental variables (Brown et al. 1995). However, a common hypothesis suggests that biotic factors predominantly constrain species' southern range boundaries, whereas abiotic factors exert more influence on northern range boundaries (MacArthur 1984).

Regardless of location, the mechanisms that limit populations at range boundaries often differ from those affecting populations within the core of the range. Range boundary populations often exist at lower densities (Brown et al. 1996) and face environmental extremes that impose physiological constraints (Sexton et al. 2009). Particularly, populations at northern range boundaries often persist at the edge of the species' climatic niche, where individuals experience environmental conditions close to their physiological limits (MacArthur 1984). For example, the northern range of the Virginia opossum (*Didelphis virginiana*) is limited by winter temperatures, which increase mortality and restrict the species' ability to persist beyond that boundary (Kanda 2005). Consequently, range boundary populations are often more vulnerable than interior populations, as they tend to occupy suboptimal habitats (Gaston 2003) and may be isolated from larger, more continuous populations (Brown et al. 1995, Thomas & Kunin 1999).

The sub-Arctic treeline

The northern treeline serves as a natural range boundary for many species, marking the northern limit for boreal forest species and the southern limit for Arctic species. This transition zone forms the boreal-tundra ecotone, where species from both ecosystems converge. In northern Manitoba, treeline is a fragmented open-canopy forest dominated by three conifer species, black spruce (*Picea mariana*) and white spruce (*Picea glauca*), interspersed with tamarack (*Larix laricina*), and an understory vegetation including willow (*Salix* sp.) and Labrador tea (*Rhododendron* sp.). The proximity of the treeline in this region to Hudson Bay results in a harsh sub-Arctic climate, with long, cold winters and short, cool summers that impose a brief growing season.

White spruce is a masting species and a primary food source for many boreal granivores, providing essential macronutrients such as carbohydrates (Downie & Bewley 2000), lipids

(Carrier et al. 1999), and proteins (Lobo & Millar 2011). As with other masting species, white spruce exhibit highly variable seed and cone production, characterized by intermittent years of hyperabundant production followed by years of little to know production. Years of excessive production are known as mast years (Kelly 1994, Archibald 2011). Masting is typically synchronous across large geographical areas and is widely hypothesized to function as a predator satiation strategy, whereby an overabundance of seeds during mast years temporarily reduces the impact of seed predators, allowing a greater proportion of seeds to escape consumption (Kelly & Sork 2002).

The masting interval for white spruce typically ranges from two to six years in ideal habitats and 10 to 12 years in less favourable habitats (Nienstaedt & Zasada 1990, Lamontagne & Boutin 2007). The extreme variability in cone production imposes significant challenges for granivorous species, which must adapt to exploit this highly pulsed resource while surviving years of scarcity. Boreal seed predators such as the American red squirrel (*Tamiasciurus hudsonicus*) have adapted many life history traits to fluctuate with cone crop size (Smith 1968).

Red squirrels

The American red squirrel is a small arboreal mammal (< 300 g) and the only arboreal squirrel species inhabiting the northern treeline (Fitak et al. 2013). The species has a wide distribution spanning much of Canada and the northern United States, extending down into the southwestern states (Smith 1968, Weigl & Hanson 1980, Steele 1998). Red squirrels are highly adaptable, occupying a variety of habitats including coniferous and mixed forests, as well as urban environments. Nevertheless, they exhibit a strong preference for dense boreal coniferous forests, which provide abundant conifer seeds and interlocking canopies for efficient travel and foraging (Smith 1981, Goheen & Swihart 2005).

Throughout their range, red squirrels maintain individual, non-overlapping territories year-round. Individuals rarely leave their territories except when searching for mates, which primarily involves males, and when pilfering food from neighbouring squirrels' caches (Stuart-Smith & Boutin 1995). Adult mortality is considerably high, ranging from 40% to 90%, and varies seasonally, annually, and geographically (Kemp & Keith 1970, Rusch & Reeder 1978).

Red squirrels are primarily granivorous, specialising in conifer seeds across most of their distribution (Steele 1998, Boutin et al. 2006, Archibald 2011). In coniferous habitats, individuals harvest and cache spruce cones in centrally located caches known as middens (Smith 1968, Gurnell 1984). The location of middens minimizes the energy expended during cone harvest (Steele 1998) and facilitates easier defense (Smith 1968). Middens are reused and can contain multiple years of cached cones. When a territory owner is replaced, the new individual will continue to use the established midden (Boutin & Larsen 1993). Middens are often easily identified by the discarded cone debris that accumulates annually as squirrels strip scales from cones to retrieve the seeds. The damp and cool microclimate of middens provides an ideal environment for storing food over winter (Smith 1968, Donald & Boutin 2011).

Squirrels typically harvest spruce cones in late summer and autumn, enabling squirrels to cache sufficient resources to survive the winter, and in many regions they are able to hoard enough cones to survive multiple winters (Gurnell 1984). Many aspects of red squirrels' life histories are tightly linked to the annual variability in conde production (Boutin 1990, Humphries & Boutin 2000, Réale et al. 2003, Boutin et al. 2006, LaMontagne et al. 2013). In northern areas, reproduction is usually limited to one litter per season, typically in late March to early June (McAdam et al. 2007). However, in mast years, squirrels can increase their reproductive output in anticipation of seed maturation in years of high cone production (Berteaux & Boutin 2000, McAdam & Boutin 2003) and may attempt to produce a second litter (Boutin et al. 2006, Lane et al. 2015). Additionally, growth in nestling red squirrels and higher overwinter survival has been positively linked to the previous year's production (McAdam & Boutin 2003). Territory size also correlates to spruce cone availability, as individuals possessing larger territories and food caches exhibit higher overwinter survival rates (Steury & Murray 2003, LaMontagne et al. 2013).

In the northernmost portion of their range, red squirrels feed almost exclusively on white spruce seeds (McAdam & Boutin 2003). Yet, as opportunistic omnivores, individuals are capable of incorporating a variety of alternative food sources, including other plants and animals, particularly in summer before the maturation of cone crops and in years of low cone production (Layne 1954, Smith 1968, Currah et al. 2000, Willson et al. 2003, Fletcher et al. 2010). The high plasticity in squirrel diet and behaviour enable individuals to cope with substantial temporal variation in food availability and climate, supporting their persistence across a broad geographic distribution.

Thesis objectives and structure

The work presented in this dissertation investigates the biotic and abiotic factors that limit red squirrels at their northern range boundary, the sub-Arctic treeline. This study comprises four data chapters, each addressing different but interconnected aspects of red squirrel ecology and the broader food web dynamics at this ecological margin.

In chapter 2, I examined how annual fluctuations in white spruce (*Picea glauca*) cone and seed production influence squirrel reliance on alternative food sources. I quantified annual cone and filled-seed production and reconstructed squirrel diets using stable isotope analysis. I predicted that 1) overall cone and filled-seed production at the treeline would be low, 2) squirrels would frequently consume alternate food sources depending on the annual abundance of filled seeds and cones, 3) their dietary niche would be broad, and 4) that autumn diet would be broader than spring diet, as alternate food sources such as fungi and berries are more abundant in autumn.

In chapter 3, I evaluated the influence of food availability, winter temperatures, and predation risk on squirrel survival and seasonal changes in body mass. I estimated squirrel survival using mark-recapture data and assessed the nutritional content of the primary food source, spruce seeds, and a common alternative food source, fungi. I hypothesised that low resource availability at the treeline would affect overwinter mortality and body mass and predicted that survival and spring body mass would fluctuate with annual food availability. Additionally, I predicted that predation risk would further affect overwinter mortality, as squirrels are one of the few prey species at the treeline that are active above the snow in winter.

Chapter 4 explored the trade-off between thermal benefits and predation risk associated with squirrel nest type. I tracked radio-collared squirrels to evaluate nest choice and quantified seasonal predation risk using motion-activated trail cameras placed at squirrels' middens. I hypothesized that 1) predator visits to middens would exhibit seasonal variation, owing to fluctuations in prey accessibility, 2) squirrels would prefer nest types based on their thermal properties, thereby enhancing their chances of overwinter survival, and 3) the choice of nest type would influence the vulnerability of squirrels to predators. I thus predicted that predator observations would increase during winter when other prey species would be largely inaccessible. Furthermore, I anticipated that ground burrows would be warmer in winter compared to ambient temperatures and tree-nest temperatures and that more burrowing predators would be observed at sites where squirrels were nesting below ground.

In chapter 5 I investigated predator-prey interactions at the treeline, focusing on the role of squirrels as a prey source for American marten, one of their major predators. Using stable isotope analysis, I reconstructed the annual and seasonal diets of American marten and quantified the abundance of key prey species at the treeline, including red squirrels, red-backed voles (*Clethrionomys gapperi*), and snowshoe hares (*Lepus americanus*). I hypothesized that the diet of martens would reflect the 1) annual abundance of their primary prey, voles, with further 2) seasonal shifts in response to changes in prey availability. Therefore, I predicted that martens would primarily feed on voles during years with high abundance, while squirrels would constitute a larger portion of the marten diet in winter, when voles are less accessible in the subnivean zone but squirrels remain active above the snow. Additionally, I used stable isotopes to investigate the annual and seasonal dietary niche breadth in marten diets. I expected a more specialised diet in years of high vole abundance and in winter when few prey species are available above the snow.

This thesis integrates field-based ecological monitoring with laboratory-based stable isotope analysis, offering a comprehensive, multifaceted approach to understanding the factors that constrain species at their geographic range boundaries. Collectively, these chapters provide insights into the ecological challenges faced by populations living at range boundaries and highlight the behavioural and dietary plasticity that facilitates persistence in resource-limited, climatically extreme environments. More broadly, understanding the factors that define species distributions and limit range expansions contributes to our understanding of latitudinal diversity gradients, food-web complexity, and species vulnerability. This knowledge is increasingly important as climate change continues to alter environmental conditions and shift species distributions worldwide.

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Chapter 2: Presumed seed specialists rely on fungi as their primary food source at the sub-Arctic treeline

Abstract

Range boundaries limit local populations, which may experience pronounced fluctuations in resource availability, particularly at higher latitudes, often seen as resource pulses. In boreal forests, conifers undergo pulses of seed production followed by intervals of low seed production**,** profoundly affecting consumers dependent on these resources. Red squirrels (*Tamiasciurus hudsonicus*) are considered seed specialists across the boreal forest. We evaluated how annual changes in white spruce (*Picea glauca*) cone production at the sub-Arctic treeline near Churchill, MB, Canada, influenced squirrels' use of alternative food sources, predicting that low cone production would increase reliance on alternate foods. Cone crops varied from 2020-2023, with a mast year in 2022 of 471 cones per tree, approximately 70-80% lower than mast years elsewhere, and lower crops in other years (6-115 cones per tree). Furthermore, the number of filled seeds (containing an embryo) per cone was low, ranging from 0.6 ± 0.03 (mean ± SE) in 2022 to 3.6 ± 2.6 in 2023. Using stable isotope ratios of hair and Bayesian mixing models, we found that squirrels primarily consumed fungi (~70% of diet), even in mast years, with other food sources varying with cone production. The dominance of fungi in squirrel diet, even in mast years, highlights the dietary plasticity of red squirrels beyond seed specialization, challenging the seed specialization paradigm. Flexible foraging strategies likely allow populations to persist in resource-limited environments and may facilitate range expansion as climate change reshapes habitats.

Key words: American red squirrel, diet, facultative specialist, stable isotopes, range boundaries, *Tamiasciurus hudsonicus*

Introduction

Species with extensive geographical distributions often have smaller, fragmented populations near their range limits, where habitats are harsher than at the core of their distributions (Gaston 2003, Lynch et al. 2014). Range boundaries can be shaped by physical barriers directly preventing species' dispersal (Gross & Price 2000) or gradients in climatic variables, which create physiological limits. In the northern hemisphere, northern range boundaries are frequently governed by abiotic factors like extreme climate, while biotic factors, such as competition, and food availability, impose additional constraints at southern range limits (Brown et al. 1995, Sirén & Morelli 2020). However, combinations of factors likely work in concert, limiting the expansion of species beyond their range. Compared to core environments, range boundary habitats often exhibit greater climatic variability (Rehm et al. 2015), which imposes physiological limits and impact population growth (Sexton et al. 2009). Although range boundary habitats can be expansive, they are often low quality or exist as fragmented patches of high-quality habitat. Climatic factors and the corresponding influences on habitat suitability, population density, and reproductive fitness strongly reinforce range limits and can be further exacerbated by inter and intraspecific interactions and resource availability. At northern range boundaries, extreme climates can reduce the availability and accessibility of food sources, particularly during winter. The variation in seasonal climates at higher latitudes dictates large annual and seasonal fluctuations in resource availability (Humphries et al. 2005), as shorter growing seasons and cooler seasonal temperatures restrict primary and secondary productivity (Gross & Price 2000, Callaghan et al. 2004). Species respond to fluctuations in food sources in multiple ways. Some species, such as the boreal woodland caribou (*Rangifer tarandus caribou*), migrate seasonally following food sources (Rettie & Messier 2000, Ferguson & Elkie 2004).

Other species, such as wolverines (*Gulo gulo*), may change activity patterns or cache food to buffer against fluctuating food availability in an overall low-productivity environment (van der Veen et al. 2020). When food is spatially and temporally variable, species may rely on pulses of food sources.

Pulsed resources are episodic events of superabundant resource production with long inter-pulse periods of normal or subnormal production. While resource pulses are short-lived, they drive the dynamics of many plant and animal communities. The bottom-up effect of pulsed resources can be seen at the level of the individual, the population, and indirectly at the community level (Yang et al. 2008, Yang et al. 2010). At the individual level, consumer diets shift in response to pulsed resources, which induces numerical responses in consumer populations. These responses then propagate across trophic levels as increased densities in consumers become secondary resource pulses for higher trophic levels (Yang et al. 2010).

The boreal forest, one of the world's largest terrestrial biomes, represents the northern range limit for many species (Kayes & Mallik 2020, Krebs et al. 2023). Conifers in the boreal forest experience periodic masting, with years of high seed production followed by long intervals of low or absent seed production (Kelly & Sork 2002, Lamontagne & Boutin 2007, Archibald et al. 2012). Mast years are often synchronous across large geographical areas (Lamontagne & Boutin 2007, Krebs et al. 2012) and overwhelm seed predators, enhancing seed survival and establishment (Kelly & Sork 2002). White spruce (*Picea glauca*) is the dominant tree species in dry habitats at the northern limit of the boreal forest (Bonan and Shugart 1989) and exhibits periodic masting (Archibald et al. 2012, Krebs et al. 2012, Krebs et al. 2023), approximately every 3-5 years (Lamontagne & Boutin 2007). Many small mammals rely heavily on spruce seeds but may switch to alternative food sources, such as fungi, which can be highly abundant but nutrient-poor, during non-mast years (Ostfeld & Keesing 2000, Fletcher et al. 2010).

The dietary strategies of species can be defined based on the breadth of their dietary niches. Facultative generalists typically have a very broad dietary niche compared to facultative specialists that exhibit a narrower dietary niche (Shipley et al. 2009, Pagani-Núñez et al. 2016). However, both groups exhibit behavioural plasticity. Facultative generalists optimize resources by switching to more abundant or accessible food sources when the availability of their usual foods declines or when seasonal or annual fluctuations alter the relative abundance of alternative resources, while facultative specialists can expand their diet when primary resources are scarce, enabling both groups to exploit fluctuating resources and maintain broad geographic distributions, making them more successful in habitats where food availability is low and heterogeneous (Shipley et al. 2009, Newbury & Hodges 2018, Szumski et al. 2023). In contrast, obligate generalists, though able to use diverse food sources, have limited specialization and may be less efficient in optimizing their diet in such environments, and obligate specialists are vulnerable due to their reliance on specific resources, which could be scarce (Shipley et al. 2009, Dehling et al. 2021). Due to the low predictability of food abundance, generalist species are common at northern range boundaries (Callaghan et al. 2004). Facultative specialists living in these habitats may exhibit behaviours more like facultative generalists when primary food sources are unpredictable and fluctuate dramatically. Individuals may also employ behavioural methods such as food hoarding and increasing litter and clutch sizes to take advantage of years of superabundant food (Callaghan et al. 2004, Boutin et al. 2006, McAdam et al. 2019).

American red squirrels (*Tamiasciurus hudsonicus*) are primarily granivorous, specialising in conifer seeds across most of their distribution (Steele 1998, Boutin et al. 2006). In the northern parts of their range, red squirrels appear to feed almost exclusively on white spruce seeds (McAdam & Boutin 2003). While seeds constitute the majority of squirrel diet, red squirrels can opportunistically consume a large variety of foods including fungi (Layne 1954, Steele 1998, Fletcher et al. 2010), berries (Benhamou 1996, Currah et al. 2000), and animal prey, including songbird eggs (Bayne & Hobson 2002, Willson et al. 2003) and young lagomorphs (Layne 1954, Sullivan & Sullivan 1982, Peers et al. 2020). These alternative food sources, however, typically make up a small proportion of annual diet (Layne 1954), and are relied on only when spruce cone availability is low. Despite observations of consumption of alternative food sources, detailed quantifications of non-seed food sources are often lacking (Currah et al. 2000, Fletcher et al. 2010). Many life history parameters of red squirrels, including reproduction (Boutin et al. 2006, Lane et al. 2015), growth (McAdam & Boutin 2003), and overwinter survival (Steury & Murray 2003, LaMontagne et al. 2013), are intimately linked to spruce cone production (Humphries & Boutin 2000, McAdam & Boutin 2003, Réale et al. 2003, Boutin et al. 2006). Assuming seed productivity is consistent, seed availability can be estimated through spruce cone availability by counting the number of cones produced within the study area (Lamontagne & Boutin 2007, Kucheravy et al. 2021). However, the quality and quantity of seeds produced each year can also vary (Waldron 1965, Zasada 1988), further influencing overall food availability.

We reconstructed the diet of red squirrels living at their sub-Arctic range boundary using stable isotope analysis and explored how spruce cone production affected squirrel diet. We hypothesised that squirrels would adopt a highly supplemented diet in low cone years and predicted that 1) overall cone production would be low, 2) squirrels would frequently consume alternate food sources, depending on the yearly abundance of cones, and 3) their dietary niche would be broad. We further predicted that 4) fall diet would be broader than spring diet, as alternate food sources such as fungi and berries are more abundant in fall.

Methods

Study area

Our study area near Churchill, MB (58°45’ N, 94°04’ W), on the western edge of Hudson Bay, is on the transition between boreal forest and Arctic tundra. The forest is primarily composed of white spruce, black spruce (*Picea mariana*), and tamarack (*Larix laricina*) (Mamet & Kershaw 2011, Mamet & Kershaw 2013) and is heavily fragmented by patches of wetlands and open tundra (Harper et al. 2011, Harper et al. 2018). The forest has a low density of mature trees (~600 stems ha^{-1}) and a sparse canopy of ca. 25% (Lafleur 1999). The proximity of the sub-Arctic treeline to Hudson Bay, which is frozen eight months of the year, exposes the region to cool summer temperatures and extreme winters (Mamet & Kershaw 2011). In our study area, mean monthly temperatures ranged from -28.4 to -15.1 ^{o}C in winter (November-April) and 1.2 to 20.6 ^{o}C in summer (June-August) from 2019-2023 (Environment Canada).

Fieldwork was conducted in boreal woodland consisting of patchy forest, transitioning to a denser forest and ending in an open-canopy forest near a large fen (Figure 1). We established sites at 250 m intervals along a 7 km transect running north to south, looking for signs of squirrel activity such as vocalisations, cone debris, and middens. Middens are large food caches containing hundreds to tens of thousands of cones (Haines et al. 2022, Wishart 2023), and are easily identified by the concentrated accumulation of cone debris, often reaching surfaces areas up to a few hundred m^2 (Steury & Murray 2003). Very few true middens, characterized by large fields of cone debris, were observed in our study area. Here, we define a ‘midden’ as any form of food cache, including true middens and burrows with cone debris that have the potential to contain cones. To account for juvenile dispersal and the possibility of squirrels taking over previously unoccupied sites, we checked all sites for signs of squirrel activity each year in early June.

Food availability

To assess annual spruce cone production, we conducted cone counts of individually marked trees each August (five trees per site; 9 sites in 2020, 26 sites in 2021-2023). Each tree was at least 5 m from the nearest sampled tree and had a minimum of 5 cm diameter at breast height (DBH), representing the approximate minimum age at which white spruce trees can bear cones (Lamontagne & Boutin 2007). At squirrel-occupied sites, we sampled the five closest trees to the midden. At unoccupied sites, we selected the five closest trees from a flagged reference point, following a bearing determined using a random number generator. Each August, we took 3-5 photographs of each tree on opposing sides. Using ImageJ image processing software, we counted the cones visible in each picture (Nygren et al. 2017). The photographs for each tree were matched to avoid double-counting cones. We calculated food availability at each site as the mean number of cones per tree (Kucheravy et al. 2021), multiplied by tree density to estimate cones per hectare. To calculate tree density at each site, we counted the number of white spruce trees (> 5 cm DBH) within four 15 m radius plots (two on each side of the flagged reference point) spaced 20 m apart. To estimate seed production, we used data from the control sites, which encompassed our sampling area, from Benjamin et al. (2024). We collected cones from the upper part of the crown using a pole pruner. The number of cones collected per tree depended on their availability but averaged 13 ± 1 cones. The cones were air-dried for at least 72 h prior to dissecting and removing the seeds. We placed the seeds in 95% ethanol to separate the filled seeds containing an embryo (sinking seeds) from empty seeds (Ho 1984, Sirois 2000).

To estimate availability of fungi, in 2022 we collected all epigeous fungal sporocarps within two randomly selected 15 m radius plots at each site. In 2023, we collected all epigeous fungi within four 1-m^2 quadrats placed 5 m from each site's centre point in the four cardinal directions. At occupied sites, the midden served as the centre, and at unoccupied sites, we used the cone count centre point. All samples were oven-dried for 48-72 hrs, and dry fungal biomass (kg ha^{-1}) was used to reflect fungus availability at each site (Derbridge & Koprowski 2019).

We monitored squirrel hoarding activity using motion-activated Browning trail cameras (model: Strike Force Extreme and Strike Force HD Pro X) installed at 17 occupied sites (one camera per site). We chose sites with previously observed middens and installed each camera facing the largest midden if a site had multiple middens. Cameras captured bursts of three photos when activated within a three-metre detection range, with a 0.5 second recovery between bursts. We recorded the date, time, location, and type of food items hoarded from August 1 to September 30, reflecting the typical harvest period for squirrels in central areas of the boreal forest (Fletcher et al. 2010, Archibald et al. 2013) and to account for the shorter growing seasons and earlier winters characteristic of the northern boreal treeline. Squirrels entering middens with food or crossing the camera's field of view carrying food, were classified as hoarding events. Observations may have underestimated activity if a midden had multiple entrances not covered by cameras.

Stable isotope sample collection and preparation

Red squirrel body hair moults in spring and fall, while tail hair moults only in the fall (Nelson 1945). We collected body hair (~3-4 clippings, ~0.5 cm behind the shoulder) and tail hair (~2-3 cm) each June, from a total of 103 squirrels (90 unique individuals, 9 recaptures, and 4 donated by local trappers) to compare seasonal diets. We captured squirrels using Tomahawk live traps (model #202). We recorded the sex and marked each squirrel with a Passive Integrated Transponder (PIT) tag injected under the skin for permanent identification and metal ear tags threaded with coloured pipe cleaners for visual identification

We collected samples of potential food sources from forested and forest-wetland regions within our study area in August 2021 (Table S2.1). Samples included white (n = 9) and black spruce seeds (n = 9), tamarack seeds (n = 6) and buds (n = 15), and the most common species of berries (n = 97), lichen (n = 22), and fungi (n = 110).

Hair samples were washed twice with mild, soapy water and rinsed thoroughly before drying in a drying oven for 48 hours. We removed seeds from spruce cones and dried the seeds, fungi, berry and lichen samples in a drying oven for a minimum of 48 hours, and ground all food samples to a powder using a mortar and pestle, washing the tools between samples with 70% ethanol. All samples were analyzed for carbon and nitrogen stable isotope ratios on a continuous-flow isotope ratio mass spectrometer (Delta V Advantage) coupled to a Costech 4010 Elemental Combustion system and a ConFlo IV universal interface.

Data analysis

We used stable isotope analysis to examine annual and seasonal variation in squirrel diets. The isotopic composition of a consumer's tissues reflects dietary sources, adjusted by trophic discrimination factors (TDFs) to account for isotopic shifts during assimilation (Phillips 2012, Parnell et al. 2013). We applied rodent-specific TDFs and standard deviations of $\Delta^{13}C = 3.3 \pm 2.2‰$ and $\Delta^{15}N = 1.9 \pm 0.2‰$ (Hobbie et al. 2017, Pauli et al. 2019) to source isotope values. Using a K nearest-neighbor (KNN) randomization test (k = 15) we classified 231 samples into five isotopically distinct food groups: berries (species n = 7), lichen (n = 2), conifer seeds and buds (not isotopically distinct: ANOVA: $\delta^{13}C$: $F_{3,35} = 0.65$, $p = 0.59$; $\delta^{15}N$: $F_{3,35} = 1.57$, p =

0.38), and fungi divided into two groups (genera n = 5) due to $\delta^{15}N$ variation across genera and habitats (forest vs forest-wetland) (Table S2.1, Figure S2.1).

We estimated the dietary contribution of each food group to squirrel diet using Bayesian isotopic mixing models with the MixSIAR and *rjags* packages (Parnell et al. 2013) in R. All models incorporated uniform prior distributions and concentration dependence using the mean elemental concentrations for each prey group. We used Markov-chain Monte Carlo (MCMC) methods to estimate the parameters of the mixing models and ran three parallel MCMC chains with a burn-in of 50,000 iterations. We generated posterior samples using 150,000 iterations of the models and a thinning rate of 50 and checked model convergence using the Gelman and Geweke diagnostic tests produced by MixSIAR (Geweke 1991, Jackson et al. 2011, Gelman et al. 2014).

Stable isotope ratios were also used to estimate dietary niche breadth, where narrower isotopic niches indicate more specialised diets (Bearhop et al. 2004, Jackson et al. 2011). For opportunistic species, niche breadth can shift seasonally and annually with food availability. Isotopic niche breadth was modelled using an ellipse-based approach with the Stable Isotope Bayesian Ellipses in R (SIBER) package in R. We compared isotopic signatures from body (spring diet) and tail (fall diet) hair samples. Previous studies suggest the fall moult occurs between late August and into September (Kranowski 1969) and the spring moult occurs between April and May (Layne 1954), although populations at higher latitudes can moult between March and June (Lepage & Parker 1988). However, we did not observe signs of moulting during our trapping efforts throughout June and into early July. We therefore assumed that all body hair collected in June was recently grown. We generated metrics for isotopic niche breadths using standard ellipse areas corrected for small sample size (SEA_c) and Bayesian Standard Ellipse

Areas (SEA_b) (150,000 MCMC iterations, 95% credible interval). We compared seasonal and annual niche breadths using the credible intervals to estimating the probability (0-1) that one group's ellipses were smaller than another (Jackson et al. 2011). We also examined the dietary niche overlap between ellipses by calculating the proportion of ellipse overlap using 1,000 draws of the posterior estimates of each ellipse.

All statistical analyses were conducted in R (version 4.0.5). We used generalised linear mixed effects models (GLMM) to determine if mean cone production varied annually, using 'site ID' as a random effect. Since the cone count data was overdispersed, we used a negative binomial link function. For fungal biomass, we used a paired t-test to determine if dry fungal biomass varied from 2022 to 2023.

We examined annual variation in filled seed counts per cone using a linear model (LM) with cone production (total cones per tree) and seed production (total number of seeds per cone, comprising both filled and empty seeds) as additional explanatory variables. To examine the effect of cone abundance on hoarded food items, we used trail camera observations to determine the daily mean cone and fungi hoarded per camera. We used a GLMM for each food source with a Poisson link and accounted for spatial variation using 'site ID' as a random effect.

We tested for annual and seasonal variation in squirrel diet, using a multivariate analysis of variance (MANOVA) to compare $\delta^{13}C$ and $\delta^{15}N$ values between years and body and tail hair. We further examined annual diet variations in MixSIAR, with 'year' (4 levels) as a fixed effect and 'site ID' (26 levels) as a random effect. Squirrels were only trapped at 26 of the 29 sites in our study area. We ran a second mixing model to estimate individual diets using 'squirrel ID' as a fixed effect. We incorporated the mean individual dietary proportions from MixSIAR into three beta regression models (berries, fungi, and lichen) with a canonical link function using the *betareg* package in R to determine if the consumption of alternate food sources fluctuated with cone abundance, using the mean cone abundance at the sites where the individuals were trapped. We used beta regression models since dietary proportions range from zero to one (McAulay et al. 2020). We paired all squirrel diets with cone production estimates from the previous fall, as tail hair reflects the previous fall's diet and body hair collected in summer reflects the spring diet when most food items such as fungi, cones, and berries were not yet available. Thus, squirrels likely relied on food cached the previous fall.

Results

White spruce density across sites averaged 483.7 stems ha^{-1} (± 54.2 SE; range 56 to 579). From 2020 to 2023, annual spruce cone production varied significantly (Table S2.2), with the greatest number of cones per tree in the mast year of 2022 (471.2 ± 33.1), low crops in 2021 (7.9 ± 0.8) and 2023 (6.2 ± 1.2), and a moderate cone year in 2020 (11.5 ± 6.0). The number of filled seeds per cone was low overall, but also varied annually (Table S2.2), with the greatest number of filled seeds per cone in 2023 (4.9 ± 1.6), the fewest number in the mast year of 2022 (0.6 ± 0.0), and a low production of filled seeds in 2021 (2.4 ± 0.6). Fungus production across sites ranged from 0.0 to 8.5 kg ha^{-1}, with greater abundance in 2022 (3.5 ± 0.5) compared to 2023 (2.5 ± 0.6) ($t_{25} = 5.51$, $p = 0.014$). Variability of fungal abundance between sites was high but lower in 2022 (coefficient of variation = 67%) compared to 2023 (coefficient of variation = 114%).

Mean daily observations of spruce cone and fungus hoarding in August and September fluctuated annually in response to cone production (GLMM cones: $z_{1,46} = 9.1$, $p < 0.001$; fungi: $z_{1,46} = -2.3$ $p = 0.004$). Cone hoarding was highest in 2022 (8.1 ± 0.2 cones day^{-1}), lowest in 2021

(0.9 ± 0.3), and relatively low in 2020 (2.3 ± 0.3) and 2023 (1.4 ± 0.6). Fungus hoarding was highest in 2021 (1.0 ± 0.2 sporocarp day^{-1}), lowest in 2020 (0.1 ± 0.0) and 2022 (0.1 ± 0.0), and relatively low in 2023 (0.7 ± 0.1).

Male and female squirrels did not differ in $\delta^{13}C$ (t = -0.44, df = 51.43, p = 0.661) or $\delta^{15}N$ (t = -0.97, df = 51.43, p = 0.543) (Table S2.3 and Figure S2.1), and neither $\delta^{13}C$ nor $\delta^{15}N$ values varied seasonally (MANOVA $F_{1,87} = 2.9$, p = 0.596). The Bayesian ellipses were also similar in size (female $SEA_b = 3.7$; male $SEA_b = 3.2$) and the proportional overlap between male and female ellipses was also high (0.862), further suggesting a similar diet. However, $\delta^{15}N$ values varied annually within each season (spring: $F_{2,86} = 18.53$, $p < 0.001$; fall: $F_{2,86} = 14.19$, $p < 0.001$), but $\delta^{13}C$ did not (spring: $F_{2,86} = 1.72$, p = 0.153; fall: $F_{2,86} = 0.99$, p = 0.135). The areas of the Bayesian ellipses reflecting dietary niche breadths in fall ($SEA_b = 2.9$) and spring ($SEA_b =3.3$ 2.8) were similar (Figure 2.2), as the proportion of posterior draws where the ellipses were smaller in fall than spring was 0.514. The proportional overlap between spring and fall ellipses was 0.815, further suggesting a high degree of similarity between seasonal diets (Table S2.3). The areas of the ellipses were largest in 2022 (spring: $SEA_b = 2.5$; fall: $SEA_b = 2.7$), following a low crop in 2021, and the smallest in 2023 (spring: $SEA_b = 1.0$; fall: $SEA_b = 1.1$), following the mast year in 2022 (Figure 2.2, Figure 2.3). Similarly, the dietary niche breadth in 2023, following the mast year, was narrower than 2022 (reflecting a low crop in 2021) for both spring and fall (Table S2.3).

Squirrel diet consisted predominantly of fungi (Figure 4) (> 57% in spring and > 62% in fall), with a small conifer cone contribution (~30% in spring and fall) (Table S2.4 and Figure S2.2). However, consumption of different food groups varied annually (Figure 2.4). Filled seed production significantly affected the consumption of most alternate food sources (fungi: z = -

4.68, $p < 0.001$, pseudo $r^2 = 0.661$; berry: $z = -4.22$, $p < 0.001$, pseudo $r^2 = 0.442$). However, lichen consumption was not affected by changes in cone production ($z = -1.31$, $p = 0.054$, pseudo $r^2 = 0.118$).

Discussion

This study demonstrated that red squirrels, often considered seed specialists, can exhibit a diet predominantly composed of fungi rather than seeds, which to our knowledge has not been found elsewhere. The sporadic and low cone production in our study area, along with a low number of filled seeds within cones, is reflected in this predominantly fungal diet. In interior boreal forest, many studies have recorded extensive white spruce cone production in mast years over thousands of cones per tree (Krebs et al. 2012, McAdam et al. 2019, Leeper & LaMontagne 2021, Krebs et al. 2023, Wishart 2023). While cone production quadrupled in our mast year (2022), the total number of cones per tree rarely exceeded 600 cones per tree, with a maximum of 1288 cones. Given the low tree density and small tree size in our study area compared to interior areas of squirrel habitat (Young et al. 2002, Sharma & Parton 2007, Boonstra et al. 2008), food availability per hectare remains limited. Although we experienced two years of low crops in our 4-year study period, low cone crops succeeding a mast year are not uncommon due to the immense energy requirements of a mast year (Krebs et al. 2012). The single masting event we observed, as well as the 2018 mast year from our study area (Kucheravy et al. 2021), did not achieve crop yields to the same extent as other studies (Table 2). Unfavourable environmental and climatic conditions at distributional edges can affect tree growth, resulting low cone production, and a reduction or failure to produce viable seeds. In particular, the dynamics of tree populations at northern latitudinal limits can be linked to the limiting effect of low seasonal temperatures and short growing seasons on pollen and seed production and the subsequent germination and establishment of seeds and seedlings (Henttonen et al. 1986, Zasada 1988, Sirois 2000). In conifers, while long-term cone production is frequently linked to tree size, for species such as white spruce, climatic limitations in the past and current years rather than endogenous factors often dictate cone crop size (Messaoud et al. 2007, Krebs et al. 2012, LaMontagne 2020). Seasonal temperature changes in our study region typically exhibit a delay of approximately one month compared to boreal forest regions farther south of the northern treeline (Environment Canada data from Haines Junction Airport, Yukon, and Churchill Airport, Manitoba). Additionally, snow cover can persist for up to two months longer (Scott et al. 1993). The variable nature of the climate at the northern boreal forest treelines reduces the likelihood of having consecutively abundant years for spruce cones (Messaoud et al. 2007). Additionally, life history theory predicts individuals will prioritize limited energy budgets toward growth, survival, and reproduction to maximize fitness. In the harsh growing conditions presented at the northern treeline, trees may allocate energy toward growth instead of reproduction.

In addition to fluctuation in spruce cone production, the quality and quantity of seeds per cone can vary with cone size and year (Waldron 1965, Zasada 1988), potentially making cone numbers a poor predictor of food availability. Without the embryonic tissues, empty seeds have little nutritional value for granivores (Verdú & García-Fayos 1998). As the proportion of empty seeds increases within cones, the handling cost of finding highly nutritious seeds also increases (Verdú & García-Fayos 1998, 2001). In our study area, the percentage of filled seeds per cone fell from 8.5% (2021) to 1% in the mast year (2022). Such observations are lower than those observed in other white spruce forests. In northern Ontario, O'Connell (2005) reported that the percentage of filled seed per cone ranged from 26% to 42% in a non-mast year. Furthermore, in contrast to our observations, Waldron et al. (1965) noted an increase in filled seed in mast years compared to non-mast years, from 12% to 58% in southern Manitoba. Reduced pollination is commonly associated with empty seeds in conifers (O'Connell et al. 2006). In many spruce species, unpollinated ovules can develop into seed coats that contain degenerated gametophytes (Owens 1995). Factors such as small stand sizes, low tree density, and environmental stresses such as temperature, water availability, and nutrients, can restrict pollination, increase inbreeding, and raise seed abortion rates (Owens 1995, O'Connell et al. 2006, Benjamin et al. 2024). For plants residing at the northern limit of their distribution, shorter and cooler growing seasons can result in low seed quality and quantity (Zasada 1992, Lavoie & Payette 1994). Specifically, studies have suggested low seasonal temperatures during spring pollination can lead to seed abortion and cone damage (Zasada 1992, Owens 1995) and inhibit seed maturation (Sirois 2000). In white spruce, multi-year reproductive cycles increase susceptibility to such factors affecting seed production. In our study, while the number of filled seeds per cone was low, the total number of filled seeds per tree exceeded those in low cone crop years due to the abundance of cones. However, extracting filled seeds from many empty cones likely increases predator handling costs, influencing seed predator abundance and foraging efficiency (Perea et al. 2013). The low seed quality in our study area may further explain why squirrels in this area predominantly consumed fungi.

In our study area, fungi appeared from late August to early September with an annual variation typical for boreal forest fungal crops (Luoma et al. 2003, Krebs et al. 2008). Mean dry biomass was comparable with other studies in coniferous forests, including interior boreal forest (2.5 kg ha^{-1}) (Krebs et al. 2008) and Douglas-fir forests in the Pacific Northwest (2-5 kg ha^{-1}) (Luoma et al. 2003). Fungal reproduction can also experience widespread synchrony (Mehus

1986). Our estimates of spruce cone and fungal abundance could be biased by squirrels sampling and caching food prior to our surveys. Despite conducting surveys at consistent times annually, fluctuations in fungal emergence due to rainfall could mean peak abundance was missed.

At the northern treeline, red squirrels must constantly adjust to fluctuating seasonal temperatures and food availability. Optimal foraging theory states that species prioritize high-energy foods to maximize fitness (Stephens & Krebs 1986), exhibiting specialized diets when preferred resources are abundant and broader diets when resources are scarce. Our results support foraging theory, with a narrower dietary niche breadth following the mast year, although broader than expected if spruce seeds were the dominant food source, likely due to the low quality of seeds at the treeline. In non-mast years, dietary niche breadths generally expanded, with broadest niche observed during the low cone crop in 2021.

Fungi were the primary contributor to squirrel diet (~70%) across all seasons and years, although other food sources, including berries, were more prominent during the low cone crop year (2021 but reflected in the 2022 diet). Although not a major component, berries increased during low cone years. Like fungi, berries are typically abundant in late summer and early fall and can act as an important source of carbohydrates when spruce seeds are scarce (Stephens et al. 2019). Lichen was negligible and showed no variation with cone abundance, consistent with red squirrel's limited use of this resource (Currah et al. 2000, Dubay et al. 2008).

Red squirrels are also known nest predator of understory-nesting birds, such as passerines, in northern coniferous forests (Reitsma et al. 1990, Sieving & Willson 1998, Willson et al. 2003). However, passerines were excluded from our stable isotopes models because they arrive for the mating season in early June, after the spring moult has occurred. Snowshoe hares were also excluded as a potential food source since predation by red squirrels is rare and mainly involves young hares (< 2 weeks old) in spring and summer (O'Donoghue 1994). In more interior northern regions of red squirrel habitat, snowshoe hares typically have three litters (late May, late June - July after the spring molt, and early August before the fall moult) (O'Donoghue 1994, Oli et al. 2020). But delayed spring conditions in our study area shift reproduction into late August, overlapping with fall molt hair growth. However, during August and September, red squirrels are intensely focused on harvesting spruce cones and fungi, reducing their likelihood of consuming alternative food sources. Further, other studies reporting hare consumption by red squirrels have observed squirrels scavenging on hare carcasses in winter (Peers et al. 2020), which was not covered by the timeline of hair growth in our study.

We observed little seasonal variation in diet during our study period. In early summer, red squirrels will feed on spruce buds and new growth as their winter food caches become depleted, shifting to primary resources, such as spruce seeds and fungi, as they become more abundant in late summer and early fall (Ren et al. 2017). As spruce buds were isotopically similar to seeds, we were unable to differentiate between the two food items in spring diet. However, squirrels were observed eating spruce buds in early summer during our study. By late summer and fall, as primary resources become available, squirrels likely consume the same food items they are caching.

Throughout their distribution, red squirrels primarily consume conifer seeds and are considered conifer specialists (Smith 1968, Fletcher et al. 2010, McAdam et al. 2019, Wishart 2023). In northern interior areas of boreal forest, red squirrels rely exclusively on white spruce seeds (McAdam & Boutin 2003, Boutin et al. 2006) with individuals hoarding up to 20,000 cones for winter (Smith 1968, Hurly & Lourie 1997). While spruce cones may not be abundant at the treeline, our results suggest squirrels still prefer conifer seeds, with seed availability influencing the consumption of alternate foods like fungi and berries. Fungi are a known alternate food source for red squirrels, often comprising over half of squirrel's diet in low cone years (Steele 1998, Currah et al. 2000, Koprowski 2005, Teron & Hutchison 2013, Derbridge & Koprowski 2019, Pauli et al. 2019). Fungal hoarding by red squirrels fluctuated with cone production, peaking during the low cone crop year (2021) and declining in the mast year (2022). In the northern boreal forest, spruce cone hoarding can be highly variable, and red squirrels will switch from hoarding cones to fungi in years of low production, hanging mushrooms in trees to dry prior to caching (Currah et al. 2000, Krebs et al. 2008). Fletcher et al. (2010) noted that fungi comprised over 60% of observed caching events when spruce cone production was low, but only 1% of cached items during abundant cone years. The availability and minimal foraging effort needed make fungi a valuable secondary food source for squirrels, and squirrels play a key role in fungal spore dispersal (Krebs et al. 2008).

Conclusion

In summary, the production of white spruce cones varies greatly, and red squirrels have had to adjust their diet according to the availability of primary resources. Compared to similar studies conducted elsewhere in the northern boreal forest (LaMontagne et al. 2005, LaMontagne 2007, Lamontagne & Boutin 2007, Krebs et al. 2012, Wishart 2023), spruce cone production was generally lower at the sub-Arctic treeline. As a consequence, red squirrels living at the sub-Arctic edge of their range rely on fungi as their primary food source, even during mast years. While the caching and consumption of fungi by red squirrels is not a new phenomenon (Currah et al. 2000, Fletcher et al. 2010, Pauli et al. 2019), fungi have not previously been documented as the primary food source for red squirrels, which emphasizes how marginal treeline is for squirrels.

Climate change is altering vegetation patterns in the sub-Arctic and within the treeline, leading to increased tree density and recruitment (Payette et al. 2001, Mamet & Kershaw 2011, Mamet & Kershaw 2013). In recent decades, changes in masting dynamics have been largely attributed to climate change. In the northern boreal forest, the intervals between mast years have shortened from 5-7 to 2-4 years since 2006 (Krebs et al. 2023). As a result, the restrictions placed on squirrels by limited food availability may lessen in the future, increasing the success of future populations and the likelihood of range expansion. However, given the low proportion of filled seed within cones, it is unclear if these shorten intervals between mast years represent increased seed availability. Understanding how factors operate within range boundaries to restrict a species' success and how species conform to these limitations can provide insight into adaptations along latitudinal gradients (Gaston 2009) and species’ potential for future range expansion.

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Tables

Table 2.1: Comparison of white spruce cone counts and the percentage of seeds that were filled seeds within spruce cones near Churchill, MB, Canada, from interior forests within red squirrel distribution.

Location	Total cones/tree	Years	Reference
Mast year			
Treeline	74 – 863	2022	this study
Treeline	0 – 407	2018	Kucheravy et al. 2021
Yukon	1,000 – 2,500	1993 – 2010	Krebs et al. 2012
Yukon	750 – 3,000	2005 – 2022	Krebs et al. 2023
Yukon	1,000 – 3,500	2008 – 2022	Wishart 2023
Michigan	2,400 – 2,700	2012 – 2017	Leeper & LaMontagne 2021
Non-mast year			
Treeline	0 – 123	2020 - 2023	this study
Treeline	0 – 93	2019	Kucheravy et al. 2021
Yukon	0 – 200	1993 – 2010	Krebs et al. 2012
Michigan	216	2012 – 2017	Leeper & LaMontagne 2021
Wisconsin	6 – 186	2012 – 2014	Corona et al. 2022
	Filled seeds/cone (%)		
Mast year			
Treeline	1.2 ± 0.1	2022	this study
Southern Manitoba	58	1954 – 1963	Waldron 1965
Alaska	60.5	1957 – 1959	Zasada & Gregory 1969
Non-mast year			
Treeline	6.4 ± 1.4	2021 – 2023	this study
Southern Manitoba	12 – 48	1954 – 1963	Waldron 1965
Northern Quebec	66	2001 – 2004	Messaoud et al. 2007
Northern Ontario	26 – 42	1994	O'Connell 2006
Alaska	22 – 62	1954 – 1963	Zasada & Gregory 1969

Figures

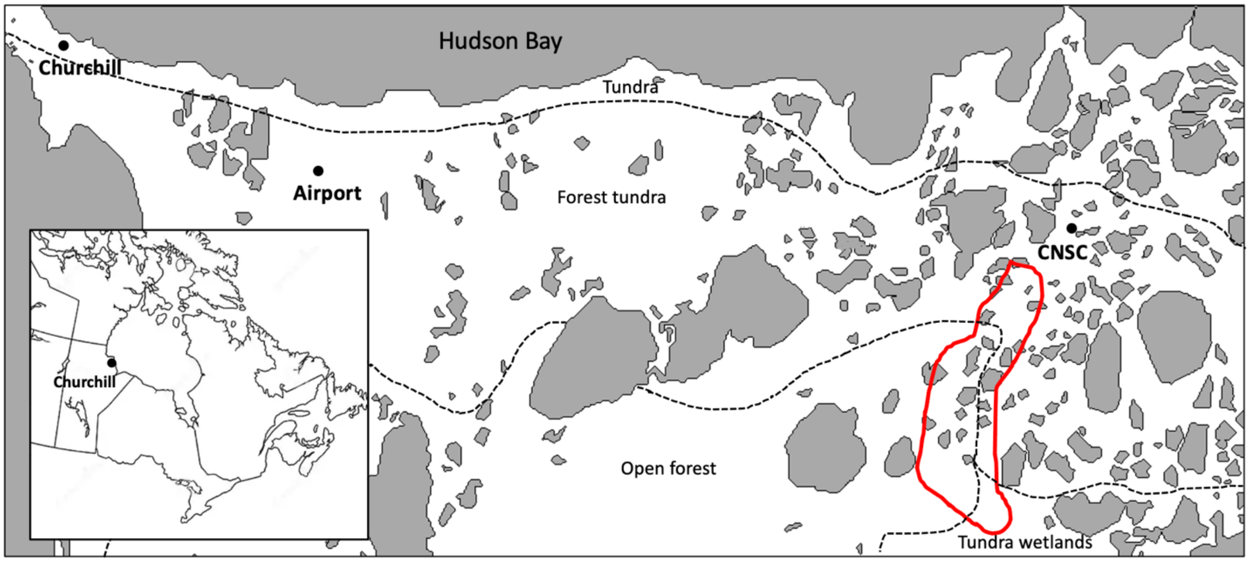

Figure 2.1: A map of the study area (outlined in red) located near Churchill, MB, Canada. Dashed lines delineate separate habitat types (Corkery et al. 2019).

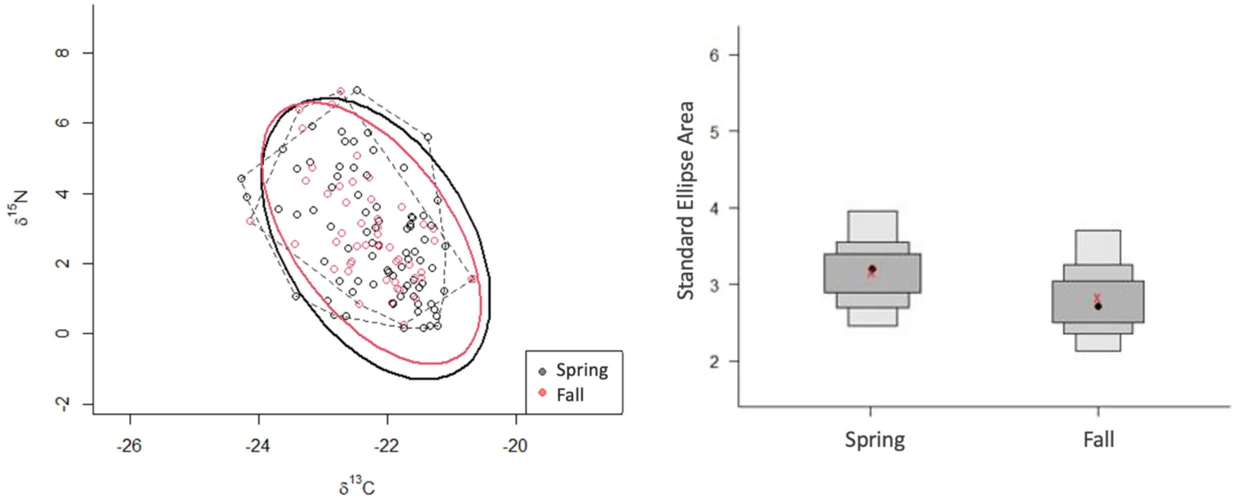

Figure 2.2: Comparison of seasonal dietary niches from 2020 – 2023 and the associated standard ellipse areas (SEA). Bayesian ellipses are generated using the SIBER package in R. The dotted lines indicate convex hulls and solid lines indicate standard ellipse areas. The black dots in the SEA represent the median, the red 'x' the mode, and the boxes indicate the 50, 75, and 95% credible intervals.

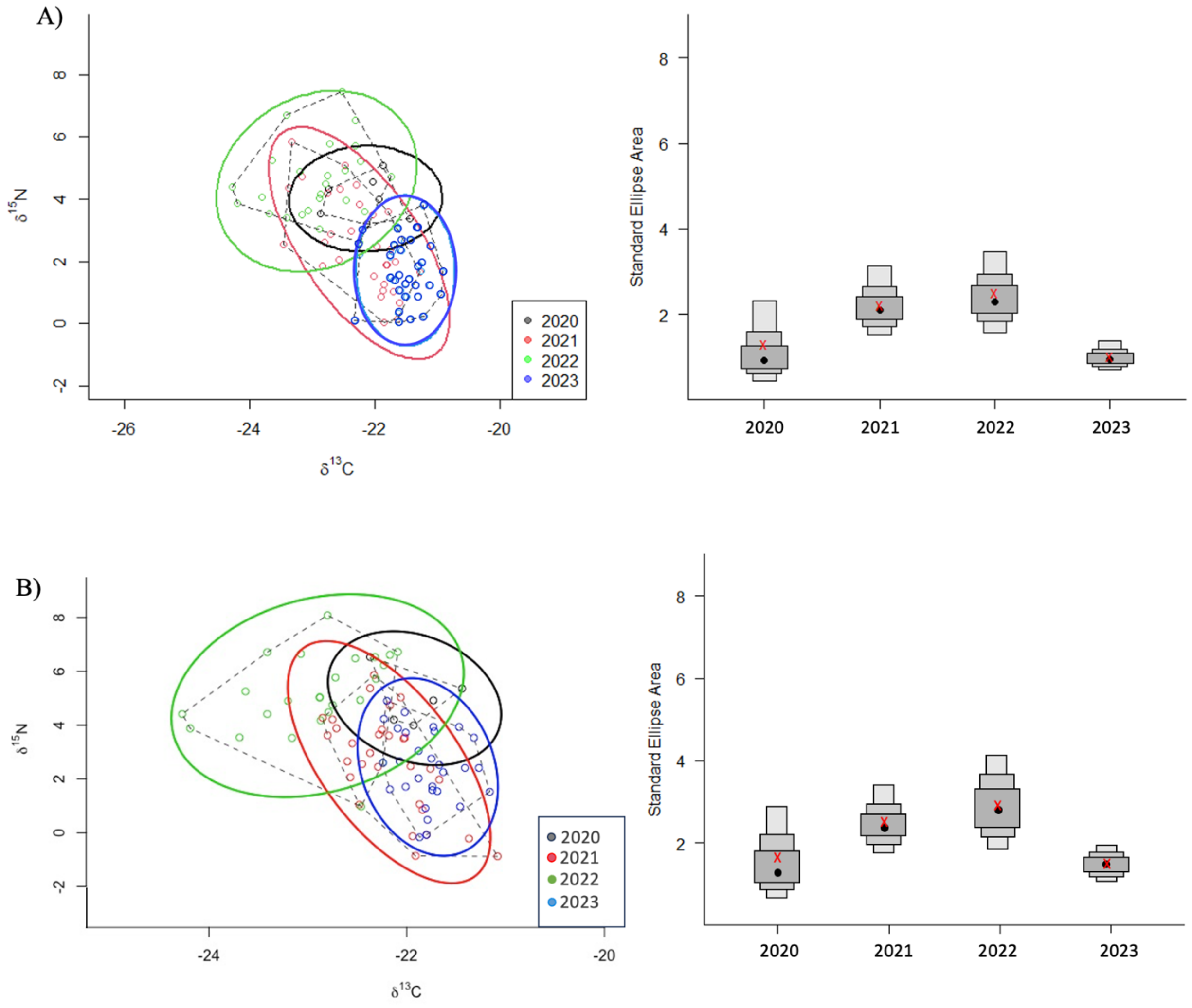

Figure 2.3: Comparison of A) spring and B) fall dietary niches from 2020 – 2023 and the associated standard ellipse areas (SEA). Bayesian ellipses were generated using the SIBER package in R. The dotted lines indicate convex hulls and solid lines indicate standard ellipse areas. The black dots in the SEA represent the median, the red ‘x’ the mode, and the boxes indicate the 50, 75, and 95% credible intervals.

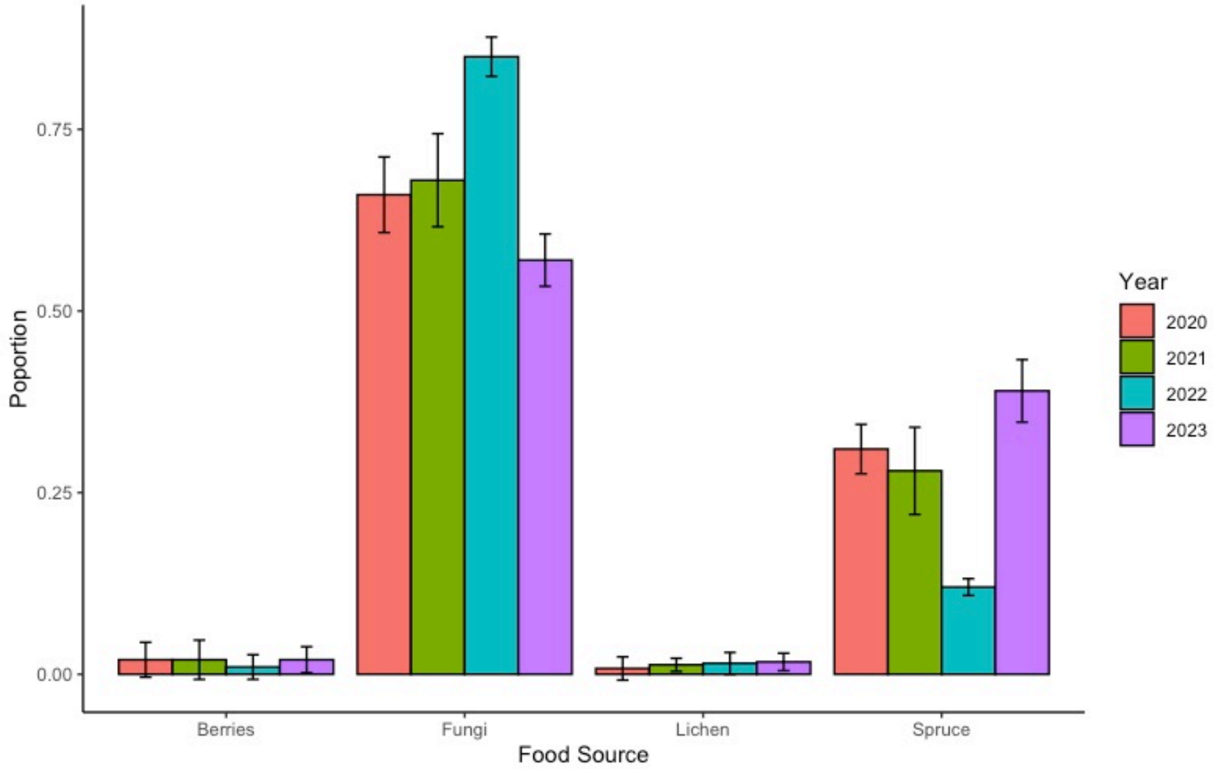

Figure 2.4: Comparison of the annual contributions of five food sources to spring squirrel diet from 2020 – 2023 (mean + SD). Proportions were estimated from Bayesian mixing models using the MixSIAR package in R. Note: 2022 diet (blue) reflects the low cone crops in 2021, and 2023 diet (purple) reflects the 2022 mast year.

Supplementary Tables

Table S2.1: Isotopic values, $\delta^{13}C$ and $\delta^{15}N$ (‰), of potential food items for red squirrels. Food samples were collected in August 2021, near Churchill, MB, Canada.

Group*	Species	$\delta^{13}C$	SE	$\delta^{15}N$	SE	n
Berries	*Geocaulon lividum*	-28.7	0.1	-5.4	0.5	16
Berries	*Ribes lacustre*	-28.1	0.3	-3.1	0.1	10
Berries	*Ribes triste*	-28.3	0.2	-3.0	1.3	19
Berries	*Vaccinium vitis idea*	-28.3	0.4	-6.5	0.2	15
Berries	*Empetrum nigrum*	-27.8	0.3	-5.0	0.4	14
Berries	*Vaccinium uliginosum*	-27.2	0.1	-3.5	0.3	12
Berries	*Shepherdia canadensis*	-28.1	0.4	-3.2	0.1	11
Conifer seeds	*Picea glauca*	-23.5	0.8	-3.2	0.5	9
Conifer seeds	*Picea mariana*	-23.5	0.3	-4.0	0.4	9
Conifer seeds	*Larix laricina*	-23.1	0.3	-3.7	0.8	6
Conifer buds	*Larix laricina*	-23.1	0.4	-3.5	0.8	15
Fungi (1)	*Russula spp.*	-26.4	0.1	4.0	0.1	16
Fungi (1)	*Cortinarius spp.*	-25.9	0.3	5.2	0.1	17
Fungi (1)	*Suillus spp.*	-26.9	0.1	5.2	0.1	17
Fungi (1)	*Lactarius spp.* (forest)	-25.9	0.2	6.4	1.1	9
Fungi (2)	*Mycena spp.*	-26.7	0.1	-2.2	0.1	9
Fungi (2)	*Lactarius spp.* (forest-wetland)	-25.4	0.1	-0.4	0.7	5
Lichen	*Usnea longissima*	-26.7	0.1	-8.8	0.2	12
Lichen	*Cladonia rangiferina*	-26.4	0.0	-8.0	0.2	10

*The two fungal groups were distinguished based on variations in $\delta^{15}N$ across genera and sample location.

Table S2.2: Model output summaries for annual variation in cones and seeds a near Churchill, MB, Canada, 2020-2023. Cone estimates are calculated from five trees per site (9 sites in 2020, 26 sites in 2021-2023). Fungal biomass was calculated from two 15 m radius plots per site in 2022 and four 1 m2 quadrats per site in 2023 (26 sites).

GLMM	Estimate	SE	z	p
Cones				
Intercept	6.15	0.29	64.96	
2020	-1.57	0.21	-71.63	< 0.001
2021	-4.64	0.77	-55.37	< 0.001
2023	-4.16	0.73	-56.83	< 0.001
LM	Estimate	SE	t	p
Filled seeds/cone				
Intercept	0.58	0.10	0.58	
2021	2.27	0.14	1.90	0.061
2023	4.35	0.42	3.08	0.013
Filled seeds/tree				
Intercept	158.43	38.62	4.10	
2021	-78.54	0.54	-1.44	0.003
2023	-117.02	0.56	-2.14	0.052

Table S2.3: Bayesian Standard Ellipse Area (SEAb) and 95% credible intervals (CI), the probability that the SEA of group 1 is smaller than that of group 2, and the proportion of seasonal and sex ellipses that overlap. Annual comparisons were made with the diet reflecting the mast year, as we predicted a smaller dietary niche breadth that year (2023 diet reflects the 2022 mast).

Model	SEA_b (95% CI)	Probability	Proportion overlap
Seasons	Spring = 3.3 (2.7 – 3.9)	Fall < Spring = 0.514	0.815
	Fall = 2.9 (2.3 – 3.6)		
Sex	Female = 3.7 (2.9 – 5.0)	Male < Female = 0.562	0.862
	Male = 3.2 (2.6 – 4.7)		
Annual spring	2020 = 1.3 (0.9 – 2.4)	2023 < 2020 = 0.613	2020:2021 = 0.312
	2021 = 2.3 (2.0 – 3.3)	2023 < 2021 = 0.708	2020:2022 = 0.165
	2022 = 2.5 (1.8 – 3.7)	2023 < 2022 = 0.826	2020:2023 = 0.238
	2023 = 1.0 (0.9 – 1.4)		2021:2022 = 0.394
			2021:2023 = 0.073
			2022:2023 = 0.048
Annual fall	2020 = 1.5 (0.8 – 2.8)	2023 < 2020 = 0.589	2020:2021 = 0.434
	2021 = 2.3 (1.9 – 3.2)	2023 < 2021 = 0.731	2020:2022 = 0.221
	2022 = 2.7 (2.0 – 4.0)	2023 < 2022 = 0.805	2020:2023 = 0.546
	2023 = 1.1 (1.0 – 2.0)		2021:2022 = 0.342
			2021:2023 = 0.269
			2023:2023 = 0.138

Table S2.1: Proportion contributions to overall diet (mean and 95% credible intervals) of four known diet items of red squirrels near Churchill, MB, Canada, 2020-2023 in a) spring and b) fall diet. Diet estimates are based on posterior probabilities from Bayesian stable isotope mixing models of δ13C and δ15N in hair samples.

a) Spring diet (95% CI)					
	Spruce seeds	Fungi (1)	Fungi (2)	Berries	Lichen
2020	0.31 (0.08-0.74)	0.54 (0.06-0.81)	0.13 (0.01-0.55)	0.02 (0.00-0.10)	0.00 (0.00-0.00)
2021	0.28(0.04-0.67)	0.31 (0.06-0.63)	0.37 (0.08-0.60)	0.02 (0.00-0.10)	0.00 (0.00-0.00)
2022	0.12 (0.04-0.60)	0.75 (0.34-0.76)	0.10 (0.00-0.41)	0.01 (0.00-0.10)	0.00 (0.00-0.00)
2023*	0.39 (0.10-0.74)	0.10 (0.02-0.52)	0.47 (0.26-0.54)	0.02 (0.00-0.08)	0.00 (0.00-0.00)
b) Fall diet (95% CI)					
2020	0.35 (0.09-0.70)	0.44 (0.03-0.60)	0.18 (0.04-0.28)	0.02 (0.00-0.04)	0.00 (0.00-0.00)
2021	0.32 (0.06-0.62)	0.54 (0.06-0.81)	0.11 (0.02-0.32)	0.02 (0.00-0.03)	0.00 (0.00-0.00)
2022*	0.17 (0.02-0.53)	0.63 (010-0.72)	0.16 (0.03-0.20)	0.03 (0.00-0.04)	0.00 (0.00-0.00)

* Reflects diet from the mast year (2022)

Supplementary Figures

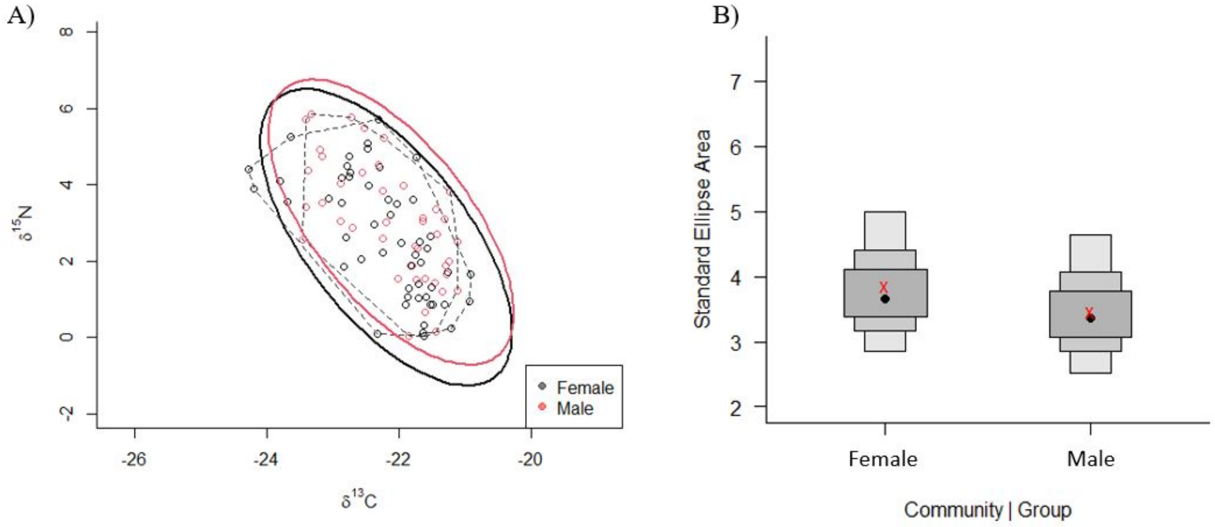

Figure S2.1: Comparison of A) dietary niche breadths of red squirrels for males and females, and B) standard ellipse areas (SEA) for males (1.1) and females (1.0). Ellipses were generated using the SIBER package in R. The dotted lines indicate convex hulls and solid lines indicate standard ellipse areas. The black dots represent the median, the red 'x' the mode, and the boxes indicate the 50, 75, and 95% credible intervals.

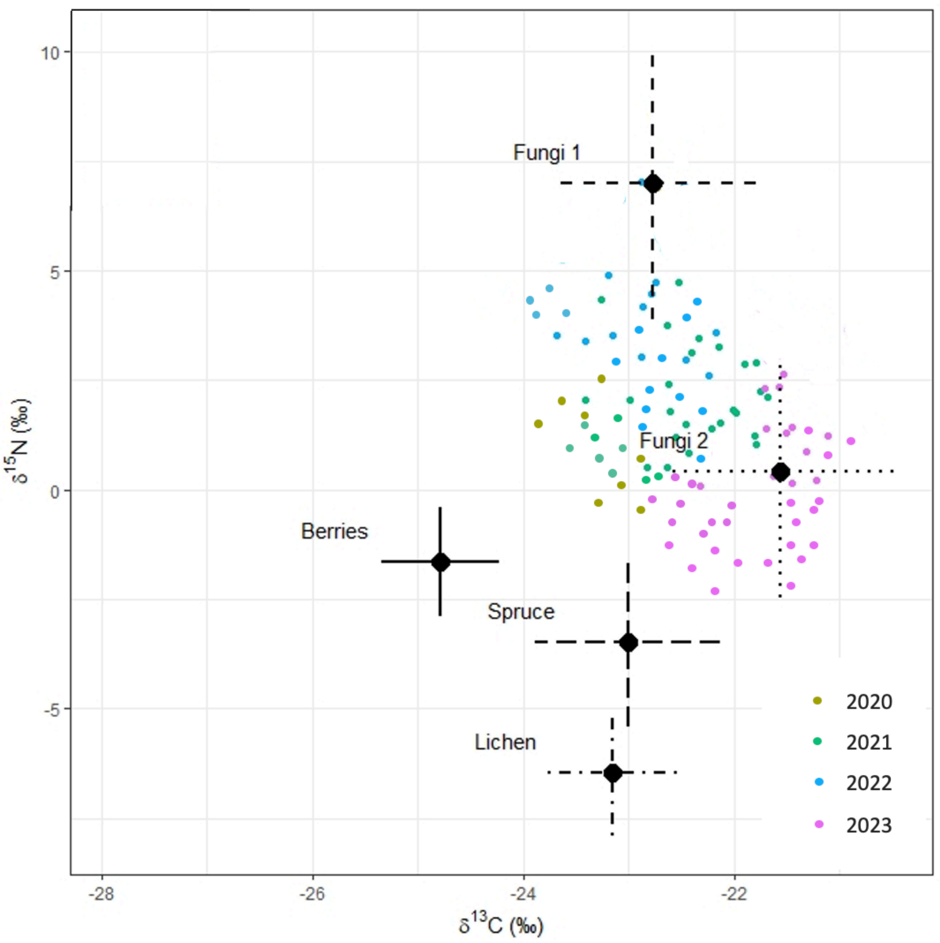

Figure S2.2: Isospace plot with isotopic signatures (δ13C and δ15N) reflecting annual spring diet for 104 squirrels trapped between 2020-2023. Food sources include berries (n = 97), conifer seeds and buds (n = 39), fungi (n = 73), and lichen (n = 22). Fungus samples were identified to genera and grouped into two fungal sources (group 1: Russula, Cortinarius, Suillus, and dry location Lactarius; group 2: Mycena and wetland location Lactarius) based on their isotopic signatures. All samples of potential food sources were collected from the field in August 2021. All source samples were corrected using TDFs of Δ13C = 3.3 ± 1.0‰ and Δ15N = 1.9 ± 1.0‰. Note: 2022 (blue) reflects the low cone crops in 2021, and 2023 (purple) reflects the 2022 mast year.

Chapter 3: Low-lipid fungal diet affects North American red squirrel survival and body condition in harsh winters in a resource-limited environment

Abstract

Resource availability shapes survival and body condition, particularly at the northern limits of a species' distribution, where winters can be harsh and high-quality foods are scarce. We examined overwinter survival and seasonal body mass changes in a population of red squirrels (*Tamiasciurus hudsonicus*) at the sub-Arctic treeline that primarily consumes fungi because of the low availability of high-quality seeds. Lipid content was much higher in spruce seeds (34.7%) compared to fungi (4.0 – 7.8%), although protein content was similar (22.2% in spruce seeds; 19.9 – 23.9% in fungi). From 2020 to 2023, squirrel body mass was consistently lower in spring than fall for both sexes and fluctuated with the previous fall's production of white spruce (*Picea glauca*) cones and seeds. Overwinter mass loss was also affected by seed abundance. Overwinter survival was low, ranging from 14% to 33%. Although squirrels in this population primarily consume fungi, survival rates were influenced mainly by abundance of filled seeds (seeds with an embryo), along with prewinter body mass and predation risk. In northern environments, organisms rely on a combination of behavioural and physiological modifications to overcome winter challenges and persist in suboptimal habitats. Our research underscores the critical role of spruce seeds for seed specialists, even when they are forced to depend on alternative food sources like fungi. This study emphasises the importance of understanding the interplay between environmental conditions, resource availability, and species plasticity and highlights the ongoing challenges faced by organisms inhabiting environments with low-quality resources at their distributional edge.

Introduction

Extreme winter temperatures constrain species' geographical distributions (Repasky 1991, Humphries et al. 2002, Rubidge et al. 2011) and strongly influence an organism's body condition and survival (Kautz et al. 2020). Winter conditions are particularly costly for endotherms, which must maintain a constant core body temperature (Pauls 1979). For species such as the Virginia opossum (*Didelphis virginiana*), winter temperatures limit their northern distribution by directly impacting mortality (Kanda 2005). Surviving overwinter in northern environments is demanding, combining the challenges of low minimum temperatures, deep snow, reduced food resources, and, often, increased predation (Hodges et al. 2006). Low temperatures can subject organisms to great physiological challenges, influencing behaviour and affecting overall fitness (Karl & Fischer 2008). Compared to large organisms, smaller organisms have a higher surface area-to-volume ratio and thus have a challenge retaining heat. Low seasonal food availability further exacerbates this dilemma, limiting winter foraging success and indirectly affecting overwinter success (Jackson et al. 2001).

Despite the constraints of low temperatures and resources, many species inhabit regions with severe winters. To mitigate the effects of winter, some animals hibernate, storing large amounts of energy as body fat, which allows them to survive prolonged periods with little or no food (Speakman & Rowland 1999, Bertile et al. 2021). Smaller organisms, however, are limited in the amount of fat that can be stored and the thickness of insulation that can be carried (Geiser 2011, 2013). Such species may instead reduce energetic requirements in winter using daily torpor, which lasts only a few hours and is often interrupted by activity and feeding. Still these species do not exhibit extensive fattening prior to winter (Ruf & Geiser 2015). Other species remain active throughout winter, although individuals typically reduce their daily activity patterns to reduce energy requirements. Such species may use behavioural strategies such as communal nesting (Koprowski 1996) or relocating to the subnivean zone (Korslund & Steen 2006), where snow provides thermal insulation by trapping heat released from the soil (Aitchison 2001).

For species that remain active in winter, individuals have a fixed amount of food available until the following growing season. Thus, limited food resources during winter can make overwinter survival heavily dependent on food availability (Rödel et al. 2004). Many species will cache extensive amounts of food prior to winter (Humphries et al. 2003, Busher et al. 2020, van der Veen et al. 2020) to mitigate low food availability. However, the amount of food available also restricts caching and can vary annually with changes in food abundance (Vander Wall 1990). The variation in seasonal climates at higher latitudes dictates large annual and seasonal fluctuations in resource availability (Humphries et al. 2005). Within northern range boundaries at high latitudes, extreme winter climates can reduce the accessibility of food sources, further limiting food availability. In these environments, overwinter declines in small mammal populations are common (Haapakoski et al. 2012).

The boreal forest is one of the world's largest terrestrial biomes, encompassing over 30% of Earth's forests (Gauthier et al. 2015, Thiffault 2019), and is characterised by long, cold winters and short, cool summers. Snow cover persists at least five months of the year in the southern region of the boreal forest, increasing up to seven to eight months farther north (Shugart et al. 1992). Cold temperatures, a short growing season, acidic soils, and an extensive permafrost layer create difficult growing conditions for vegetation (Deluca & Boisvenue 2012, Safford & Vallejo 2019), resulting in a low diversity of trees, dominated largely by conifers (Saucier et al.

2015). Conifer species commonly experience brief periods of excessive seed production, followed by long intervals of low or absent seed production (Kelly & Sork 2002, Archibald et al. 2012), a pattern known as masting that is often synchronous across large geographic scales (Lamontagne & Boutin 2007, Krebs et al. 2012). The abundance of seeds during mast years satiates seed predators, ensuring more seeds escape predation and germinate (Kelly & Sork 2002). White spruce (*Picea glauca*) is a masting conifer species commonly found in the boreal forest and experiences large fluctuations in annual cone crops (Archibald et al. 2012, Krebs et al. 2012, Krebs et al. 2023), with masting intervals typically occurring every 3-5 years (Lamontagne & Boutin 2007). The variable nature of the northern climate in the boreal forest reduces the likelihood of having consecutively abundant years for cones (Messaoud et al. 2007).

Spruce seeds are an essential source of carbohydrates, lipids, and proteins for many birds and small mammals (Humphries et al. 2003, Lobo & Millar 2013) and are easily digested, yielding a greater energy return per item than other food sources (Gordon 1992a, Angell 1993, Frank 2009). Seeds are also adapted to persisting in a state of dormancy for long periods, making them ideal for hoarding (Gordon 1992b). Red squirrels (*Tamiasciurus hudsonicus*) are specialised seed predators found throughout the boreal forest. In much of the northern parts of their distribution, red squirrels feed almost exclusively on white spruce seeds (Boutin & Larsen 1993, LaMontagne et al. 2013), and seed abundance can affect squirrel behaviour, habitat selection, and reproduction patterns (Becker et al. 1998, Réale et al. 2003, Steury & Murray 2003, Boutin et al. 2006, Fletcher et al. 2010, LaMontagne et al. 2013). Red squirrels harvest mature cones from late summer to fall, storing them in central food caches or middens (Steele 1998, Archibald et al. 2013). Squirrels depend heavily on cached cones for overwinter survival and spring reproduction (Larsen & Boutin 1994, Boutin et al. 2006, Larivée et al. 2010, LaMontagne et al. 2013). The number and size of middens within a squirrel's territory can vary depending on food availability and habitat (Gurnell 1984). Squirrels must obtain territories before or in synchrony with cone maturation to maximise their cache. Individuals who acquire territories late will have less time to secure resources before winter (Fisher et al. 2019).

Red squirrels do not hibernate or go into daily torpor (Brigham & Geiser 2012), making them the smallest arboreal mammal that exhibits activity throughout winter above the snow (Pruitt & Lucier 1958), which exposes individuals to frigid temperatures. Red squirrels have very little insulative fat (Johannesdottir 2017) and usually rely on hoarded cones as their primary food source over winter (Steele 1998). For a small mammal, red squirrels have low field metabolic rates in winter, a possible adaptation to low resource availability that minimises energy expenditure (Humphries et al. 2005). Red squirrels also reduce energy costs in winter by spending most of their time in well-insulated nests and limiting outside activities to the warmest time of day (Pruitt & Lucier 1958, Pauls 1979). In the sub-Arctic, red squirrels may overwinter in subterranean and subnivean habitats, using extensive burrow systems (Pruitt & Lucier 1958). By adopting an energy-conservative lifestyle, individuals prolong the life of winter food caches and reduce exposure to winter temperatures and predators (Humphries et al. 2005). Despite such behavioural strategies, Williams et al (2014) suggested food availability is more crucial to overwinter squirrel success and survival, superseding the effect of climate.

In interior areas of the boreal forest, spruce trees produce thousands of cones in mast years (Archibald 2011, Krebs et al. 2012, McAdam et al. 2019, Leeper & LaMontagne 2021, Krebs et al. 2023, Wishart 2023), a magnitude that is often able to satiate squirrel hoarding activity (Gerhardt 2005, Fletcher et al. 2010, Williams et al. 2014). In contrast, mast years at the sub-Arctic treeline do not achieve such high crop yields (Kucheravy et al. 2021), with a mean of only 471 cones per tree produced in a recent mast year (Windsor et al. 2025). Additionally, the quality and quantity of seeds per cone is limited at treeline, with >90% of the seeds unfilled (Benjamin et al. 2024, Windsor et al. 2025), or lacking a gametophyte, which provides little nutritional value to consumers (Verdú & García-Fayos 1998). In response to reduced spruce seed production, red squirrels at the treeline have adopted a predominantly fungal diet (Windsor et al. 2025). However, as a seed specialist, this dietary shift could potentially impact various life history traits, as these resources may differ in nutritional value and, therefore, may not be perfectly substitutable.

We examined the impacts of food availability on overwinter survival and spring body mass of red squirrels at their sub-Arctic range boundary. We hypothesised that low resource availability at the treeline would affect overwinter mortality and body mass. Although squirrels at the treeline consume primarily fungi, which are a good source of protein, their energy return may be lower than spruce seeds. We thus predicted that survival and spring body mass would fluctuate with annual food availability. Additionally, we predicted that predation risk would further contribute to overwinter mortality, as squirrels are one of the few prey species at the treeline that are active above the snow in winter.

Methods

Study area

Our study area near Churchill, MB, Canada (58°45’ N, 94°04’ W) lies on the transition between boreal forest and Arctic tundra. The forest is heavily fragmented and primarily composed of white spruce, black spruce (*Picea mariana*), and tamarack (*Larix laricina*) (Mamet & Kershaw 2013), with a low density of mature trees (approximately 600 stems/ha) and a sparse canopy (estimated at 25%) (Lafleur 1999). Cone production in our study area is highly variable (Kucheravy et al. 2021, Windsor et al. 2025). During our study period, cone abundance peaked at 185,849 cones/ha in 2022, a mast year, and was lowest in 2023, with only 3,029 cones/ha. Fungi are a common alternative food source for red squirrels in years of low cone production, and fungus production averaged 2.5-3.5 kg/ha (dry biomass) from 2022-2023 (Windsor et al. 2025). Potential predators of red squirrels in this area include red fox (*Vulpes vulpes*), American marten (*Martes americana*), short-tailed weasel (*Mustela erminea*), and rough-legged hawks (*Circus hudsonius*). Most of our fieldwork was conducted along a 7 km stretch of boreal woodlands running north to south, with sampling sites every 250 m (Windsor et al. 2025).

Climate

We obtained daily temperature records from Environment Canada's online historical weather database for the Churchill Airport weather station (Climate ID: 5060595), approximately 20 km west of our study area, to assess annual variation in winter temperatures, which can impact body condition due to increased thermoregulatory demands in cold temperatures. For this study, we considered winter to be months with persistent snow cover (Nov-Apr). We used the daily temperature records (recorded hourly) to calculate the mean daily and monthly winter temperatures each year. We also examined the number of extreme cold days (< -30°C) each year.

Food availability and nutritional content

Food availability for red squirrels is often reflected by the number of spruce cones produced. However, the number of filled seeds in spruce cones is markedly lower at the treeline than in other white spruce forests and may also vary between years, independent of cone abundance (Windsor et al. 2025). To examine the impact of food availability on body mass and survival, we used food estimates from the previous August, as squirrels rely on food harvested in fall as a winter food source. Cone abundance was estimated at the site where each squirrel was trapped, and filled seed abundance was estimated from nearby sites (within 500 m). Both were converted to units per hectare based on tree density estimated at the site where each squirrel was captured (Windsor et al. 2025). We also estimated fungus abundance at each sampling site in August 2022 and 2023 (Windsor et al. 2025) as fungi are a common supplemental food source for squirrels in years of low cone production (Currah et al. 2000, Fletcher et al. 2010).

To compare the nutritional composition of the two main food sources for red squirrels in our study area, we analysed the crude lipid and protein content of white spruce seeds and fungi. We collected five spruce cones at four different sites, chosen randomly, from 2-3 trees per site separated >5 m from each other (20 cones total). Fungal samples were collected for three genera commonly consumed by squirrels (*Russula, Lactarius*, and *Cortinarius*) (Currah et al. 2000), with one to two samples collected per genus from four separate sites, selected randomly.

Prior to nutrient analyses, we dried all samples in a drying oven for at least 48 hours before homogenising using a mortar and pestle. To estimate crude lipid content, we weighed 3-5 g of half of the seed (n = 10) and fungal samples (n = 3 per genus) and placed samples in a Soxhlet apparatus for 12 hours to extract lipids using petroleum ether as a solvent. After removing the samples and evaporating the solvent in a drying oven, we calculated the mass difference between samples before and after lipid removal and expressed this difference as a percentage of the original dry mass to estimate crude lipid content. We then estimated the crude protein content of the remaining samples (seeds: n = 10; fungi *Russula*: n = 3, *Lactarius*: n = 4, *Cortinarius*: n = 4) using the Bradford assay method (Bradford 1976). The Bradford reagent binds to proteins within a sample, causing a colour change that can be read on a spectrophotometer. We prepared an initial 25 g of each sample and created a standard curve solution using protein standard, sodium hydroxide (NaOH) and Bradford reagent. Each test tube of standard curve solution contained varying ratios of protein standard and NaOH for a total of 100 µL per tube. We read the absorbances of each sample and the standard curve (595 nm). We plotted the absorbances of each protein sample against the standard curve in R, fitted a line of best fit and used the slope and intercept values to determine the quantity of protein in each assay (protein = intercept + slope * absorbance).

Predation risk

We installed Browning trail cameras (model: Strike Force Extreme and Strike Force HD Pro X) at 17 active squirrel sites (one camera per site) across our study area (7 installed in August 2020, three more in June 2021, and the remainder in June 2022). We only chose sites with previously observed middens and set up each camera facing the midden. At sites with multiple middens, we placed the camera facing the largest midden (based on surface area). At three sites, when squirrel activity changed, we relocated the trail camera to the next largest midden or feeding site in June. The cameras were installed approximately 1 to 1.5-m above the ground on a tree or reinforcing bar. Cameras were programmed to take a burst of three photos each time they were activated within a 3-m detection range, with a 0.5-second recovery period between bursts. We checked the cameras each April, June, and August from August 2020 to August 2023, and additionally, in January 2021 and 2022, to exchange memory cards and replace batteries, if necessary. Observations of known squirrel predators were recorded, and we calculated the total number of predator observations per camera and the number of trap days each camera was operational from August to May to estimate predation risk overwinter. To account for cameras being unavailable due to deep snow or dead batteries, we used the total number of predator observations and trap days operable to calculate an observation frequency per 100 trap days. Predator observations were considered independent if 1) they were at least 30 minutes apart, 2) consecutive photos of the same species could be identified as different individuals (e.g., based on pelage), or 3) photos of the same species were separated by photos of other species (Montalvo et al. 2019, Montalvo et al. 2023).

Body mass and survival

We systematically captured squirrels in June 2021-2023 and in late August 2020, 2022, and 2023 using Tomahawk live traps (model #202). We trapped at each site for five consecutive days and checked traps daily at 4-hour intervals from approximately 6:00 until 21:00. We weighed squirrels at each capture to the nearest 1 g with a 500 g Pesola spring scale and recorded their body measurements. We visually inspected each squirrel to verify sex and assess general body condition. We marked individuals with a unique metal ear tag threaded with coloured pipe cleaners for visual identification and injected them with a subcutaneous Passive Integrated Transponder (PIT) tag for permanent identification. We expected females to give birth late June to mid-July based on observations of mate searching and copulating. Juvenile red squirrels in the northern boreal forest typically emerge from their nests approximately 45 days after birth, remaining with their mothers for an additional 25 days before dispersing, at which time they weigh < 160 g (Berteaux & Boutin 2000). Since our June trapping period coincided with the breeding season, we assumed all squirrels trapped in June were adult; any offspring from the previous year would have reached adult size and mass by this time. In August, all captured squirrels weighed over 160 g. Additionally, we did not trap multiple squirrels at the same site in August, suggesting these individuals were not sharing a territory, a characteristic more common among juveniles. Consequently, we presumed that all August-trapped squirrels were also adults.

Red squirrels are highly territorial and rarely move to new territories (Larsen & Boutin 1994). Therefore, if new individuals were trapped in a territory belonging to a different squirrel the previous year, and the original individual was not recaptured the following year, that individual was considered deceased. We calculated overwinter survival as the proportion of squirrels caught in August each year that were recaptured the following June. In June, male squirrels were frequently trapped at multiple sites, likely due to their movement between territories in search of mates, while females were consistently trapped at the same site. In August, male and female squirrels were consistently trapped at just one site, presumably in their home territory. We did not estimate June-to-August survival within the same year, as failing to capture a male in August may reflect territory-based movements rather than mortality. We also estimated annual survival as the proportion of squirrels caught in June that were recaptured the following June.

Statistical analysis

All statistical analyses were conducted in R (version 4.0.5). We used Analyses of Variance (ANOVAs) and post-hoc Tukey pairwise comparisons to assess differences in crude lipid and protein content between white spruce and fungal genera, as well as annual variation in winter temperatures and predator observations.

We used linear mixed models (LMM) to examine squirrel mass change over summer (June to August) and overwinter (from August to the following June), with sex and year as predictors and 'squirrel ID' as a random effect. We also used LMMs to analyse the effect of fall food availability on spring mass using the number of cones and filled seeds per hectare and the abundance of fungi (kg per hectare) as food indices, with sex as an additional predictor. All models included 'squirrel ID' (104 levels) and 'site ID' (26 levels; no squirrels were captured at

3 of the 29 sites) as random effects. We selected the best model using Akaike's information criterion corrected for small sample size (AICc).

To test the effect of food availability on overwinter survival, we used Generalised Linear Mixed Models (GLMMs) with a 'binomial' family and a logit link function. Additional explanatory variables included August body mass, sex, and predation risk, with 'site ID' (17 levels) as a random effect, and we again selected the best model using AICc.

Similarly, we used GLMMs to test the effect of food availability on annual survival, with sex and predation risk as additional explanatory variables. Since this dataset encompassed more years than our overwinter survival models (2021-2023), we further included the number of days < -30°C as an explanatory variable. In June, squirrels still rely on the remnants of cached food from the previous fall, as new food sources such as cones and fungi are not yet available. As a result, June body mass does not accurately reflect the prewinter body mass of individuals. Therefore, mass was not included in these models. We selected the best-fitting model using AICc.

To ensure a valid comparison of model performance for all spring body mass and overwinter survival models, we restricted these analyses to the two years (2022-2023) for which data on both cone and filled seed production were available. All mixed models were analysed using the packages 'lme4' and 'lmer'. The continuous predictor variables were standardised and all models were checked for multicollinearity by calculating the variance inflation factors (VIF).

Results

Climate

Mean monthly ambient winter temperatures (November to April) ranged from -28°C to -15°C, with an average of 16.4 ± 9.5 (SE) days per winter where the mean daily temperature was < -30°C. Mean daily temperatures varied monthly (2-way ANOVA $F_{11,36} = 57.2$, $p < 0.001$), but neither year ($F_{2,36} = 0.4$, $p = 0.662$) nor the interaction between month and year ($F_{22,36} = 0.3$, $p = 0.950$) were significant. The number of days < -30°C was highest in 2022-2023 (n = 38) and lowest in 2020-2021 (n = 9) (Table 3.1).

Nutrients

Crude protein content did not differ between white spruce seeds (range 19.7-25.2%) and fungi (range 18.3-27.4%; $F_{4,35} = 1.2$, $p = 0.33$; Table 3.2). In contrast, lipid content was much higher in spruce seeds (range 32.7-37.6%) than in fungi (range 3.7-8.3%; $F_{4,35} = 3211$, $p < 0.001$; Table 3.2).

Predation risk

Our trail cameras recorded 223 independent observations of four predator species (87 red fox, 75 American marten, 59 short-tailed weasel, and two rough-legged hawks) in 11,733 trap days (Table 3.1). Three trail cameras were near active red fox natal dens, so red fox kits were excluded. Mean monthly predator observations varied annually ($F_{3,15} = 6.7$, $p < 0.001$), with overall annual predator frequency highest in 2022-2023 and lowest in 2020-2021 (Table 3.1).

Body mass

Squirrel body mass was higher in August than in June (Table 3.1). Spring body mass was greatest for both sexes in 2023 following the mast year, and lowest in 2022 following the 2021 low cone crop year (Table 3.1). Mass increased over the summer for squirrels captured in June and August of the same year (2022 and 2023) ($t_{18} = 8.8$, $p < 0.001$) and the increase was similar each year ($t_{16} = 0.2$, $p = 0.543$). Females gained 12.5 ± 2.5g and males 23.6 ± 3.1g, although the sex difference was not significant ($t_{16} = 2.6$, $p = 0.068$). Individuals initially trapped in August and again the following June experienced a significant mass loss overwinter ($t_{13} = -4.6$, $p < 0.001$), which did not vary by year ($t_4 = 0.6$, $p = 0.600$). Females lost 23.3 ± 3.3g and males lost 45.0 ± 2.9g overwinter, although this difference was not statistically significant ($t_4 = 0.9$, $p = 0.321$).

The model with the best support for explaining spring body mass included filled seed abundance from the previous fall and sex (Figure 3.1 and Table S3.1). The model explained 70% of the variation in spring body mass (conditional $r^2 = 0.70$, marginal $r^2 = 0.62$) and was significantly affected by filled seed abundance and sex (Table 3.3).

Survival

Overwinter survival was estimated for two years and was lowest in 2022 – 2023 (14.2%) and highest in 2020 – 2021 (33.3%). The best model for overwinter survival included filled seed abundance and pre-winter body mass (Table 3.4 and Table S3.2). Our model was only based on one year of survival data, as data on the number of filled seeds was only available for 2021-2023, and our overwinter survival data was only available for 2020-2021 and 2022-2023. There was a significant effect of filled seed abundance and mass (Figure 3.2 and Table 3.4). The next best model had a ΔAICc <2 and included filled seed abundance, pre-winter body mass and predation risk as explanatory variables (Table S3.2).

Our annual survival models were based on data from 2021-2023. The best-fitting model for annual survival included filled seed abundance, predation risk, and the number of days < -30°C (Table S3.3). There was a significant effect of filled seed abundance and predation risk and a marginal effect of the number of days < -30°C (Table S3.4). The next best model had a ΔAICc <2 and included filled seed abundance and predation risk as explanatory variables (Table S3.3).

Discussion

Organisms living in highly seasonal environments face many challenges in winter as reduced food availability is paralleled by low ambient temperatures, increasing thermoregulatory costs. At the sub-Arctic treeline, cone production, red squirrel body mass, and overwinter survival fluctuated annually. Overwinter survival during our study period (14-33%) was lower than previously published overwinter survival rates in Alberta (33-61%) (Kemp & Keith 1970), British Columbia (20-30%) (Haughland & Larsen 2004), and the Yukon (67-71%) (Larsen & Boutin 1994). Mean spring body mass (males 182-207g, females 194-217g) was also relatively low compared to other red squirrel populations in Alberta (males 239g, females 219g; Patterson et al. 2015), British Columbia (235g; Ransome & Sullivan 1997), Yukon (257g; Descamps et al. 2008), and Arizona (220-230g; Koprowski 2005). Spring body mass was highest in 2023 following the mast year and lowest in 2022 following the low crop year and declined overwinter for both sexes. In contrast, other studies have found that female red squirrels maintain a consistent winter body mass while males peak over winter after initiating testicular activity (Layne 1954, Koprowski 2005). Under such circumstances, males likely increase their fat stores in anticipation of breeding (Arbetan 1992), whereas food availability in our study area was likely insufficient to support overwinter breeding preparation.

Influence of seed and cone abundance on survival and body mass

For red squirrels, overwinter survival is often affected by the number of cones stored in their cache (LaMontagne et al. 2013). Territory size can further reflect the quantity of food sources available to a squirrel, and higher mortality rates are often observed in individuals with smaller territories (Steury & Murray 2003). Food supplementation experiments with red squirrels have shown improved winter survival and reproduction with access to additional food sources

(Boutin 1990). However, fungi, the main alternative food source available late in the season, are approximately 88% water (estimated from wet and dry biomass calculations), and therefore may provide little nutritional value, limiting squirrels' ability to compensate for reduced seed availability. Our top survival model showed a significant effect of filled seed abundance (Figure 3.2) and overwinter survival was lowest following the mast year, likely due to the drastically reduced number of filled seeds produced that year (Windsor et al. 2025). Although overall cone production was higher during the mast year, resulting in more filled seeds per hectare, this benefit would only apply to squirrels occupying areas with high tree density. In contrast, in low tree-density areas where cone abundance per hectare remains low even in mast years, the sharp decline in filled seeds per cone would directly reduce food availability. Consistent with this, filled seed production was six times higher in 2023 than in the mast year 2022, and overwinter survival in 2020-2021 was double that of 2022-2023.

Throughout their distribution, squirrels primarily consume conifer seeds (Smith 1968, Fletcher et al. 2010, McAdam et al. 2019, Wishart 2023), and at higher latitudes, individuals feed exclusively on white spruce seeds (McAdam & Boutin 2003, Boutin et al. 2006). However, at the treeline, in addition to low spruce cone production, the quality and quantity of seeds per cone is limited, with the mean percentage of filled seeds per cone ranging from 1-9% (Windsor et al. 2025). Unfilled seeds are empty husks lacking a gametophyte, which can account for over 80% of a filled seed's total lipid content (Bornman et al. 2003), and have little nutritional value for granivores (Verdú & García-Fayos 1998). Other white spruce forests typically produce higher proportions of filled seeds. O'Connell (2005) reported that the percentage of filled seed per cone ranged from 26% to 42% in a non-mast year in northern Ontario. Furthermore, in contrast to our observations, Waldron et al. (1965) noted an increase in filled seed in mast years compared to non-mast years, from 12% to 58% in southern Manitoba. In our study area, the low production of filled seeds per cone likely amplified the effects of low cone production on food availability at the treeline, particularly in the year following the mast year.

However, squirrel diet is highly plastic, and as opportunistic consumers, squirrels can consume a wide variety of alternative foods (Bayne & Hobson 2002, Willson et al. 2003, Teron & Hutchison 2013, Pauli et al. 2019). After conifer seeds, fungi are an important secondary food source commonly consumed in years of low cone abundance (Currah et al. 2000, Boonstra et al. 2008, Maser et al. 2008, Fletcher et al. 2010). In our study area, fungi were the largest contributor to squirrel diet (~ 70%) from 2020-2023, whereas spruce seeds contributed approximately 25% of squirrel diet (Windsor et al. 2025). Fungus dry biomass ranged from 2.5 to 3.5 kg/ha in 2022 and 2023 in our study area (Windsor et al. 2025). However, our results suggest that consumption of a substantial amount of fungi at the treeline likely reflects a reliance on a lower-quality food source when preferred resources like spruce seeds are scarce. The strong influence of seed availability on both survival and body mass highlights the critical role of conifer seeds in supporting overwinter condition, a pattern also reflected in our model comparisons, where seed-based models consistently outperformed fungal abundance models. Although consumption of alternate food sources, like fungi, may enable some squirrels to survive in years of low cone abundance, these food items may not provide sufficient nutrient quality to maintain body condition, particularly in harsh winters.

Lower squirrel survival following the mast year may result from winter caches full of barren cones and few alternate food sources. However, despite the low number of filled seeds, some squirrels may improve survival probability by hoarding pilfered cones, expanding their territories, or consuming alternate foods. Trail camera data from our study area showed fungus hoarding activity fluctuated with cone production, peaking during the low cone crop (2021) and was lowest during the mast year (Windsor et al. 2025). Compared to other years, the higher body masses of squirrels trapped the spring following the mast year suggests some squirrels had access to an adequate supply of spruce seeds. Previous studies have shown a positive association between red squirrel territory size and resource abundance (Smith 1968, Steury & Murray 2003, LaMontagne et al. 2013) and a significant effect of territory size on overwinter survival (Steury & Murray 2003). Similarly, Eurasian red squirrels (*Sciurus vulgaris*) will increase territory sizes in habitats with fewer food resources (Lurz et al. 2000). While we did not examine territory size, surviving squirrels may have owned larger territories and thereby more resources than other squirrels in the study area. Squirrels are also known pilferers (Gerhardt 2005, Donald & Boutin 2011) and may increase their probability of survival by stealing from the middens of neighbouring squirrels.

Reduced food availability prior to winter can also influence survival, as reflected in pre-winter body condition, and we found prewinter mass was an important influence on overwinter survival. For many species, overwinter survival has been linked to larger body mass (Murie & Boag 1984, Michener 1989, Loison et al. 1999, Côté & Festa-Bianchet 2001, Rödel et al. 2004, Korslund & Steen 2006, Schorr et al. 2009). Body mass plays a crucial role in the overwinter survival of small mammals, providing insulation and energy reserves. In European red squirrels (*Sciurus vulgaris*), heavier individuals have a higher survival rate (Wauters et al. 2007, Santicchia et al. 2018). Body mass is particularly important for juveniles, who typically have lower fat reserves than adults, and there is often a critical mass that must be reached to survive winter (Larivée et al. 2010, Hendrix et al. 2020). Although red squirrels typically give birth late March to early May (Humphries & Boutin 2000), based on field observations, we expect females to breed later in this region. Considering the body measurements and mass recorded in the field, we considered all squirrels trapped in June to be adults.

For many animals, body mass commonly declines in winter when food availability is reduced. While this pattern is especially common in hibernating species, which must rely on fat reserves for energy in addition to cached food stores (Michener 1983, Murie & Boag 1984, Michener & Locklear 1990, Korslund & Steen 2006, Schorr et al. 2009), the trend is also observed in non-hibernating species such as voles and flying squirrels (Rödel et al. 2004). Throughout their distribution, red squirrels often rely on fungi in years of low cone abundance. However, these alternative food items provide lower nutritional content and are composed of complex carbohydrates that are largely indigestible for many mammals, resulting in low availability of nutrients to consumers (Cork & Kenagy 1989, Luoma et al. 2003). During a food supplementation experiment, Luoma et al. (2003) noted squirrels could maintain body mass on a diet of either pine seeds or rodent chow but lost body mass when diet was replaced with truffles (Luoma et al. 2003). Similar results were also observed in studies on golden-mantled ground squirrels (*Callospermophilus lateralis*) (Cork & Kenagy 1989), western red-backed voles (*Myodes californicus*), and northern flying squirrels (*Glaucomys sabrinus*) (Claridge et al. 1999), where individuals were unable to maintain body mass on a fungus diet. Protein is an essential element crucial for various functions in an organism's body including tissue repair and growth, immune function, enzyme and hormone production, and molecule transportation and storage (Wu 2014). Overall, crude protein content was similar between fungus and spruce seed samples. Fungus protein ranged from 10-15% in our study area and was comparable with other studies (16 - 27%) (Pederson et al. 1987, Frank 2009, Yu et al. 2020). Our spruce seed protein content (0-15%) was also similar to previously published results, 8-25% (Gifford & Tolley 1989, Fletcher et al. 2013, Lobo & Millar 2013). In contrast, crude lipid content was much higher in spruce seeds than in our fungus samples. Lipids are also essential as they are necessary for hormone production, vitamin absorption, and cell membrane structure, and are an important source of energy (Gurr 1983). Seeds, in general, have high lipid content, and our spruce seed samples (21-25%) were comparable to reported values from other spruce species 21.2-38.4% (Wolff et al. 1996, Wajs-Bonikowska et al. 2016). However, fungi had a much lower crude lipid content (1-5%) than spruce seeds. Other studies have reported similarly low lipid contents for fungi, ranging from 3.5-4.6% (Frank 2009). Without a stable source of lipids for energy, individuals must rely on fat reserves as a fuel source, which can eventually lead to reduced body mass over a prolonged period, especially in species like red squirrels, which have very little insulative fat (Johannesdottir 2017). Lower squirrel body mass in spring may suggest that modifications like reduced activity and alternate food sources are not sufficient to completely buffer against the low overall abundance of lipid-rich foods at the treeline in winter.

Winter temperature and survival and mass declines

The severe winter temperatures characteristic of higher latitudes can be challenging for organisms, increasing thermoregulatory costs and energetic requirements. Mammals use metabolic heat to regulate body temperature irrespective of fluctuating environmental temperatures (McNab 2002). Although this ability enables individuals to withstand extended periods of activity, the process is energetically expensive. When faced with temperatures below a species' critical temperature, individuals must increase their energy expenditure and metabolism to maintain body functions (Humphries et al. 2005). Previous studies describe the geographical temperature range for red squirrels as from approximately -30ºC, the coldest average temperature at the northern edge of the species' distribution, to around 30ºC, the highest average temperature at the southern edge of the species' distribution (Johannesdottir 2017). Our study area's mean monthly temperatures ranged from -28ºC to -15ºC in winter (November to April) from 2020 to 2023. Annual winter temperatures were similar during our study period, but there was a large variation in the number of days below -30ºC. In our best model for annual survival, the number of days below -30ºC emerged as a predictor variable. While the effect was not statistically significant at the conventional threshold, the near significance indicates that extreme cold events could exacerbate the challenges posed by limited food availability. Although winter temperatures were not included in our overwinter survival model, survival was lowest in 2022-2023 following the mast year. Not only was the number of filled seeds lowest that year, the same winter also experienced the highest number of days below -30°C. This temporal coincidence further suggests that extreme cold may contribute to reduced survival, particularly when combined with limited food resources.

Many organisms can mitigate the effects of winter temperatures through behavioural and physiological modifications. In small mammals, brown adipose tissue is responsible for non-shivering thermogenesis and fluctuates in quantity with ambient temperature (Aleksiuk 1971, Merritt 1986, Merritt & Zegers 2002). High proportions of brown adipose tissue enables individuals to survive winter temperatures in northern environments (Dew et al. 1998). Aleksiuk (1972) observed a marked increase in brown adipose tissue in red squirrels in the fall and peaking in winter, correlating negatively to ambient winter temperatures. Some species may also reduce body mass in response to cold temperatures to reduce energy requirements. Many mammals, including pikas, microtines, and mustelids, intentionally reduce body and brain mass to reduce their basal energy expenditure, the amount of energy needed to carry out fundamental metabolic functions (Heldmaier 1989, Wang & Wang 1996, Lovegrove 2005, Wang et al. 2006, Taylor et al. 2013, LaPoint et al. 2017).

Various behavioural strategies can also be used to cope with winter temperatures. Nest sharing is one such strategy to mediate winter's high thermogenic costs. Communal nesting involves sharing a refuge, often to share duties such as raising offspring. However, sharing a nest can also reduce the costs of thermoregulation (Williams et al. 2013). Communal nesting may benefit younger individuals who have not obtained peak pre-winter body mass. In tree squirrels, communal nesting is often observed during winter and is usually involved in non-territorial species (Layne & Raymond 1994, Koprowski 1996). Unlike many tree squirrel species, red squirrels are highly territorial and aggressive towards conspecifics (Smith 1968, Price et al. 1986). During June, we often trapped multiple squirrels at the same site. However, these occurrences always involved a single female and one or more male squirrels, and only the females were trapped at the same sites in August of the same year. In August, squirrels were only trapped individually at sites, but on several occasions where adult female squirrels were trapped, a noticeably smaller, likely juvenile squirrel was observed and heard in a nearby tree throughout the trapping session. Further, a subset of squirrels trapped during our study were fitted with radio collars, and successfully tracked squirrels were never tracked to the same site as another squirrel (Chapter 4). Red squirrels typically breed from May to July (Boutin & Larsen 1993), when male squirrels temporarily expand their territories to overlap with neighbouring females (Lane et al. 2009). While it is possible squirrels in our study area communally nest, males were most likely trapped in a neighbouring squirrel's territory while looking for a mate. Although we did not observe signs of communal nesting overwinter from trail camera images, communal nesting has been previously observed in red squirrels (Williams et al. 2013). Williams et al. (2013) observed that nest sharing among red squirrels was higher in colder winters and typically involved mothers and daughters. Communal nesting has also been observed in other asocial tree squirrels, such as Abert's squirrels (*Sciurus aberti*) (Edelman & Koprowski 2007), with increased proportions of shared nests with decreasing ambient temperatures. Overwintering strategies like communal nesting, more commonly observed in a single sex, may lead to sex-specific differences in survival and body condition.

Sex bias in survival and seasonal body mass

In many mammalian species, males are typically larger than females, which can influence survival rates and seasonal changes in body mass. Individuals with a higher body mass typically have higher metabolic rates and thus require more energy to sustain themselves through periods of limited resources (Nagy 1994, Speakman 2005). Male red squirrels generally are larger than females, ranging in mass from 180 g to 260 g, while females range from 180 g to 250 g (Koprowski 2005, Wheatley 2007). In our study, male red squirrels had a similar body mass to female red squirrels in August but were smaller in mass than females in June (Table S3.6). Regarding survival, larger individuals in both hibernating and non-hibernating species would have increased pressure to accumulate body fat and cache food for winter. For example, Loisson et al. (1999) noted during two abnormally harsh winters, male red deer had to be one kilogram heavier, relative to their mean body mass, than females to have the same chance of survival. While previous studies have reported higher survival rates in female red squirrels (Steury & Murray 2003, LaMontagne et al. 2013), our analysis did not reveal significant differences in survival between males and females, which was particularly surprising given the more extreme mass loss experienced by males overwinter. On average, male squirrels lost 20.6 ± 3.0% of their body mass compared to 10.8 ± 8.0% in females. Male squirrels may hoard larger amounts of alternate food sources, such as fungi. The larger food cache could increase their survival odds, but the lower lipid content would contribute to the greater overwinter mass loss experienced by male squirrels. Although the mechanisms responsible for the sex-specific mortality rates in red squirrels are largely unidentified, male squirrel mortality may relate to their increased activity away from their territories during the breeding season (Smith 1968, LaMontagne et al. 2013), increasing their exposure to predators and aggressive male conspecifics.

In contrast to our survival models, sex contributed to variation in seasonal body mass. Although males were lighter than females in spring, they were similar in mass by the end of August, and females maintained more body mass over winter than males. Sex-specific seasonal mass changes may result from differences in food acquisition strategies. Rodents often exhibit sexual variation in food caching behaviours, specifically in preparation for winter. Male common hamsters (*Cricetus cricetus*) allocate more time to accumulating body fat prior to hibernation, whereas females rely mostly on cached food, resulting in a higher proportion of body fat in males emerging from hibernation in spring (Siutz et al. 2012). In contrast, male Richardson's ground squirrels (*Urocitellus richardsonii*) survive winter by relying on fat reserves and cached food, whereas females infrequently hoard food and rely solely on fat reserves (Michener 1992). For red squirrels, previous studies have shown sex-specific variation in cached resources, where females harvested a higher proportion of cones, but males hoarded more cones in middens (Donald & Boutin 2011, Haines et al. 2022, Wishart 2023). However, reconstructed diets for squirrels in our study area show little difference between males and females, with both sexes consuming mostly fungi (Windsor et al. 2025).

Differences in spring body mass may also reflect variations in energy expenditure and the timing of reproductive energy requirements. For example, male Richardson's ground squirrels are often more active than females in spring, engaging in activities like fighting competitor males, monitoring neighbouring females and defending larger home ranges. As a result, males often lose mass between emerging from hibernation and the onset of breeding, whereas females typically increase body mass (Michener 1983). Like the Richardson's ground squirrel, male red squirrels will also occupy larger territories in spring, often expanding to overlap with neighbouring females (Lane et al. 2009). Red squirrels are promiscuous, with males travelling large distances to mate with as many nearby females as possible (Lane et al. 2010). Thus, the peak energetic investment in reproduction for male red squirrels is during the mating season, when they rely entirely on cached resources. In contrast, female red squirrels have higher reproductive energy demands in summer, during lactation, when food resources become more available (Haines et al. 2022). Although many males in our study were trapped prior to observations of mate-seeking behaviour, males were often observed travelling far distances, sometimes >2 km, to rendezvous with females (pers. obs.). Thus, the increased energy expenditure by males during and just prior to our trapping sessions in June may have contributed to their lower spring body mass.

Influence of predation risk on overwinter survival

Low resource availability in winter can increase foraging time and thus exposure to predators (McNamara et al. 1994, Sweitzer 1996, Mónus & Barta 2016). Additionally, some predators may rely more on small mammals as a food source in winter, when other prey may be scarce. Red squirrels are not known as a major food item for predators in the boreal forest. However, squirrels can be an important alternative prey when preferred prey species like red-backed voles (*Myodes rutilus*) winter in the subnivean zone (Cumberland et al. 2001, Selonen et al. 2016) and other prey species like snowshoe hares decline (O'Donoghue et al. 1998). Since squirrels do not hibernate or use torpor (Brigham & Geiser 2012), individuals must travel to and from their middens for food, increasing their vulnerability and exposure to predators. Although our best-fitting survival model included predation risk, the observed number of predators near squirrel middens was low, with little annual variation. Previous studies on squirrel predation provide conflicting results. While some studies have noted increased overwinter mortality in association with predator abundance (lynx *Lynx canadensis*, marten, and weasels) (Stuart-Smith & Boutin 1995, Haapakoski et al. 2012, Hendrix et al. 2020), others have found no strong effect on squirrel populations (Boonstra et al. 2001). In our study area, squirrels use an extensive network of subterranean tunnels in which they live and access food caches (Windsor et al. 2025). Tunnel systems may reduce the time squirrels spend above ground in winter, reducing their exposure to winter temperatures and predators. However, mustelid predators, like marten and short-tailed weasels (*Mustela erminea*), are adept at hunting in burrows and are known to enter squirrel nests and tunnels (Studd et al. 2015), as well as use squirrel middens as entrances into the subnivean zone to hunt for other small rodents (Sherburne 1993, Pearson & Ruggiero 2001). Although squirrels are active throughout winter, individuals may be more vulnerable to predators in other seasons, such as fall, when adults are busy transferring harvested cones to their midden and juveniles become independent and disperse. Dispersal is risky as individuals are more vulnerable to predators and exposed to environmental conditions (Garrett & Franklin 1988, Yoder et al. 2004, Rödel et al. 2015). In European rabbits (*Oryctolagus cuniculus*), higher fall dispersal rates in males led to higher mortality estimates in males (Rödel et al. 2004). For red squirrels, juveniles may disperse in the fall to acquire territory, although mothers will often bequeath a portion of their territory to offspring (Price & Boutin 1993, Berteaux & Boutin 2000). Hendrix et al. (2020) suggested dispersed juveniles unable to obtain territories in the fall were more susceptible to predators and winter temperatures and consequently had lower survival rates. In addition to low body mass, low food availability may also indirectly affect survival rates, as individuals in poor body condition have a higher likelihood of predation (Wirsing et al. 2002).

Conclusion

In seasonal environments, organisms rely on behavioural and physiological modifications to overcome the challenges of winter. However, the limited resources and harsh environment of the sub-Arctic treeline may exceed the plasticity of individuals. Our results show that squirrels at the northern edge of their distribution are faced with reduced availability of high-quality food, affecting overwinter survival and seasonal body mass, and highlight the ongoing challenges faced by organisms in low-resource and range-boundary environments. More research is needed to evaluate whether the abundance of alternate food resources can mitigate the challenges of winter by influencing overwinter survival or if squirrels use other strategies at their northern range boundary not previously observed in more robust, core-habitat populations.

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Tables

Table 3.1: Annual variation in environmental characteristics and red squirrel body mass (mean ± SE, sample size in parentheses) near Churchill, MB, Canada. Cones, seeds, and fungi were assessed in August of the designated year (Windsor et al. 2025), winter temperature reflects the following Nov-April, and predator frequency is number observed on trail cameras per 100 trap days over winter (Aug-May; 7 cameras in 2020, 10 cameras in 2021, and 17 cameras in the other years).

Variables	2020	2021	2022	2023
# of cones (per ha)	34,158 ± 3,027	2,485 ± 1,181	91,827 ± 27,627	2,110 ± 947
# of seeds (per ha)	--	6,461 ± 5,023	55,096 ± 16,664	7,596 ± 6,456
# seeds (per cone)	--	2.6 ± 1.6	0.6 ± 0.02	3.6 ± 2.6
# of days < -30°C	9	16	38	21
Fungus abundance (kg per ha)	--	--	3.5 ± 0.5 (29)	2.5 ± 0.6 (29)
Predator frequency	1.2 ± 0.1	1.3 ± 0.4	1.7 ± 0.2	--
Mean trap days (per camera)	283.0 ± 8.1	359.6 ± 1.6	362.1 ± 0.8	--
June body mass (g)				
Males	--	197.7 ± 5.6 (11)	182.3 ± 3.3 (12)	207.1 ± 4.8 (15)
Females	--	205.8 ± 4.7 (15)	193.8 ± 4.8 (13)	207.2 ± 7.5 (17)
August body mass (g)				
Males	220.0 ± 6.7 (6)	--	212.9 ± 3.8 (7)	207.3 ± 3.4 (9)
Females	210.0 ± 2.2 (6)	--	207.3 ± 3.4 (9)	216.3 ± 5.4 (8)

Table 3.2: Crude protein and lipid content (%) in white spruce seeds and three fungus genera (mean ± SE, sample sizes in parentheses). Samples were collected near Churchill, MB, Canada in August 2022.

Sample	Protein	Lipid
Spruce seeds	22.2 ± 0.6 (10)	34.7 ± 0.6 (10)
Fungi		
Russula	19.9 ± 0.3 (3)	4.0 ± 0.1 (3)
Lactarius	23.6 ± 0.3 (4)	4.8 ± 0.1 (3)
Cortinarius	23.9 ± 0.7 (4)	7.8 ± 0.1 (3)

Table 3.3: Results from the top linear mixed model to explain spring body mass of red squirrels captured near Churchill, MB from 2022-2023.

Predictors	Estimate	Standard Error	z	p-value
Intercept	5.28	0.30	14.03	0.408
Filled seeds per ha	4.31	0.81	1.52	< 0.001
Sex: Male	-7.02	0.20	-3.33	< 0.001
Random Effects	Variance			
Squirrel ID	0.00			
Site ID	0.00			

Table 3.4: Results from the top generalized linear mixed model for overwinter survival of red squirrels captured near Churchill, MB, Canada, from 2022-2023.

Predictors	Estimate	Standard Error	z	p-value
Intercept	-0.81	0.12	-1.17	0.611
Filled seeds per ha	3.01	0.44	3.05	0.008
Mass in August	4.77	0.37	3.94	< 0.001
Random Effects	Variance			
Squirrel ID	0.01			
Site ID	0.01			

Figures

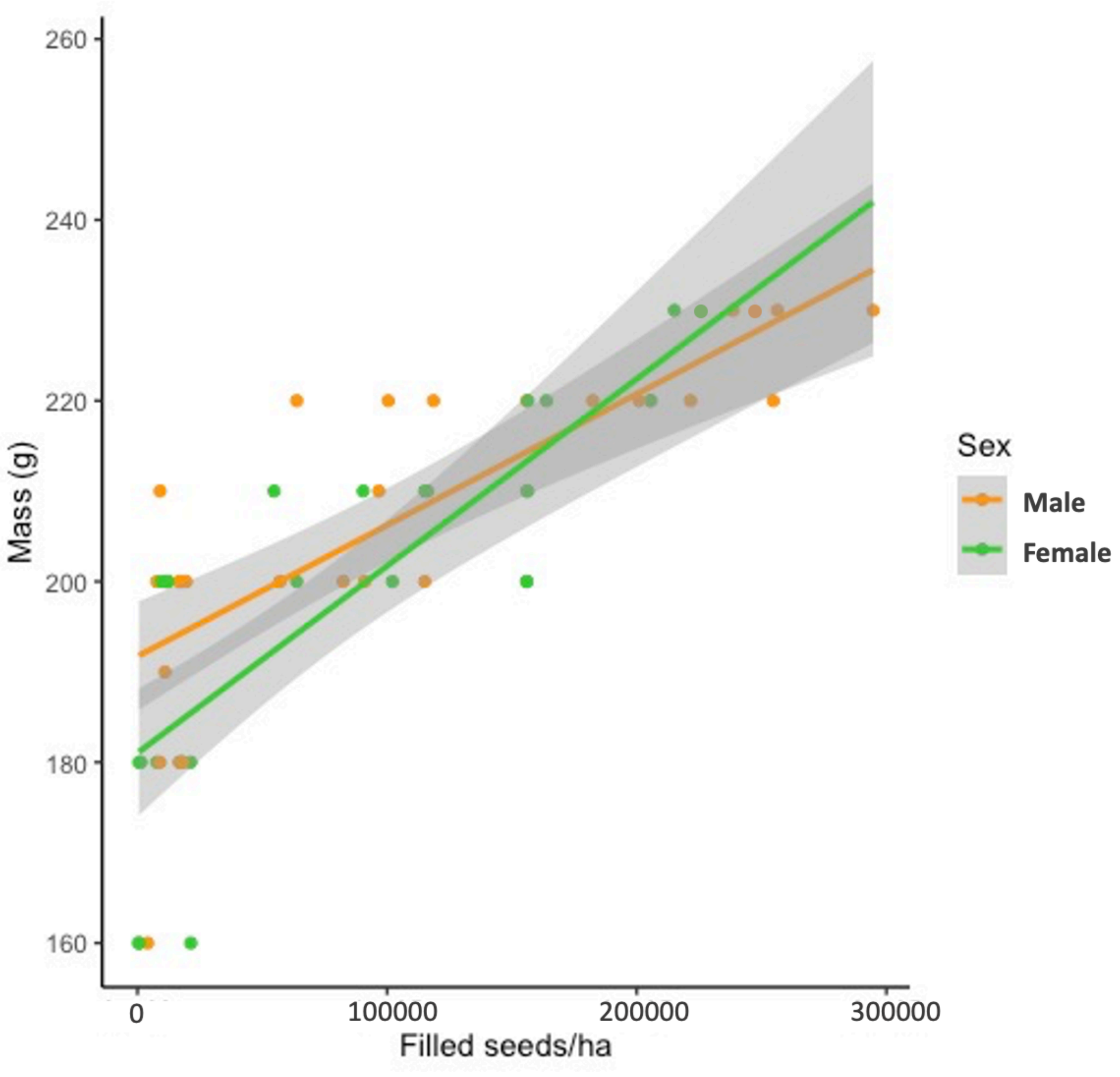

Figure 3.1: Spring body mass of male (n = 27) and female (n = 29) squirrels related to the number of filled seeds available (per hectare) at the same site the previous fall. Data were collected from Aug 2022 to June 2023 near Churchill, MB, Canada.

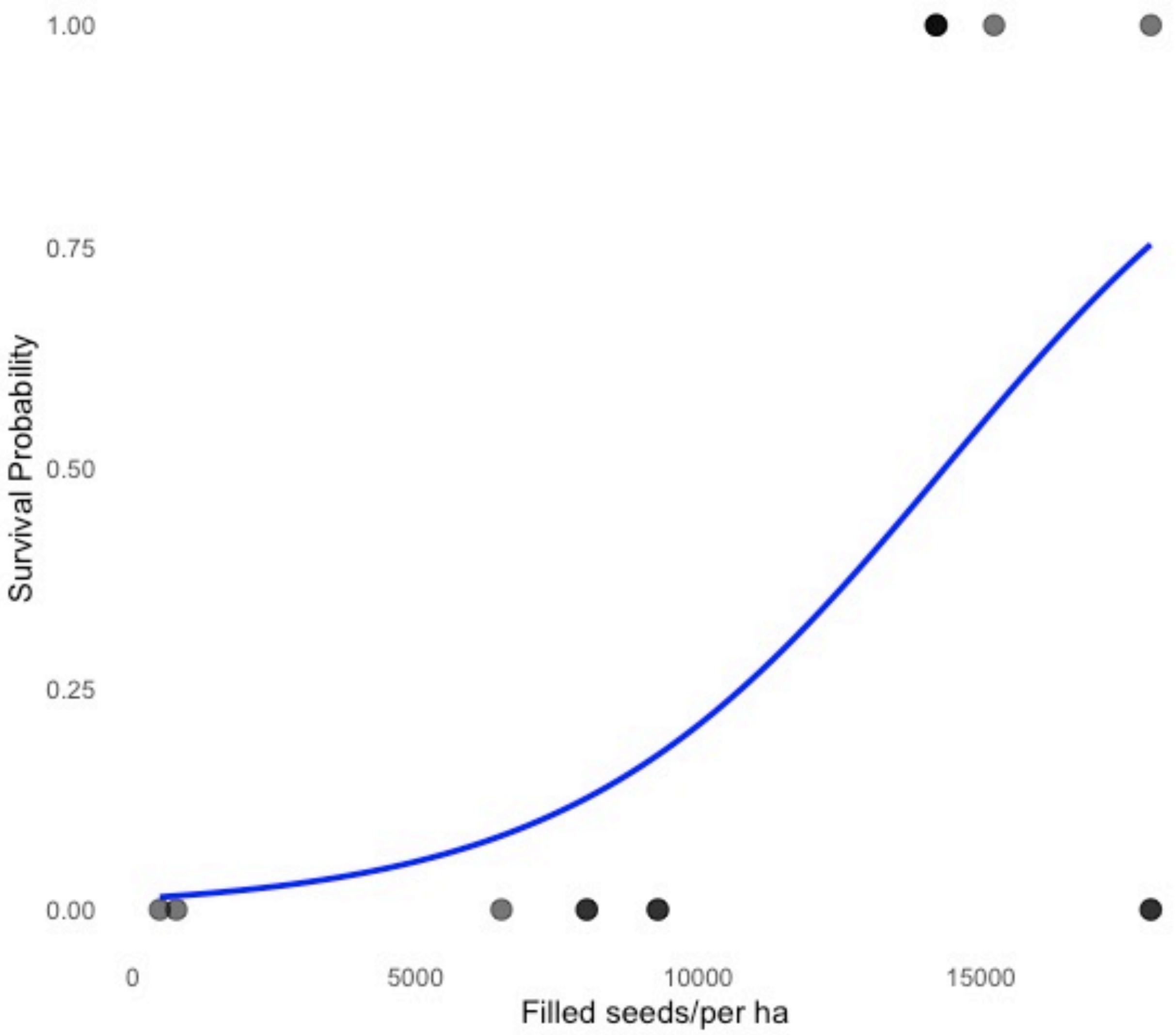

Figure 3.2: Overwinter survival of squirrels trapped near Churchill, MB, Canada, based on a logistic regression using filled seed abundance as a predictor variable. Survival was estimated from mark-recapture data from August 2022 to June 2023.

Supplementary Tables

Table S3.1: Comparison of linear mixed models explaining spring body mass in red squirrels trapped 2022-2023 near Churchill, MB, Canada, sorted by corrected Akaike Information Criterion (AICc) and AICc weight (wi). All models included 'site ID' and 'squirrel ID' as random effects. Predictor variables included the previous year's filled seed and cone abundance (per ha) and fungus abundance (kg per hectare), and sex.

Model	**AIC_c**	**ΔAIC_c**	**w_i**
Filled seeds + sex	458.6	0.0	0.45
Cones + sex	460.3	1.8	0.19
Filled seeds	461.1	2.3	0.14
Cones	461.9	2.5	0.13
Fungi + sex	463.0	4.4	0.05
Sex	464.6	6.0	0.02
Fungi	465.0	6.4	0.02

Table S3.2: Comparison of generalized linear mixed models explaining overwinter survival in squirrels trapped each June from 2022-2023 near Churchill, MB, Canada, sorted by corrected Akaike Information Criterion (AICc) and AICc weight (wi). All models included ‘site ID’ and ‘squirrel ID’ as random effects. Predictor variables included the previous year’s cone and filled seed abundance (number per hectare) and fungus abundance (kg per hectare), sex, predation risk, and pre-winter body mass.

Model	**AICc**	**ΔAICc**	**w_i**
Filled seeds + mass	17.9	0.0	0.12
Filled seeds + mass + predation risk	19.2	0.8	0.08
Filled seeds	19.7	1.4	0.08
Cones + mass	20.4	1.6	0.07
Cones + mass + predation risk	20.6	2.4	0.07
Cones	21.3	2.6	0.07
Fungi + mass	21.5	3.3	0.06
Mass	22.2	3.7	0.06
Mass + predation risk	22.6	4.2	0.06
Filled seeds + mass + sex	23.2	4.6	0.05
Filled seeds + mass + sex + predation risk	23.8	5.3	0.05
Cones + mass + sex	24.2	5.8	0.05
Fungi	24.7	6.0	0.04
Cones + mass + sex + predation risk	25.0	6.7	0.04
Fungi + mass + predation risk	25.4	7.2	0.02
Fungi + mass + sex + predation risk	25.8	7.6	0.02
Fungi + mass + sex	26.3	8.0	0.02
Mass + sex	26.8	8.7	0.01
Mass + sex + predation risk	27.6	9.1	0.01
Filled seeds + sex	28.0	9.5	0.01
Filled seeds + sex + predation risk	29.3	10.2	0.01
Cones + sex	29.8	10.9	0.00
Cones + sex + predation risk	30.5	11.1	0.00
Fungi + sex + predation risk	31.2	11.4	0.00
Fungi + predation risk	31.8	12.3	0.00
Filled seeds + predation risk	32.2	13.2	0.00
Cones + predation risk	32.8	13.7	0.00
Sex + predation risk	33.5	14.2	0.00
Fungi + sex	34.0	15.0	0.00
Sex	34.6	15.8	0.00
Predation risk	35.1	16.3	0.00

Table S3.3: Comparison of generalized linear mixed models explaining annual survival in squirrels trapped each June from 2021-2023 near Churchill, MB, Canada. Models are sorted by corrected Akaike Information Criterion (AICc) and AICc weight (wi). All models included ‘site ID’ and ‘squirrel ID’ as random effects. Predictor variables included the previous year’s filled seed and cone abundance (per ha), sex, predation risk, and the total number of days below -30°C.

Model	**AIC**$_c$	**ΔAIC**$_c$	**w**$_i$
Filled seeds + predation + days < -30°C	25.9	0.0	0.18
Filled seeds + predation	27.1	0.7	0.15
Cones + predation + days < -30°C	29.5	1.4	0.10
Cones + predation	29.7	1.7	0.10
Filled seeds	30.1	2.1	0.08
Filled seeds + sex + predation + days < -30°C	30.8	2.3	0.08
Filled seeds + days < -30°C	32.1	2.9	0.07
Cones + sex + predation + days < -30°C	33.4	3.4	0.06
Cones + days + < -30°C	33.9	3.6	0.06
Cones	35.1	4.1	0.04
Filled seeds + sex + days < -30°C	36.2	4.5	0.04
Cones + sex + days < -30°C	36.8	4.8	0.02
Filled seeds + sex < -30°C	38.0	5.6	0.01
Cones + sex + < -30°C	41.4	6.2	0.01
Filled seeds + sex	42.3	6.9	0.00
Cones + sex	44.1	7.3	0.00
Predation	45.6	7.7	0.00
Days < -30°C	45.9	8.1	0.00
Sex	46.0	8.6	0.00

Table S3.4: Results from the top generalized linear mixed model to explain annual survival of red squirrels near Churchill, MB, Canada.

Predictors	**Estimate**	**Standard Error**	**z**	**p-value**
Intercept	-0.98	0.88	-1.33	0.585
Filled Seeds	1.66	0.67	1.16	0.023
Predation risk	-1.79	0.58	-2.31	0.007
Days < -30°C	-0.82	0.46	-1.26	0.051
Random Effects	Variance			
Squirrel ID	0.02			
Site ID	0.07			

Table S3.5: Body mass and measurements for squirrels trapped near Churchill, MB, Canada from 2020-2023.

Year	Squirrel ID	Sex	Mass (g)	Spine length (cm)	Tail length (cm)
2020					
	RS-20-CH-01	Female	250	14.5	13.0
	RS-20-CH-02	Female	250	11.0	11.0
	RS-20-CH-03	Male	220	12.0	14.0
	RS-20-CH-04	Male	220	10.5	12.5
	RS-20-CH-05	Female	200	14.0	15.0
	RS-20-CH-06	Female	130	14.0	15.5
	RS-20-CH-07	Male	200	14.5	13.0
	RS-20-CH-08	Female	220	8.5	10.5
	RS-20-CH-09	Male	240	12.5	11.0
2021					
	RS-21-CH-01	Female	250	12.0	13.5
	RS-21-CH-02	Female	220	12.5	11.0
	RS-21-CH-03	Female	250	11.5	14.0
	RS-21-CH-04	Male	200	11.0	13.0
	RS-21-CH-05	Female	200	11.0	10.0
	RS-21-CH-06	Female	210	11.0	15.0
	RS-21-CH-07	Female	230	13.5	14.0
	RS-21-CH-08	Female	180	10.5	15.0
	RS-21-CH-09	Female	200	11.0	13.5
	RS-21-CH-10	Female	160	9.5	14.0
	RS-21-CH-11	Male	220	12.0	16.5
	RS-21-CH-12	Male	200	12.0	10.5
	RS-21-CH-13	Male	230	11.0	13.0
	RS-21-CH-14	Female	200	9.0	10.0
	RS-21-CH-15	Female	180	9.0	12.0
	RS-21-CH-16	Male	200	13.0	13.5
	RS-21-CH-17	Male	180	11.0	15.0
	RS-21-CH-18	Male	200	12.0	15.0
	RS-21-CH-19	Male	210	11.0	14.0
	RS-21-CH-20	Male	220	10.0	13.0
	RS-21-CH-21	Male	160	8.5	14.0
	RS-21-CH-22	Female	200	10.5	15.0
	RS-21-CH-23	Female	180	10.0	11.0
	RS-21-CH-24	Female	210	11.0	13.5
	RS-21-CH-25	Male	200	10.0	11.5
	RS-21-CH-26	Male	220	9.5	12.0
	RS-21-CH-27	Female	160	9.5	14.0

2022					
	RS-22-CH-01	Female	200	9.5	9.0
	RS-22-CH-02	Male	200	10.5	9.0
	RS-22-CH-03	Female	200	12.0	9.5
	RS-22-CH-04	Male	180	11.5	14.0
	RS-22-CH-05	Male	180	11.5	12.0
	RS-22-CH-06	Male	200	10.0	9.0
	RS-22-CH-07	Female	160	9.5	12.0
	RS-22-CH-08	Female	210	11.5	11.5
	RS-22-CH-09	Male	180	9.0	12.5
	RS-22-CH-10	Female	200	9.5	10.5
	RS-22-CH-11	Female	200	10.5	11.5
	RS-22-CH-12	Female	200	12.5	12.0
	RS-22-CH-13	Female	220	10.0	10.5
	RS-22-CH-14	Male	200	10.0	9.0
	RS-22-CH-15	Female	180	9.0	11.0
	RS-22-CH-16	Male	200	10.0	11.0
	RS-22-CH-17	Female	180	8.5	12.5
	RS-22-CH-18	Male	180	9.5	13.0
	RS-22-CH-19	Male	180	9.5	10.0
	RS-22-CH-20	Female	220	10.0	9.5
	RS-22-CH-21	Male	180	11.0	10.5
	RS-22-CH-22	Female	200	11.0	10.0
	RS-22-CH-23	Male	180	11.5	10.0
	RS-22-CH-24	Male	160	10.0	12.5
	RS-22-CH-25	Female	180	10.0	12.0
2023					
	RS-23-CH-01	Female	140	14.5	14.0
	RS-23-CH-02	Female	250	17.0	16.0
	RS-23-CH-03	Female	210	14.0	13.5
	RS-23-CH-04	Male	200	13.0	15.5
	RS-23-CH-05	Male	210	14.5	14.0
	RS-23-CH-06	Female	220	16.5	15.0
	RS-23-CH-07	Female	200	13.5	13.5
	RS-23-CH-08	Female	200	13.0	13.5
	RS-23-CH-09	Male	190	15.0	14.0
	RS-23-CH-10	Female	180	14.0	13.0
	RS-23-CH-11	Male	170	12.0	14.5
	RS-23-CH-12	Male	210	12.5	14.0
	RS-23-CH-13	Female	210	15.0	13.5
	RS-23-CH-14	Male	200	13.5	16.0
	RS-23-CH-15	Male	190	16.0	14.0
	RS-23-CH-16	Male	170	12.5	11.0
	RS-23-CH-17	Male	190	13.0	14.0
	RS-23-CH-18	Male	180	14.5	13.0
	RS-23-CH-19	Female	210	14.0	14.0

RS-23-CH-20	Male	180	11.5	13.0
RS-23-CH-21	Female	200	13.5	14.0
RS-23-CH-22	Male	180	14.0	15.5
RS-23-CH-23	Male	200	14.0	15.0
RS-23-CH-24	Female	210	15.0	14.0
RS-23-CH-25	Male	210	14.5	15.0
RS-23-CH-26	Female	220	14.0	15.0
RS-23-CH-27	Male	160	13.0	15.0
RS-23-CH-28	Female	150	13.0	13.5
RS-23-CH-29	Female	160	11.0	13.0
RS-23-CH-30	Female	210	13.0	15.0
RS-23-CH-31	Female	200	15.0	16.0

Chapter 4: Beneath the surface: assessing the seasonal predation risks faced by ground-nesting tree squirrels at the sub-Arctic treeline

Abstract

Predation is a crucial mechanism that regulates prey populations and can vary in intensity over time. At higher latitudes, seasonal fluctuations in environmental conditions can impose significant energetic costs on organisms, leading them to reduce their daily activity and seek shelter in thermal refuges to mitigate such costs. However, selecting such microhabitats may increase an individual's vulnerability to predation. We investigated the trade-off in nest selection by red squirrels (*Tamiasciurus hudsonicus*) in relation to seasonal predation risk at the sub-Arctic treeline. From 2020-2023, we tracked 43 radio-collared squirrels to their nests. We evaluated the seasonal predation risk to squirrels by using images captured from 17 motion-activated trail cameras placed at squirrels' middens and compared the frequency of predator observations with the observed frequency of squirrel activity and the nest type used by each site's inhabitant. Of the 43 nests, we observed only four dreys, all in 2020. The remaining nests were all burrows, likely due to their thermal properties, as temperatures were approximately 15°C warmer than ambient temperatures in winter and nearly 3°C cooler in summer. Observations of red foxes (*Vulpes vulpes)* and American martens (*Martes americana*) were highest in summer and autumn when squirrels are most active, and juveniles emerge and disperse from nests. Conversely, the observation frequencies of short-tailed weasels (*Mustela erminea*) were highest in winter when squirrels spend most of their time in burrows. The structure of burrows thus protects squirrels against larger predators, but it leaves squirrels vulnerable to predation from smaller burrowing predators. As climate change reshapes vegetation patterns and modifies the thermal landscape of

the sub-Arctic treeline, understanding the nesting behaviours of species in these northern environments becomes crucial for predicting how climate-driven changes will impact the availability and suitability of shelters and microhabitats in the future.

Introduction

Predators serve as crucial regulators of populations in ecosystems (Ferreira & Faria 2021). At the local level, predation pressure can influence population dynamics, life history traits, and behavioural strategies, while at a global scale, it can determine the geographical distribution of species (Case et al. 2005, Holt & Barfield 2009, Ferreira & Faria 2021). The extent and degree of predation risk can vary over spatial and temporal scales. In many ecosystems, food availability fluctuates seasonally. Resources in spring and summer can be plentiful due to the increased vulnerability of prey during reproduction and caring for offspring (Norrdahl & Korpimäki 2000). Alternatively, prey refuges may change seasonally, affecting an individual's exposure and vulnerability to predators. For instance, in summer, small prey species may use dense foliage and vegetation to hide from predators. During winter, some northern species may seek shelter in the subnivean zone, thereby reducing their vulnerability to above-ground predators (Hansson 2002). The abundance and efficacy of refuges are influenced by habitat quality, with the structural complexity of a habitat playing a pivotal role in determining a species' predation risk by providing cover and refuge (Denno et al. 2005, Shepard 2007, Arnan et al. 2014), nesting sites, and a diverse prey community (Mena & Medellín 2017, Sukma et al. 2019, Chang & Todd 2023).

The availability of suitable nest sites and nesting substrate plays a crucial role in assessing the quality of habitats, as they provide not only protection from predators but also shelter from environmental conditions, a place to raise young, and for many small rodents, a storage cache for food (Fancy 1980, Steele 1998, Merrick et al. 2007, Catall et al. 2011, Edelman 2011, Deeming 2023). The type and location of nests can differ significantly across species, and even within species, nest type and location can vary among individuals, populations, and habitats (Deeming 2023). In highly seasonal environments, species may prioritize thermal needs over protection from predators by selecting nest sites or constructing nests that provide optimal conditions for survival in adverse climatic seasons. Such nests may offer protection from the elements, suitable microclimates, and resources necessary for successful winter survival, but may be more vulnerable to predator attacks. Thus, there is a trade-off in balancing between competing priorities: seeking shelter that will buffer against extreme temperatures, and shelter that will provide protection from predators. For instance, in northern environments, small ground-dwelling mammals burrow beneath the snow in winter, where extensive snow provides thermal relief and additional protection from most above-ground and avian predators (Gilg et al. 2009, Duchesne et al. 2011, Bilodeau et al. 2013). But under the snow in the subnivean zone, small mustelids can present a great predation threat, their narrow body size being well-adapted for hunting in tunnel systems (King & Powell 2006, Jung et al. 2021).

Red squirrels (*Tamiasciurus hudsonicus*) are a small arboreal mammal with a wide distribution across North America (Arbogast et al. 2001). Although they occupy a diverse array of habitats, nest selection is typically limited to cavity nests, leaf-litter nests known as dreys, and ground burrows (Young et al. 2002, Merrick et al. 2007, Deeming 2023). Nest selection is critical for winter survival and thermoregulation in northern regions of the squirrels' distribution (Smith 1968). In southern mixed forests, red squirrels commonly select tree cavities as preferred nests (Hamilton 1939, Layne 1954, Stevens et al. 1988, Young et al. 2002), although individuals will also construct dreys. Typically, large-diameter trees with a dense branch structure are chosen (Stevens et al. 1988). Compared to dreys, the solid structure of cavity walls provides better protection from climate and terrestrial and avian predators (Young et al. 2002). In coniferous forests, where tree cavities can be scarce, dreys are preferred (Klugh 1927, Hatt 1929, Fancy 1980, Steele 1998) and are usually found in the dense crown foliage of a spruce tree within a cluster of trees, where the interlocking branches provide numerous travel pathways (Rothwell 1979, Stevens et al. 1988). On rare occasions, red squirrels also use burrow nests (Hatt 1929, Mayfield 1948, Pruitt & Lucier 1958, Fancy 1980, Stevens et al. 1988). Burrows typically consist of multiple cavities connected by tunnels leading to food caches and numerous entrances.

Unlike chipmunks (*Tamias striatus*) (Munro et al. 2005) and other small boreal rodents, squirrels do not engage in hibernation or daily torpor (Brigham & Geiser 2012), nor do they accumulate high levels of seasonal body fat for insulation or energy reserves (Johannesdottir 2017) like Arctic ground squirrels (*Urocitellus parrygii*) (Buck & Barnes 1999) and thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) (Sonsalla et al. 2021). As a result, red squirrels are often important alternative prey for predators in winter, as they remain active above the snow over winter. For example, red-backed voles (*Clethrionomys gapperi*), a preferred prey for many small carnivores, overwinter beneath the snow and are less available during winter (Thompson & Colgan 1990, Selonen et al. 2016). In northern forests, American martens (*Martes americana*), whose climbing agility matches that of squirrels, are an important squirrel predator (Poole & Graf 1996, Bull 2000). Other known predators include weasels (*Mustela spp.)* (Lisgo 1999, Edwards & Forbes 2003), birds of prey, fishers (*Pekania pennanti*) (Powell et al. 1997, Weir et al. 2005), red foxes (*Vulpes vulpes*) (Jędrzejewski & Jędrzejewska 1992, Smith et al. 2023), and lynx (*Lynx canadensis*) (Ivan and Shenk 2016, Squires and Ruggerio 2007). While squirrels are not typically a primary prey species for these predators, they are opportunistically hunted and frequently consumed when main prey are scarce. In this study, we considered any mustelid species, as well as lynx, red foxes, and birds of prey, as potential squirrel predators.

The objective of this study was to quantify the predation risk faced by red squirrels inhabiting the sub-Arctic treeline and examine how this risk varied seasonally and in relation to squirrel nest selection. We used a network of 17 trail cameras deployed at squirrel middens near Churchill, MB, to capture images from August 2020 to 2023 and determined the frequency of predator observations seasonally. Additionally, we tracked radio-collared squirrels to record their nest preferences and the observed predator frequency at each site. We hypothesized that (1) predator visits to middens would exhibit seasonal variation, owing to fluctuations in prey accessibility, (2) squirrels would prefer nest types based on their thermal properties, thereby enhancing their chances of overwinter survival, and (3) the choice of nest type would influence the vulnerability of squirrels to predators. We thus predicted that predator observations would increase during winter when other prey species would be largely inaccessible. Furthermore, we anticipated that ground burrows would be warmer in winter compared to ambient temperatures and drey temperatures and that more burrowing predators (mustelids) would be observed at sites where squirrels were nesting below ground.

Methods

Study area

Our study area near Churchill, MB (58°45’ N, 94°04’ W), on the western coast of Hudson Bay, is on the transition between boreal forest and Arctic tundra. The forested parts of this area are primarily composed of white spruce (*Picea glauca*), black spruce (*Picea mariana*), and tamarack (*Larix laricina*) (Mamet & Kershaw 2011, Mamet & Kershaw 2013) and are heavily fragmented (Payette et al. 2001). The proximity of the sub-Arctic treeline to Hudson Bay, which is frozen nine months of the year, exposes the region to cool summer temperatures and extreme winters (Mamet & Kershaw 2011, Mamet et al. 2015). We established sites by setting up a 7 km transect running north to south, marking each 250 m interval with coloured flagging tape. At each flagged interval, we walked 50 m perpendicular (east and west) away from both sides of the transect, looking for signs of squirrel activity such as vocalisations, small piles of cone debris at the base of trees, and middens. Middens are large food caches used by squirrels as a winter food source and are easily identified by the concentrated accumulation of cone debris (Finley 1969, Frank & Cox 2009). Sites with signs of squirrel activity or visual confirmation of squirrels in trees were considered inhabited, and sites where no squirrel presence was observed were considered uninhabited. Red squirrels are highly territorial and rarely move to new territories, but their territory sizes are highly variable and are often linked with food availability. In interior areas of squirrel habitat, territory size ranges from 0.25 to 1.99 ha (Vlasman & Fryxell 2002, Steury & Murray 2003), although farther north, territory size ranges from 0.09 to 0.86 ha (LaMontagne et al. 2013). The size of squirrel territories can also fluctuate seasonally. During the mating season, male squirrels will temporarily expand their territories into neighbouring female territories (Vlasman & Fryxell 2002, Steury & Murray 2003) and in late summer and autumn, squirrel territories can contract, reflecting increased hoarding activity and defense of middens (Gurnell 1983, Vlasman & Fryxell 2002). However, dispersed juveniles must acquire a territory in autumn to survive over winter. To account for the possibility of squirrels taking over previously uninhabited sites, we checked all sites for signs of squirrel activity each year in early June.

Trapping and nest selection

We used Tomahawk live traps (model #202) to systematically trap squirrels throughout August 2020 and June 2021-2023 and restrained individuals in a cloth handling bag (Koprowski 2002, Windsor et al. 2025). We trapped at each site for five consecutive days, from approximately 6:00 until 21:00, and checked traps at four-hour intervals. Each squirrel was weighed to the nearest 1 g with a 500 g Pesola spring scale and sexed prior to being marked with a unique metal ear tag threaded with coloured pipe cleaners for visual identification and injected with a subcutaneous Passive Integrated Transponder (PIT) tag (Windsor et al. 2025). Based on observations of mate searching and copulating during the trapping period, we expected female squirrels gave birth June to mid-July. Therefore, we additionally examined trapped females for signs of pregnancy, looking for enlarged nipples and palpating their abdomens. Following capture, we fitted adult squirrels with radio collars (model PD-2C, Holohil Systems Limited Carp, ON, Canada) weighing <5% of squirrels' body mass. We tracked collared individuals at randomly predetermined times between 6:00 and 20:00. A nest was deemed 'located' when a squirrel was successfully tracked or observed running into the same nest or nest type two or more times. For each observation we recorded the location and nest type. Each successfully tracked squirrel was located two to three times per week over a two-week period in June and a one-week period in August. Each August, we trapped for two days at sites where squirrels were originally collared. We verified captured squirrels still had PIT and ear tags and removed radio collars.

Nest temperatures

In late August in 2021 and 2022, we deployed 12 data loggers (Maxim Integrated Thermochron iButton model: DS1921G-F5) annually, programmed to record temperatures in four-hour intervals. To measure burrow temperatures, we chose six burrows previously located from tracking individuals and installed the logger as far inside the tunnels as we could reach (approximately 20 cm). To mimic the thermal properties of dreys, we constructed three artificial nests made of moss, peat, and grasses, held together with netting, and hung in spruce trees approximately two metres off the ground, placing the logger in the centre of the nest. We recorded ambient temperature by securing 3 dataloggers to trees where trail cameras were installed, approximately 1-2 m above the ground at eight active squirrel sites. We collected the dataloggers at the end of June in 2022 and 2023 and uploaded the temperature data using 1-Wire® Viewer (Maxim Integrated).

Predator and squirrel observations

We installed a total of 17 Browning trail cameras (model: Strike Force Extreme and Strike Force HD Pro X) at sites across our study area (2020 n = 8; 2021 n = 10; 2022-2023 n = 17). We selected sites with previously observed active middens and set each camera facing the midden. We chose to place the cameras at the middens as squirrels are vulnerable when traveling on the ground to and from their middens. At sites with multiple middens, we placed the camera facing the largest midden, as determined by surface area. Here, we define a 'midden' as any form of food cache, including true middens and burrows with food debris, as very few middens were observed in our study area. We secured each camera to a tree or piece of rebar 1-1.5 m above the ground. Cameras were programmed to take a burst of three photos each time they were activated within a 3-m detection range, with a 0.5-second recovery period between bursts. The cameras were checked each year in April, June, and August, changing batteries and SD memory cards when necessary. Additionally, cameras were checked in January in 2021and 2022. Observations of predators were considered independent if 1) they were at least 30 minutes apart, 2) consecutive photos of the same species could be identified as unique individuals (e.g., based on pelage variation), or 3) photos of the same species were separated by photos of another species (Montalvo et al. 2023). In contrast, for squirrels we intended to assess prey activity and exposure rather than individual counts. Therefore, we treated each squirrel observation that occurred at least five minutes apart as an independent indication of activity. To compare seasons, which were different lengths, we calculated a seasonal observation frequency, standardised by sampling effort to account for variation in camera functionality due to deep snow, dead batteries, or full memory cards. For each camera and season, we divided the total number of squirrel and known squirrel predator observations by the number of trap days the camera was operational and standardised per 100 days.

Statistical analyses

All statistical analyses were conducted in R (version 4.0.5) using the packages ‘lme4’ and ‘lmer’. To examine annual variation in nest type observed, we used a generalized linear mixed model (GLMM) with a ‘binomial’ logit function, as squirrels’ nest type was marked as 0 = absent or 1 = present. We fitted ‘squirrel ID’ (47 levels) as a random effect to account for non-independent repeated observations of nest type from the same individual and ‘site ID’ (17 levels) as a random effect to account for variability between sites. To assess if mean daily temperatures differed between burrows, artificial tree nests, and ambient we used a linear mixed-effects model (LMM) with ‘year’ (two years) as a fixed effect and ‘site ID’ (eight levels) as a random effect. To compare all combinations of nest types and ambient temperatures, we ran post-hoc pairwise comparisons using the *emeans* package in R. Comparisons were adjusted for multiple testing using the Tukey’s method. We confirmed model assumptions using graphical analysis of residuals.

We examined variation in observed predator frequency using a GLMM with a ‘Gamma’ distribution and an inverse link function and included ‘season’ (four levels), ‘year’ (three levels), and squirrel activity (frequency of squirrel observations) as fixed effects and ‘site ID’ (17 levels) as a random effect. We additionally ran separate GLMM models for each predator species. We defined seasons as spring (May – June), summer (July – August), autumn (September – October), and winter (November – April). For this study, we considered winter equivalent to months when there was persistent snow cover on the ground for the entire month and summer as snow-free months. Although snow cover persisted through May and partially into June in 2021, this year was an anomaly and so we retained our seasonal definition to maintain consistency across all four years. To evaluate predator frequency differences among seasons we ran a Tukey’s post-hoc test.

To evaluate the effect of squirrel nest type on predator activity in 2020 (the only year with multiple nest types), we used a generalized linear model with a ‘Gamma’ distribution and an inverse link function, with ‘nest type’ (two levels) and squirrel activity as fixed effects. We also included an interaction between nest type and squirrel activity.

Results

Nest type selection and temperature

From 2020 to 2023, we radio-collared 47 squirrels and successfully tracked 43 individuals to nests. Each squirrel was consistently tracked to the same nest type, either a drey or a burrow system, although the specific burrow entrances varied. For squirrels observed using different burrow entrances, new entrances were always within 1-2 metres of the previously recorded entrances. On separate occasions, squirrels were observed entering one burrow and exiting or peeking out of a nearby burrow. This behaviour strongly suggests that the burrows are part of an extensive and interconnected burrow system. We only observed multiple nest types in 2020 (burrows = 5, dreys = 4), leading to significant differences between 2020 and the subsequent years in observed nest type (2021: $z = -0.58$, $p = 0.009$; 2022: $z = -0.65$, $p = 0.008$; and 2023: $z = -0.62$, $p = 0.009$. The choice of burrows as a nest type based on radio tracking squirrels did not vary annually, with most squirrels ($n = 5$) tracked to burrows in 2020 and all squirrels in subsequent years ($n = 34$) tracked to burrows (Table 4.1). The dreys observed in 2020 were a hollowed-out bolus of peat and moss, approximately 50 cm tall (estimated from a destroyed tree nest found on the ground) and nestled in the crowns of large white spruce trees (mean $\pm$ SE: 18.6 ± 2.1 cm DBH).

Temperatures were significantly warmer in 2021-2022 than 2022-2023 ($t = 6.00$, $p < 0.001$). Post-hoc comparisons showed burrow dataloggers had significantly higher temperatures than ambient dataloggers ($t = 2.62$, $p < 0.001$) and tree nest dataloggers ($t = 1.77$, $p < 0.001$), but temperatures did not differ between ambient and tree nest dataloggers ($t = 0.57$, $p = 0.573$). In winter, burrows were on average 15.3°C (± 1.4 SE) warmer than the ambient temperature and 10.8°C (± 0.2) warmer than the artificial nests ($n = 3$). In contrast, in summer, burrows averaged 2.6°C (± 0.9) cooler than ambient temperatures and 1.0°C (± 0.8) cooler than the artificial nests (Figure 4.1).

Predator observations and squirrel activity

Predator observations varied seasonally ($x^2 = 18.5$, $p < 0.001$) but not annually ($x^2 = 1.1$, $p = 0.355$). Post-hoc comparisons revealed significantly higher predator observations in autumn compared to spring and winter, and in summer compared to spring and winter, but no differences between autumn and summer or spring and winter (Table 4.2 and Figure 4.2). Predator frequency was also positively associated with squirrel activity ($z = 3.03$, $p < 0.001$), which peaked in summer and autumn (Figure S4.1).

When predator species were considered separately seasonal variation in predator activity was also evident for weasels ($x^2 = 12.1$, $p = 0.005$), foxes ($x^2 = 32.5$, $p < 0.001$), and marten ($x^2 = 19.1$, $p < 0.001$) (Figure 4.2), but no significant differences occurred among years for any predator species (weasels: $x^2 = 3.3$, $p = 0.172$; foxes: $x^2 = 2.0$, $p = 0.413$; martens: $x^2 = 6.4$, $p = 0.172$). Post-hoc comparisons revealed significantly higher weasel observations in winter compared to spring, autumn, and summer, with no other seasonal differences (Table 4.2). Fox observations followed a similar pattern to the combined predator model, with significantly higher observations in autumn compared to spring and winter, and in summer compared to spring, and winter, and no other seasonal differences (Table 4.2). Marten activity was also higher in autumn compared to spring and winter, and in summer compared to spring and winter, with no additional seasonal differences (Table 4.2). Similar to the cumulative predator species model, the frequency of squirrel observations was positively associated with observations for all predator species (weasels: $z = 1.60$, $p = 0.004$); foxes: $z = 4.13$, $p < 0.001$; marten: $z = 1.71$, $p < 0.001$).

In 2020, neither nest type ($t = 0.80$, $p = 0.124$) nor the interaction between squirrel activity and nest type ($t = 0.83$, $p = 0.113$) had a significant effect on combined predator observations, although squirrel activity still positively affected predator observations ($t = 3.27$, $p = 0.002$). When predator species were modelled separately, squirrel activity positively affected the frequency of fox ($t = 3.29$, $p = 0.001$), marten ($t = 2.41$, $p = 0.005$) and weasel ($t = 2.20$, $p = 0.008$) observations. The significant interaction between nest type and squirrel activity for weasels ($t = 9.21$, $p < 0.001$) indicated weasel observations increased with squirrel activity only at burrows, not dreys (Figure 4.3). There was also some evidence of a similar interaction effect for foxes (t = 1.18, p = 0.069) and martens (t = 0.91, p = 0.061), but the main effect of nest type was not significant for any species (fox: t = 0.72, p = 0.485; marten: t = 0.21, p = 0.088; weasel: t = 0.71, p = 0.085).

Discussion

We found that burrows are the preferred nest type of red squirrels at the sub-Arctic treeline, whereas relatively few observations of burrow nests compared to drey and tree cavity use have been made for red squirrels elsewhere. Most previous observations have included the use of burrows by a small number of individuals within a population of cavity or drey-using squirrels (Yeager 1937, Gurnell 1984, Edelman et al. 2009, Guillemette et al. 2009, Leonard & Koprowski 2009). Although we did not demonstrate a significant effect of nest type on predation risk by larger predators, we did observe more weasels at burrow nests when squirrel activity was high, and in winter when squirrels are settled in their burrows.

Red squirrels construct a variety of different nests across their geographical range (Deeming 2023). In the southern regions of their distribution, red squirrels show a strong preference for cavity nests (Stevens et al. 1988, Young et al. 2002), while there is a shift to dreys in northern coniferous forests (Gurnell 1984, Steele 1998). The difference in nest preference is likely the result of differences in the physical structure and complexity of a habitat (Andruskiw 2003, Merrick et al. 2007). Tree diameter, branching structure, and density are essential proximate factors influencing nest selection (Rothwell 1979, Steele 1998). Tree cavities are a common nest type in old-growth forests where trees generally have a large diameter and decayed trees and snags are more abundant (Holloway & Malcolm 2006, 2007, Patterson et al. 2007). In these habitats, tree basal area, the amount of area covered by tree stems, is larger (approximately

75 m^2/ha) (Edelman et al. 2009) than forests where dreys are preferred (approximately 40-60 m^2/ha) (Young et al. 2002). Dreys are commonly found in coniferous forests with a canopy cover exceeding 70% and tree density greater than 600 stems/ha (< 20 cm DBH). In such areas, squirrels choose dense areas of forests for drey construction, using trees with interlocking crown branches with at least two other neighbouring trees (Young et al. 2002). The dense tree canopies and interlocking branches in high tree densities associated with coniferous forests provides plentiful opportunities for squirrels to escape predators (Smith 1968, Young et al. 2002, Goheen & Swihart 2005, Merrick et al. 2007). In contrast, the forest at the sub-Arctic treeline is heavily fragmented with a low tree density and open canopy (Lafleur 1999, Payette et al. 2001). Compared to other regions of squirrel's distribution, our study area has a low basal area (8.3 ± 8.1 m^2/ha, mean ±SE; data from Windsor et al. 2025), mean DBH (8.5 ± 2.3 cm), and tree density (483.7 ± 54.2 stems/ha, DBH > 5 cm) (Table S4.1), although the four dreys we observed were in sites with above average tree density (558.2 ± 26.5 stems/ha). Therefore, burrows may be a preferred nest type in a habitat that is not conducive for cavity and drey nests.

Additional field observations also support the idea that squirrels rely heavily on underground burrows. Although we did not directly quantify year-round burrow occupancy, our telemetry tracking frequently located squirrels during early morning and post-dusk periods, times when squirrels are typically inside nests, indicating that they were likely sleeping in the burrows. Trail camera footage further showed squirrels repeatedly entering and exiting multiple burrow entrances around middens during summer and autumn while caching and retrieving cones, yet in winter, squirrels were only observed briefly protruding from burrow openings and were never recorded entering or exiting burrows at the surface. This pattern suggests the presence of interconnected subterranean tunnels that squirrels live in and use without exposing themselves above snow. Additionally, annual site inspections revealed that dreys were only present in 2020 and were never observed again, despite their large size and conspicuous construction from peat in an open-canopy, low-density forest. Given the thorough annual surveys, it is unlikely that additional dreys were constructed and not detected. These combined observations strongly suggest that squirrels depend extensively on underground burrows throughout the year.

Specific nest types may also be selected based on the thermal benefits they provide. For example, flying squirrels shift their nest preference from dreys to cavity nests in northern populations, as tree cavities can provide a more stable nest temperature than tree nests (Holloway & Malcolm 2007). In a study testing artificial rodent nests, researchers noted a greater use of black nest tubes by white-footed mice (*Peromyscus leucopus*) in winter. In comparison to white nest tubes, the black tubes reached a higher daily maximum temperature in winter (approximately 2.5°C), suggesting that mice selected for nests with higher thermal properties in winter (Catall et al. 2011). Burrows are widely used by a range of taxa world-wide. In arid climates, many species rely on burrows to create a cooler microclimate and reduce the energetic costs associated with thermoregulation (Bulova 2002, Walde et al. 2009, Whittington-Jones et al. 2011, Pike & Mitchell 2013). Desert tortoises, for instance, can spend over 90% of their time inside burrows, which provide a cooler and more consistent thermal shelter (Pike & Grosse 2006). Similarly, in northern environments during winter, many small mammals, including hibernating species, rely on the insulative properties of nests to reduce thermogenic costs and survive the colder months (Milling et al. 2018, Altamirano et al. 2019, Gubert et al. 2022). The thermal environment of an individual's nest or burrow can be a major factor in determining the energetic costs associated with survival. For example, studies have shown that burrows' thermal properties can reduce rabbits' thermoregulatory costs by over 50% in winter (Milling et al. 2018).

While energetic costs of thermoregulation were beyond the scope of this study, we did observe the mean seasonal winter temperature in squirrel burrows was approximately 15°C higher than ambient temperature. Further, like the burrows of desert species, squirrel burrows were approximately 3°C lower than mean ambient temperature in summer. Other studies have observed similar patterns in winter burrow temperatures. For example, Arctic ground squirrels' burrows have been found to maintain soil temperatures approximately 10°C warmer than ambient temperatures (Barnes 1989, Buck & Barnes 1999). Similar results have been observed in the winter burrows of pygmy rabbits (*Brachylagus idahoensis*) (Milling et al. 2018), Chinese pangolins (*Manis pentadactyla*) (Bao et al. 2013), and kangaroo rats (*Dipodomys spectabilis*) (Edelman 2011).

In our study area, where winter temperatures can reach -40°C and summer temperatures 30°C, squirrels face a high thermoregulatory cost due to their small size and high surface-to-volume ratio (Johannesdottir 2017). To reduce energy costs, squirrels spend most of their time during winter in well-insulated nests and limit their outdoor activities to the warmest times of the day (Pruitt & Lucier 1958, Pauls 1979). For example, in Alaska, squirrels are rarely observed being active above the snow when ambient temperatures reach -30°C (Pruitt & Lucier 1958). Similarly, Humphries et al. (2005) documented female red squirrels increased out-of-nest activity on warmer days, with a higher proportion of females observed outside nests as ambient temperatures increased. In our study area, trail camera data revealed a marked reduction in squirrel activity during winter. The decreased activity is likely attributed to the squirrel's natural tendency to reduce out-of-nest activities in winter and their reliance on extensive burrow systems. Burrows provide easy access to food stores without the need for exposure to harsh winter conditions. The soil and snow around burrows act as natural insulators, which buffers individuals against fluctuations in surface temperatures and humidity (Burda et al. 2007). Soil maintains a more stable temperature inside burrows than the fluctuating temperatures on the surface, and the daily fluctuations in ambient temperatures have little effect on soil temperatures past 30-40 cm (Reichman & Smith 1990, Van Wambeke 1992). Thus, deeper burrows can provide better insulation to inhabitants. Although permafrost likely limits burrow depth in northern environments, studies suggest that Arctic ground squirrels select sites with a deeper than average permafrost table to enable individuals to excavate deeper burrows (Buck & Barnes 1999). We did not quantify burrow depth in this study, but previous research in our study area estimates the permafrost layer is 10-15 m thick (Brown 1970, Sladen et al. 2009), and the active layer above the permafrost can extend less than one metre (Zhang et al. 2012, Macrae et al. 2014). The organic layer above burrows plays an important role in insulating burrows by reducing heat transfer between the burrow and the external environment (Buck & Barnes 1999). A thick organic layer increases the distance heat needs to travel to reach the surface, thus reducing the rate of heat transfer (Wierenga & De Wit 1970). Snow can provide additional insulation for burrows by decreasing thermal conductivity (Sturm et al. 1997) and burrow temperatures in winter have been shown to correlate positively with snow depth (Buck & Barnes 1999). To further increase the ambient temperature within nests, squirrels will collect various materials to insulate their nests (Patterson et al. 2007). In dreys and cavities, squirrels often use soft bark, tree leaves, moss, dried grass, and feathers (Layne 1954, Deeming 2023). Plant fibres particularly have shown to reduce the energy expenditure of other squirrel species in cold temperatures (Stapp et al. 1991). Although we did not excavate burrows in our study, squirrels were observed in the trail camera images carrying clumps of moss and peat into their burrows along with polyester bedding stolen from the live traps.

Winter predators in our study area are mostly limited to terrestrial predators, as most bird species have since migrated south. Thus, we anticipated squirrels overwintering in burrows would be more susceptible to predation, particularly to mustelid predators adept at burrowing and hunting in the subnivean zone (Studd et al. 2015). Although we did not find a significant effect of nest type on the overall number of predators observed at sites, when predator species were considered separately, short-tailed weasels exhibited a significant positive interaction between squirrel activity and nest type: weasel observations increased with squirrel activity at sites where squirrels used burrows, but not at sites with dreys (Figure 5.3C). Foxes and martens showed similar patterns, with predator observations appearing to increase more steeply with squirrel activity at burrow sites than drey sites. These patterns likely reflect predator-specific hunting abilities and constraints in winter conditions. In winter, foxes and martens search for prey by listening for sounds emanating from the subnivean zone. Foxes will pounce on the snow to scare prey to the surface or break through the snow layers to reach prey tunnels (Ables 1969, Bilodeau et al. 2013), whereas martens will enter the subnivean through breaks in the snow around woody debris and vegetation (Ruggiero & Forest 1994). While snow depth impedes hunting success, martens are generally more successful than foxes at hunting in the snow (Willebrand et al. 2017). Nevertheless, the structural design of squirrel burrows may protect from larger predators like foxes and martens. Red foxes are medium-sized predators, whose head width (measured by zygomatic breadth) measures approximately seven centimetres (Commandant 2018) and chest girth measures approximately 35.1 cm (Cavallini 1995). Martens are smaller predators, measuring approximately four centimetres head width (Proulx et al. 1997) and 10-12 cm chest girth (Schulte-Hostedde et al. 2011). The diameters of squirrel burrow entrances were relatively small (5-7 cm), though larger than the entrances of vole burrows (2.5-

3.5 cm) (Herman 1977, Gervais et al. 2010, Mougeot et al. 2020). In contrast, weasels are small enough to hunt in squirrel burrows, with a head width of approximately two centimetres (Elsasser & Parker 2008). Weasels are small, agile hunters, perfectly adapted for hunting small underground rodents (Sittler 1995). Thus, although choosing to nest over winter in burrows may provide thermal relief for squirrels, there is likely a trade-off with easier access for small predators like weasels.

The protection provided by the burrow structure from most predators is likely also a contributing factor to the seasonal variation in predator observations. For larger predators, observation frequency was lowest in winter (November-April) and peaked in summer and autumn (July-October). Martens primarily hunt voles and small rodents, but in winter, when such prey species move to the subnivean zone, the proportion of squirrels in marten diets often increases (Thompson & Colgan 1990, Gosse & Hearn 2005). Unlike many other small prey species, squirrels are active year-round. Even in winter, they remain active above the snow, travelling between their nests and food caches (Brigham & Geiser 2012). Hence, squirrels are a frequently consumed winter prey (Thompson 1987, Nagorsen et al. 1989, Thompson & Colgan 1990, Gosse & Hearn 2005), comprising up to 35% of marten diets (Thompson & Colgan 1990, Lachowski 1997). Moreover, predators such as martens may also select for squirrels and other larger prey in winter to meet increased energetic demands (Harlow 1994, Cumberland et al. 2001, Fargallo et al. 2020). However, at the treeline, squirrels are infrequently consumed by martens in winter, and instead, contribute largely to summer and autumn marten diets (20-40%) (Chapter 4). We observed less squirrel activity and fewer large predators like martens in winter. In colder environments, squirrels will restrict their activity outside of the nest to the warmest parts of the day (Pruitt & Lucier 1958, Pauls 1979). This, in conjunction with the limited entry access to burrows, likely reduces squirrels' vulnerability to larger predators in winter. In comparison to its larger competitors, weasel observations peaked in winter and were lowest in summer and autumn. Weasels' small body size enables individuals to easily enter both the subnivean zone and subterranean burrows. We see a similar trend reflected in the increased presence of squirrels in the winter diet of weasels (Lisgo 1999). Weasels are mainly a terrestrial predator. Although they can climb, their agility to climb trees is superseded by their prowess of hunting in burrows (Studd et al. 2015). Thus, in warmer seasons, when squirrels are spending most of their time in trees, they are a trickier prey to catch. Correspondingly, weasel observations were positively influenced by the interaction between nest type and squirrel activity. Weasel presence increased at burrow nests when squirrel activity increased, potentially reflecting higher foraging effort near burrows where prey are more accessible. For martens and red foxes, although no statistically significant effect was detected the results cannot be discounted. We observed more than one nest type in only one of the four years of our study, limiting our ability to fully assess the potential influence of nest type on predator observations. A study spanning additional years with more frequent observations of multiple nest types would provide a stronger basis for determining whether martens and red foxes are affected similarly to weasels.

In contrast to our predictions, predator observations were highest in summer and autumn for most predators. The increase in predator observations is likely due to the increased abundance and availability of squirrels. Squirrels and other small prey are more prevalent in the summer after the breeding season. During this time, juveniles emerge from their nests and become more independent, increasing their exposure to predators (Zielinski et al. 1983, Larsen & Boutin 1994). Squirrels typically breed from May to July, and in late summer, juveniles will disperse from their natal areas to find their own territories (Larsen & Boutin 1994, Berteaux & Boutin

2000, Haughland & Larsen 2004, Cooper et al. 2017). Juveniles' small stature and inexperience make them particularly vulnerable to predation while dispersing (Rödel et al. 2015, Hendrix et al. 2020). Squirrels are also vulnerable to predators during the harvesting period. In late summer and into autumn, juvenile and adult squirrels harvest spruce cones for their winter food caches. Individuals clip cones from the tops of trees and carry the cones back to their midden, often centralized within the individual's territory (Smith 1968, Archibald et al. 2013). The increased amount of time squirrels travel on the ground transferring the cones to their middens increases their vulnerability to predators (Yoder et al. 2004, Rödel et al. 2015) and compromises their vigilance (Higginson et al. 2012). Additionally, the low tree density of our study area exacerbates squirrel's vulnerability by forcing squirrels to spend more time travelling on the ground. In open areas, foraging individuals often have increased vulnerability to predators, in comparison to covered areas, or areas with dense vegetation, where individuals have increased access to shelter (Abdulwahab et al. 2019). Our findings on frequency of predator observations are supported by seasonal diet reconstructions of martens in our study area. The winter diets of martens showed little to no contributions of squirrels, in comparison to late summer diet, where squirrels contributed approximately 24% of marten diets (Chapter 3). Overall, the fluctuations in predator observations can be attributed to seasonal changes squirrels' behaviour and accessibility.

Conclusion

Red squirrels acclimatize seasonally by reducing activity and energy expenditure at cold temperatures in winter (Brigham & Geiser 2012). Our research suggests squirrels at the sub-Arctic treeline select burrows as year-round nests due to their increased thermal benefits in winter. Although burrow use is not novel for red squirrels, to our knowledge, our study is the first to highlight burrow use as the primary nest preference for a population of squirrels. However, our research further suggests that while burrows protect squirrels from the harsh winters at the treeline, this nest type increases squirrels' vulnerability to burrowing predators.

Climate change is altering the vegetation patterns and modifying the thermal environment within the sub-Arctic treeline (Payette et al. 2001, Mamet & Kershaw 2011). Understanding why species exploit specific nest types in northern environments can help to understand the extent to which climate-based changes will influence the value of shelters and microhabitats in the future.

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Tables

Table 4.1: Annual nest types used by radio-collared squirrels (n = 47) from 2020-2023 tracked near Churchill, MB

Year	Radio-collars deployed	Successfully tracked squirrels	Burrow nests	Dreys
2020	9	9	5	4
2021	12	11	11	0
2022	13	11	11	0
2023	13	12	12	0

Table 4.2: Results of post-hoc Tukey tests comparing seasonal and annual variation in predator observations from a total of 17 trail cameras (2020 n = 8; 2021 n = 10; 2022-2023 n = 17). Data was collected from August 2020 to August 2023 near Churchill, MB, Canada.

Model	Estimate	SE	z	p-value
All predators				
Autumn:Spring	-0.58	0.14	-1.21	0.020
Autumn:Summer	0.01	0.13	0.14	0.774
Autumn:Winter	-1.31	0.14	-2.33	0.001
Summer:Spring	-0.53	0.13	-3.72	0.021
Summer:Winter	-1.51	0.12	-4.62	< 0.001
Winter:Spring	-0.06	0.14	-0.44	0.075
Foxes				
Autumn:Spring	-0.80	0.10	-0.61	0.030
Autumn:Summer	0.09	0.11	0.20	0.782
Autumn:Winter	-1.32	0.13	-1.61	0.002
Summer:Spring	0.71	0.12	-0.91	0.031
Summer:Winter	-1.51	0.22	-2.21	0.011
Winter:Spring	-0.04	0.14	-0.34	0.476
Marten				
Autumn:Spring	-0.51	0.11	-0.42	0.021
Autumn:Summer	0.07	0.13	0.13	0.508
Autumn:Winter	-1.06	0.25	-1.17	0.007
Summer:Spring	-0.20	0.13	-0.24	0.025
Summer:Winter	-0.13	0.20	-0.17	0.036
Winter:Spring	-0.06	0.11	-0.11	0.738
Weasels				
Autumn:Spring	0.02	0.15	0.09	0.070
Autumn:Summer	-0.05	0.10	-0.13	0.310
Autumn:Winter	2.13	0.18	1.25	0.003
Summer:Spring	0.14	0.13	0.41	0.421
Summer:Winter	1.73	0.15	1.52	< 0.001
Winter:Spring	1.33	0.11	0.93	< 0.001

Figures

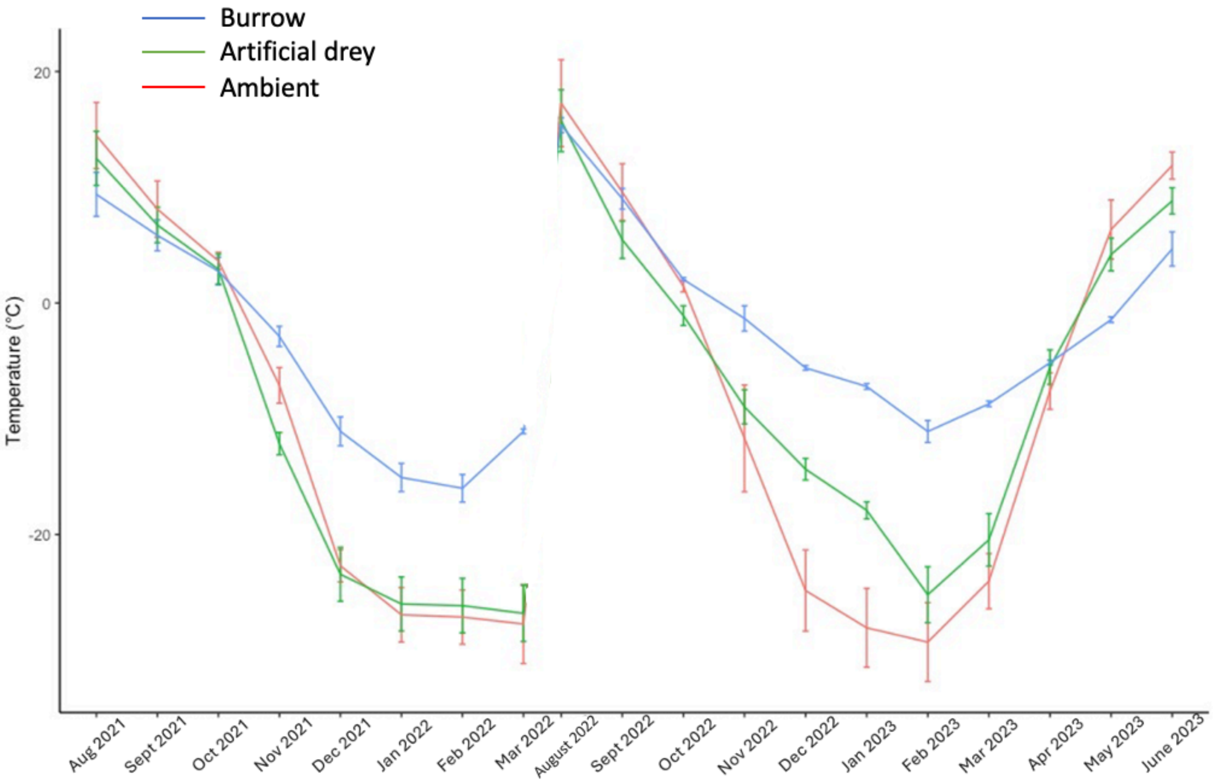

Figure 4.1: Monthly means (SE) of dataloggers recording the temperatures inside burrows (n = 6) and artificial dreys (n = 3) and ambient temperature (n = 3) from August 2021 to March 2022 and August 2022 to June 2023 near Churchill, MB, Canada.

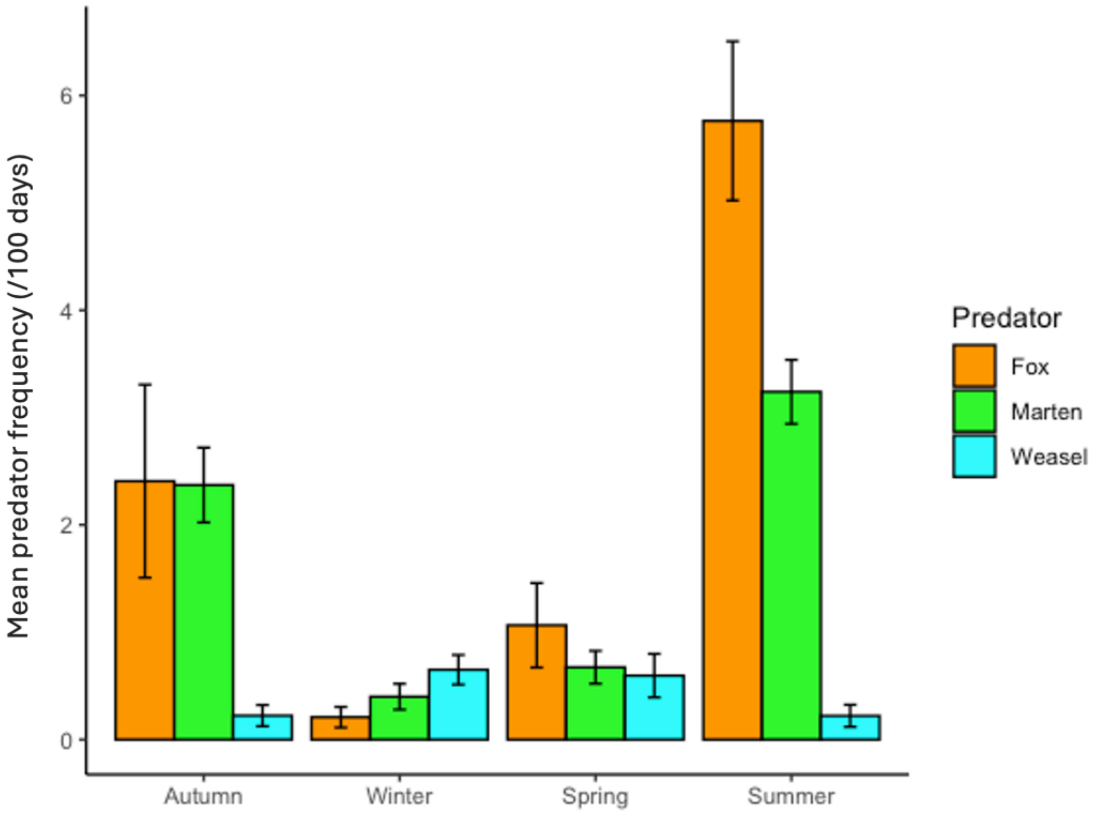

Figure 4.2: Seasonal mean (+SE) predator observations, standardized per 100 trap days per camera, for red foxes, American martens, and short-tailed weasels. Seasonal observation frequency was calculated from the images collected by 17 trail cameras (n = 8 2020-2021; n = 10 2021-2022; n = 17 2022-2023) deployed facing a midden at inhabited squirrel sites near Churchill, MB, Canada from August 2020 to August 2023.

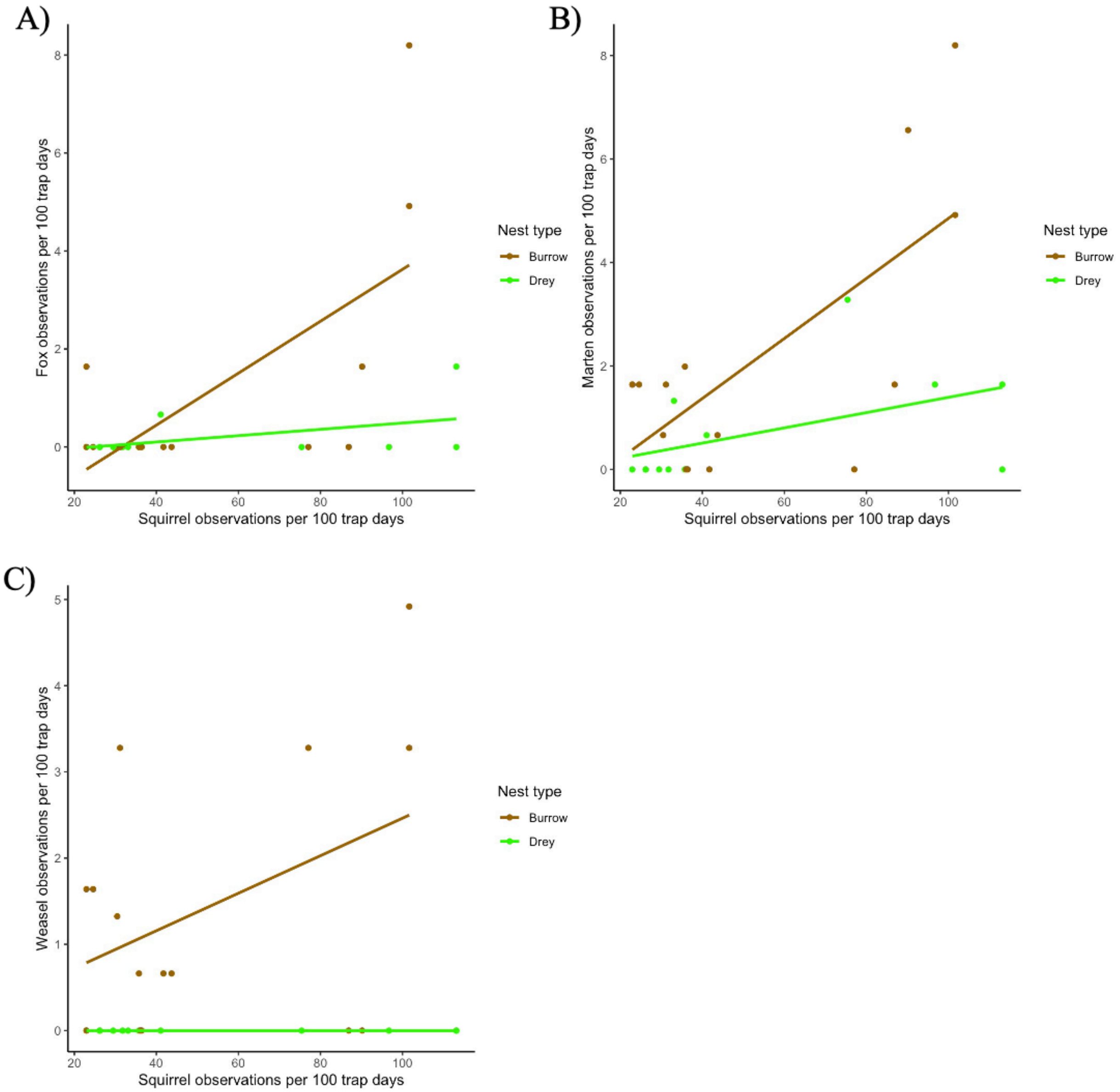

Figure 4.3: The effect of squirrel activity and nest type on observations of A) red foxes, B) American martens, and C) short-tailed weasels at seven camera trap sites near Churchill, MB, Canada. Data were collected from August 2020 to June 2021 and observation frequencies were standardized to number per 100 trap days across three seasons (autumn, winter, and spring).

Supplementary Tables

Table S4.1: Habitat variables measured at study sites (n = 29). Habitat surveys were conducted within 4 circular (15 m radius) plots per site. Within each plot, all trees > 5 cm diameter at breast height (DBH) were counted and classified by species (black spruce, white spruce, or tamarack), and converted to density. The number of dead trees was also recorded. The site-level mean DBH was calculated using values from all four plots. Basal area was calculated for each site using mean DBH and tree density. Vegetation composition for each site (% of stems that were black spruce, white spruce, tamarack, or dead) was calculated using total stem counts pooled across the four plots. Shrub cover (%) was visually estimated within four equal subsections of each circular plot and averaged across the four plots to derive site-level values.

Characteristic	Mean (± SE)
Tree density (stems > 5 cm DBH ha^{-1})	642.7 (71.3)
Mature tree density (stems > 20 cm DBH ha^{-1})	4.7 (1.6)
DBH (cm)	8.5 (2.3)
Basal area (m^2/ha)	3.8 (8.1)
Black spruce (%)	0.1 (0.1)
White spruce (%)	73.6 (3.7)
Tamarack (%)	26.3 (3.7)
Dead trees (%)	2.5 (0.8)
Shrub cover (%)	36.1 (1.5)

Supplementary Figures

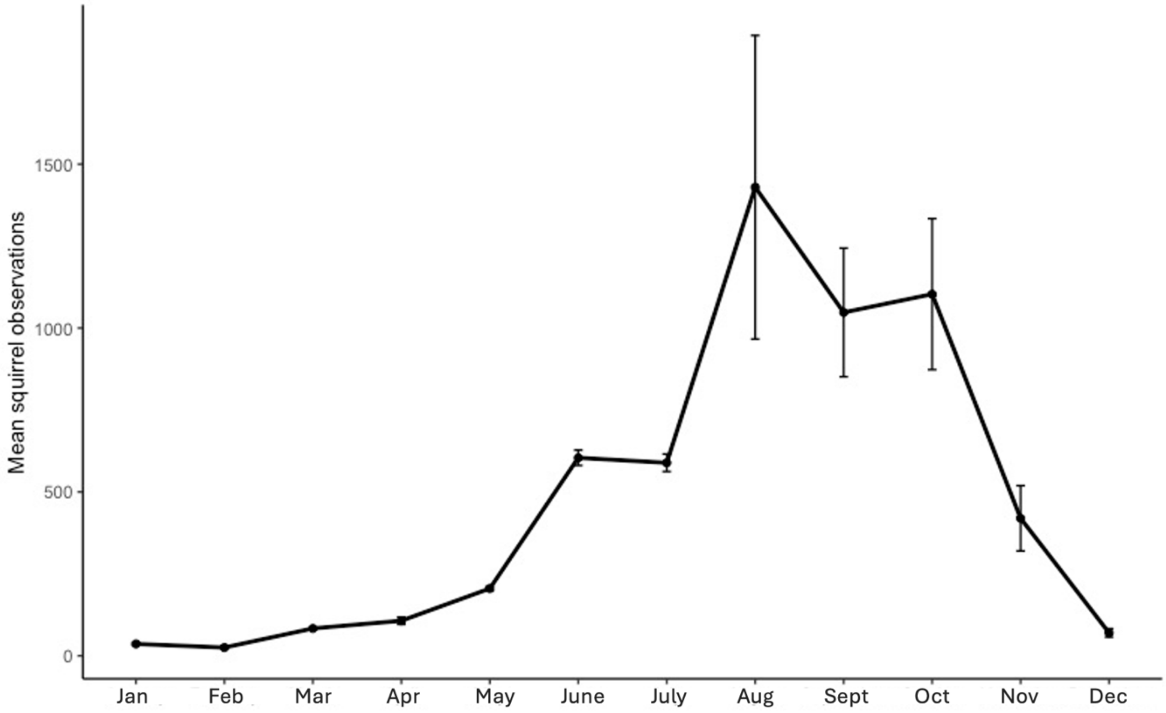

Figure S4.1: Monthly means (SE) of squirrel observations, estimated from motion activated trail cameras (n = 17) installed facing squirrel middens from August 2020 to August 2023, near Churchill, MB, Canada. Observations varied seasonally ($F_{3,23} = 16.33$, $p < 0.001$) but not annually ($F_{3,23} = 1.14$, $p = 0.355$), so monthly data were averaged across all years.

Chapter 5: Dynamic foraging: Seasonally specialist behaviours in a generalist predator at the sub-Arctic treeline

Abstract

Optimal foraging theory suggests that organisms select food sources that maximize their energy gain, while minimizing the costs of acquisition, and switch to alternative sources when their preferred food items are not readily available. Therefore, dietary niche breadths can be dynamic, and predators may exhibit both generalist and specialist strategies in response to annual and seasonal changes in resource diversity and availability. At the sub-Arctic treeline, few species remain year-round and many small mammals retreat into the protection of the subnivean zone during winter, and this reduced prey availability in winter may restrict the dietary niche of predators. We reconstructed the diet of American martens (*Martes americana*) at the sub-Arctic treeline using stable isotope analysis and examined whether seasonal and annual prey availability influenced the diets of martens in late summer and winter. We collected 341 marten carcasses from local fur trappers near Churchill, MB, Canada, from 2020 to 2023, along with samples of their prey. Red squirrels (*Tamiasciurus hudsonicus*) were important late-summer prey for marten, but snowshoe hares (*Lepus americanus*) were their primary prey year-round. The dietary niche breadth of martens was smallest in winter when they primarily consumed snowshoe hares (~ 80%). In contrast, late-summer diets were more diverse, with a larger niche breadth, and included a significant proportion of hares, squirrels, and voles (approximately 27%, 23%, and 28%, respectively). Prey abundance fluctuated throughout the study period, with variation differing among species. Squirrels remained the most numerous prey, followed by voles and snowshoe hares, suggesting a possibly seasonal selection for larger, less abundant prey. Climate change is altering sub-Arctic vegetation, potentially increasing prey abundance. Understanding

marten diets in low-resource environments offers insights into behavioural and dietary plasticity along latitudinal gradients and can aid in managing this important boreal fur-bearing species.

Introduction

Foraging theory posits that organisms employ foraging behaviours or decisions that maximize energy gain, while minimizing costs of acquisition (Pyke 1984, Baudrot et al. 2016, Whitney et al. 2018). Predators often forage opportunistically and base their foraging on prey abundance and accessibility (Slauson & Zielinski 2017). Changes in prey abundance can alter predator foraging strategies, resulting in prey-switching behaviour, where a predator's reliance on specific prey species increases as the prey species' population rises. As the abundance of a particular prey species declines, predators will switch to foraging on more abundant alternative prey (Ben-David et al. 1997, Kjellander & Nordström 2003, Sundell et al. 2003). Thus, a species' dietary strategy is driven by how they respond to changes in resource availability. Facultative generalists have a broad dietary niche and will prey-switch when specific prey is more abundant (Shipley et al. 2009). In contrast, facultative specialists exhibit narrower dietary niches but will expand their dietary niche breadth when primary prey are scarce (Pagani-Núñez et al. 2016). However, dietary niche breadths can be dynamic, and predators may exhibit both generalist and specialist strategies in response to annual and seasonal changes in resource diversity and availability.

While prey abundance is a commonly used predictor of predator diet, the presence of prey does not guarantee their accessibility to predators (Andruskiw et al. 2008). Seasonal shifts in prey availability can force predators to modify their prey selection, particularly those that remain active throughout the winter (Whitney et al. 2018). In North America's boreal forest, small mammals such as voles, shrews, and mice retreat under the snow in winter to seek thermal refuge and are relatively inaccessible to most predators (Aitchison 2001, Duchesne et al. 2011, Poirier et al. 2023). Although inhabiting the subnivean zone does not entirely protect small mammals from smaller predators such as short-tailed weasels (*Mustela erminea*), it can reduce the efficacy of larger predators (Lima 1990, Jedrzejewska & Jedrzejewski 1998, Duchesne et al. 2011, Penczykowski et al. 2017). Above the snow, generalist predators such as red foxes (*Vulpes vulpes*), American martens (*Martes americana*), Pacific martens (*Martes caurina*), and bobcats (*Lynx rufus*) focus on more accessible prey such as tree squirrels (O'Donoghue et al. 2001, Newbury 2013), which remain active year-round and are often on the snow surface (Steele 1998, O'Donoghue et al. 2001), and ungulate carrion (Randa et al. 2009, Needham et al. 2014). The sub-Arctic treeline at the edge of the boreal forest has a low diversity of resident (non-migratory) prey species.

American martens are a small-bodied mustelid species primarily found in North America's mature boreal and mixed forests (Andruskiw et al. 2008, Yom-Tov et al. 2008, Twining et al. 2019). They are considered a generalist predator, feeding on various small mammals, fish, birds, eggs, invertebrates, and vegetation, although voles are widely considered the primary prey of martens (Martin 1994, Zhou et al. 2011). Martens will also opportunistically scavenge on ungulate carrion such as white-tailed deer (*Odocoileus virginianus*) (Cumberland et al. 2001), moose (*Alces alces*), and caribou (*Rangifer tarandus*) (Gosse & Hearn 2005). The selection of prey by martens has been debated in the literature. Some studies suggest that martens prefer certain prey species, particularly voles (Thompson & Colgan 1990, Buskirk 1992), while others argue that they hunt based on prey abundance (Andruskiw 2003) and availability (Coffin et al. 1997, Gosse & Hearn 2005). However, the need for larger prey to meet winter energetic demands may also play a role in marten foraging strategy (Cumberland et al. 2001). Winter is a challenging season for mammals, particularly for those that do not hibernate or undergo periods of torpor (Taylor & Buskirk 1994, Boonstra 2004). For predator species, larger body sizes (Tamburello et al. 2015, Portalier et al. 2019), high resting metabolic rates (Muñoz-Garcia & Williams 2005, Gilbert et al. 2009), and the increased effort needed to locate and capture prey in the snow ultimately increases their energetic demands in winter (Bull & Heater 2000, Menzies et al. 2022). Foraging for larger prey species can provide a greater energy gain for predators (Cumberland et al. 2001, O'Brien et al. 2005, Slauson & Zielinski 2017), although more energy may be expended to catch larger prey (Bull 2000).

This study assessed the annual and seasonal variation in American marten diet in an area of low prey diversity in response to variations in prey availability. We used stable isotope analysis (δ^{13}C and δ^{15}N) to reconstruct martens' late-summer and winter diets. We quantified the density and biomass of known marten prey species, including red squirrels (*Tamiasciurus hudsonicus*), southern red-backed voles (*Clethrionomys gapperi*), and snowshoe hares. We hypothesized that the diet of martens would reflect the 1) annual abundance of their primary prey species, voles, with further (2) seasonal shifts in response to changes in prey availability. Therefore, we predicted that martens would primarily feed on voles during years with high abundance, while red squirrels would constitute a larger proportion of the marten diet in winter, when voles are less accessible in the subnivean zone but squirrels remain active above the snow (O’Donoghue et al. 2001, Newbury 2013, Selonen et al. 2016). Additionally, we used stable isotopes to investigate the annual and seasonal dietary niche breadth in marten diets. We expected a more specialized diet in years of high vole abundance and in winter when few prey species are available above the snow.

Methods

Study area

Our study area was the region of treeline near Churchill, MB (58°45’ N, 94°04’ W) on the western edge of Hudson Bay. This open-canopy forest at the edge of the boreal forest and Arctic tundra has a low tree density of mature trees (approximately 600 stems/ha) primarily composed of white spruce (*Picea glauca*), black spruce (*Picea mariana*), and tamarack (*Larix laricina*) (Lafleur 1999). The region is characterized by long winters and cool summers, with snow cover lasting approximately eight months of the year (Scott et al. 1993, Duguay et al. 2002, Da Silva 2023). In our study area, the potential prey and carrion base for martens includes southern red-backed voles, red squirrels, snowshoe hares, willow ptarmigan (*Lagopus lagopus*), and moose.

Estimating prey abundance

We estimated the annual abundance of southern red-backed voles using mark-recapture data collected each June from 2019 to 2023, and of red squirrels using data collected from 2020-2023, with the exception of 2020, when trapping occurred in August. Voles were trapped along two 300 m transects with 20 stations each at 15 m intervals. We deployed two Sherman and one Longworth live-traps within 2 m of each station, baited with peanut butter and whole oats. Traps were set for 72 hours and checked every eight hours (McDonald et al. 2017). Squirrels were trapped using Tomahawk live-traps (model #202) at sites located every 250 m along a 7 km transect (4 traps per site, n=26 sites) (Koprowski 2002). The traps were opened every morning at 6:00, checked every four hours, and closed every evening at 21:00 for five consecutive days. We used the rMARK package (version 3.0.0) in R (R Core Team 2021), which uses the program MARK, version 11, to estimate the annual abundance of voles and squirrels using a closed population model (White & Burnham 1999, Schwarz & Arnason 2014). We considered four models in our analysis: M_0 (capture probability was the same for all individuals at all trapping events), M_h (capture probability varied among individuals), M_t (capture probability varied among trapping events), and M_b (capture probability differed as a behavioural response to previous trap experiences). We selected the best fitting model using Akaike's Information Criterion corrected for small sample size (AICc) (Akaike 1998). The squirrel mark-recapture data were collected from fewer sites in 2020 ($n = 9$). Program MARK returns an estimate of the number of individuals (N) present within the area sampled during trapping, which we then converted to density. For voles, we assumed each trapping station sampled the same area, so the area sampled by the two transects was 0.90 ha. For squirrels, we assumed an effective sampling width of 50 m along the 7 km transect, for an effective sampling area of 35 ha. To ensure that the increase in sampling sites did not bias the estimates, we repeated the analysis using only data from sites common to all years.

We estimated annual snowshoe hare density from 2019 to 2023 using faecal pellet counts (Murray et al. 2002, Warret Rodrigues 2023). Each year we counted and removed all faecal pellets from eight transects, each with ten 1 m^2 circular plots placed 30 m apart (Ewacha et al. 2014, Freeth et al. 2016). We averaged the number of pellets from the 10 plots for each transect and estimated hare density (#/ha) using a regression equation developed by comparing mark-recapture estimates of snowshoe hares and faecal pellet counts in a similar plot design ($y = 0.398 + 0.060x$, where x represents the mean number of pellets) (McCann et al. 2008).

To account for differences in prey size (Cumberland et al. 2001), we used the annual density estimates and estimated mean body mass for each prey species (hare 1400 g, (Hodges et al. 2006); voles 19 g, this study; and squirrels 212 g, Windsor et al. 2025) to estimate annual biomass (kg per hectare).

Sample collection and preparation

We collected samples of marten and potential prey for diet reconstruction using stable isotope analysis. We obtained marten carcasses (n = 341) legally harvested from local trappers in Churchill, MB at the end of the trapping season (November-February) from 2019-2020 to 2022-2023 (Manitoba 2024a). The carcasses were kept frozen at the Churchill Northern Studies Centre until processing each April. For each carcass we determined sex and measured body mass and length. We classified martens as juvenile (< 1 year) or adult (> 1 year) by measuring the length of temporal muscle coalescence. Individuals with complete temporal muscle separation or a dividing point >1 mm were considered adults (> 1 year old) (Poole et al. 1994, Flynn & Schumacher 2016). We collected samples of hair (approximately three to four clippings each 0.5 cm from the hind feet) and muscle (two to three strips approximately 0.5-1 cm long from the bicep femoris and gluteus) for stable isotope analysis. Growth of their winter pelage begins in late summer and is complete by October (Pauli et al. 2009, Carlson et al. 2014, Witt et al. 2020), so hair samples represent diets assimilated in late summer into autumn. Muscle samples collected from martens harvested during the winter trapping season approximated diet assimilated from October to February.

We also obtained red squirrel and moose muscle samples from local hunters and trappers and used stable isotope data for snowshoe hare hair samples, and willow ptarmigan and red-backed vole muscle, as well as berries, previously collected from the study area (Friesen 2013, Warret Rodrigues 2023, Windsor et al. 2025).

All samples were kept frozen until preparation for stable isotope analysis. Hair samples were washed with mild soapy water twice to remove surface oils, rinsed thoroughly with water, and dried for 48 hrs in a drying oven. All muscle samples were freeze dried for at least 48 hrs and lipid extracted using petroleum ether in a Soxhlet apparatus, then homogenized (Elliott et al. 2017). We did not remove lipids from the berry samples, since vegetation contains low amounts of lipids (Ben-David 1996). We weighed 0.4-0.6 mg of hair and muscle samples and 2.5-3.0 mg of berry samples in a tin capsule using a Sartorius microbalance (Model Cubis II). We sent the prepared samples to the Great Lakes Institute for Environmental Research, University of Windsor, for analysis of carbon and nitrogen stable isotope ratios on a continuous-flow isotope ratio mass spectrometer (Delta V Advantage) coupled to a Costech 4010 Elemental Combustion system and a ConFlo IV gas interface.

Data analysis

All statistical analyses were conducted in R (version 4.0.5) (R Core Team 2021), and model assumptions were assessed and met for all tests. We used a 2-way Analysis of Variance (ANOVA) to test for differences in prey density and biomass among species and years. To determine if age structure of martens differed by sex, we used chi-square (a separate test each year). We used a linear model to test for variation in marten body mass with year, age, sex, and their interactions. To compare individual years, we ran post-hoc pairwise comparisons using the *emmeans* package in R and adjusted for multiple testing using the Tukey's method.

We used stable isotope analysis to recreate marten diet and examine annual and seasonal variation in diet. The isotopic composition of a consumer's tissues reflects the composition of its diet, weighted by the proportions of the dietary items. Stable isotope ratios typically increase during the assimilation of dietary sources into consumer's tissues due to fractionation and isotopic routing, but the amount of increase varies with tissue type and diet source (e.g., plant vs animal (Caut et al. 2009)). Trophic discrimination factors (TDFs) are therefore used in diet reconstruction to account for these predictable isotopic shifts (Phillips 2012, Parnell et al. 2013). Trophic discrimination factors have not been measured for martens, and previously published studies have used TDFs estimated for red foxes (Roth & Hobson 2000, Carlson et al. 2014, Kirby et al. 2018, McAulay et al. 2020, Breault et al. 2021b) or factors estimated from captive mink feeding experiments (Ben-David 1996, Ben-David et al. 1997, Flynn et al. 2004). We estimated TDF values for marten muscle and hair from the SIDER (Stable Isotope Discrimination Estimate in R) package (Healy et al. 2018) in the program R (R Core Team 2021), which uses a Bayesian phylogenetic regression to estimate the TDF of a consumer using known values in phylogenetically related species (Healy et al. 2018). For models using marten muscle, we applied $\Delta^{13}C = 1.24 \pm 1.0‰$ and $\Delta^{15}N = 3.12 \pm 1.0‰$ to all prey source values. For models using marten hair, TDFs for prey were estimated as $\Delta^{13}C = 2.36 \pm 1.0‰$ and $\Delta^{15}N = 3.23 \pm 1.0‰$. For berries, we used previously published TDFs used for hair of Pacific marten consuming plant-based sources (Breault et al. 2021b) of $\Delta^{13}C = 2.0 \pm 0.3‰$ and $\Delta^{15}N = 5.6 \pm 0.3‰$ (Hilderbrand et al. 1996, Mowat & Heard 2006, Adams et al. 2017). However, TDFs for plant-based food sources have not previously been estimated for marten muscle, so we applied the difference between the SIDER-estimated TDFs for marten hair and muscle and used $\Delta^{13}C = 0.88 \pm 1.4‰$ and $\Delta^{15}N = 5.49 \pm 1.4‰$ for berries in the marten hair model. For prey hair samples (collected from snowshoe hares), we first subtracted 1.5‰ from $\delta^{13}C$ to reflect muscle values before adding the TDFs (Codron et al. 2007).

We used six food sources in our diet models: voles, squirrels, hares, ptarmigan, moose, and berries, and employed a K nearest-neighbour (KNN) randomization test to investigate whether their stable isotope ratios differed significantly. Pairwise comparisons were performed between all food sources, assessing whether the isotopic composition of one source was distinct from another. A Bonferroni correction was applied to account for the increased risk of Type 1 errors due to multiple comparisons (Rosing et al. 1998). We found six source groups were isotopically distinct (Table 5.1, Table S5.1).

We used Bayesian mixing models in the MixSIAR package (version 3.1.12) in R (R Core Team 2021) to estimate the relative proportions of food sources in marten diet (Parnell et al. 2013). We ran separate MixSIAR models for late summer and winter, with 'year' (4 levels) as a fixed effect, to test for annual variation in marten diet each season. We also ran additional seasonal MixSIAR models with 'age-sex class' (4 levels) as a fixed effect, to test for diet variation based on age and sex. All models used uniform prior distributions. We ran three parallel Markov-chain Monte Carlo (MCMC) chains (iterations = 1,000,000, burn-in = 500,000, thinning = 500) and checked model convergence with a visual assessment and the Gelman and Geweke diagnostic tests produced by MixSIAR (Geweke 1991, Jackson et al. 2011, Gelman et al. 2014). Variables for the Gelman-Rubin diagnostic were <1.01 and between -1.96 and 1.96 for the Geweke diagnostics. Additionally, the effective sample size (ESS) for all variables was above 800 and multiplicative error values for Epsilons 1 and 2 were <1. We used the *rjags* package (version 416) in R (version 1.4.1103) (R Core Team 2021) through MixSIAR to calculate the posterior distributions of the estimated proportions of each food source. We compared diet proportions seasonally using the mean and 95% Bayesian credibility intervals (CI) of proportional diets estimated using two tissue types, hair and muscle.

We also used stable isotope analyses to estimate dietary niche breadth, a measure of diet diversity. Organisms with a narrow dietary niche likely have a highly specialised diet, whereas individuals with a broad dietary niche likely consume a wide range of resources (Bearhop et al. 2004, Jackson et al. 2011). For generalist species like martens, dietary niche breadth may shift with seasonal and annual changes in food availability. Isotopic niche breadth can be modelled using Bayesian ellipses, which represent the variability in the distribution of the isotopic compositions of samples (Jackson et al. 2011, Jackson et al. 2019). We estimated dietary niche breadths for martens based on age-sex class, and season using an ellipse-based approach in the SIBER (Stable Isotope Bayesian Ellipses in R) package (version 2.1.9) in R (Jackson et al. 2011, Manlick et al. 2019, R Core Team 2021). We measured isotopic niche breadth for marten hair and muscle samples, representing late-summer and winter diets, from 2020 to 2023 using standard ellipse areas (SEA) (Jackson et al. 2011). For each analysis, SIBER generated the Bayesian Standard Ellipse Area (SEAb) by running 10,000 MCMC iterations and generating a 95% credible interval (CI) for the mode for each ellipse. To compare niche breadths between groups, we compared the posterior distributions of Bayesian standard ellipse areas (SEAb). For each pairwise comparison, SIBER calculated the proportion of posterior draws where one ellipse's area was smaller than the other (Jackson et al. 2019). Probabilities of differences in isotopic niche breadths (P) range from zero to one, where values close to 0.5 indicate similar ellipse sizes, and values near 0 or 1 suggest distinct differences in ellipse sizes (0 indicating the first ellipse is smaller, and 1 indicating it is larger).

Results

Prey abundance

Our best fitting model for squirrel and vole abundance was the behavioural (M_b) model. The next closest model was the individual heterogenic model (M_h) (Table S5.2). Abundance estimates varied among prey species ($F_{2,6} = 31.38$, $p < 0.001$), with a significant interaction between species and year ($F_{2,6} = 4.18$, $p = 0.014$). Similarly, biomass estimates varied between prey species ($F_{2,6} = 11.87$, $p < 0.001$), with a significant interaction between prey species and year ($F_{2,6} = 2.88$, $p = 0.006$). On average from 2019-2023, hares had the highest density (mean ± SE) (4.2 ± 1.5 ha^{-1}) and biomass (5,895 ± 1,633 g/ha). Voles (1.1 ± 0.6 ha^{-1}) and squirrels (0.8 ± 0.2 ha^{-1}) had similar density estimates, although squirrels had a higher mean biomass (207 ± 38 g/ha) compared to voles (20 + 12 g/ha) (Table 5.2). Density estimates from the reduced-site model were similar to those from the full model across all years, suggesting that the subset from one year was not biased (Table 5.2).

Marten demographic and body size variation

From 2020 to 2023, we collected 341marten carcasses (147 adult males, 46 adult females, 40 juvenile males, 108 juvenile females). Age class distributions differed by sex each year (2020: $x^2 = 17.6$, $p < 0.001$; 2021: $x^2 = 15.7$, $p < 0.001$; 2022: $x^2 = 7.4$, $p = 0.006$; 2023: $x^2 = 33.3$, $p < 0.001$). Marten mass varied significantly by age ($F_{1,1} = 29.62$, $p < 0.001$), sex ($F_{1,1} = 251.00$, $p < 0.001$), and year ($F_{1,3} = 28.72$, $p < 0.001$). Adult males (mean ± SE, 1005 ± 13 g) were heavier than females (862 ± 37 g), while the mass of juvenile males (677 ± 13 g) and females (619 ± 17 g) were more similar, but the interaction between sex and age was not significant ($F_{1,1} = 1.33$, $p = 0.251$).

Post-hoc comparisons showed that martens collected in 2020 were heavier than in 2021 ($t = -2.89$, $p < 0.001$), 2022 ($t = -2.04$, $p < 0.001$), and 2023 ($t = -1.67$, $p < 0.001$). Mass did not differ significantly between 2021-2022 ($t = 0.42$, $p = 0.731$), 2021-2023 ($t = 0.19$, $p = 0.418$), or 2022-2023 ($t = 0.99$, $p = 0.226$). We found no significant interactions between year and sex ($t = 1.02$, $p = 0.149$) or year and age class ($t = 0.75$, $p = 0.283$).

Variation in marten diet

Stable isotope ratios of muscle suggested martens consumed primarily snowshoe hare (mean: 79.1%; CI: 65.3-100.0%) in winter with small contributions from squirrels (mean: 7.4%: CI: 3.2-12.4%), voles (mean: 8.8%; CI: 0.0-5.9%) and moose carrion (mean: 3.1%; CI: 0.0-16.1%) (Figure 4.1). Stable isotope ratios of hair suggested that marten diets were more diverse in late summer. In late summer, martens largely consumed hares (mean: 41.3; CI = 6.4-66.3%) and similar proportions of voles (mean: 23.1; CI = 0.0-34.5%) and squirrels (mean: 20.6; CI: 0.0-32.8%). Ptarmigan (mean: 1.2; CI: 0.0-3.5%) and berries (mean: 4.8; CI: 0.0-10.2%) were present in late-summer diet but less prominent (Figure 5.1).

Adult female martens consumed more squirrels in winter than other age-sex classes, but consumption of other foods did not differ between age-sex classes (Figure S5.1, Table S5.3). In 2022, winter consumption of squirrels and voles decreased compared to other years. Hare, moose, ptarmigan and berry consumption were similar across years (Figure 5.2 and Table S5.4).

Summer diets were similar among age-sex classes (Figure S5.1, Table S5.3). We found squirrel and vole consumption varied annually, while hare consumption did not. Moose, berry, and ptarmigan consumption remained consistently low in late summer across all years (Figure 5.2 and Table S5.4).

Dietary niche breadth

Dietary niche breadths based on the Bayesian ellipses were similar between male (SEAb = 2.65; CI: 2.35-2.90) and female (SEAb = 2.35; CI = 2.12-2.63) adult martens (P adult male < adult female = 0.62). Juveniles had smaller niche breadths than adults (female SEAb = 1.50; CI = 1.34-1.62; male: SEAb = 1.98; CI = 1.79-2.13) and juvenile females had the smallest niche breadth compared to other age-sex classes (juvenile female < adult female = 0.34; P juvenile male < adult male = 0.27; P juvenile female < juvenile male = 0.41). The dietary niche breadth was larger for late-summer diet (SEAb = 1.78; CI = 1.71-1.86) compared to winter diet (SEAb = 1.22; CI = 1.17-1.27) with a high probability that the niche breadth of winter diets was smaller than late-summer diets (P = 0.18) (Figure 5.3).

The dietary niche breadth for winter was similar between 2019-2020 and 2020-2021 (P 2019-2020 < 2020-2021 = 0.35) and similar (P 2021-2022 < 2022-2023 = 0.56) but smaller in 2021-2022 (P 2019-2020 < 2021-2022 = 0.97; P 2020-2021 < 2021-2022 = 0.76) and to a lesser extent 2022-2023 (P 2019-2020 < 2022-2023 = 0.98; P 2020-2021 < 2022-2023 = 0.65) (Figure 5.4, Table S5.5). Annual late-summer ellipses were similar between 2021and 2022 (P 2021< 2022 = 0.59), and smaller in 2020 (P 2020 < 2021 = 0.37; P 2020 < 2022 = 0.00) and 2023 (P 2023 < 2020 = 0.17; P 2023 < 2021 = 0.04; P 2023 < 2022 = 0.07) (Figure 5.4, Table S5.5).

Discussion

While martens consume a variety of prey species across their distribution, voles are considered a primary food source (Thompson & Colgan 1990, Martin 1994, Buskirk & Zielinski 1997, Flynn et al. 2004, Hales et al. 2008). However, the diversity of marten diet decreases with increasing latitude (Martin 1994, Zhou et al. 2011). Similarly, other mustelids like ermines (*Mustela erminea*) have shown a shift from semi-generalists to specialists in response to prey abundance at higher latitudes (Hanski et al. 1991, Hanski & Henttonen 1996). We found that snowshoe hares were the primary food source for martens from 2020 to 2023. In winter, martens' diet almost entirely consisted of hares. In late summer, martens' diet primarily consisted of a combination of hares, voles, and squirrels.

While we did not observe significant variation in diet between age-sex classes of martens. other studies have documented sex-based dietary differences, with males consuming larger prey such as ruffed grouse and snowshoe hare, while females tend to consume more small rodents and birds (Nagorsen et al. 1989, Poole & Graf 1996, Bull 2000). These differences have been attributed to sexual dimorphism in body size, as adult female martens are typically 20-40% smaller in mass than males (Moors 1980, Buskirk & McDonald 1989, Schulte-Hostedde et al. 2011), which may influence prey selection. However, other studies have not found significant sex-based dietary variation (Thompson 1987, Ben-David et al. 1997, Andruskiw et al. 2008, Hales et al. 2008), consistent with our results. The similarity in diet across age-sex classes may reflect the ability of both male and female martens to efficiently exploit a shared set of prey types. Thomas and Colgan (1990) suggested that female martens were equally proficient hunters despite their smaller size, and a similar explanation may apply to martens in our study area.

Similarly, we did not observe any significant diet variation between adult and juvenile martens, supporting previously published studies (Thompson & Colgan 1990, Ben-David et al. 1997, Andruskiw et al. 2008, Hales et al. 2008). By winter, juveniles may have grown and developed sufficient hunting skills and experience to hunt the same prey as adults (Thompson & Colgan 1987, Hales et al. 2008). Nagorsen et al. (1989) further suggested low dietary partitioning between juvenile and adult martens can result from low prey diversity. The diversity of mammalian prey generally decreases with increasing latitude (Chernov 1995, Hanski & Henttonen 1996, Willig et al. 2003, Callaghan et al. 2004). At the sub-Arctic treeline, summer sees an influx of prey species such as shorebirds and geese who have migrated north for the breeding season. However, few prey species remain year-round and of the small mammals who remain, many retreat into the protection of the subnivean zone during winter, thus limiting available prey diversity in winter.

According to optimal foraging theory, generalist predators tend to forage for prey with high abundance and switch to alternative prey species when preferred prey are scarce or less profitable. While martens typically forage on voles in abundant years, they switch to alternative prey such as mice, squirrels, and shrews when voles become scarce (Thompson & Colgan 1990, Coffin et al. 1997). In our study area, squirrel biomass increased moderately from 2020-2023, from 98-273 g/ha. In contrast, vole biomass ranged from 0-65 g/ha, and snowshoe hare biomass peaked in 2019 (12,500 g/ha) then remained consistent (3,000-5,100 g/ha) from 2020-2023. Annual prey density estimates in our study area were similar to previously published estimates of low-cycle years for snowshoe hares (Freeth et al. 2016, Oli et al. 2022) and southern red-backed voles (Fauteux et al. 2015, Tisell et al. 2019). Although our study spanned four years, the typical length of a vole cycle, we observed persistently low variation in prey abundance. Freeth et al. (2016) similarly reported consistently low hare densities in our study area over a longer time span, suggesting that both voles and hare populations exhibit low-amplitude cycles and that prey densities in our system are generally low, rather than temporarily in a low phase of a typical population cycle.

While some studies have observed a large proportion of smaller prey, primarily mice and voles, in winter diets (Poole & Graf 1996, Bull 2000, Hales et al. 2008), martens commonly rely on larger prey in winter, including snowshoe hare and tree squirrels, which can comprise up to 35% of their diet (Thompson & Colgan 1990, Lachowski 1997). In our study area, snowshoe hares made up approximately 80% of marten winter diet, contributing to a narrower dietary niche breadth. Squirrels, by contrast, comprised a smaller proportion of the diet, ranging from 1.5% to

10.7%, suggesting that even when squirrels were more abundant than hares, martens still primarily selected hares.

This prey selection pattern likely reflects both prey availability, since small prey often seek refuge under the snow, and energetic demands. Although martens typically reduce activity in winter (Gilbert et al. 2009), they cannot accumulate substantial body fat (Harlow 1994, Zielinski 2000), and cold climates can exacerbate energy requirements. Martens farther south require 709 kJ/d to meet their energetic demands (Gilbert et al. 2009), approximately 4-5 voles per day (Carlson et al. 2014), making larger prey, such as hares, a more efficient energy source (Kujawa et al. 2014, Slauson & Zielinski 2017, Fargallo et al. 2020). In colder northern latitudes like our study area, where mean winter temperatures range from -5 to -30°C and extreme temperatures reach as low as -50 °C (Environment-Canada 2023), martens may have stronger incentive to target the most profitable prey. Hares provide an estimated 1,350 Kcal, compared to 470 Kcal for squirrels, and just 30-35 Kcal for voles and mice (Powell 1981, Thompson 1987). Even when there is a high abundance of small prey, larger prey can contribute over 85% of caloric intake in winter diets (Thompson 1987, Cumberland et al. 2001), consistent with our findings.

The low contribution of squirrels to marten winter diets is likely due to low density and decreased availability. Squirrels are often found in higher proportions of martens' winter diet, compared to smaller prey like voles, as they are more accessible above the snow (Brigham & Geiser 2012). However, squirrels in our study area spend much time underground. Data from trail cameras and radio-tracked squirrels suggest that these squirrels live in extensive underground burrows year-round (Chapter 5). Typically, squirrels are active throughout the winter above the snow and are particularly vulnerable while travelling to winter food caches

(Pruitt & Lucier 1958, Brigham & Geiser 2012). Owning a territory and, in turn, having access to nests and tunnels act as a crucial refuge for squirrels, reducing their vulnerability to predators (Larsen & Boutin 1994, Hendrix et al. 2020). Additionally, in response to low spruce seed production at the treeline, squirrels in our study area have a lower body mass (males 182-207g, females 194-217g) (Chapter 3) than other populations of red squirrels in in Alberta (males 239g, females 219g) (Patterson et al. 2015), British Columbia (235g) (Ransome & Sullivan 1997), Yukon (257g) (Descamps et al. 2008), and Arizona (220-230g) (Koprowski 2005). Therefore, snowshoe hares may represent an abundant and critical energy source when seasonal energy demands are high and smaller prey like squirrels and voles are less readily accessible.

Compared to winter, martens in late summer exhibited a broader dietary niche, with more diversity, mainly due to the increased consumption of voles and squirrels. Contrary to our predictions, squirrels were more prevalent in marten diets in late summer than winter, likely due to their increased accessibility. Squirrels and other small prey like voles become more available to predators in the summer and autumn, after the breeding season. Red-backed voles can have multiple litters, breeding from April until October. Young develop quickly and become independent approximately 20 days after birth (Hanski & Henttonen 1996). Red squirrels breed from May to July, usually producing a single litter, although multiple litters can occur during mast years (Boutin & Larsen 1993). Juveniles are usually independent and disperse in late summer to obtain territories and create a satisfactory food cache before winter (Berteaux & Boutin 2000, Haughland & Larsen 2004, Cooper et al. 2017). During this time, predators likely encounter juveniles at a higher rate (Zielinski et al. 1983, Larsen & Boutin 1994, Anderson & Boutin 2002). Due to their small size and lack of experience, juveniles are particularly vulnerable to predation while dispersing and looking for territories (Berteaux & Boutin 2000, Rödel et al.

2015, Hendrix et al. 2020). Additionally, in the late summer and into autumn, both juvenile and adult squirrels are busy harvesting spruce cones from trees for their winter food caches. The increased amount of time squirrels spend travelling on the ground transferring clipped cones to their caches further increases their vulnerability to predators. Therefore, the increased availability of squirrels in the summer and autumn likely makes them attractive seasonal prey for martens.

We observed modest interannual fluctuations in the late-summer diets of martens that coincided with species-specific changes in prey availability and biomass. Hare consumption was lowest in 2021 and 2022, which reflects prey availability in 2020 and 2021 respectively, while moose consumption peaked in 2021. Overall prey biomass was also lowest in 2020 compared to other years. Squirrel consumption was also lower in 2021 compared to 2020. The decrease in squirrel consumption is likely due to low squirrel density and biomass in 2020. Hare density and biomass were consistently low from 2020 – 2023 with a noticeable peak in 2019. The elevated hare consumption in 2020, which reflects prey availability in 2019, likely reflects a response to an increased hare density. Hare density estimates were inferred from pellet counts conducted in June, which predominantly reflect winter activity. As a result, there may be a time lag between the estimated hare density and the availability of hare as prey during late summer, which may confound our interpretation of marten response to prey abundance. Although vole consumption peaked in 2023, vole density and biomass were consistently low (peaking in 2020). As a common primary prey species for martens, the abundance of voles may have influenced the reduced consumption of hares.

Although moose were only a small contributor to marten diet (< 7.5% in winter and < 1.7% in summer), ungulate carrion has appeared in the diets of other marten populations

(Nagorsen et al. 1989, Ben-David et al. 1997, Cumberland et al. 2001, Powell et al. 2003, Gosse & Hearn 2005, Breault et al. 2021a, Pauli et al. 2022). According to Wilmers et al. (2003), natural deaths, predation by predators, and hunting by humans are the primary factors that make ungulate carcasses available to scavengers. The presence of carrion while hunting alternative prey is a low-cost opportunity that requires only the stripping of the carcass and fighting off possible competitors. As a result, carrion is an appealing and opportunistic food source for predators (Wilmers et al. 2003, Moleón et al. 2014). In northern Manitoba, the moose hunting season is from August to December, and hunting is limited to one moose tag license per two hunters (Manitoba 2024b). The increased presence of moose carrion in late-summer diets of martens and specifically in 2020, is likely due to the scavenging on remains left behind from predator kills and hunter harvests. While we have information on annual variations in moose densities, declines in moose populations in Manitoba (Shura & Roth 2013, Timmermann & Rodgers 2017) have led to provincial limits on hunting tags, which in turn could affect the availability of moose carcasses consumed opportunistically by martens as carrion, with fluctuations influenced by both hunting intensity and local predator populations, such as wolves.

While we observed little berry consumption by martens, berries are often a crucial seasonal food item in the summer and autumn diets of marten when they are more abundant (Nagorsen et al. 1989, Baker 1992, Ruggiero & Forest 1994, Twining et al. 2019) and can comprise up 34% of marten diets (Ben-David et al. 1997, Helldin 2000, Gosse & Hearn 2005). Berry yield fluctuates dramatically in the boreal forest and is strongly influenced by snow cover, as well as summer temperature and rainfall from up to two years prior (Krebs et al. 2009, Keogh et al. 2022). While we did not have data for berry abundance, berries are typically abundant in the boreal forest from July to September, although abundance can fluctuate dramatically as annual berry crops are largely determined by climatic factors (Krebs et al. 2009). Although ptarmigan did not contribute substantially to marten diets, previous studies indicate that Arctic and sub-Arctic ptarmigan populations may be influenced by snowshoe hare cycles through an alternative prey mechanism (Krebs et al. 2014, Boonstra et al. 2016).

Conclusion

Martens at the sub-Arctic treeline primarily consumed snowshoe hares, which were the most abundant and highest-biomass prey species across all years. In addition, we documented seasonal shifts in marten foraging behaviour. During winter, martens focused on larger, more abundant and high-return prey, likely reflecting the need to maximize energetic gain when metabolic demands are high and smaller prey are less accessible. In late summer, martens employed a more diverse foraging strategy, incorporating a broader range of prey as availability and accessibility increased. We suggest that the seasonal changes in marten diet reflect their capitalization of seasonal variation in availability of prey species and their reliance on more energetically profitable prey.

In general, prey diversity decreases with increasing latitude. At the sub-Arctic treeline prey populations including red squirrels and voles are limited likely due to limited primary resources. However, climate change is altering vegetation patterns in the sub-Arctic and within the treeline, which may increase food availability, thereby increasing the abundance of future populations. An increased understanding of marten diet in a low-resource environment can provide insight into adaptations along latitudinal gradients and enhance our ability to manage important fur-bearing populations effectively.

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Tables

Table 5.1: $\delta^{13}C$ and $\delta^{15}N$ (‰) of muscle tissue of American marten and their diet sources near Churchill, MB, Canada, 2020-2023. For hares, the hair samples were measured and the $\delta^{13}C$ values were adjusted to reflect muscle.

		$\delta^{13}C$		$\delta^{15}N$	
Species	n	Mean	SE	Mean	SE
American marten	341	-25.28	0.05	7.45	0.04
Red squirrel	3	-23.59	0.18	3.42	1.07
Southern red-backed vole[1]	6	-24.63	0.33	2.24	0.96
Snowshoe hares[2]	2	-27.31	0.15	3.02	0.33
Moose	3	-25.51	0.39	3.07	0.18
Willow ptarmigan	6	-23.62	0.35	2.85	0.65
Berries[3]	97	-28.09	0.04	0.88	0.09

[1] Friesen 2013

[2] Warret Rodrigues 2023

[3] Windsor et al. 2025

Table 5.2: Annual estimates of density (ha^{-1}) and biomass (g/ha, based on average weights for each species) for marten prey species from 2019-2023 (95% CI in parentheses). Abundances were estimated from mark-recapture and faecal pellet counts near Churchill, MB, Canada.

	2019	2020	2021	2022	2023
Red squirrel					
Density	--	0.3 (0.3-0.5)	1.0 (0.9-1.1)	0.9 (0.8-1.0)	1.1 (1.0-1.2)
Biomass	--	98 (77-125)	241 (216-269)	217 (200-253)	273 (247-301)
Vole					
Density	0.0 (0.0-0.3)	3.4 (2.9-3.7)	1.4 (1.0-1.9)	0.0 (0.0-0.3)	0.5 (0.0-1.0)
Biomass	0 (0-6)	65 (55-108)	27 (19-36)	0 (0-6)	10 (0-19)
Snowshoe hare					
Density	8.9 (5.8-11.9)	3.2 (2.9-3.4)	2.1 (1.7-2.6)	3.6 (2.7-4.6)	3.3 (2.8-3.7)
Biomass	12,401 (8,180-16,230)	4,421 (4,050-4,801)	3,001 (2,400-3,590)	5,101 (3,811-6,391)	4,551 (3,950-5,151)
Reduced-site squirrel model (n = 9 of 26)					
Density	--	0.4 (0.3-0.5)	0.6 (0.4-0.8)	0.5 (0.4-0.6)	0.7 (0.5-0.9)
Biomass	--	105 (85-132)	148 (108-189)	120 (95-162)	172 (122-232)

Figures

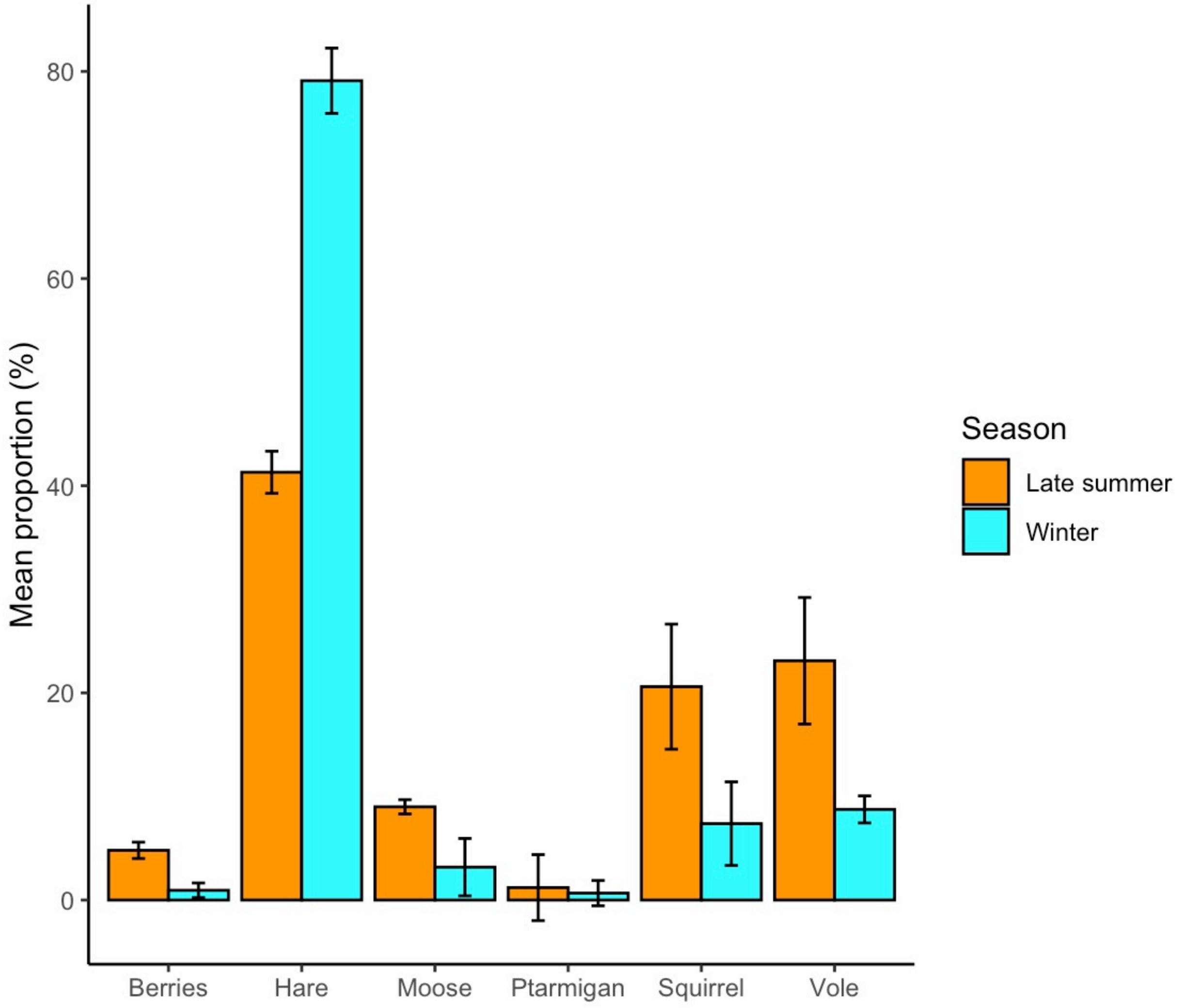

Figure 5.1: Seasonal proportions of dietary sources in marten diets (n = 341) from 2020 to 2023. Martens were trapped near Churchill, MB. Diet estimates (mean ± SD) are based on posterior distribution from Bayesian stable isotope mixing models of δ13C and δ15N in muscle (late-summer diet) and hair (winter diet) samples.

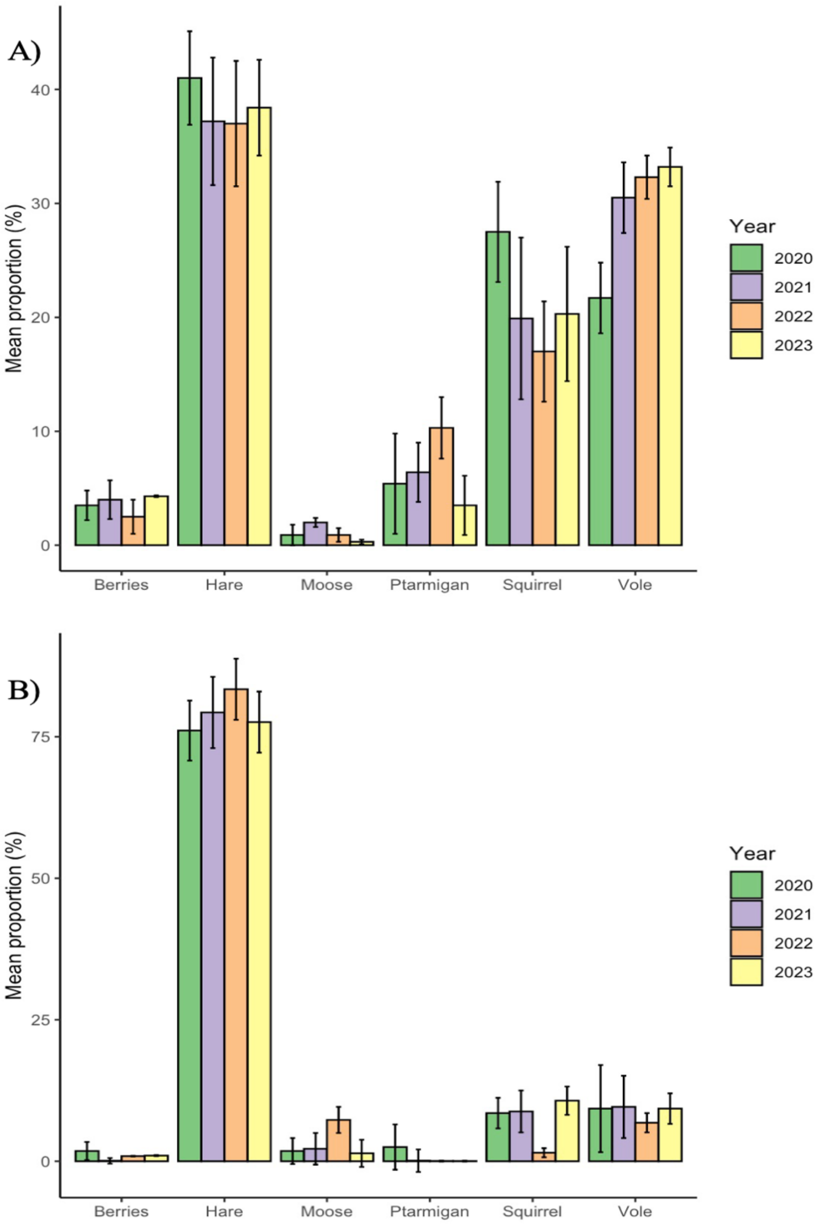

Figure 5.2: Annual proportions of dietary sources estimated from marten samples (n = 341) (mean ± SD) collected from 2020 to 2023 reflecting diet in A) late summer of the previous year and B) winter. Martens were trapped near Churchill, MB. Diet estimates are based on posterior estimates from Bayesian stable isotope mixing models of δ13C and δ15N in muscle (late-summer diet) and hair (winter diet) samples.

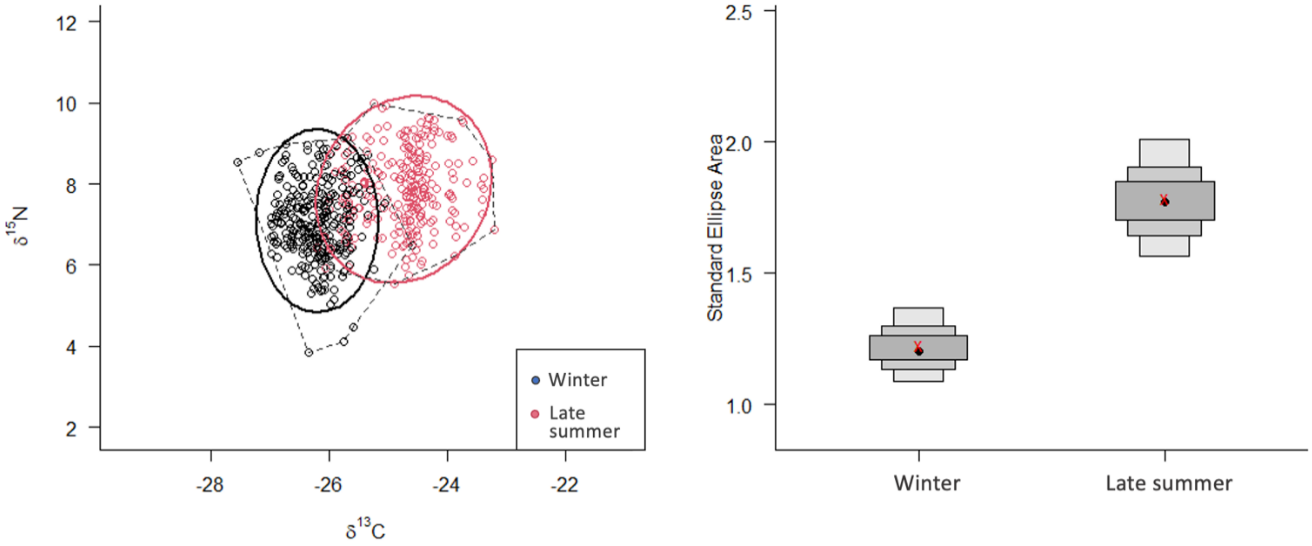

Figure 5.3: Seasonal variation in dietary niche breadths and standard ellipse areas of martens (n = 341). Ellipses were generated using the SIBER package in R. The dotted lines indicate convex hulls and solid lines indicate standard ellipse areas. The black dots represent the mode, the red 'x' the maximum likelihood of the SEAc, and the boxes indicate the 50, 75, and 95% credible intervals.

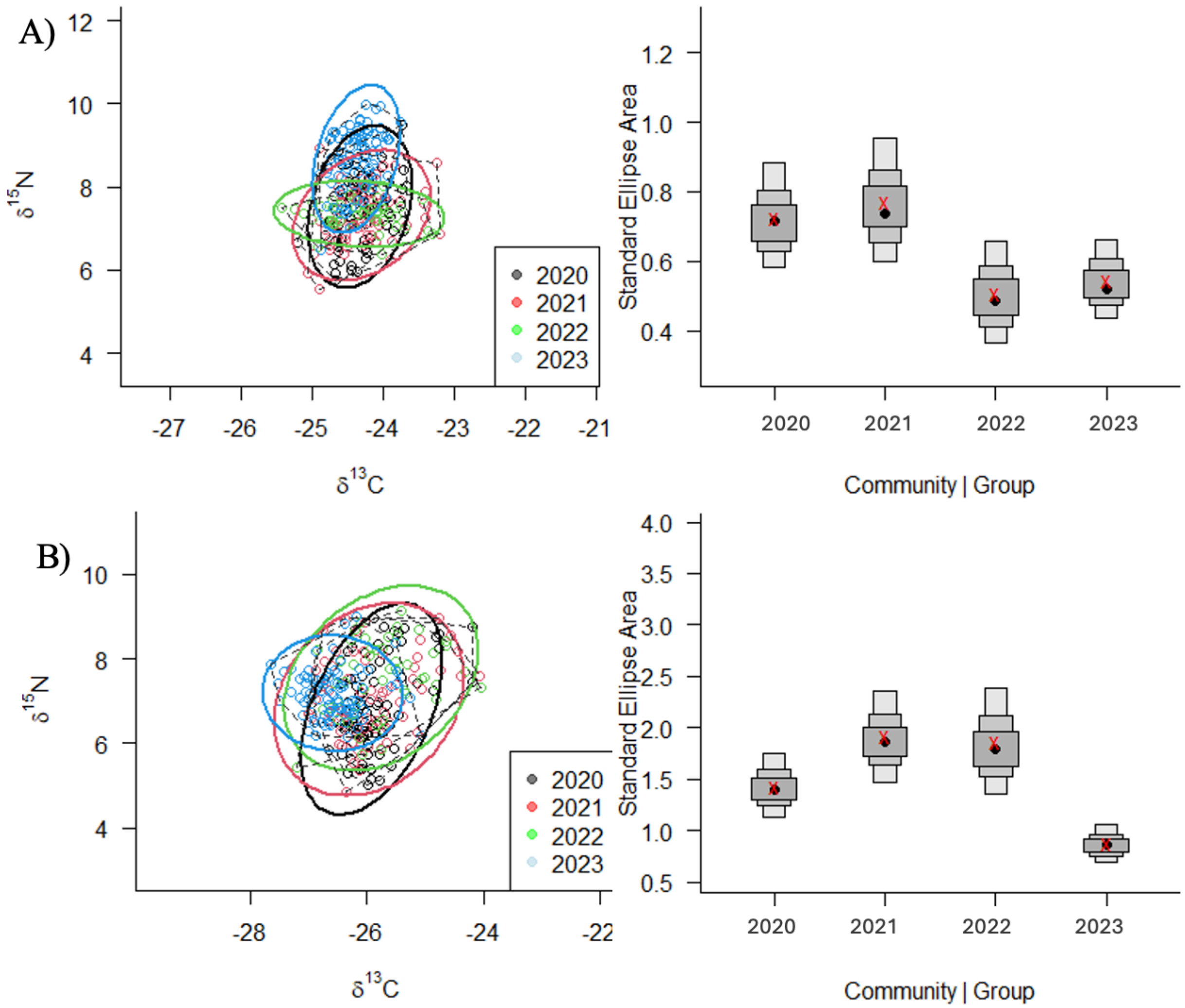

Figure 5.4: Annual variation in A) late summer and B) winter dietary niche breadths and standard ellipse areas of martens (n = 341). Ellipses were generated using the SIBER package in R. The dotted lines indicate convex hulls and solid lines indicate standard ellipse areas. The black dots represent the mode, the red ‘x’ the maximum likelihood of the SEAc, and the boxes indicate the 50, 75, and 95% credible intervals.

Supplementary Tables

Table S5.1: Results of the KNN pairwise comparisons of different food sources. The significance threshold was adjusted using the Bonferroni correction to account for multiple comparisons. Misclassification rates reflect the proportion of observations incorrectly assigned to a source group.

Source	N	Misclassification rate	p-value	Corrected p-value
Berries	97	0.05	0.036	0.045
Hare*	2	0.23	0.053	0.056
Moose*	3	0.20	0.045	0.058
Ptarmigan	6	0.05	< 0.001	0.017
Squirrel	6	0.12	0.009	0.020
Vole	7	0.15	< 0.001	0.030

*Although the corrected p-values for these species slightly exceeded our threshold of significance, the small sample sizes likely contributed to these results, and therefore we chose to include them as separate sources in the mixing models.

Table S5.2: Comparison of abundance models to examine annual abundances of red squirrels and red-backed voles, trapped 2020-2023 near Churchill, MB, Canada. Models are sorted by corrected Akaike Information Criterion (AICc) and AICc weight (wi).

Model	AICc	ΔAICc	w_i
M_b	399.9	0	0.4
M_h	401.7	1.7	0.2
M_t	403.9	1.8	0.2
M_0	404.1	4.1	0.0

Table S5.3: Relative mean contribution (95% credible intervals in parentheses) of food items to winter and late-summer diets of different age-sex classes of martens trapped near Churchill, MB, Canada, from 2019 – 2023 (n = 341). Diet estimates are based on posterior estimates from Bayesian stable isotope mixing models of δ13C and δ15N in marten muscle (late-summer diet) and hair (winter diet) samples.

Food source	Adult male (%)	Adult female (%)	Juvenile male (%)	Juvenile female (%)
Winter				
Hare	80.6 (1.7-100.0)	77.9 (1.5-100.0)	74.5 (1.4-100.0)	73.6 (1.2-100.0)
Squirrel	5.1 (0.0-7.1)	9.7 (0.0-20.1)	5.3 (0.0-6.2)	7.7 (0.0-11.3)
Vole	10.2 (0.0-24.6)	12.2 (0.0-31.6)	13.4 (0.0-35.1)	11.0 (0.0-24.3)
Moose	3.1 (0.0-4.9)	3.8 (0.0-5.5)	6.3 (0.0-8.3)	6.7 (0.0-9.0)
Berries	0.0 (0.0-3.2)	0.1 (0.0-3.5)	0.6 (0.0-3.4)	0.7 (0.0-5.1)
Ptarmigan	0.2 (0.0-2.0)	0.3 (0.0-2.1)	0.2 (0.0-2.4)	0.4 (0.0-2.1)
Summer				
Hare	42.6 (1.2-57.0)	37.7 (1.1-44.5)	36.1 (0.0-46.0)	36.8 (0.0-43.1)
Squirrel	20.7 (0.0-41.8)	19.0 (0.0-30.2)	21.6 (0.0-39.2)	20.9 (0.0-38.7)
Vole	26.5 (1.0-43.3)	30.6 (1.2-48.8)	31.4 (1.2-47.6)	30.0 (0.8-44.2)
Moose	0.9 (0.0-2.7)	0.7 (0.0-2.1)	1.3 (0.0-3.3)	1.2 (0.0-3.2)
Berries	2.3 (0.0-10.0)	2.5 (0.0-9.9)	3.4 (0.0-12.1)	3.5 (0.0-12.1)
Ptarmigan	7.0 (0.0-21.2)	9.5 (0.0-22.4)	6.2 (0.0-15.4)	7.6 (0.0-12.5)

Table S5.4: Annual relative mean contribution (95% credible intervals in parentheses) of food items to winter and late-summer diets of martens, trapped near Churchill, MB, Canada, from 2019 – 2023 (n = 341). Diet estimates are based on posterior estimates from Bayesian stable isotope mixing models of δ13C and δ15N in marten muscle (late-summer diet) and hair (winter diet) samples.

Food source	2020	2021	2022	2023
Winter				
Hare	76.1 (8.4-100.0%)	79.3 (9.3-100.0%)	83.4 (10.3-100.0)	77.6 (8.4-100.0)
Squirrel	8.5 (0.0-15.0)	8.8 (0.0-18.3)	1.5 (0.0-6.3)	10.7 (0.0-22.1)
Vole	9.3 (0.0-20.5)	9.6 (0.0-21.3)	6.9 (0.0-12.3)	9.3 (0.0-20.6)
Moose	1.8 (0.0-7.4)	2.2 (0.0-7.0)	7.3 (0.0-13.6)	1.4 (0.0-5.9)
Berries	1.8 (0.0 -7.0)	0.1 (0.0-0.0)	0.9 (0.0-4.1)	1.0 (0.0-4.8)
Ptarmigan	2.5 (0.0-8.7)	0.1 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.1)
Summer				
Hare	41.0 (0.0-88.1)	37.2 (0.0-85.5)	37.0 (0.0-83.8)	38.4 (0.0-87.0)
Squirrel	27.5 (0.0-72.3)	19.9 (0.0-56.4)	17.0 (0.0-38.7)	20.3 (0.0-52.9)
Vole	21.7 (0.0-40.5)	30.5 (0.0-66.0)	32.3 (0.0-76.1)	33.2 (0.0-80.7)
Moose	0.9 (0.0-2.5)	2.0 (0.0-6.7)	0.9 (0.0-5.6)	0.3 (0.0-2.8)
Berries	3.5 (0.0-12.4)	4.0 (0.0-14.0)	2.5 (0.0-8.9)	4.3 (0.0-17)
Ptarmigan	5.4 (0.0-18.0)	6.4 (0.0-22.6)	10.3 (0.0-25.4)	3.5 (0.0-16.5)

Table S5.5: Bayesian Standard Ellipse Area (SEAb, ‰2) and 95% credible intervals (CI) for isotopic niche breadth of marten diets from 2020-2023.

Year	SEAb	95% CI
Winter		
2019-2020	0.73	0.67-0.78
2020-2021	0.77	0.67-0.78
2021-2022	0.51	0.46-0.56
2022-2023	0.55	0.51-0.59
Summer		
2020	1.43	1.32-1.53
2021	1.91	1.75-2.06
2022	1.86	1.68-2.02
2023	0.87	0.80-0.93

Supplementary Figures

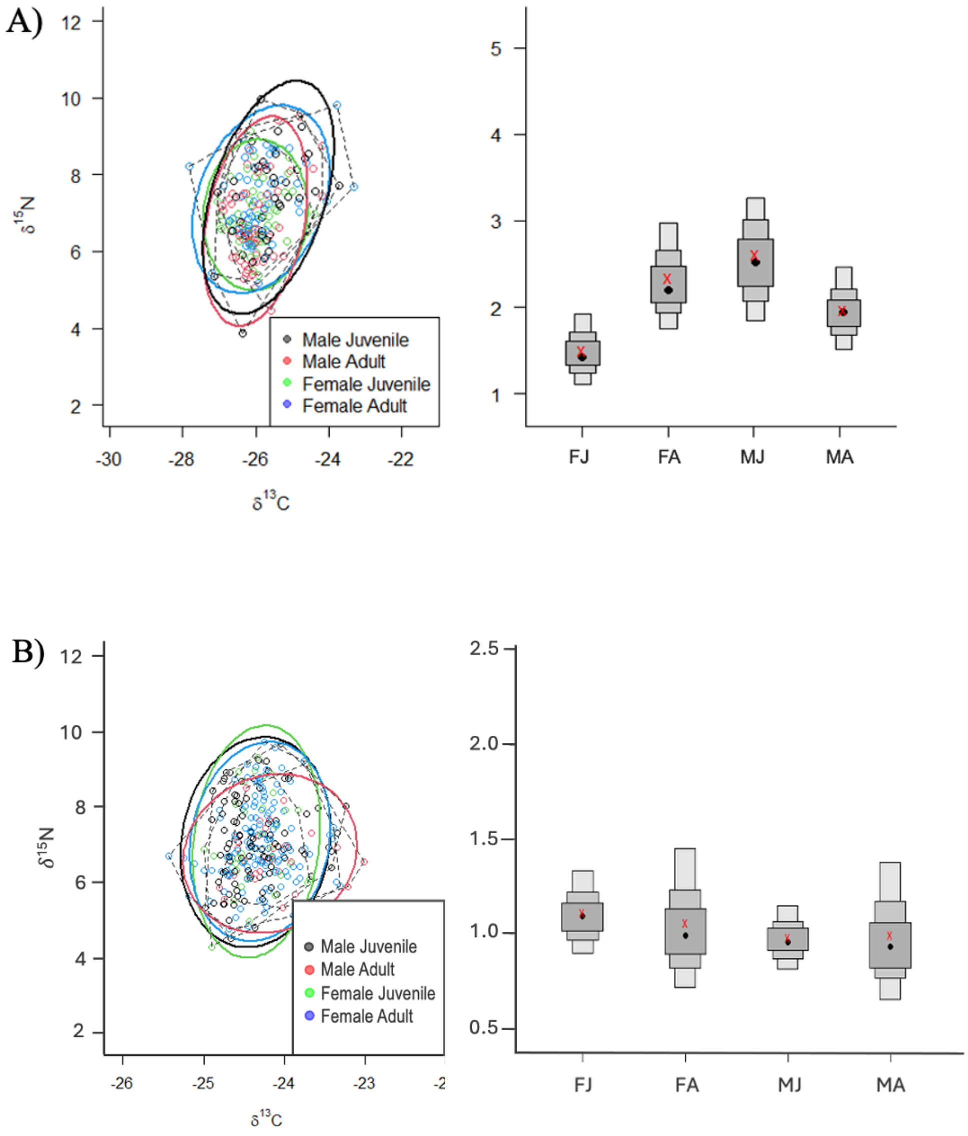

Figure S5.1: Comparison of A) winter and B) late-summer dietary niche breadths and standard ellipse areas of juvenile male (MJ n = 40) and female (FJ n = 108) and adult male (MA n = 147) and female (FA n = 46) martens. Ellipses were generated using the SIBER package in R. The dotted lines indicate convex hulls and solid lines indicate standard ellipse areas. The black dots represent the median, the red ‘x’ the mode, and the boxes indicate the 50, 75, and 95% credible intervals.

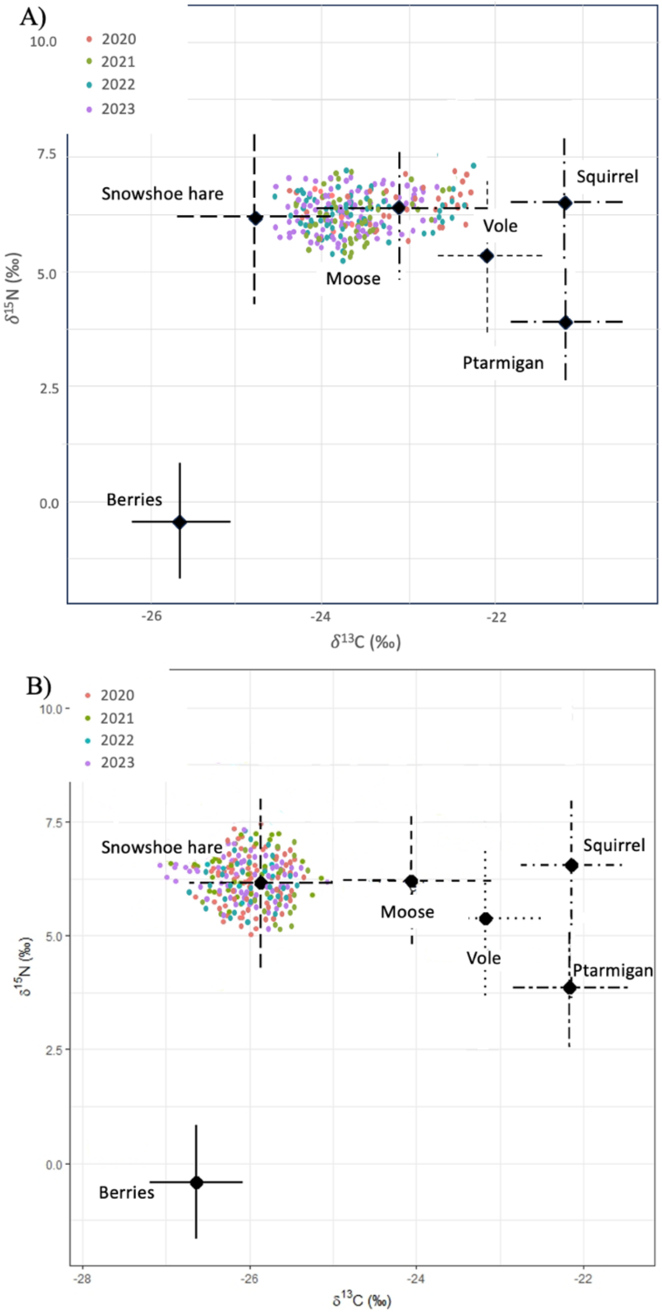

Figure S5.2: Isospace plot with stable isotope ratios (δ13C and δ15N) reflecting A) late-summer and B) winter diet for martens (n = 341) trapped between 2020-2023 near Churchill, MB, Canada.

Chapter 6: Conclusion

Geographic range boundaries delineate the spatial limits of a species' distribution. Within these boundaries, various biotic and abiotic factors impose restrictions on populations, ultimately preventing individuals from expanding their distribution (Kirkpatrick & Barton 1997, Holt 2003, Kubisch et al. 2014). Using a comprehensive approach that combines lab and field methods, along with tools from ecology and forest productivity, we evaluated key factors that restrict red squirrels at their northern range boundary. This study provides invaluable insights into the plasticity of red squirrels concerning diet, survival, and nesting behaviour. Here, I will briefly summarize our findings from each chapter and discuss future research directions.

Across their range, red squirrels predominantly feed on conifer seeds, establishing them as conifer specialists (Smith 1968, Fletcher et al. 2010, McAdam et al. 2019, Wishart 2023). However, in Chapter 2, stable isotope mixing models revealed that red squirrels consume mostly fungi at the sub-Arctic treeline, regardless of mast year. An analysis of annual spruce cone and seed production indicated that yields at the treeline were generally lowered compared to interior habitats (Waldron 1965, O'Connell et al. 2006, O'Connell et al. 2007). Despite an increase in cone production during the mast year of 2022, the cone crop was still subpar compared to interior regions. Factors such as a short growing season and harsh winters at the treeline likely hinder pollination and seed production, affecting seed and cone quantities (Owens 1995, García et al. 2000, Sirois 2000). Although spruce seeds were not a major part of squirrel diet, the quantity of filled seeds strongly predicted fungus and berry consumption. Additionally, our study found that the frequency of fungi hoarding varied with cone production, peaking during years of poor cone crops and declining during mast years.

Reduced food availability can pose significant challenges for organisms, especially in highly seasonal environments. In Chapter 3, we found low cone and filled-seed abundance notably impacted spring body mass and survival in squirrels. Our study observed that the mean spring body mass of squirrels was lower compared to other populations (Ransome & Sullivan 1997, Koprowski 2005, Patterson et al. 2015) and fluctuated with cone and seed production. Over winter, squirrels tended to lose mass, likely due to the nutritional differences between spruce seeds and fungi. While both food sources had similar protein content, spruce seeds contained much higher levels of lipids. Without a consistent lipid source, squirrels must rely on fat reserves for energy, leading to a decline in body mass over time, particularly since squirrels naturally possess minimal insulative fat (Johannesdottir 2017). We also identified a sex bias in spring body mass, with males being lighter than females, although they equalized by late summer. Females retained more body mass from August through the following spring, which was further reflected in our seasonal mass models. Prewinter mass and seed production were critical factors for overwinter survival, with survival rates declining after the mast year. Although cone production peaked during the mast year, filled-seed production was lowest that year, underscoring the direct connection between food availability and survival. Even in years with higher cone production and in turn higher filled-seed availability, individual survival may be compromised if winter food caches are filled with barren cones.

In Chapter 4 our research indicated that squirrels at the treeline predominantly use burrows as year-round nests. Although burrow use is known among red squirrels, our study is the first to identify burrows as the primary nest preference for a squirrel population. Red squirrels are known to construct various nest types across their range (Deeming 2023). However, the sub-Arctic treeline is characterized by an open canopy and fragmented forest with low tree density

(Lafleur 1999, Payette et al. 2001), likely making burrows a preferred nest choice in a habitat that is not conducive to cavity nests or dreys. We also observed warmer winter temperatures in burrows, suggesting burrows may also be preferred by squirrels for thermal benefits. While burrows offer protection against harsh winters, they also increase vulnerability to smaller burrowing predators like short-tailed weasels. Larger burrowing predators like martens are too large to effectively hunt in the narrow squirrel burrows. Thus, sightings of larger predators were highest in summer and autumn when juvenile squirrels and other small prey are more independent and exposed. In contrast, short-tailed weasels observations peaked in winter, when their small body size allows easy access to both the subnivean zone and burrows for hunting.

In the final data chapter, our study revealed that martens predominantly consumed snowshoe hare, despite the higher annual abundance of other primary prey species. In winter, martens focused on larger prey, while their foraging strategy diversified in late-summer and autumn. During this time, marten diet comprised of a mix of hare, voles, squirrels, and moose, although snowshoe hare remained the largest contributor. These seasonal dietary shifts likely reflect martens' adaptations to variations in prey accessibility and availability, as well as their preference for more energetically profitable prey (Carlson et al. 2014, Kujawa et al. 2014). The increased occurrence of such smaller prey in late-summer and autumn corresponds with their higher abundance and availability post-breeding season, when juveniles become independent. Additionally, squirrels are more vulnerable in late summer as they harvest spruce cones for their winter food caches.

Climate change is significantly impacting vegetation patterns in the sub-Arctic and treeline regions, leading to increased tree density and altering masting dynamics, which in turn affects wildlife populations and their behaviours. At the northern edge of their distribution, red squirrels face challenges like reduced food availability. But as the intervals between mast years shorten, potentially increasing food availability and the success of treeline species and enhancing the likelihood of range expansion. Thus, a comprehensive understanding of how species adapt to changing conditions along latitudinal gradients is crucial for predicting their potential for future range expansion and survival in the face of ongoing climate change.

Future directions

Our research has underscored the challenges that red squirrels face at their northern range boundary and highlighted their plasticity in adapting to this suboptimal environment. Given that squirrels are seed specialists, our study concentrated on spruce cone and seed production. However, our diet reconstructions revealed fungi as a main food source in this area. Future studies should focus on understanding the annual variation and availability of fungi at the treeline to provide a more comprehensive picture of food availability.

In terms of predation, while we primarily examined American martens, we discovered that martens favoured snowshoe hares over squirrels, and squirrel burrows provided protection from larger predators like martens. Instead, our findings suggest that smaller bodied predators like short-tailed weasels might be significant predators of red squirrels at the treeline. Future research should include reconstructing the diet of weasels to better assess their impact as primary squirrel predators.

Our fieldwork also faced inconsistencies with data collected sporadically from August and September 2020, June 2021, and both June and August of 2022 and 2023. This inconsistency may limit the interpretation of our findings on annual and seasonal mass change and survival rates. Future research would benefit from more consistent and comprehensive field seasons to enhance data reliability.

As a pilot project, we have only begun to explore how red squirrels persist at the treeline and the challenges they encounter. To fully understand the restrictive nature of the treeline environment, future studies should investigate other aspects of squirrel ecology such as reproduction, territory size, and the genetic diversity of the population. While red squirrel reproduction is well documented in interior habitats, the unique climate and habitat conditions of the treeline, result in a brief and delayed breeding season for many species. Further, given the dependency of squirrel reproduction on spruce cone production, the reduced cone and filled-seed production at the treeline may limit reproductive success.

Territory sizes, which are often linked to habitat quality and food availability, should also be examined. In low-resource environments, squirrels often possess larger territories to encompass sufficient resources. However, at the treeline, the forests' heavy fragmentation and low tree density might make larger densities less favourable due to increased exposure to predators.

To track squirrels to their nests, we used VHF collars, but these can be challenging to operate in forested environments, and the fast movements of squirrels make tracking difficult. Future research could benefit from the use of satellite GPS collars to track territory sizes and movement patterns more accurately. Satellite collars would also enable continuous tracking beyond the limitations of field seasons.

By expanding research to these areas, we can gain a deeper understanding of the ecological dynamics at the treeline and the adaptive strategies red squirrels employ to persist in this challenging environment.

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