

# **Variation in facultative diet specialization of Canada lynx**

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## Thesis Abstract

The facultative specialist hypothesis predicts that niche expansion by specialist predators is advantageous when preferred prey are limited and alternative prey are available, yet patterns of niche variation differ across spatiotemporal gradients and it remains unclear how environmental characteristics such as prey availability and community structure affect foraging strategies of free-living organisms. Understanding of facultative foraging has been constrained by narrow spatial or temporal scales because of methodological limitations; however, increasing refinement of diet reconstruction using stable isotope analysis enables examination of foraging dynamics across large geographic areas. Canada lynx (*Lynx canadensis*) are facultative specialists of snowshoe hare (*Lepus americanus*) and both experience cyclic fluctuations in abundance, but snowshoe hare fluctuations are attenuated towards southern latitudes where availability of alternative resources for predators is higher. Using stable isotope analysis, I tested the effect of biotic interactions on foraging strategies of lynx across spatiotemporal scales to elucidate a broader perspective on adaptive foraging decisions in free-living organisms. In northern populations, where availability of alternative prey in winter is largely restricted, declining hare abundance prompted differential niche expansion among individuals, whereby newly independent lynx used alternative prey but adults maintained highly specialized diets. The relative consumption of alternative prey was consistently higher and less variable in southern lynx populations, regardless of fluctuations in hare abundance, indicating that a more generalized foraging strategy is obligate for all individuals in these areas. Although southern lynx fed more broadly than northern conspecifics, their diet breadth remained narrow when compared with sympatric bobcat (*Lynx rufus*), a closely-related generalist species that also prefers lagomorphs. Across four different communities, lynx diets overlapped completely with bobcat, but over half of bobcat fed differently from lynx, suggesting

high potential for competition and supporting the generality of the specialist-generalist paradigm of foraging strategies at large spatial scales. Finally, I investigated potential consequences of niche expansion to individual stress physiology, but found no relationship between chronic stress and prey choice. Collectively, my thesis demonstrates how prey availability and community complexity differentially affect the facultative foraging strategies of a free-living specialist predator, substantiating the facultative specialist hypothesis at an exceptionally large geographic scale.

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## General Introduction

The ecological niche concept and foraging theory are central to our understanding of natural processes in a time of rapid global change because they provide a framework to explain and predict species interactions and population dynamics. Specialist species are particularly vulnerable to environmental change because of their focus on exploiting a few resources well (Devictor et al. 2010), and many specialist species are experiencing population declines and greater extinction risk than generalist species with broader ecological tolerances (Clavel et al. 2011). Increasing evidence indicates major gaps in knowledge of how specialization is expressed and modified in response to changing environmental conditions. While the foundational research into specialization focused on broad patterns of species, Bolnick et al. (2003) spurred a prolific advancement in research on the incidence and implications of individual specialization that has identified numerous ways that individual foraging decisions can differentially affect population dynamics (Agashe and Bolnick 2012) and community dynamics (Bolnick et al. 2011). The state of the art seeks to explain the causes and consequences of facultative specialization across spatiotemporal scales (Pagani-Núñez et al. 2016), within levels of biological organization (individual, population, community; Devictor et al. 2010), and across levels of biological organization (Bolnick et al. 2011).

The ecological niche concept is a cornerstone of biological thinking seeking to describe the range of abiotic and biotic factors that ultimately govern the geographic extent and population dynamics of a species (Holt et al. 2009). Several niche concepts have been formalized to guide ecological inquiry into the environmental elements necessary for the existence of a species (Grinnell 1917), the functional role of a species in a community (Elton 1927), and the elements moderating species coexistence (Gause 1936; MacArthur and Levins 1967). The Hutchinsonian

niche concept (1957) unifies the above goals into a framework that allows consideration of both used and prospective niche elements to gain insight to how ecological constraints such as resource availability, interspecific competition, habitat use, and barriers to dispersal moderate the population dynamics and evolution of the species (Futuyma and Moreno 1988; Holt et al. 2009). Thus, the ecological niche concept informs all branches of biology and is foundational to our understanding of the genetics, dynamics, and evolution of species (Futuyma and Moreno 1988).

Hutchinson (1957) defined the ecological niche as a multi-dimensional hypervolume of environmental variables (or niche axes) that include the abiotic and biotic factors within which a species can exist indefinitely. Each niche axis is thought of as a spectrum of conditions ranging from minimum requirements at either extreme, with optimal conditions in between. The full suite of adequate environmental conditions, called the fundamental niche, is an abstract idea to collect a list of alternative resources and/or behavioural responses an organism might exploit to endure changing environmental conditions. In practice we measure the realized niche, the subset of resources in the fundamental niche that are used by the organism, population, or species, but can develop an understanding of the fundamental niche through observation of resource use across spatiotemporal scales. Defining axes for a species' habitat and diet requirements are critical to understanding and predicting ecosystem dynamics in a changing world as they impact trophic interactions, interspecific interactions, and spatiotemporal patterns of abundance (Chase and Leibold 2003).

Classically, resource use strategies have been categorized dichotomously as “specialist” or “generalist” depending on the breadth of resource types used, and yet considerable variability within a species' realized niche may arise from individual responses to change in resource abundance, resource diversity, and interspecific competition for shared resources (Devictor et al.

2010; Bolnick et al. 2011). Observations of dietary plasticity have prompted scientists to further qualify foraging strategies as “facultative” to accommodate for environmental factors that prompt spatiotemporal change in resource use (Pagani-Núñez et al. 2016). Thus, resource use strategies are better conceptualized as a spectrum that ranges from extreme specialization to generalist strategies along a continuum and intuitively suggests that a strategy may change in accordance with environmental change. Another advantage of the specialist-generalist spectrum is that it can be applied to multiple levels of biological organization to address specialization within individuals, between individuals, among populations, and among species (Devictor et al. 2010).

Dietary specialists faced with environmental change, such as increased competition and changes in prey community dynamics, must change their behaviour to fulfill their energy requirements or face extinction (Poisot et al. 2011). Optimal foraging theory predicts that specialists may expand their dietary niche (Van Valen 1965) or disperse to patches with reduced levels of resource competition to maximize their rate of energy intake (Stephens and Krebs 1986; Svänback and Bolnick 2007). Lab studies have significantly advanced our understanding of ecological niche dynamics and foraging decisions of specialist consumers by rigorously controlling environmental conditions (eg. Svänback and Persson 2004; Hooper et al. 2008; Agashe and Bolnick 2012); however, these studies are limited in scope to small organisms that are easily reared in a lab setting, require good *a priori* knowledge of relevant environmental conditions to free-living populations, and may not capture rare events (Holt et al. 2009). Observations of variability in specialization in free-living organisms are increasingly abundant, but generally limited by spatial or temporal scale (e.g. Inger et al. 2006; Herbst and Mills 2010).

Herein, I investigate the effect of variable environmental conditions on facultative diet specialization of free-living consumers at a large spatial scale, using Canada lynx (*Lynx*

*canadensis*) as a model. Lynx are a specialist consumer of snowshoe hare (*Lepus americanus*) whose evolutionary history of specialization is clear; they are morphologically adapted to pursue hares in winter with long hind legs and large paws that allow them to run quickly without sinking in deep snow (Murray and Boutin 1991), and they exhibit density-dependent population cycles following the abundance of hares (Elton and Nicholson 1942). Yet, there is evidence for temporal dietary niche expansion by lynx populations in northern regions associated with declining hare abundance (Brand et al. 1976; O'Donoghue et al. 1998), and decreasing diet specialization towards southern latitudes (Roth et al. 2007), emphasizing the facultative potential of their foraging strategy across spatiotemporal scales to meet environmental conditions. Furthermore, lynx are broadly distributed and populations experience different environmental conditions in terms of snowshoe hare abundance and cyclicity, the diversity and abundance of alternative prey, the diversity of intraguild competitors, and human influence (Murray et al. 2008), creating ample opportunity for contrasts among populations. To address ecological questions at a continental scale, a cooperative relationship was established between principal investigators at Trent University and the two fur auction houses that grade and auction most furbearer pelts harvested in North America each year. Annual sampling trips to the auction houses since 2009 have amassed a collection of over 16 500 samples of lynx skin and hair for use in genetic, stable isotope, and hormone analyses.

This thesis is written in manuscript format and contains four papers that, together, extend our understanding of how preferred prey availability, the diversity of alternative prey, and intraguild competitors affect the extent of diet specialization by Canada lynx. **Chapter 1** investigates intrapopulation variation in diet through a fluctuation in hare abundance, testing whether niche expansion is differentially expressed among individuals or whether use of



alternative prey is a ubiquitous response of all lynx to prey limitation. **Chapter 2** tests the facultative specialist hypothesis at a continental scale and highlights how preferred prey abundance and the diversity of alternative prey available affects specialization. **Chapter 3** quantifies diet breadth and overlap of sympatric lynx and bobcat to assess the degree of niche differentiation expressed by closely-related species with contrasting foraging strategies along the specialist-generalist spectrum. Finally, niche expansion is a response to preferred prey limitation moderated by the stress axis, but whether dietary plasticity mitigates consequences of chronic stressors is unclear. **Chapter 4** compares the degree of specialization expressed by lynx with concurrent accumulation of cortisol in the body to identify the impact of foraging plasticity on individual physiology. Collectively, these manuscripts address important knowledge gaps of how specialization is expressed and modified by free-living organisms in response to changing environmental conditions.

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## **Chapter 1: Demographic differences in diet breadth of Canada lynx during a fluctuation in prey availability**

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Contributions of Authors:

C. M. Burstahler was responsible for design, analysis, interpreting the data, and writing the manuscript. R. J. Gau and J. D. Roth conceived of the project and coordinated sample collection. D. L. Murray and J. D. Roth contributed to critical revision of the work, including interpretation of the data and editing the manuscript.

## Abstract

Population dynamics of specialist carnivores are closely linked to prey availability, but the extent of variability in diet breadth of individual carnivores, relative to natural variability in the abundance of their primary prey, is not well understood. Canada lynx (*Lynx canadensis*) specialize on snowshoe hares (*Lepus americanus*) and exhibit cyclic fluctuations in abundance that lag 1-2 years behind those of snowshoe hares. Declining hare densities spur demographic changes in lynx, but it is unclear whether a corresponding increase in diet breadth occurs: i) broadly across a lynx population; ii) only among individuals who are able to effectively switch to alternative prey; or iii) only among individuals who cannot capture sufficient primary prey. We measured stable isotope ratios of lynx muscle tissue spanning a cyclic increase and decline in hare density (1998-2001) in Fort Providence, NT, Canada. We found that lynx cohorts responded differently to hare population change, with yearling animals having broader diets at low hare densities, while adults and dependent juveniles maintained a constant diet through the initial decline in hare density. This result was consistent irrespective of lynx sex, and indicates that yearling lynx likely are forced to adopt a broader diet when primary prey densities decline. Our results imply that select cohorts of specialist carnivores can exhibit high dietary plasticity in response to changes in primary prey abundance, prompting the need to determine whether increased diet breadth in young lynx is a successful strategy for surviving through periods of snowshoe hare scarcity. In this way, cohort-specific niche expansion could strongly affect the dynamics of organisms exhibiting population cycles.

## Introduction

Food limitation is a critical environmental factor shaping the dynamics of consumers, and organisms may expand their dietary breadth to contend with limited food availability (Van Valen 1965). Yet, individuals vary in age, size, sex, and foraging experience, meaning that certain segments of the population are likely predisposed to lower success when acquiring limiting resources (Polis 1984; Kramer et al. 2015). This means that individuals with reduced ability to acquire scarce resources must use alternative prey, disperse, or die. Prey switching may enhance resilience of the population to environmental stressors by reducing intraspecific competition, mitigating consequences to productivity, and thus may curb the rate of population decline. In this way, dietary plasticity may buffer fluctuations in population size by reducing mortality and enabling continued reproduction under marginal conditions; thus, it is essential to measure both population-wide and demographic responses to limiting resources in order to understand the impacts of prey limitation on population dynamics (Wennersten & Forsman 2012).

Dietary niche expansion of consumer populations may be driven by increased variability within individual diets or by divergence of individual diets. First, if alternative prey are easy to catch relative to primary prey, then all members of a population may exhibit dietary plasticity when primary prey are scarce (Roughgarden 1972). On the other hand, if alternative prey is especially difficult for consumers to capture, then diet switching may be restricted to effective or experienced hunters (i.e., adults). For example, as recruitment of Eurasian perch (*Perca fluviatilis*) increases, adults are able to switch from feeding on benthic invertebrates to cannibalizing energy-rich juveniles, but subadults maintain a diet of invertebrates (Persson, Byström & Wahlström 2000). Alternatively, if prey switching occurs among individuals who are unable to effectively capture primary prey at low densities, then young, free-living animals should

have broader diets during primary prey shortages. Ungulates are the preferred prey of cougars (*Puma concolor*) and, during summer, ungulates comprise the largest proportion of cougar diet for all segments of the population. During winter, availability of ungulates diminishes and subadult diets expand to include a variety of non-ungulate prey while adults maintain a considerable proportion of ungulate prey in their diet (Knopff et al. 2010).

Canada lynx (*Lynx canadensis*) are specialist predators in a prey-limited system, providing a useful model for examining effects of food limitation on diet breadth and its variability in a population (Figure 1.1). Lynx in the core range exhibit population cycles following one to two years behind that of their preferred prey, snowshoe hare (*Lepus americanus*), which cycle in abundance every 9-10 years (Krebs et al. 2013). Lynx cycles have been attributed ultimately to changes in snowshoe hare density (Krebs et al. 2001) and proximately to changes in juvenile recruitment (Brand & Keith 1979; Mowat, Slough & Boutin 1996; Slough & Mowat 1996). Additionally, lynx can increase their use of alternative prey in their winter diets when hare densities decline (Brand, Keith & Fischer 1976; O'Donoghue et al. 1998a), meaning that the influence of primary prey can be dampened during periods of food shortage. During winter in the boreal forest, red squirrel (*Tamiasciurus hudsonicus*) comprise the greatest available alternative resource for lynx (Mowat, Poole & O'Donoghue 2000), and ruffed grouse (*Bonasa umbellus*), small mammals (*Peromyscus* spp., *Microtus* spp.) and ungulate carrion also are consumed (Van Zyll de Jong 1966; Brand, Keith & Fischer 1976).

Alternative prey may be important to lynx populations during cyclic lows (O'Donoghue et al. 1998b; Roth et al. 2007), but it remains unclear whether variability in diet breadth is pervasive across a population, or whether certain individuals within select cohorts are more likely to switch prey. We examined lynx diet breadth variability among cohorts from a harvested population near



Fort Providence, Northwest Territories, Canada. Our previous work (Roth et al. 2007) revealed that snowshoe hare are depleted in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  compared to alternative prey species, facilitating inference of lynx diet from stable isotope ratios. Because hare densities varied threefold during our study, we predicted that diet breadth would vary through time, with stable isotope ratios from tissue samples being more characteristic of those indicative of alternative prey, during periods of hare paucity (greater  $\delta^{13}\text{C}$  and/or  $\delta^{15}\text{N}$ ). In following with the three alternative mechanisms of intrapopulation niche expansion outlined above, we predicted that lynx niche expansion during periods of low hare abundance would occur either: 1) across the population (if alternative prey are profitable and easily captured); 2) only among adults (if alternative prey are difficult to capture); or 3) only among independent subadults (if alternative prey are not profitable and represent a food of last resort for individuals that are unable to capture sufficient primary prey when at low densities).

## **Methods**

To examine annual variability in lynx diets, we measured stable isotope ratios of lynx harvested by fur trappers within 100 km of Fort Providence, Northwest Territories, Canada (61.35N, 117.65W). The study region is within the southern portion of the Taiga Plains ecological region (Ecosystem Classification Group 2007), and is mostly flat with large lakes and stands of coniferous and mixed boreal forest. Winters have mean temperature of  $-19^{\circ}\text{C}$ , while summers have a mean temperature of  $13^{\circ}\text{C}$ ; annual precipitation ranges from 350-450 mm (Poole 1989). Throughout the territory, lynx and snowshoe hare populations undergo periodic 10-year cycles in abundance (Murray, Steury & Roth 2008; Krebs et al. 2013).

We collected muscle samples from lynx harvested from November through March in four winters from 1997-1998 to 2000-2001 (hereafter referred to as winters 1998 through 2001). Lynx age was determined by counting cementum annuli of canine teeth (Matson's Laboratory LLC, Milltown, MT). Lynx were grouped into four age classes for analysis: juveniles (<1 year old), yearlings (1 year old), subadults (2 years old), and adults ( $\geq 3$  years old). We considered subadults separately because the age of first reproduction increases when snowshoe hare are scarce (Parker et al. 1983; Slough & Mowat 1996). Hair of snowshoe hare and red squirrel were collected in 2001 to provide estimates of prey stable isotope ratios in the study area (see Roth et al. 2007).

Harvested lynx samples underestimate the number of kits (36%) and individuals  $\geq 2$  years old (4%) while overestimating the number of yearlings (40%) (Slough & Mowat 1996). We applied this correction factor to our harvest sample to provide a better estimate of population age structure. We calculated annual recruitment as the ratio of juveniles to adults ( $\geq 3$  years old), based on population estimates corrected for harvest bias.

Snowshoe hare abundance was estimated each year using fecal pellet counts (Krebs et al. 2001) from seven sites throughout the Northwest Territories. At each site 4-6 transects with 70-100 plots each (5.08 x 305 cm, spaced at 25 m intervals; see Poole 1989) were surveyed. Hare density was estimated from pellet counts using a regression equation developed for Yukon Territory (Krebs et al. 2001). Estimates reflect hare density of the previous year, biased heavily towards winter hare abundance (Murray, Ellsworth & Zack 2005), thus pellet counts from June 1998 reflect hares available for lynx trapped in winter 1997-1998.

To prepare samples for stable isotope analysis, muscle tissue was freeze dried and powdered with a mortar and pestle. Since carbon stable isotope ratios of lipids differ significantly from that of other compounds (DeNiro & Epstein 1977), lipids were removed using a Soxhlet

apparatus with petroleum ether for over eight hours, then samples were dried in an oven to eliminate the solvent. Hair tissue of prey was washed with soap and water, thoroughly rinsed, dried, homogenized, and wrapped in tin capsules. Sample stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were measured on a continuous flow isotope ratio mass spectrometer at the University of Central Florida. Stable isotope ratios are expressed using conventional delta notation (Ben-David & Flaherty 2012). As muscle tissue has a turnover rate of approximately 1-3 months (Boecklen et al. 2011) our samples reflect an average of winter diet and coincide with the temporal period of prey availability reflected in snowshoe hare pellet counts. Both snowshoe hare and red squirrel moult in autumn, so hair stable isotope ratios reflect prey during October-November of 2000 (coincident with lynx winter diet 2001).

### *Data Analysis*

Snowshoe hare availability is an important predictor of lynx numerical and behavioural responses (O'Donoghue et al. 1997, 1998a); however, dietary niche expansion is expected at the onset of snowshoe hare decline when predator density is high and access to prey is diminishing. This critical point of predicted dietary niche dynamics is not likely to coincide with the lowest hare density value but rather follow a time lag, thus a linear model of diet and hare density was not considered appropriate. Further obscuring the story, measures of snowshoe hare density in Fort Providence and territory-wide trends were incongruous, leading us to seek additional proxy factors to define the quality of environmental conditions experienced by the lynx population. It is well established that lynx recruitment responds strongly to prey availability and is more reliable than other measures of productivity such as placental scars or corpora lutea (Mowat, Slough & Boutin 1996). We characterized each year of study dichotomously as marginal or good by first

considering local snowshoe hare density, second considering local recruitment of lynx, and finally confirming our designation by referring to mean hare density and lynx harvest for the Territory. We consider these as indicators of environmental conditions experienced by lynx and relevant at both broad and local spatial scales.

Population-level diet responses are reflected in mean stable isotope ratios. Together,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  provide a two-dimensional measure of diet and thus are ideally modeled together using MANOVA; however, lack of homogeneity of variance-covariance and weak linear association between response variables raised concerns against use of MANOVA for these data. Linear mixed models allow for unequal variance structures but are not yet accessible for multiple response variables. As such, we considered  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  separately for population-level diet responses. Note that predictions regarding each remain the same: both are expected to increase during marginal conditions as use of alternative prey increases.

The influence of environmental condition, age class, and sex on mean lynx stable isotope ratios was evaluated using standard model selection procedures (Burnham & Anderson 2002) from a set of *a priori* linear mixed models fit by maximum likelihood. Fixed effects included the interaction of environmental condition (*EC*) and age class (*A*), as well as main effects of *EC*, *A*, and sex (*S*). Red squirrel stable isotope ratios are variable between seasons, years, and among individuals depending on conifer masting events, composition of food caches, and use of animal protein (Roth et al. 2007, Appendix D). Red squirrels comprise the greatest available alternative resource to lynx during winter in northern latitudes (Mowat, Poole & O'Donoghue 2000) and could strongly influence stable isotope ratios measured in lynx. As such, year of collection (*Y*) was included in all models as a random effect to account for unmeasured variation in prey community stable isotope ratios. Separate intercepts were fit for each year but slopes of

parameters were held constant. Raw values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were analyzed as no transformations improved model fit. A null model was included to provide a baseline comparator for other models in the suite. The difference between a model's AIC value and the minimum AIC of the model ( $\Delta\text{AIC}$ ) provides a measure of Kulback-Leibler information loss relative to competing models, and thus a measure of each model's plausibility relative to others in the set (Burnham & Anderson, p. 70). Models with  $\Delta\text{AIC}$  smaller than two were considered competitive, unless they differed by one parameter with little change in maximized loglikelihood (Arnold 2010; Burnham & Anderson 2002, p. 131). The variance explained by each model was summarized as the marginal and conditional  $R^2$ , where marginal  $R^2$  considers variance explained by fixed effects only while conditional  $R^2$  reflects variance explained by both random and fixed effects (Nakagawa & Schielzeth 2013). Coefficients for all models were then recalculated using restricted maximum likelihood (REML) to improve estimation of random effects and balance unequal variance among predictors (Bolker et al. 2009). The relative importance of predictor variables to each of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was measured by summing the model weights ( $w_i$ ) of all models in which a predictor appears.

Individual-level responses are reflected in the variability among individual stable isotope ratios, or diet breadth of a demographic group. We examined changes in diet breadth of cohorts based on the dispersion of stable isotope ratios in two-dimensional space (i.e., the bi-plot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values; Bearhop et al. 2004). The standard ellipse area ( $SEA$ ) characterizes spatial variability in the stable isotope data as a two-dimensional standard deviation, governed by the covariance matrix between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and containing approximately 40% of the data (Jackson et al. 2011; see also Batschelet 1981). More diverse individual diets within a demographic group are characterized by greater spread in stable isotope ratios, and thus a larger

standard ellipse. We used the *SEAc* metric, which improves estimation for small sample sizes (Jackson et al. 2011). Our large annual sample permitted partitioning of the data into age classes without compromising precision of our *SEAc* estimates.

We used a linear mixed effect model based on a split plot design to examine differences in *SEAc* associated with environmental condition (between-plot factor) and age class (within-plot factor), including interactions between environmental condition and age class. Intercepts were allowed to vary by year of collection (random effect) to control for potential differences of prey community stable isotope ratios between winters. In its classical form, the split plot design assumes a single datum per cell and thus is appropriate for the single *SEAc* estimate per grouping per year. We used  $\Delta AIC$  to assess split plot model fit in comparison with simpler variations of the model, including a null model that considered only the random intercepts for year of collection. Explained variance was summarized as the marginal and conditional  $R^2$ . Confidence intervals for parameter estimates of the selected model were calculated using a basic bootstrap of 999 simulations (Thai et al. 2013). All analyses were conducted using the ‘lme4’ (Bates et al. 2015) and ‘siar’ (Parnell & Jackson 2013) package in R (R Core Team 2014).

## **Results**

The study period was characterized by variable snowshoe hare densities and lynx recruitment rates in Fort Providence (Table 1.1). Territory-wide mean hare densities and total lynx harvest suggest a relatively low peak abundance for both lynx and hares, but estimates are highly variable across the landscape (Table 1.1). Good years include data collected winter of 1999 and 2000, represented by higher snowshoe hare densities (mean local hare densities: 0.53 and 0.90 hares/ha, respectively) and high lynx recruitment (mean recruitment rate: 1.58 and 1.87

juveniles/adult, respectively). Marginal years include data collected in 1998 and 2001, representing low hare densities (mean local hare densities: 0.45 and 0.62 hares/ha, respectively) and minimal recruitment (mean recruitment rate: 0.62 and 0.40 juveniles/adult, respectively).

We collected 505 lynx carcasses comprising 112 lynx in 1998, 199 lynx in 1999, 93 lynx in 2000, and 165 lynx in 2001. Lynx age, estimated from tooth cementum annuli, ranged from juveniles (<1 year) to 12 years old, and the sample included 14.0% juveniles, 49.4% yearlings, 19.8% subadults, and 16.7% adults overall. The sex ratio of sampled lynx was 58.4% male and the age distribution of the population (corrected for harvest bias) fluctuated between years (Table 1.1).

Stable isotope ratios of snowshoe hares ( $n = 6$ ,  $\delta^{13}\text{C} = -26.28 \pm 0.93\text{‰}$  SD,  $\delta^{15}\text{N} = 3.25 \pm 1.44\text{‰}$  SD) and red squirrels ( $n = 6$ ,  $\delta^{13}\text{C} = -20.70 \pm 0.53\text{‰}$  SD,  $\delta^{15}\text{N} = 9.91 \pm 3.21\text{‰}$  SD) collected in 2001 were distinct and encompassed the range in stable isotope ratios observed for lynx.

Model selection suggests that all hypothesized parameters were potentially important predictors of mean  $\delta^{15}\text{N}$ , but had little effect on mean  $\delta^{13}\text{C}$  (Table 1.2). Supported models of mean  $\delta^{15}\text{N}$  suggest that an interaction between environmental condition and age class, and/or the main effects of *EC* and *A*, influenced lynx population stable nitrogen ratios. While both Model 1 and the Global model are within two  $\Delta\text{AIC}$  values, the log-likelihoods are essentially equivalent, indicating that the addition of sex does little to improve predictive performance of the model. Random intercepts for Model 1 were: 1998 = 4.33, 1999 = 4.57, 2000 = 4.54, and 2001 = 4.77. Note that variance in  $\delta^{15}\text{N}$  explained by fixed effects is considerably better than models of  $\delta^{13}\text{C}$  and that inclusion of variance explained by the random effect of year improves the explained variance only slightly. Cumulative Akaike weights (CW) suggest that environmental condition

(CW = 0.90), age class (CW = 0.73), and their interaction (CW = 0.58) were important predictors of  $\delta^{15}\text{N}$ , but sex was of little importance (CW = 0.29). Coefficients for all models are presented in Table 1.3.

In contrast, supported models for  $\delta^{13}\text{C}$  included the null, suggesting that hypothesized predictors had no measurable effect on lynx stable carbon ratios. Indeed, the variance explained by fixed effects alone ( $R_m^2$ ) was almost negligible for  $\delta^{13}\text{C}$  while the variance explained by year of collection ( $R_c^2$ ) was considerably higher. Fit of the global model for  $\delta^{13}\text{C}$  satisfied the assumptions of normality and homogeneity of variance. Random intercepts were: 1998 = -24.76, 1999 = -24.95, 2000 = -25.75, and 2001 = -25.14. Cumulative AIC weights (CW) for predictors of  $\delta^{13}\text{C}$  were:  $EC:A = 0.01$ ,  $EC = 0.31$ ,  $A = 0.19$ , and  $S = 0.46$ .

Stable isotope ratios and  $SEAc$  estimates of lynx grouped by environmental condition and age class are presented in Figure 1.1. Diet breadth estimates ( $SEAc$ ) of cohorts during good years were: juveniles =  $0.64\%{}^2$ , yearlings =  $0.64\%{}^2$ , subadults =  $0.52\%{}^2$ , and adults =  $0.62\%{}^2$ . Diet breadth estimates ( $SEAc$ ) of cohorts during marginal years were: juveniles =  $0.59\%{}^2$ , yearlings =  $1.22\%{}^2$ , subadults =  $1.12\%{}^2$ , and adults =  $0.61\%{}^2$ . The global split plot model of diet breadth fit better than simpler variations (Table 1.4). Diet breadth of yearlings expanded when environmental condition declined, but juveniles, subadults, and adults maintained largely similar diets across environmental conditions (Figure 1.2). The coefficient for subadult niche expansion under marginal environmental conditions may suggest a tendency towards niche expansion, but confidence intervals of our model overlap zero (Figure 1.2). Random intercepts for the global model were: 1998 = 0.60, 1999 = 0.50, 2000 = 0.72, and 2001 = 0.37. Model fit improved considerably with addition of random effects ( $R_m^2 = 0.37$  and  $R_c^2 = 0.77$ ). Visual inspection of



fitted values and residuals indicated compliance with assumptions of normality and homoscedasticity.

## **Discussion**

Our results indicate that, at the onset of a cyclic decline in snowshoe hare abundance, it is those segments of the lynx population that were unable to effectively capture primary prey that drove niche expansion. In our study, yearling lynx increased their use of alternative prey when faced with marginal environmental conditions; however, adults and their dependent juveniles maintained a narrow diet of snowshoe hare throughout the initial period of declining hare abundance. As our study only captures the beginning of the cyclic decline we cannot speak to dietary plasticity of the population during cyclic lows when recruitment has collapsed and adults predominate. Indeed, O'Donoghue et al. (1998b) describe the greatest dietary niche expansion three years following peak hare density in Kluane, YT suggesting that adults do, ultimately, use alternative prey, becoming sufficiently skilled at hunting squirrels that they do not immediately resume hunting hares at the onset of increasing hare densities. Thus it is possible that the yearling lynx forced to seek out alternative prey during the initial decline of snowshoe hare abundance develop critical hunting skills that increase survivorship throughout nadirs of the cycle.

The age class effect demonstrated herein suggests that the facultative nature of lynx specialization is more nuanced than previously described (O'Donoghue et al. 1998b; Roth et al. 2007; Murray, Steury & Roth 2008). If prey switching incurred immediate benefits to lynx, then all segments should be equally likely to expand their diet breadth in immediate response to their environment; yet, our data only provide evidence for those animals that are naïve hunters spending their first winters as independent, free-ranging individuals. The observed changes in diet

breadth among newly-independent lynx may reflect hunting inexperience (Polis 1984), limited access to good hare habitat, or responses to intraspecific competition (Svanbäck & Bolnick 2007). Collectively, our data suggest that use of alternative prey is not easy (otherwise we would expect a population-wide response), nor do alternative prey present a more profitable food source (in which case more experienced, adult lynx would use them), but rather that the switch to alternative prey is likely a last resort for select individuals, following limitation of their primary prey. Juvenile lynx forage in mother-kit groups during their first winter (McCord & Cardoza 1982; Mowat, Slough & Boutin 1996), and so it is not surprising that juvenile diet coincided with that of adults. Subadult lynx show a tendency towards niche expansion, which may reflect differences in speed of acquisition of hunting skills among developing lynx.

Prey switching is a form of behavioural plasticity that allows rapid responses to environmental change, yet it is not always clear whether switching is beneficial or not (Tuomainen & Candolin 2011). One- to two-years following snowshoe hare decline, lynx populations experience increased mortality largely due to human trapping, intra- and inter-specific strife, and starvation (Slough & Mowat 1996; O'Donoghue et al. 1997). Assuming that use of alternative prey is advantageous to young lynx, the tendency towards more variable diets may contribute to the resilience of certain individuals to survive through nadirs of the snowshoe hare cycle. While recruitment and mortality remain the prime drivers of the lynx cycle, it is possible that niche expansion of younger age classes may curb the rate of population decline through enhanced survivorship of some individuals (Agashe & Bolnick 2012). Thus, there remains a need to determine more fully if use of alternative prey by young lynx is a successful strategy for surviving through periods of hare scarcity.

A number of factors distinguish our study period as an unconventionally low fluctuation in the history of lynx-hare population cycles in the Northwest Territories (Figure 1.1; Elton & Nicholson 1942, see Mackenzie Basin in Fig. 8, p. 232). Peak snowshoe hare density was roughly 5-fold less than historic peaks in snowshoe hare abundance for Fort Providence (7-9 hares/ha, Poole 1994; 5.3-6.2 hares/ha, Poole 1997). Previous age class estimates from harvested lynx in Fort Providence (1989-1993) found juveniles to range from zero during periods of low hare density to 49% when hares were abundant (Poole 1994); similar figures were reported in other parts of the lynx range (Brand & Keith 1979; Parker et al. 1983; Slough & Mowat 1996). In contrast, our study comprised a small proportion of juveniles (14%) over the four-year period and a lower peak recruitment (1.9 juveniles/adult).

Environmental condition was a good predictor of lynx stable isotope ratios, although models of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  did not fully agree (Table 1.2). Our best explanation for this disjunction is that stable isotope data are inherently noisy because so many environmental and physiological factors affect them. We speculate that the disagreement between stable-carbon and -nitrogen models may be explained by noise generated via several competing inputs to the data set. First, our sample represents harvested lynx only, which means that we lack data for lynx that died from starvation and it is probable that a portion of our sample represents dispersers moving through the area from another region with a different prey base. Prey stable isotope ratios vary geographically (Roth et al. 2007) and thus lynx originating from other regions would reflect these differences, adding noise to the system. Second, red squirrel stable isotope ratios vary temporally in both carbon and nitrogen (Roth et al. 2007), further contributing to noise. Finally, it is possible that some lynx were using a different species of alternative prey that does not differ greatly from hares in  $\delta^{13}\text{C}$ , thus we see a clear signal for diet switching in  $\delta^{15}\text{N}$  only. Because of these confounding

influences we chose to analyze variability of lynx stable isotope ratios in addition to mean diet explained by the linear mixed models, to clarify differences in diet breadth in the absence of adequate prey stable isotope data.

The lynx of Fort Providence, NT appear to be highly sensitive to even minor changes in snowshoe hare densities, demonstrating measurable responses in both resource use and reproductive output. The cumulative evidence suggests that greater availability of snowshoe hare coincides with greater consumption of hares by all segments of the population and enables increased recruitment to the population. At the population level, our data support a trade-off of investing in reproduction when resources are abundant and prioritizing survival when resources are limiting. Demographically, our data demonstrate an age class effect whereby young, naïve individuals exploit alternative resources to contend with limited availability of snowshoe hare, while adults and their kits are able to maintain constant diets during initial hare decline. The age class effect provides a more precise definition for the degree of dietary flexibility exhibited by a specialist consumer, and implies a link between diet, survivorship, and population dynamics.

Lynx at the southern periphery of their range have demonstrated population declines and have been federally protected as a Threatened species in the contiguous United States (USFWS 2000; Murray, Steury & Roth 2008). The southern periphery is characterized by lower snowshoe hare densities and dampened population cycles (Hodges 2000; Murray 2000). Intrapopulation variation can potentiate larger range size and reduce extinction risk (Wennersten & Forsman 2012), thus the propensity of young lynx to use alternative prey may be a pivotal characteristic to maintain lynx populations in regions with chronically low snowshoe hare abundance. Future studies addressing such complexities will be critical to conservation and harvest management

planning for lynx, as well as for other carnivores that also demonstrate spatiotemporal variability in prey choice and diet breadth.

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**Table 1.1** Summary of factors considered at local (Fort Providence, NT) and broad (territory-wide) spatial scales for designation of environmental condition category (*EC*: G = good, M = marginal). Estimated age class structure of the study population is corrected for harvest bias based on Slough and Mowat (1996). Recruitment was calculated from age structure data corrected for harvest bias. The total number of lynx pelts harvested per winter in the Northwest Territories from 1997-1998 through 2000-2001 and the mean (and SD) estimated density of snowshoe hare from seven sites across the Northwest Territories from June 1998-2001 indicate broad scale environmental conditions.

Year	<i>n</i>	Local measures				Territory-wide measures				<i>EC</i>
		Juvenile (0 yrs)	Yearling (1 yr)	Subadult (2 yrs)	Adult (3+ yrs)	Lynx recruitment (juv. adult <sup>-1</sup> )	Snowshoe hare density (hares ha <sup>-1</sup> )	Lynx harvest (# pelts)	Snowshoe hare density (hares ha <sup>-1</sup> )	
1998	112	0.13	0.33	0.33	0.21	0.62	0.04	709	0.09 (0.27)	M
1999	199	0.35	0.32	0.11	0.22	1.58	0.14	1491	1.12 (1.65)	G
2000	93	0.33	0.34	0.15	0.18	1.87	0.49	1330	1.24 (0.67)	G
2001	165	0.07	0.38	0.37	0.18	0.40	0.23	711	0.78 (0.55)	M

**Table 1.2** Linear mixed models compared to identify predictors of mean Canada lynx population diet reflected in stable isotope ratios for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (n=505). Hypotheses included an interaction between environmental condition (*EC*) and age class (*A*), their main effects, and sex (*S*) as fixed effects. Random intercepts were fit for each year of collection (*Y*) to account for annual variation in stable isotope ratios of the prey community. For each model: *K* refers to the number of parameters; log-likelihood (*L*) indicates the probability of observed values;  $\Delta\text{AIC}$  is the difference between each model and the minimum AIC; model weight ( $w_i$ ) is the relative likelihood of each model ( $\exp(-0.5 \Delta\text{AIC})$ ) divided by the sum of relative likelihoods of all models in the set;  $R_m^2$  represents the marginal variance explained (fixed effects only); and,  $R_c^2$  represents the conditional variance explained by both fixed and random effects.

Model No.	Model	<i>K</i>	$\delta^{13}\text{C}$					$\delta^{15}\text{N}$				
			<i>L</i>	$\Delta\text{AIC}$	$w_i$	$R_M^2$	$R_C^2$	<i>L</i>	$\Delta\text{AIC}$	$w_i$	$R_M^2$	$R_C^2$
Global	EC:A + EC + A + S + (1 Y)	11	-196.10	10.57	0.00	0.03	0.18	<b>-545.47</b>	<b>1.72</b>	<b>0.17</b>	0.12	0.16
1	EC:A + EC + A + (1 Y)	10	-196.86	10.10	0.00	0.02	0.18	<b>-545.62</b>	<b>0.00</b>	<b>0.41</b>	0.12	0.16
2	EC + A + S + (1 Y)	8	-196.32	5.01	0.02	0.03	0.18	-550.18	5.12	0.03	0.11	0.15
3	EC + A + (1 Y)	7	-197.05	4.47	0.03	0.02	0.18	-550.24	3.24	0.08	0.11	0.15
4	EC + S + (1 Y)	5	<b>-197.72</b>	<b>1.82</b>	<b>0.12</b>	0.02	0.17	-552.59	3.95	0.06	0.10	0.14
5	A + S + (1 Y)	7	-196.48	3.34	0.06	0.01	0.17	-552.23	7.23	0.01	0.01	0.14
6	EC + (1 Y)	4	<b>-198.60</b>	<b>1.57</b>	<b>0.14</b>	0.02	0.17	<b>-552.61</b>	<b>2.00</b>	<b>0.15</b>	0.10	0.14
7	A + (1 Y)	6	-197.21	2.79	0.07	0.01	0.17	-552.30	5.36	0.03	0.01	0.14
8	S + (1 Y)	4	<b>-197.94</b>	<b>0.26</b>	<b>0.26</b>	0.00	0.17	-554.74	6.24	0.02	0.00	0.14
Null	1 + (1 Y)	3	<b>-198.81</b>	<b>0.00</b>	<b>0.30</b>	0.00	0.17	-554.76	4.30	0.05	0.00	0.14

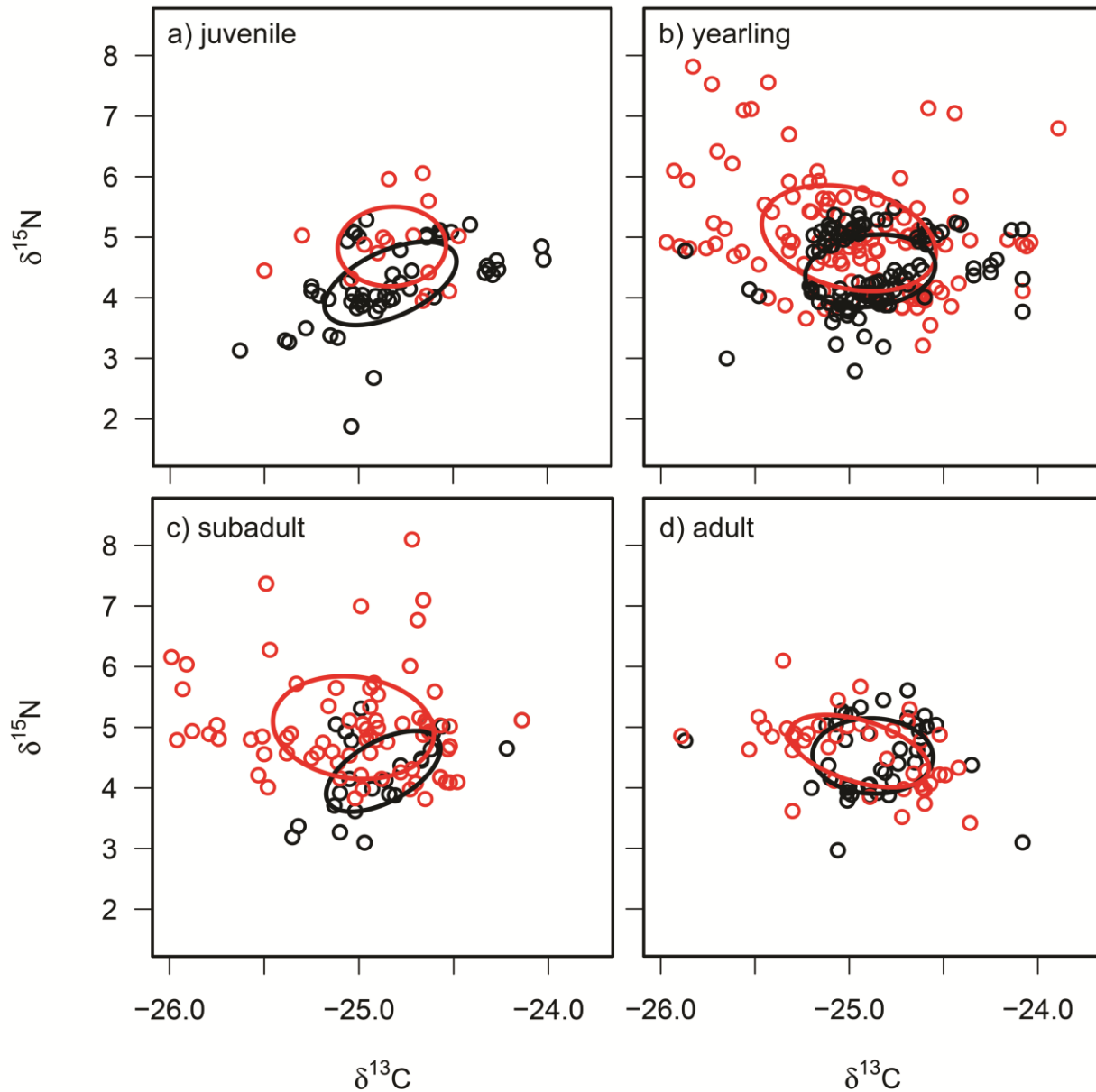
Models with  $\Delta\text{AIC} < 2$  were supported by the data and are highlighted in bold.

**Table 1.3** Parameter estimates of fixed effects for linear mixed models of mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of Fort Providence lynx. Models are presented in descending order from highest model weight to lowest from model selection (see Table 1.2). Deflections for environmental condition are from good to marginal in all cases. Deflections for age class are from juveniles to the age class in parentheses: yearlings (*y*), subadults (*s*), and adults (*a*). Deflections for sex are from male to female.

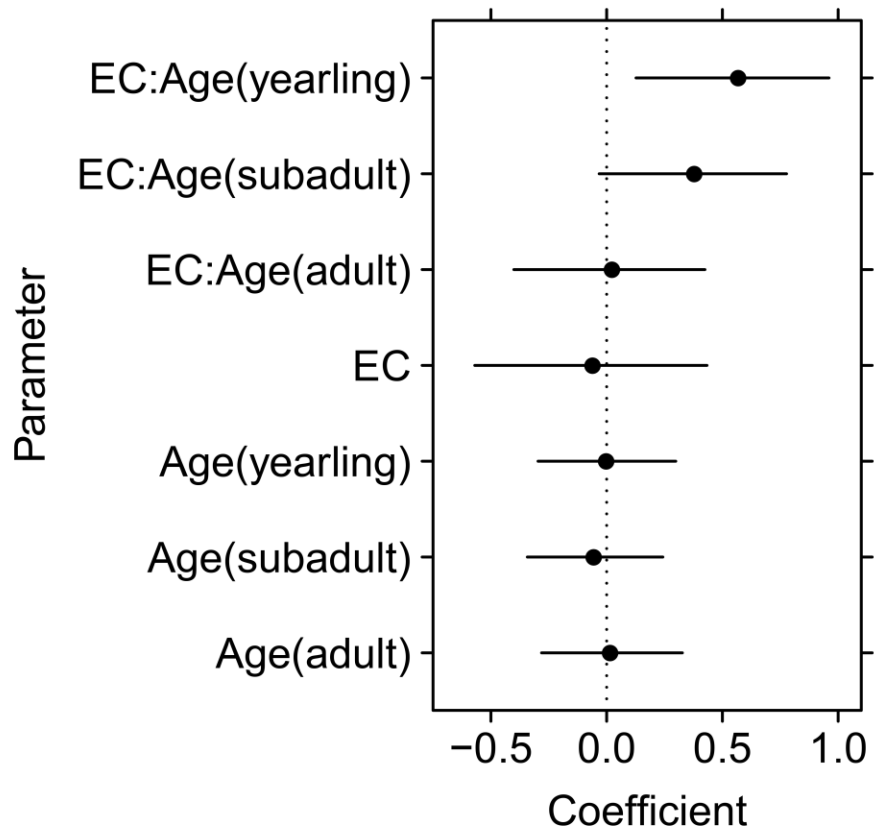
a) $\delta^{13}\text{C}$										
Model No.	Model	Intercept	<i>EC</i> : <i>A(y)</i>	<i>EC</i> : <i>A(s)</i>	<i>EC</i> : <i>A(a)</i>	<i>EC</i>	<i>A (y)</i>	<i>A (s)</i>	<i>A (a)</i>	<i>S</i>
Null	1 + (1  <i>Y</i> )	-24.90								
8	<i>S</i> + (1  <i>Y</i> )	-24.92								0.04
6	<i>EC</i> + (1  <i>Y</i> )	-24.90				-0.07				
4	<i>EC</i> + <i>S</i> + (1  <i>Y</i> )	-24.92				-0.07				0.04
7	<i>A</i> + (1  <i>Y</i> )	-24.84					-0.07	-0.09	-0.05	
5	<i>A</i> + <i>S</i> + (1  <i>Y</i> )	-24.86					-0.07	-0.09	-0.05	0.04
3	<i>EC</i> + <i>A</i> + (1  <i>Y</i> )	-24.84				-0.06	-0.07	-0.09	-0.05	
2	<i>EC</i> + <i>A</i> + <i>S</i> + (1  <i>Y</i> )	-24.86				-0.06	-0.07	-0.09	-0.05	0.04
1	<i>EC</i> : <i>A</i> + <i>EC</i> + <i>A</i> + (1  <i>Y</i> )	-24.83	-0.03	-0.05	-0.04	-0.03	-0.09	-0.10	-0.06	
Global	<i>EC</i> : <i>A</i> + <i>EC</i> + <i>A</i> + <i>S</i> + (1  <i>Y</i> )	-24.84	-0.03	-0.06	-0.04	-0.03	-0.08	-0.09	-0.06	0.04
b) $\delta^{15}\text{N}$										
Model No.	Model	Intercept	<i>EC</i> : <i>A(y)</i>	<i>EC</i> : <i>A(s)</i>	<i>EC</i> : <i>A(a)</i>	<i>EC</i>	<i>A (y)</i>	<i>A (s)</i>	<i>A (a)</i>	<i>S</i>
1	<i>EC</i> : <i>A</i> + <i>EC</i> + <i>A</i> + (1  <i>Y</i> )	4.55	-0.11	0.02	-0.41	0.45	0.13	0.05	0.00	
Global	<i>EC</i> : <i>A</i> + <i>EC</i> + <i>A</i> + <i>S</i> + (1  <i>Y</i> )	4.54	-0.11	0.02	-0.42	0.45	0.13	0.06	-0.01	0.03
6	<i>EC</i> + (1  <i>Y</i> )	4.63				0.34				
3	<i>EC</i> + <i>A</i> + (1  <i>Y</i> )	4.50				0.32	0.18	0.16	0.05	
4	<i>EC</i> + <i>S</i> + (1  <i>Y</i> )	4.62				0.34				0.01
Null	1 + (1  <i>Y</i> )	4.63								
2	<i>EC</i> + <i>A</i> + <i>S</i> + (1  <i>Y</i> )	4.49				0.32	0.18	0.16	0.05	0.02
7	<i>A</i> + (1  <i>Y</i> )	4.50					0.19	0.17	0.06	
8	<i>S</i> + (1  <i>Y</i> )	4.62								0.01
5	<i>A</i> + <i>S</i> + (1  <i>Y</i> )	4.48					0.19	0.17	0.06	0.02

**Table 1.4** Model selection results from an analysis of lynx diet breadth (estimated as  $SEAc$ ;  $n = 16$ ) as predicted by environmental condition ( $EC$ ), age class ( $A$ ), the interaction between environmental condition and age class ( $EC:A$ ), and random intercepts for year of collection ( $Y$ ). For each model:  $K$  refers to the number of parameters; log-likelihood indicates the probability of observed values;  $\Delta AIC$  is the difference between each model and the best fit model; model weight is the relative likelihood of each candidate model ( $\exp(-0.5 \Delta AIC)$ ) divided by the sum of relative likelihoods of all models in the set;  $R_m^2$  represents the marginal variance explained (fixed effects only); and,  $R_c^2$  represents the conditional variance explained by both fixed and random effects.

<b>Model</b>							
<b>No.</b>	<b>Model</b>	<b><math>K</math></b>	<b><math>L</math></b>	<b><math>\Delta AIC</math></b>	<b><math>w_i</math></b>	<b><math>R_m^2</math></b>	<b><math>R_c^2</math></b>
1	$SEAc = EC:A + EC + A + (1 Y)$	10	8.9	0.0	0.9	0.5	0.8
2	$SEAc = EC + (1 Y)$	4	0.4	4.9	0.1	0.1	0.3
3	$SEAc = A + (1 Y)$	6	2.4	5.0	0.1	0.2	0.5
Null	$SEAc = 1 + (1 Y)$	3	-0.2	6.4	0	0	0.3
4	$SEAc = EC + A + (1 Y)$	7	3.0	8.0	0	0.3	0.5



**Figure 1.1** Age-structured differences in isotopic niche breadth (*SEAc*) of lynx during marginal environmental conditions when snowshoe hare densities and lynx recruitment were low (red), and during good environmental conditions when snowshoe hare densities and lynx recruitment were high (black) for: a) juvenile lynx, b) yearling lynx, c) subadult lynx, and d) adult lynx.



**Figure 1.2** Parameter estimates of a split plot linear mixed effects model describing variation in *SEAc* estimates of lynx diet breadth by environmental condition, age class, an interaction of environmental condition and age class, and random intercepts for year of collection. Deflections for environmental condition are from good to marginal in all cases. Deflections for age class are from juveniles to the age class in parentheses. Error bars represent the 95% confidence interval of a basic bootstrap on parameter estimates.



## **Chapter 2: Are Canada lynx facultative specialists or obligate generalists when snowshoe hare are scarce?**

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C. M. Burstahler was responsible for study design, sample collection and preparation, analysis and interpretation of the data, and writing the manuscript. D. L. Murray and J. D. Roth contributed to critical revision of the work, including interpretation of the data and editing the manuscript.

### **Abstract**

Fluctuations in prey availability and geographic gradients in species diversity can affect foraging strategies of broadly-distributed predators. The facultative specialist hypothesis predicts that niche expansion by specialist predators is advantageous when preferred prey are limited and alternative prey are available, as might be expected of populations towards lower latitudes.

Canada lynx (*Lynx canadensis*) are a broadly-distributed species that rely on snowshoe hare (*Lepus americanus*) as primary prey and other species as alternative prey when snowshoe hare densities are low. We used stable isotope analysis of lynx hair to measure spatiotemporal changes in consumption of hare and other prey and predicted greater dietary breadth in southern populations and during low snowshoe hare densities in the north. Irrespective of space and time, hares were the primary food item in all lynx populations. However, the relative consumption of

alternative prey was consistently higher and less variable in southern lynx diets, while northern lynx only exhibited high dietary niche breadth during hare declines. As snowshoe hare fluctuations are dampened towards lower latitudes, our results suggest that the observed consistency in generalized feeding among southern lynx likely represents overall lower reliance on the species' primary prey. Thus, our results provide strong support for the facultative specialist hypothesis and highlight that traditional specialist-generalist feeding dichotomies are variable across spatiotemporal scales depending on the absolute and relative abundance of different prey types in the system.

## **Introduction**

There is broad interest in how predator resource use varies through space and time, consequent either to shifts in the abundance of preferred resources or the overall availability of alternative resources (Futuyma and Moreno 1988; Araujo and Costa-Pereira 2013). Resource use strategies are typically categorized as “specialist” or “generalist” depending on the breadth of resource types used, yet considerable variability within a species' realized niche may arise from changes in resource abundance, resource diversity, and interspecific competition for shared resources (Bolnick et al. 2011). Whether each strategy is obligate (fundamental) or facultative depends on how they respond to changes in resource availability (Figure 2.1). Obligate specialists have a narrow realized niche that equates to the fundamental niche because they rely invariably on one resource type (Shipley et al. 2009). Likewise, organisms that use many resources consistently through space and time are considered obligate generalists (Pagani-Núñez et al. 2016). Facultative consumers respond to changes in availability of resources across spatiotemporal scales by modifying their niche breadth to optimize energy intake, reproductive

success, and survival. For example, temporal fluctuations in prey abundance can prompt specialization by generalist consumers when prey are abundant (e.g., Akre and Johnson 1979; Pech et al. 1992), and niche expansion by specialist consumers when prey availability is restricted (e.g., Korpimäki et al. 1991; Herbst and Mills 2010). In broadly-distributed consumers, variable diversity of resource types across the landscape can also constrain certain populations to different realized niches, despite a common fundamental niche for the species (Shipley et al. 2009). Varying degrees of facultative niche variation along spatiotemporal gradients fill the spectrum between obligate specialists and obligate generalists.

Broadly-distributed species are often facultative foragers as they must adjust to a wide range of environments and inherently span a range of prey assemblages along the latitudinal diversity gradient (Hillebrand 2004). The facultative specialist hypothesis predicts that niche expansion by specialist consumers is advantageous when preferred prey are limited and adequate alternative resources are available (Glasser 1982), as might be expected of populations towards lower latitudes. When preferred prey are abundant, facultative specialists will narrow their realized niche exclusively to preferred resources. However, as preferred prey become limited, some individuals may expand their niche by consuming alternative prey or dispersing to another area that may have more prey or fewer competitors (Van Valen 1965). Glasser (1982) predicted that niche expansion by facultative specialists would manifest as increased within-individual diet variation when the consumer population is near carrying capacity and subject to unpredictable fluctuations in density, or as between-individual niche partitioning if the consumer population density remains stable near carrying capacity. Thus, we might expect to see greater within-individual diet variation at the periphery of a species' distribution where environmental conditions are less favourable and populations tend to be smaller; and, greater between-

individual variation in the core range of a species' distribution where environmental conditions are most suited to the organism and population sizes are large.

Canada lynx (*Lynx canadensis*) occur across the boreal forest of North America, where they exhibit 8-10 year population cycles with their primary prey, snowshoe hare (*Lepus americanus*; Krebs 2001). As hare populations undergo a cyclic crash and reach densities that can be 10-50 fold lower than peak numbers (Krebs et al. 2014), lynx exhibit lower reproductive output (Brand and Keith 1979), higher starvation (Poole 1994), and increased home range size and dispersal (Ward and Krebs 1985). Concurrent with these demographic changes, lynx respond to hare declines by killing more alternative prey species, including red squirrels (*Tamiasciurus hudsonicus*), grouse (*Bonasa spp.*, *Falcapennis spp.*), and small mammals (*Peromyscus spp.*, *Clethrionomys spp.*) (Brand et al. 1976; O'Donoghue et al. 1998a). These changes imply that lynx largely rely on their primary prey but exhibit some degree of dietary plasticity under extreme primary prey shortage (Burstahler et al. 2016). However, at the southern edge of their distribution in Canada and the contiguous United States, where hare abundance has been likened to cyclic lows of the core boreal range (Apps 2000), lynx must presumably navigate longer distances through a mosaic of habitats to access preferred prey (Murray et al. 2008). Our previous work showed that southern lynx rely less on hares than their northern counterparts (Roth et al. 2007), leaving open the question of whether southern lynx are less specialized because of geographic differences in their preference for hares versus plastic responses to variability in prey abundance.

In this paper, we reconstructed autumn lynx diets in select areas across their geographic range and over time, to test whether animals at lower latitudes are obligate or facultative in their foraging strategy relative to snowshoe hare availability. We predicted that northern lynx

populations would demonstrate facultative niche expansion with declining hare availability, as we have demonstrated previously (Burstahler et al. 2016). If southern lynx populations are facultative specialists, like their northern counterparts, then we predicted temporal fluctuations in prey use associated with changes in hare abundance and marked niche expansion as hare availability declines. In contrast, if southern lynx are obligate generalists, then we predicted continuous consumption of alternative prey and little change in diet breadth over time, regardless of changes in hare abundance.

## **Methods**

We used lynx hair from winter-harvested animals to capture the long-term integrated diet during autumn moult (4-6 weeks in late October-early November; Anderson and Lovallo 2003). During 2009-2012, we collected lynx hair for stable isotope analysis from harvested animals housed at North American Fur Auctions (Toronto, ON) and Fur Harvesters Auction Inc. (North Bay, ON). We cut a piece of skin and hair from the hind leg of each pelt, stored the sample in a coin envelope, and thoroughly cleaned all equipment between specimens. Following sample collection from auction houses, we acquired geolocations and year of harvest from trapping records available from provincial governments and auction houses.

We chose regions with at least 20 samples harvested within 250km of each other, over multiple years. Five study populations were selected: Alaska (centroid: 64.1730°N, 144.8074°W), Yukon Territory (centroid: 63.1248°N, 137.9848°W), Manitoba (centroid: 55.0097°N, 99.5403°W), Ontario (centroid: 49.6306°N, 92.0341°W), and Québec (centroid: 48.2348°N, 71.5529°W, Figure 2.2). Each centroid represents the centre of the minimum convex polygon drawn around the harvest geolocations, reported by registered trapline in Manitoba and

Ontario, and by management unit in Alaska, Yukon, and Québec. When subsets of available samples were large, we randomly selected 30 individuals per region per year for processing. To exclude juvenile lynx (i.e., < 1 years) from our analysis, we used samples with pelt lengths greater than 90 cm for northern regions (Slough 1996), and greater than 81 cm for southern regions (Quinn and Gardner 1984).

Within each region we sought samples from 2-3 winters each, where hare densities were deemed either 'high' or 'low' based on a combination of snowshoe hare abundance indices and lynx harvest statistics (Figure 2.3). We procured snowshoe hare abundance estimates from the literature and colleagues near each study region (Figure 2.2). Peak snowshoe hare abundance was observed in Bonanza Creek, AK during winter 2008-2009, and in Mayo, YT during winter 2006-2007 (Figure 2.3a,b; Krebs et al. 2014), followed by steep declines in abundance. In Voyageurs National Park, MN (the closest available hare data for our Ontario lynx population), winter 2008-2009 represented the highest hare abundance observed over the previous five years, although data for 2010-2011 are not available (Figure 2.3d; Moen 2009). As per the methods in Kawaguchi et al. (2015), collaborators provided hare abundance indices for Montmorency, QC that indicate a 280% increase in hare abundance from winter 2008-2009 to winter 2012-2013 (Figure 2.3e). No hare abundance indices were available for Manitoba during our study period (D. Berezanski *pers. comm.*), so designation was based on lynx harvest statistics alone.

Government harvest statistics suggest declines in lynx abundance from winter 2008-2009 to winter 2010-2011 by 30% in Alaska, 25% in Manitoba, 32% in Ontario, and by 18% in Yukon Territory from winter 2006-2007 to winter 2008-2009 (years for which we had adequate lynx samples) (Statistics Canada for 1980-2009, [www.statcan.gc.ca](http://www.statcan.gc.ca); Fur Institute of Canada for 2010-2014, [www.fur.ca](http://www.fur.ca)). Lynx harvest in Québec fluctuated between the three time points measured

over 6 years, declining by 13% from winter 2008-2009 to winter 2010-2011, then increasing by 35% to winter 2012-2013. Although the increasing trend in Québec hare abundance seems to contradict the initial decline in lynx harvest between time periods, we have not corrected for trapper effort, which may bias fur harvest statistics (Fortin and Cantin 2004). Accordingly, for our purposes we consider hare abundance more informative than lynx harvest statistics. Based on the above evidence, we assigned high hare availability to lynx harvested during winter 2008-2009 in Alaska, Manitoba, and Ontario, to lynx harvested during winter 2011-2012 in Québec, and from winter 2006-2007 in Yukon Territory; likewise, we assigned low hare availability to lynx harvested during 2010-2011 in Alaska, Manitoba, and Ontario, to lynx harvested during winter 2008-2009 in Yukon Territory and Québec, and included lynx harvested during winter 2010-2011 in Québec as an intermediate time point for comparison.

Interpretation of lynx stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) depends on those of predominant prey. As snowshoe hare and red squirrel comprise the largest proportion of lynx diets across their range (Aubry et al. 2000; Ivan and Shenk 2016), and preference for hares and red squirrels is evident even when other prey abundances are high (O'Donoghue et al. 1998b), we considered only snowshoe hares and red squirrels as primary prey of lynx. We acquired snowshoe hare fur from each region (except for Ontario), and obtained red squirrel hair during collections at the North American Fur Auctions (except for Alaska). In all cases, we collected hair from the haunch of the animal. Body hair of snowshoe hare and red squirrel moult in autumn (Layne 1954), coinciding with the autumn moult of lynx and, as hair is metabolically inert beyond the growth phase (Schwertl et al. 2003), thus our winter collected samples therefore reflect stable isotope ratios integrated during autumn moult. Guard hair and underfur may differ isotopically in some animals (e.g., Jones et al. 2006), but are not isotopically distinct in lynx

winter pelage (paired *t*-tests,  $n = 10$ ,  $P = 0.79$  for  $\delta^{13}\text{C}$  and  $P = 0.73$  for  $\delta^{15}\text{N}$ ). To prepare hair samples for measurement of stable isotope ratios we cut full strands of hair from the skin for each species, we washed hair samples with soap and water, rinsed thoroughly with water, and then dried in an oven at  $60^\circ\text{C}$  for 48 hours. Samples were homogenized with scissors, wrapped in tin capsules, and sent to the Chemical Tracers Laboratory, University of Windsor, Windsor, ON, for measurement using an isotope ratio mass spectrometer.

### *Data Analysis*

Analysis of stable isotope ratios can illuminate diet shifts and niche variation at the population level, as well as intrapopulation variation in prey use. First, diet reconstruction using mixing models identifies the proportional contribution of predominant prey to the consumer's diet and thus may identify the reliance on a single prey type (Phillips 2012). Second, variability of consumer stable isotope ratios can be interpreted to identify the degree of dietary specialization and intrapopulation variation in prey use (Bearhop et al. 2004; Newsome et al. 2007). Consistent with our conceptual model (Figure 2.1), a population of specialists will have the narrowest isotopic niche synonymous with that of their prey (Mazumder et al. 2016), while generalists will have a broad isotopic niche due to inputs from multiple prey types in different dietary proportions among individuals and perpetuated by variance in stable isotope ratios of prey (Bearhop et al. 2004). When used in conjunction with mixing models, the isotopic niche breadth can identify how similar individual diets are: closely clustered stable isotope ratios indicate that individuals have consumed the same prey types in similar proportions, while increasing isotopic niche breadth indicates divergence of individual diets (increasing between-individual variation).



Snowshoe hare stable isotope ratios are distinct from red squirrel (Table 2.1) and other alternative prey species (Roth et al. 2007), facilitating interpretation of lynx stable isotope ratios. The years from which lynx were harvested (2011 and earlier) did not align with prey samples available (2011 and afterwards); however, snowshoe hare stable isotope ratios do not vary inter-annually (Roth et al. 2007), and we pooled large regional samples of red squirrel across years to capture a conservative estimate of variability in stable isotope ratios. We confirmed that our snowshoe hare stable isotope ratios did not vary inter-annually using MANOVA (Yukon Territory:  $F_{1,16} = 0.14$ ,  $P = 0.87$ , Manitoba:  $F_{2,6} = 0.81$ ,  $P = 0.54$ , Québec:  $F_{1,10} = 1.14$ ,  $P = 0.36$ ), and assumed that stable isotope ratios of red squirrel measured in Yukon Territory were an adequate surrogate for lynx diet reconstruction of the Alaska population.

We used Bayesian stable isotope mixing models to reconstruct lynx diets from high to low hare availability in each region (R package “MixSIAR” v.3.1.7; Semmens et al. 2009). Mixing models for diet reconstruction use the mass-balance between a consumer’s stable isotope ratios and those of its prey to deduce feasible solutions for the proportion of each source in consumer diet (Parnell et al. 2013). Although lynx occasionally consume other species (Van Zyll de Jong 1966, Brand et al. 1976), inclusion of trace items in mixing models can lead to overestimates of prey contribution (Phillips et al. 2014). We corrected for differences between stable isotope ratios of prey hair (collected) and prey muscle (consumed by lynx) using discrimination factors measured in European rabbits for snowshoe hare ( $\Delta^{13}\text{C} = -1.5\text{‰}$ ,  $\Delta^{15}\text{N} = -0.6\text{‰}$ ; Neilson et al. 2005), and discrimination factors measured in deer mice for red squirrel ( $\Delta^{13}\text{C} = -1.0\text{‰}$ ,  $\Delta^{15}\text{N} = -0.8\text{‰}$ ; Miller et al. 2008). Then, we corrected for trophic enrichment of stable isotope ratios from prey muscle to lynx hair using a correction factor measured in Canada lynx ( $\Delta^{13}\text{C}: 2.4\text{‰}$ ;  $\Delta^{15}\text{N} 3.3\text{‰}$ ; Parnig et al. 2014). The trophic discrimination factor was

calculated for a single captive lynx, and so we assigned conservative standard deviations based on the highest range reported for other felids ( $\Delta^{13}\text{C}$ :  $\pm 0.5$  ‰,  $\Delta^{15}\text{N}$ :  $\pm 0.2$  ‰; Parnig et al. 2014). We used uninformative priors for all models. We ran three chains of 300 000 iterations, discarded the first 200 000 iterations, and thinned every 100 iterations (the “long” procedure specified by MixSIAR), providing 3000 feasible solutions of dietary proportions for each lynx population during high and low hare availability. Model convergence was verified by visual inspection of posterior chains, Gelman-Rubin statistics, and Geweke diagnostic z-scores. We calculated the probability that lynx dietary proportions differed between time periods by summing the number of times a pairwise comparison of posterior draws yielded greater consumption of red squirrel during low hare availability, then dividing by the total number of posterior draws.

We used Bayesian estimation to compare mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of Ontario lynx between time periods, as prey stable isotope ratios were not available for diet reconstruction. We used the  $t$  distribution to model our observations of each element and applied the same normality parameter (determining the heaviness of the tails) across time periods for each element (Kruschke 2013). A posterior predictive check confirmed that our posterior distributions represented the observed data well (mean + SD). We then subtracted the posterior distribution from high to low hare availability for each element to determine the credible difference in mean niche position.

The standard ellipse area of bivariate stable isotope data provides a measure of isotopic niche breadth for a population (Bearhop et al. 2004). We used a Bayesian resampling procedure – Stable Isotope Bayesian Ellipses in R (SIBER) – to assess statistical differences in lynx isotopic niche breadth from high to low hare availability within each region, based on the

standard ellipse area ( $SEA_B$ ) characterized by lynx stable isotope ratios (R package “SIBER” v.2.0.3, Jackson et al. 2011). Again, pairwise comparison of posterior draws determines the probability that niche breadth is larger when hare availability is low. All analyses were conducted using R version 3.3.2 (R Core Team 2016).

## Results

Lynx diets shifted to include more alternative prey with declining hare availability in Alaska, Yukon, and Manitoba, but mean niche position was similar among years in Ontario and Québec, despite changes in both hare abundance and lynx harvest (Figure 2.4). All mixing models converged well: Gelman-Rubin statistics for all variables in each model neared 1.0 (all chains < 1.01), and z-scores of Geweke diagnostics all fell within two standard deviations. The Yukon lynx population demonstrated the greatest reliance on snowshoe hare during high and low hare availability periods (median diet proportion = 85% and 74% snowshoe hare, respectively), followed by Alaska (high = 76% and low = 72% snowshoe hare), Manitoba (high = 72% and low = 65% snowshoe hare), and Québec (high = 70% (2012-2013), low = 70% (2008-2009), and intermediate = 69% (2010-2011) snowshoe hare; Figure 2.5a). Based on pairwise comparison of posterior draws, the probability that lynx consumed a greater proportion of red squirrel during low hare availability was 99% for Alaska, 100% for Yukon Territory, 100% for Manitoba, and 71% for Québec. Bayesian estimates of mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of Ontario lynx were similar between time periods ( $\delta^{13}\text{C}$ : 2009 = -24.6‰, 2011 = -24.8‰;  $\delta^{15}\text{N}$ : 2009 = 3.3‰, 2011 = 3.7‰), and the 95% credible intervals for difference between means of both elements spanned zero ( $\delta^{13}\text{C}$ : -0.4‰  $\leq$  -0.2‰  $\leq$  0.02‰;  $\delta^{15}\text{N}$ : -0.2‰  $\leq$  0.1‰  $\leq$  0.5‰).

Isotopic niche breadth ( $SEA_B$ ) of lynx populations in Alaska and Yukon Territory expanded from periods of high to low hare availability, narrowed in Ontario, and did not change in Manitoba or Québec lynx populations (Figure 2.5b). The probability that  $SEA_B$  during high hare availability was smaller than  $SEA_B$  during low hare availability was 99% for Alaska, 100% for Yukon Territory, 45% for Manitoba, 11% for Ontario, and 34% for Québec, based on pairwise comparison of posterior draws. Accordingly, we infer that latitudinal gradients not only increase reliance of lynx on alternative prey, but also that diet breadth of southern lynx populations does not fluctuate closely with the number of hares available and all individuals feed similarly on hares and alternative prey.

## **Discussion**

We found that temporal fluctuations in preferred prey availability did not affect lynx populations equally across their distribution, and that changes in prey availability corresponded with changes in foraging strategy at both the individual- and population- level. Northern lynx populations responded to declining hare availability by increasing between-individual variation, evident from increased proportional contribution of alternative prey to population diet estimates alongside isotopic niche expansion, thus supporting our previous observation that subadults shift to alternative prey while adults remain specialized on snowshoe hare (Burstahler et al. 2016). In contrast, different environmental conditions towards lower latitudes increased reliance of this “specialist” consumer on alternative prey such that all individuals adopted a more generalized foraging strategy (greater within-individual variation), evident from consistently higher consumption of alternative prey alongside a narrow and constant isotopic niche breadth. The diets of southern lynx remained temporally constant throughout fluctuations in hare availability,

indicating that a more generalized foraging strategy is obligate for lynx in these areas (albeit still considerably more specialized than other generalist consumers). Thus, our data provide strong support for the facultative specialist hypothesis and highlight that the pattern of niche expansion depends on the abundance of preferred prey and availability of alternative prey.

This latitudinal shift in lynx foraging strategies contrasts with the general observation that most species increase individual specialization (between-individual variation) towards southern latitudes (Araujo and Costa-Pereira 2013). The increasingly generalized feeding strategy of southern lynx suggests: 1) hare densities are sufficiently low that specialization is not possible, and/or 2) niche expansion is constrained by other environmental characteristics. Snowshoe hare abundances are markedly lower towards southern latitudes with cyclic peaks only 2-20 fold greater than nadirs of the cycle (Murray 2000); whereas, core boreal regions often reach peaks >4 hares/ha with cyclic amplitudes 10-50 fold higher (Krebs et al. 2014). Therefore, even though we have observed changes in hare abundance indices in southern regions during our study (Figure 2.3), the peak density may not meet the threshold density required for lynx to specialize completely on snowshoe hare. Furthermore, other environmental constraints in the southern periphery, like habitat fragmentation and a larger intraguild community of predators contending for the same resources, undoubtedly increase the search time (and risk) required to locate snowshoe hare (Murray et al. 2008) and thus favouring wider individual diet breadths.

The facultative specialist hypothesis, as defined by Glasser (1982), emphasizes the importance of predator-prey ratios to facultative foraging strategies, where diminishing prey availability limits the carrying capacity of the predator community and forces niche expansion. In highly variable environments, greater individual niche widths buffer against unpredictable changes in prey availability, whereas relatively stable environments allow for individuals to

partition resource types. The long duration of lynx-hare cycles in northern regions means that consecutive years present little flux in predator-prey ratios (excluding the 1-2 years following peak hare density), and therefore some niche partitioning is possible among lynx (Burstahler et al. 2016). We do not yet know the extent of intrapopulation diet variability of northern lynx populations at the nadir of hare cycles, but we predict that it will remain a mix of individual hare specialists and alternative prey specialists based on the facultative specialist hypothesis (Glasser 1982). In contrast, southern lynx exist at much lower densities and can fluctuate irruptively (Murray et al. 2008), meaning that they experience greater variance in predator-prey ratios from year-to-year and likely persist near carrying capacity. These demographic differences coupled with greater constraints on population growth from limited hare abundance, increased search time costs due to habitat fragmentation, and greater potential for interspecific competition (Murray et al. 2008), are likely responsible for the comparatively generalized foraging strategy and warrants further investigation.

The diet reconstruction mixing models herein are improved upon previous efforts by use of region-specific prey stable isotope ratios and species-specific diet-tissue discrimination factors. First, the spatial origin of prey aligns closely with those of consumers, despite the large geographic range considered, meaning we have greater precision when estimating dietary proportions because source inputs reflect trophically integrated stable isotope ratios from each environment that include inherent variability from stress, starvation, or other bottom-up influences on predator stable isotope ratios (Phillips et al. 2014). Second, diet-tissue discrimination factors vary considerably among species, tissues, and diets making use of generic correction factors problematic (Caut et al. 2008). Following the diet-specific argument for choosing trophic discrimination factors, Parnig et al. (2014) recommended using the correction

factors measured in bobcat for all wild felids because bobcat were fed a diet similar to what would be encountered in the wild (whole body rabbits instead of lean horse meat). The argument for diet composition suggests that lipids should not be extracted from lipid-rich prey when consumer hair is used for diet reconstruction because keratin is derived from carbon backbones of dietary lipids (Newsome et al. 2010). Thus, organisms eating prey with higher fat content should synthesize hair tissue reflecting lipid stable-carbon isotope ratios. However, the success of our mixing models demonstrates that species-specific trophic discrimination is preferred over surrogate species in this instance (using the bobcat trophic enrichment factor on our lynx data would have displaced the consumer outside of the prey isospace, see Phillips 2014).

Some caveats must be considered before directly interpreting patterns of stable isotope ratios as per our conceptual model (Figure 2.1), as they affect the variability of integrated consumer stable isotope ratios and may preclude spatial or temporal comparisons of consumer populations. First, high within-individual generalization can produce narrow isotopic niche widths that might imply specialization without further investigation because consuming multiple prey types in similar proportions will converge consumer stable isotope ratios to similar values (Matthews & Mazumder 2004). Our study uses this averaging effect to differentiate within- versus between-individual variation in diet, but requires concurrent measures of proportional diet to compare individual signatures against the population mean (Newsome et al. 2007). Second, spatial and temporal variation in prey stable isotope ratios (Flaherty and Ben-David 2010) and differences in prey community diversity contributing to consumer diets (Newsome et al. 2007) can imply differences in consumer isotopic niche breadth, even though the variation originates from the isotopic niche breadth of the prey. Our study controls for spatial variation in prey stable isotope ratios through region-specific measurements and temporal variation in red squirrel stable

isotope ratios through augmented sample sizes. It is likely that a greater diversity of prey species may contribute to southern lynx diets and, for this reason, we cannot directly compare isotopic niche breadths between regions. Ultimately, good *a priori* knowledge of important prey types, spatially and temporally aligned measurement of prey stable isotope ratios, and combining multiple diet metrics are essential to interpreting consumer foraging strategies from stable isotope data.

Populations of specialist species are declining throughout the world and ecological communities are increasingly comprised of generalists (Clavel et al. 2011). The generalist strategy is a response to lack of stability in the environment (Futuyma and Moreno 1988) and so it is reasonable to predict a shift towards more generalized behaviours at southern latitudes as the combined influence of prey community diversity, dynamics, and anthropogenic influence increases (Devictor et al. 2010). Canada lynx have demonstrated population declines, range constriction, and considerable disruption in the cyclicity of their density-dependent population cycles in the southern range (Murray et al. 2008). Currently, lynx occurrence is linked to the presence of boreal forest and the density of snowshoe hare, both of which already show signs of range constriction as climate changes (Peers et al. 2014). Peers et al. (2014) found an improvement in projected persistence of southern lynx populations when red squirrel were included in species distribution models, suggesting that the propensity of lynx to use greater proportions of alternative prey may help alleviate extinction risk. However, increasing mismatch in spatial distributions of snowshoe hare and red squirrel under climate change models may necessitate even greater modification of the realized niche for lynx to continue exploiting the areas where squirrels are anticipated to be abundant (Peers et al. 2014). Over the long run in their southern range, some individual lynx may need to become “specialists” on red squirrel (like



subadults during cyclic declines in northern regions, see Burstahler et al. 2016) or other alternative prey if they are to persist in their current distribution.

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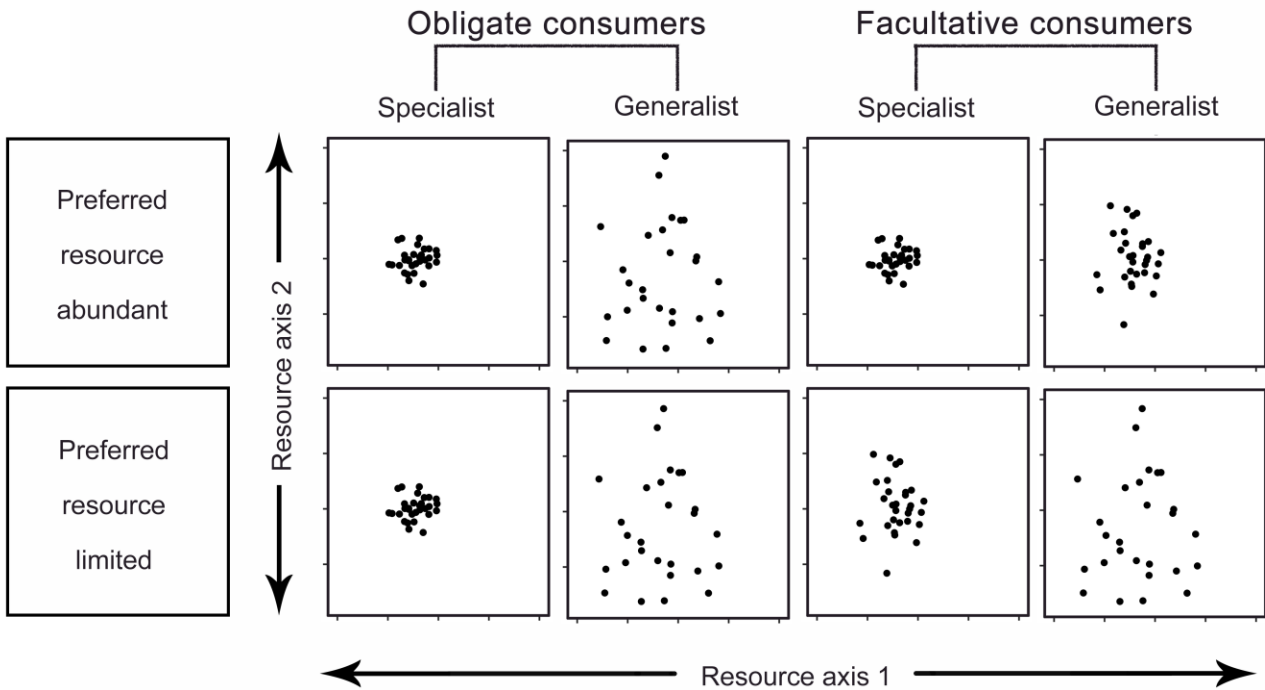
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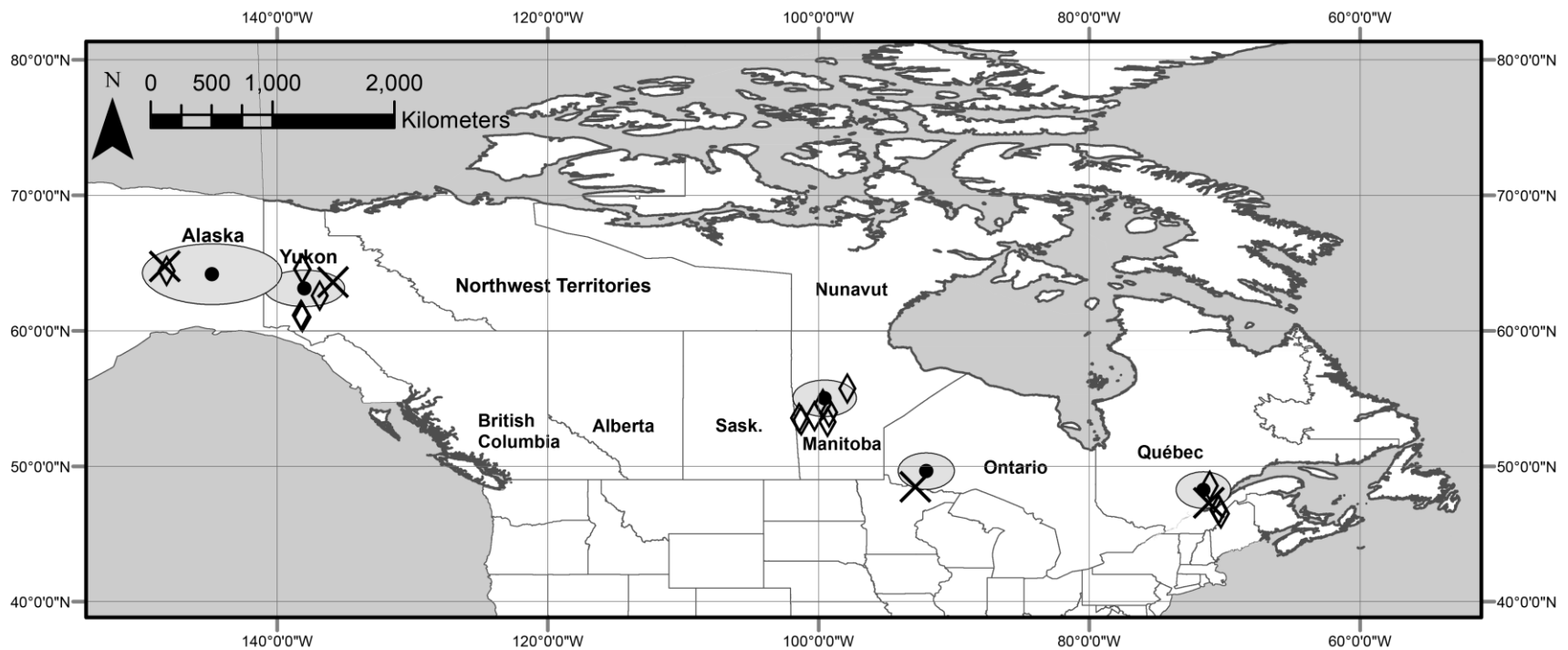


**Table 2.1:** Stable isotope ratios (mean and SD) of Canada lynx (*Lynx canadensis*), snowshoe hare (*Lepus americanus*), and red squirrel (*Tamiasciurus hudsonicus*) measured in each region. Lynx are subset into temporal periods designated as high or low snowshoe hare availability. Year reflects the winter trapping season (November-March).

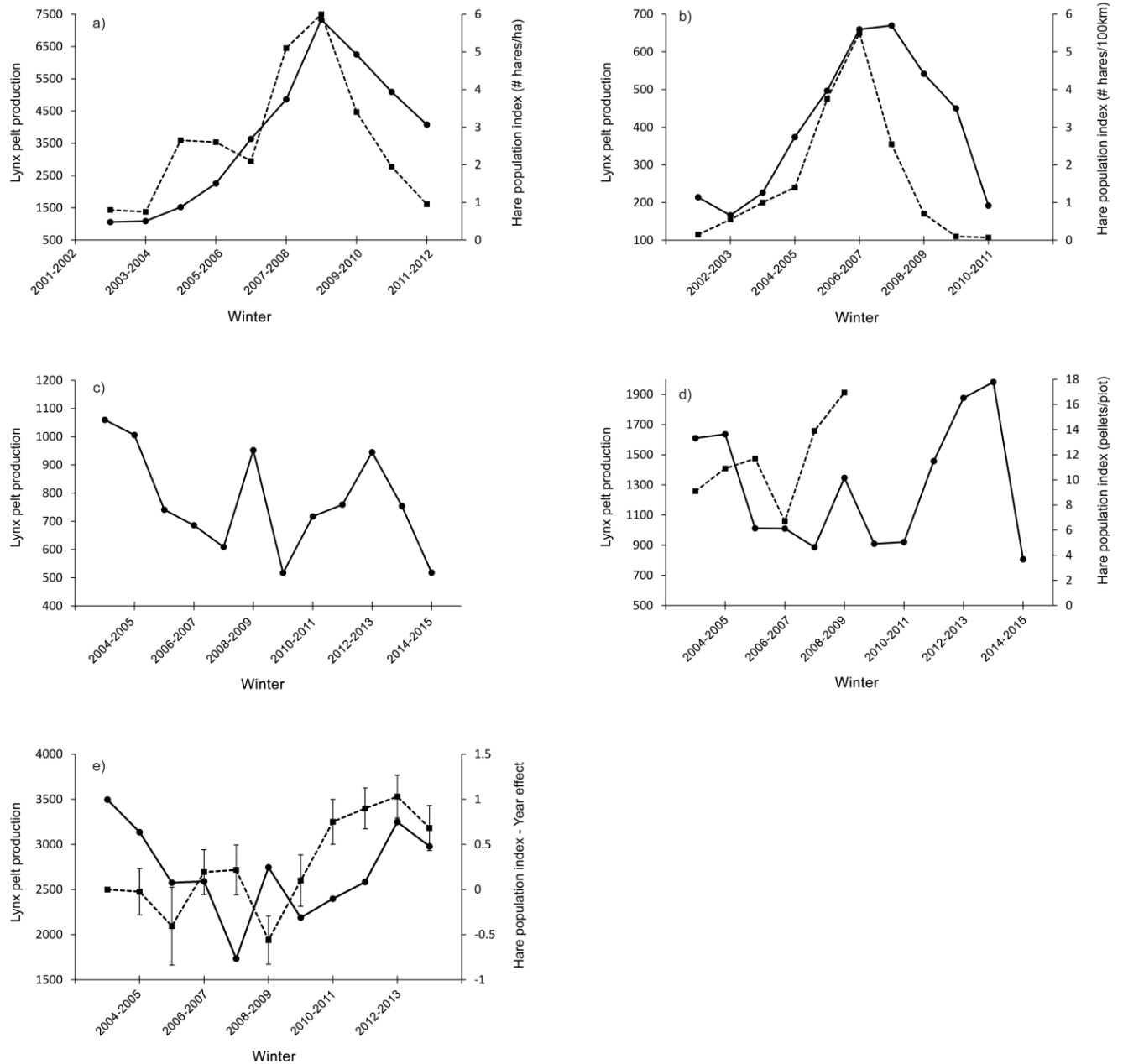
<b>Region</b>	<b>Species</b>	<b>Year</b>	<b><i>n</i></b>	<b><math>\delta^{13}\text{C}</math></b>	<b>SD</b>	<b><math>\delta^{15}\text{N}</math></b>	<b>SD</b>
Alaska	Lynx (high)	2008-2009	21	-24.3	0.32	5.2	0.93
	Lynx (low)	2010-2011	36	-24.1	0.51	5.6	1.22
	Snowshoe hare	2012-2013	14	-27.2	0.70	2.2	1.30
Yukon Territory	Lynx (high)	2006-2007	31	-24.7	0.30	5.4	0.68
	Lynx (low)	2008-2009	17	-23.9	0.55	5.8	0.56
	Snowshoe hare	2013-2014	18	-26.7	1.74	0.6	1.02
	Red squirrel	2011-2014	79	-20.4	1.04	7.2	5.34
Manitoba	Lynx (high)	2008-2009	28	-24.5	0.25	6.0	0.65
	Lynx (low)	2010-2011	30	-24.0	0.33	6.6	0.53
	Snowshoe hare	2013-2015	9	-27.4	0.80	3.3	1.48
	Red squirrel	2011-2013	58	-20.8	1.19	4.6	1.68
Ontario	Lynx (high)	2008-2009	28	-24.6	0.38	3.6	0.71
	Lynx (low)	2010-2011	30	-24.8	0.39	3.7	0.61
Québec	Lynx (high)	2008-2009	30	-24.7	0.33	4.5	0.43
	Lynx (low)	2010-2011	30	-24.7	0.38	4.5	0.48
	Snowshoe hare	2013-2014	9	-27.9	1.02	0.9	1.08
	Red squirrel	2011-2014	52	-21.1	1.13	5.3	2.47



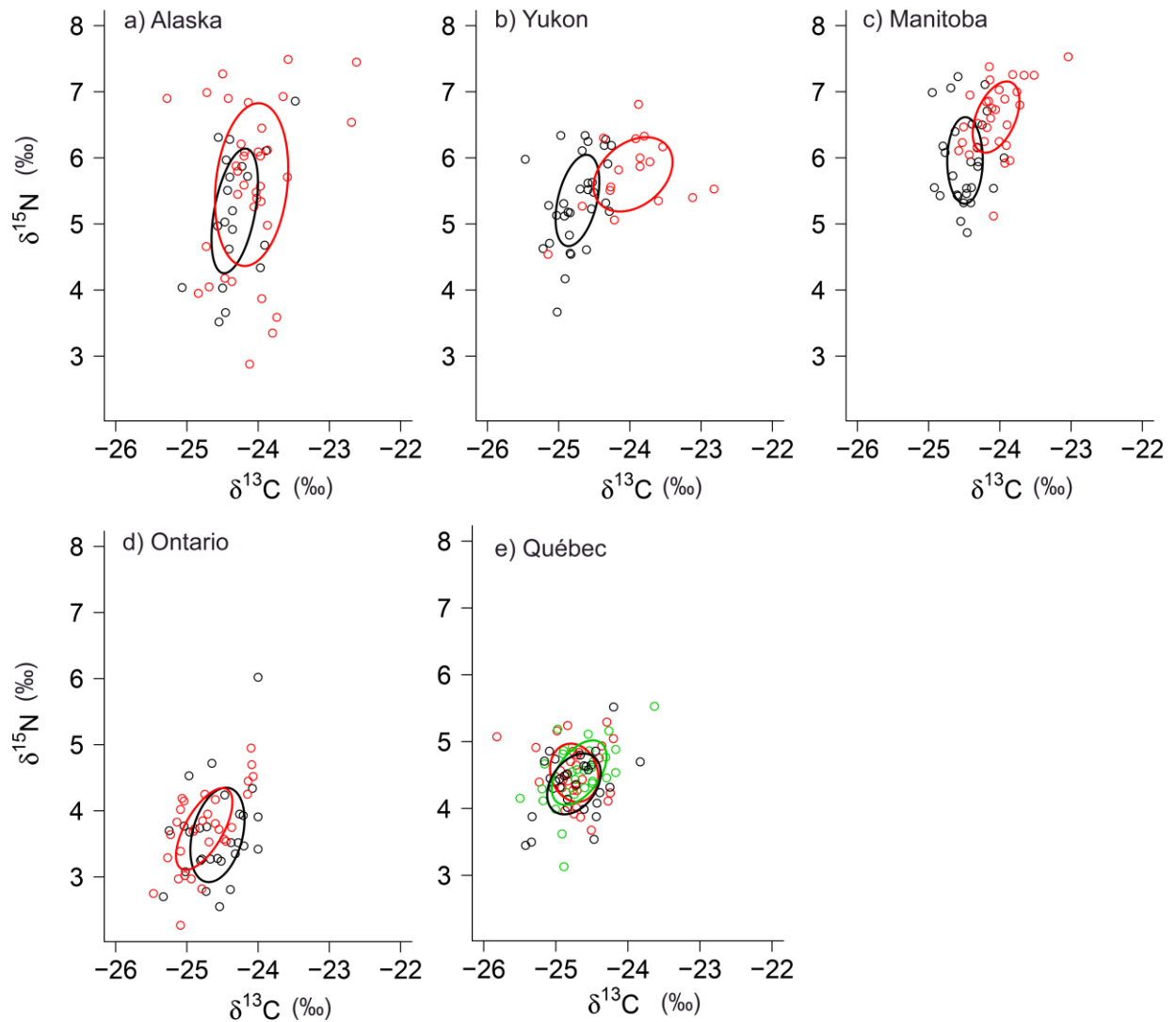
**Figure 2.1** Predicted dispersion of individual resource use along two related resource gradients in response to high and low availability of preferred resources for obligate and facultative organisms. Obligate niche strategies have consistently narrow (specialist) or broad (generalist) dispersion of individual resource use regardless of availability, while facultative specialists modify their niche breadth according to the relative abundance of one resource type. Specialists have the narrowest clustering of individual signals, as all individuals are using the same resource type. Towards generalized feeding strategies, clustering of individual resource use broadens due to inputs from multiple resources in variable proportions among individuals.



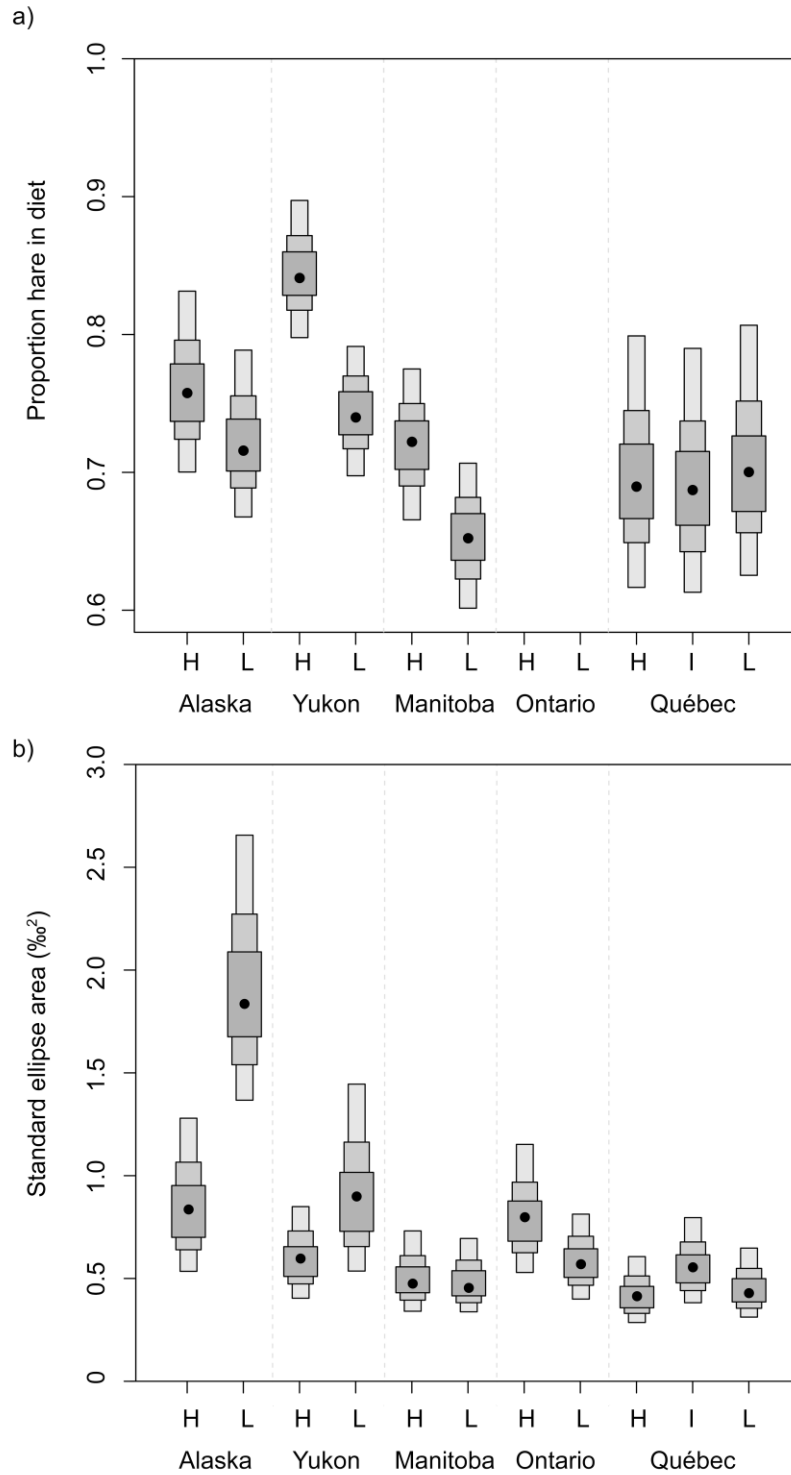
**Figure 2.2** Lynx harvest regions (point and ellipse), snowshoe hare sample origin (diamonds), and locations of snowshoe hare abundance indices (x mark). Pale green buffers indicate the range from which lynx samples were selected: 150 km from the centroid for Yukon, Manitoba, Ontario, and Québec; and, 200km from the centroid for Alaska. Snowshoe hare abundance indices come from: Bonanza Creek Experimental Forest, AK and Mayo, YT (Krebs et al. 2014); Voyageurs National Park, MN (Moen 2009); and Forêt Montmorency, QC (Kawaguchi et al. 2015).



**Figure 2.3** Lynx pelt harvest (solid lines) and snowshoe hare availability indices (dashed lines) in a) Alaska, b) Yukon Territory, c) Manitoba, d) Ontario, and e) Québec. The number of lynx harvested per winter in each province and territory is collected by Statistics Canada (up to 2009) and the Fur Institute of Canada (2010-present) based on The Census of Wildlife Pelt Production questionnaire. Hare abundance indices from Bonanza Creek Experimental Forest, Alaska were estimated using mark-recapture methods, and using pellet count transects from Mayo, Yukon Territory (Krebs et al. 2014). Snowshoe hare data overlaid with Ontario lynx harvest are pellet counts from Voyageurs National Park, MN (Moen 2009). The year effect estimates of hare abundance in Forêt Montmorency, QC represents the difference in mean winter track counts (per 200m) relative to 2004 based on GLM models after controlling for environmental covariates (see Kawaguchi et al. 2015 for details).



**Figure 2.4** Stable isotope ratios of Canada lynx populations in a) Alaska, b) Yukon Territory, c) Manitoba, d) Ontario, and e) Québec during high (black points) and low (red points) availability of snowshoe hare. Green points represent the intermediate year for Québec, and ellipses are the standard ellipse for each time period.



**Figure 2.5** Boxplots of posterior distributions for a) proportion of snowshoe hare in lynx diets and b) isotopic niche breadth ( $SEA_B$ ) for each study region during higher (H), lower (L), and intermediate (I) snowshoe hare availability. Boxes represent the 50%, 75%, and 95% credible intervals, and central tendency is the median. Dietary proportions for Ontario lynx were not estimated because region-specific snowshoe hare stable isotope ratios were not available.

## Chapter 3: Diet overlap and breadth of closely-related sympatric carnivores

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Contributions of Authors:

C. M. Burstahler was responsible for study design, sample collection and preparation, analysis and interpretation of the data, and writing the manuscript.

D. L. Murray and J. D. Roth contributed to critical revision of the work, including interpretation of the data and editing the manuscript.

### Abstract

Shared preference for a limited resource requires niche partitioning for species to coexist, and the dominant competitor for a preferred resource is determined by a trade-off between hunting proficiency and proclivity to use alternative resources. However, we are often restricted in our ability to accurately predict outcomes of niche partitioning because observations are typically limited in scale, lack replication, and therefore do not inform on the fundamental niche and full potential for adaptive responses among species in different environments. We used stable isotope analysis of samples collected from fur auction houses to investigate diet breadth and overlap of sympatric Canada lynx (*Lynx canadensis*) and bobcat (*Lynx rufus*) at a large spatial scale. We predicted that dietary niches of sympatric lynx and bobcat would overlap due to shared preference for lagomorphs, and that diet breadth of lynx (a facultative specialist) would be narrower than bobcat (a generalist). Patterns of variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in both species

were comparable across regions, with lynx occupying a narrow dietary niche that overlaps fully with bobcat, whereas >50% of bobcats feed on prey that is entirely different than lynx. This pattern of dietary overlap suggests that the potential for competition between lynx and bobcat is relatively high and that bobcat can readily adopt different prey choices, which may reflect either: 1) distinct individual feeding strategies related to local diversity in prey abundance; or 2) dietary displacement through competitive interactions. To conclude, we highlight the need to conduct field studies to better understand how diet overlap is maintained at finer spatial and temporal scales, as well as the drivers of dietary breadth and intrapopulation niche variation of sympatric specialist and generalist carnivores.

## **Introduction**

Most ecological communities conform to the shared preference hypothesis, which states that competing species will have overlapping fundamental niches that must be partitioned for these species to coexist (Wisheu 1998). Niche partitioning may occur through division of resource types used by each species, space used, or timing of resource exploitation, and the dominant competitor for a preferred resource is determined by a trade-off between superior exploitation and tolerance to using suboptimal resources (MacArthur and Levins 1964; McGill et al. 2006). Foraging theory predicts that the higher hunting proficiency of specialists should exclude generalists from preferred resources when availability is limited, but also that generalists can exclude specialists over time in highly variable or heterogeneous environments because their proclivity to use alternative resources supports higher consumer densities through periods of preferred prey scarcity (Stephens and Krebs 1986). Furthermore, within any environment we expect species to adopt a range of foraging strategies along the specialist-generalist spectrum,



and even within a species some individuals may exhibit dietary choices that are entirely inconsistent with the species' norm (Dennis et al. 2011). Thus, circumstance can favour either specialist or generalist foraging strategies when preferred resources are chronically limited, depending on the relative strength interspecific interactions, and will be highly specific to the circumstance of local environmental conditions.

Despite widespread appreciation for the spatiotemporal flexibility of foraging strategies to accommodate coexistence of species (Devictor et al. 2010), studies examining niche partitioning are often limited in scale and lack replication, leaving considerable uncertainty regarding the generality of population-level dietary choices and highlighting the need to contrast communities at broad spatial scales (Nakagawa and Parker 2015). For example, Pfennig et al. (2006) found differential outcomes of competition between two species of spadefoot toads (*Spea* spp.) depending on the relative availability of resource types in natural ponds: resource-rich ponds enabled coexistence and overlap in resource use, while limitation of detritus or shrimp consistently excluded one species of toad or the other. Complementary experiments confirmed these outcomes of character displacement due to resource availability; however, observations in other regions have identified ponds where the inferior competitor presides despite resource availability suited to the other species. Thus, even with considerable replication within a study area or set of conditions, there is need to test biotic interactions across broad spatial scales to determine the extent to which well-defined ecological patterns hold and what other environmental characteristics affect these relationships. Herein, we contrast the pattern of niche breadth and overlap between free-living consumers with shared preference for a limited resource across the continent to determine the extent to which niche partitioning and foraging strategy are preserved at broad spatial scales.

Canada lynx (*Lynx canadensis*) and bobcat (*Lynx rufus*) are closely-related species that have typically been classified as “specialist” and “generalist” consumers, respectively (Anderson and Lovallo 2003). These species occupy largely discrete geographic distributions, with lynx occurring throughout the boreal forest and bobcat occurring throughout the contiguous United States and Mexico (Peers et al. 2012). Yet, widespread increases in bobcat abundance support the suggestion that northward range expansion has established considerable overlap and competition with lynx at the intersect of their distributions (Roberts and Crimmins 2010; see also Peers et al. 2013). Species distribution models of lynx and bobcat have challenged the conventional generalist-specialist paradigm by identifying similar or higher peak performance of bobcat in most climatic and geographic conditions (Peers et al. 2012). Moreover, lynx and bobcat occupy similar climatic niches when allopatric, but lynx are constrained to a narrow, non-overlapping subset of their fundamental niche when sympatric with bobcat, suggesting that intraguild competitors may spatially exclude lynx from optimal niches at finer scales (Peers et al. 2013). The areas where lynx persist are associated with extreme climates for which they are well adapted (colder temperatures, deep snow, etc.), unlike bobcat, and suggests that spatial niche partitioning plays an important role in species coexistence. However, how presence of either predator affects the foraging strategy of the other remains unclear.

While both lynx and bobcat feed on lagomorphs, the former are recognized as being specialized on snowshoe hare (*Lepus americanus*) due to their ability to forage on this species in deep snow (Murray and Boutin 1991). Yet, lynx are facultative specialists that vary their foraging strategy according to prey availability (O’Donoghue et al. 1998). In northern regions, all individuals specialize on snowshoe hare until cyclic declines in hare abundance spur distinct niche expansion driven by subadults (Burstahler et al. 2016). Towards southern latitudes,

reliance on alternative prey is increasingly pronounced as all individuals consistently consume greater proportions of alternative prey despite fluctuations in hare availability (Chapter 2). It remains unclear whether the increasingly generalized feeding strategy of southern lynx reflects chronically low hare densities, increased availability of alternative prey, or constraint to suboptimal resources by interspecific competition. Bobcat also prefer lagomorphs as prey, including snowshoe hare (Anderson and Lovallo 2003), but can exhibit high dietary plasticity as evidenced by the variety and frequency of prey types encountered in bobcat diets across their distribution (Koehler and Hornocker 1991; McLean et al. 2015). Where both species occur, snowshoe hare fluctuations are attenuated (Murray 2000), enhancing the potential for competitive interactions through chronic limitation of preferred prey.

In this paper, we measured dietary breadth and overlap of sympatric lynx and bobcat occurring in four different regions (southern British Columbia, western Ontario, central Ontario, and southern Québec) to investigate the generality of niche partitioning and the generalist-specialist paradigm at large spatial scales. Consistent with the shared preference hypothesis and specialist-generalist foraging strategies, we predicted that dietary niches of sympatric lynx and bobcat would overlap and that diet breadth of lynx would be narrower than bobcat. Dietary overlap should be consistent across regions, unless fine scale outcomes of competitive interactions affect broad-scale patterns of niche overlap and partitioning.

## **Methods**

To compare niche breadth and overlap between species, we measured stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of lynx and bobcat hair. Canada lynx and bobcat share similar hair growth patterns, moulting once in spring (April-May) and once in autumn (October-November; Quinn

and Parker 1987; Anderson and Lovallo 2003); therefore, our stable isotope measurements from winter-harvested animals reflect the autumn diet for both species. During 2009-2012, we cut lynx hair from the hind leg of harvested animals collected at the North American Fur Auctions (Toronto, ON) and Fur Harvester Auction Inc. (North Bay, ON). All hair samples were washed with soap and water, rinsed thoroughly, and then oven-dried at 60°C for 48 hours. Samples were homogenized to a fine powder using a ball mill, wrapped in tin capsules, and sent to the Chemical Tracers Laboratory, Windsor, ON, Canada for measurement using a continuous-flow isotope ratio mass spectrometer.

Following sample collection, we acquired harvest geolocations for pelts from government offices and fur auction houses to identify the registered trapline or wildlife management unit where each animal originated. We targeted samples from four study regions where lynx and bobcat distributions were previously described to intersect (Peers et al. 2012): 1) southeastern British Columbia (centroid = 50.355°N, 119.212°W; area = 55 027 km<sup>2</sup>), 2) west of Lake Superior in Ontario and Minnesota (centroid = 48.987°N, 92.534°W, area = 57 544 km<sup>2</sup>), 3) the interlake region of Sault Ste. Marie, Ontario and Michigan (centroid = 46.820°N, 86.365°W, area = 6 808 km<sup>2</sup>), and 4) southern Québec along the St. Lawrence river (centroid = 47.365°N, 71.602°W, area = 55 000 km<sup>2</sup>). Centroids represent the centre of the polygon drawn around the outermost harvest geolocations of all individuals in each study region, hereafter referred to as British Columbia, western Ontario, central Ontario, and Québec.

Interpretation of predator stable isotope ratios depends on those of predominant prey, which may vary spatially and temporally. We assumed that predators occupying the same region had access to the same prey assemblage, and therefore variation in predator stable isotope ratios reflects differences in prey use. Differences in mean stable isotope ratios indicate reliance on

different types of resources consumed by each consumer population (Phillips 2012), while niche breadth can be interpreted from the variability in predator stable isotope ratios (Bearhop et al. 2004). Greater variability in stable isotope ratios indicates divergence of individual diets (i.e., individuals consumed different prey types and/or in different proportions), and closely clustered stable isotope ratios indicate similarity in individual diets (Matthews and Mazumder 2004).

Observations of each species per region were pooled across years because some annual samples were small and visually indistinguishable from years with larger sample sizes.

MANOVAs failed to detect annual differences in stable isotope ratios of lynx or bobcat within each region ( $P > 0.05$ ), with one exception: in British Columbia,  $\delta^{15}\text{N}$  of lynx was lower in 2011 (mean  $\pm$  SE =  $5.03 \pm 0.20\text{‰}$ ,  $n = 7$ ) compared with 2009 ( $5.95 \pm 0.23\text{‰}$ ,  $n = 9$ , Tukey's HSD  $P = 0.04$ ).

We compare niche breadth and overlap of lynx and bobcat within each region using the R packages "SIBER" (Jackson et al. 2011) and "nicheROVER" (Swanson et al. 2015; Lysy 2015), whereby the isotopic niche region defined by each niche axis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) is treated as a trait probability density function that can be compared against other groups (Carmona et al. 2016). Uncertainty is incorporated into niche region estimates using a Bayesian framework, whereby the 95% highest density interval for each niche axis is used as a continuous distribution from which to sample iteratively. We verified multivariate normality of each population graphically using qq-plots of Mahalanobis distances (Legendre and Legendre 2012, p.193), to meet the assumptions of both SIBER and nicheROVER. Three bobcat were statistical outliers that were excluded from analysis: one from British Columbia with very low  $\delta^{15}\text{N}$  ( $-0.32\text{‰}$ ), one from British Columbia with very high  $\delta^{13}\text{C}$  ( $-17.5\text{‰}$ ), and one from Québec that was enriched in both  $^{13}\text{C}$  and  $^{15}\text{N}$  ( $\delta^{13}\text{C} = -18.5\text{‰}$ ,  $\delta^{15}\text{N} = 9.14\text{‰}$ ). These outliers could represent dispersing

individuals whose stable isotope ratios reflect prey signatures from their autumn foraging region which may differ from prey signatures in the region where they were harvested. Their stable isotope ratios are consistent with the range of values reported for North American mammals (Roth et al. 2007), suggesting these are not necessarily biological outliers.

Niche regions based on the mean and variance of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for each species were estimated using an uninformative, normal Inverse Wishart prior ( $\alpha = 95\%$ ). We ran two chains of 10 000 iterations, burning the first 1000 draws and thinning every 10 draws. The breadth of resource use along both diet axes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) can be summarized by the standard ellipse area (*SEA*) in SIBER, and further broken down into each niche axis for analysis of niche position and overlap in nicheROVER. Posterior distributions represent the full range of feasible parameter values, given the observed data and prior probabilities (which we have kept vague to minimize influence on the posterior). Thus, probabilistic difference in niche position and variance among species is inferred from overlap of posterior distributions. As probability density functions integrate to 1, the density of posterior solutions is directly proportional to the relative proportion of that trait value in the population (Carmona et al. 2016) and thus may be used as a proxy for exploitation proficiency of the population (Devictor et al. 2010). Niche overlap is then calculated as the probability of locating a random individual from one species within the 95% highest density niche region of the competitor species.

## **Results**

Stable isotope ratios showed that bobcat used a greater variety of prey types and demonstrated greater disparity among individual diets in all four regions (Figure 3.1). Overall,

the isotopic niche breadth of bobcat was >7 times larger than lynx in Québec, 3 times larger than lynx in British Columbia, and 2 times larger in western and central Ontario (Figure 3.2).

The niche position of lynx and bobcat differed along one niche axis ( $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ ) in all study regions (Figure 3.3). Mean  $\delta^{13}\text{C}$  of British Columbia bobcat was 1.7‰ higher than lynx, but similar in  $\delta^{15}\text{N}$ . Bobcat had higher mean  $\delta^{15}\text{N}$  than lynx in Québec (0.7‰), western Ontario (0.9‰) and central Ontario (0.8‰), but shared similar mean  $\delta^{13}\text{C}$  with lynx. The niche regions of bobcat were consistently wider than lynx, indicating greater variety in food types were used by bobcat, while niche breadth of lynx was narrow, indicating limited variability in food types used.

Lynx diets overlapped significantly with the 95% niche region of bobcat in all regions (Figure 3.4). In contrast, the probability of an individual bobcat's diet overlapping with the dietary niche of the sympatric lynx population was considerably lower in all regions (Figure 3.4), due to the large breadth of resources used by bobcat (Figure 3.3). The probabilities of lynx occurring within the niche region of bobcat were highly skewed towards 100%, emphasizing the likelihood of complete niche overlap by bobcat populations with sympatric lynx. Bobcat from western Ontario had the greatest probability of overlapping with lynx (median = 50%), followed by Québec (median = 38%), central Ontario (median = 35%), and British Columbia (median = 34%). The degree of diet overlap between species was less certain in central Ontario, as indicated by broad credible intervals, undoubtedly due to the relatively small sample of bobcat from the region (n=8). Notwithstanding, the remarkable consistency with which these species' dietary niches overlapped suggests that general patterns of niche partitioning operate at large spatial scales.

## Discussion

Our results point to surprising consistency in the pattern of diet overlap and niche width between sympatric carnivores when examined at a large spatial scale. In each study region, our measures of integrated autumn diets support the traditional view that lynx are specialists that exploit a narrow range of resources with high efficiency, whereas bobcat are generalists that use a broader range of resources. High variance among individual bobcat diets meant that over 50% of the population was feeding differently from lynx in all regions, while lynx niches overlapped completely with the dietary niche of bobcat. This pattern of dietary overlap suggests that potential for exploitation competition between lynx and bobcat is relatively high at finer spatial scales. The variability of bobcat diets highlights their propensity to adopt different prey choices and may reflect either: 1) distinct individual feeding strategies related to local diversity in prey abundance; or 2) dietary displacement through exploitation competition. That the same pattern of niche differentiation is repeated, despite likely differences in community structure across regions, highlights the generality of the specialist-generalist paradigm at large spatial scales and adds to the growing repertoire of examples supporting the shared preference hypothesis (e.g., Wisheau 1998; Pither and Aarssen 2005).

In addition to differential prey use, coexistence of species can be maintained through partitioning space and timing of resource exploitation. For example, dolphins (*Tursiops truncatus*) and bull sharks (*Carcharhinus leucas*) in the same estuary have overlapping diets and use the same foraging regions, but they visit shared foraging sites at different times and thus coexistence is enabled through temporal partitioning of resource use (Matich et al. 2017). Likewise, fine-scale habitat partitioning can allow shared access to resources in patchy environments without interference between consumers (Palomares et al. 2016). The abiotic niche



models of lynx and bobcat suggest that spatial niche partitioning occurs to some extent at finer scales (Peers et al. 2012, 2013), which might explain how certain bobcat share the dietary niche of sympatric lynx at the broad spatial scale considered herein. Additionally, lynx home ranges can be very small ( $<10 \text{ km}^2$ ) or large ( $>200 \text{ km}^2$ ) (Burdett et al. 2007), creating ample opportunity for spatial and temporal niche partitioning of foraging areas with interspecific competitors.

Diet overlap of sympatric organisms is a precursor to exploitation and interference competition (Donadio and Buskirk 2006), and competitive interactions between lynx and bobcat are expected to intensify if snowshoe hare abundance is severely limited. Increasing evidence suggests that lynx in southern extent of their distribution are more generalized in their foraging strategy (Chapter 2; Aubry et al. 2000; Roth et al. 2007). Not only do these populations consume greater proportions of alternative prey, but individual diet breadths are consistently wide and do not track fluctuations in hare abundance (Chapter 2), indicating that hares are sufficiently scarce to force all individuals to consume alternative resources (Svanbäck and Bolnick 2005). Complete niche overlap with bobcat further suggests that interspecific competition may contribute to the obligate generalist strategy of lynx in these areas by further reducing availability of preferred prey (Chapter 2). In contrast, bobcat can clearly adjust if they are excluded from snowshoe hare by consuming other prey. We hypothesize that diet overlap at this broad spatial scale is maintained through finer scale constraints on space use by lynx (e.g., Peers et al. 2013), which would allow for certain bobcat to maintain diets similar to lynx in the region. Future investigations considering snowshoe hare availability and fine scale habitat use of sympatric lynx and bobcat could disentangle whether prey availability or competition is driving prey use patterns by lynx.

Our results provide compelling evidence for the generality of the specialist-generalist paradigm at large spatial scales in predator communities. Considering the propensity for organisms to adjust their foraging strategies to meet a variety of environmental constraints, lynx and bobcat demonstrated remarkable consistency in the pattern of diet overlap across the continent. We conclude that lynx and bobcat demonstrate shared preference for snowshoe hare, but that bobcat can adopt alternative resources when necessary. The underlying biological processes determining niche partitioning between these species remain unknown and require consideration of niche overlap at finer spatial and temporal scales with concurrent measures of prey availability.

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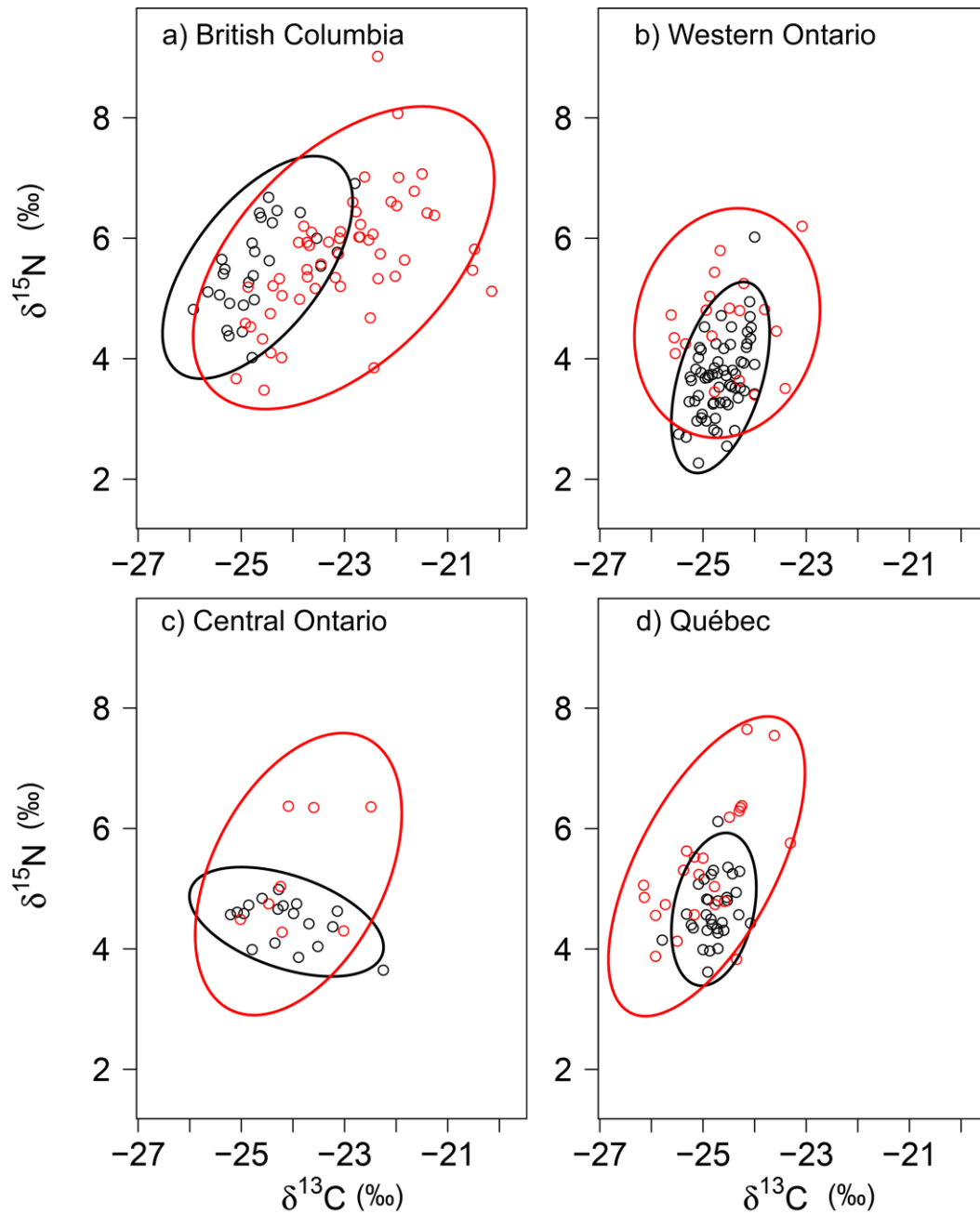
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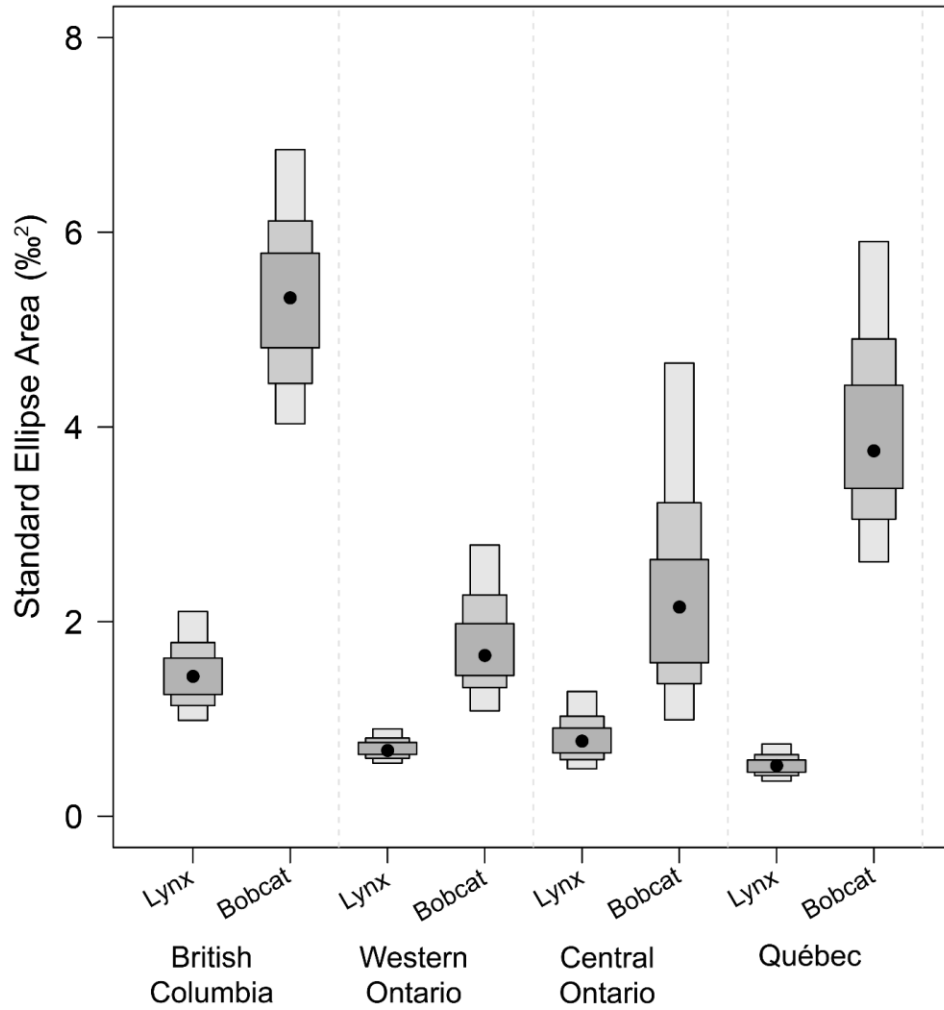
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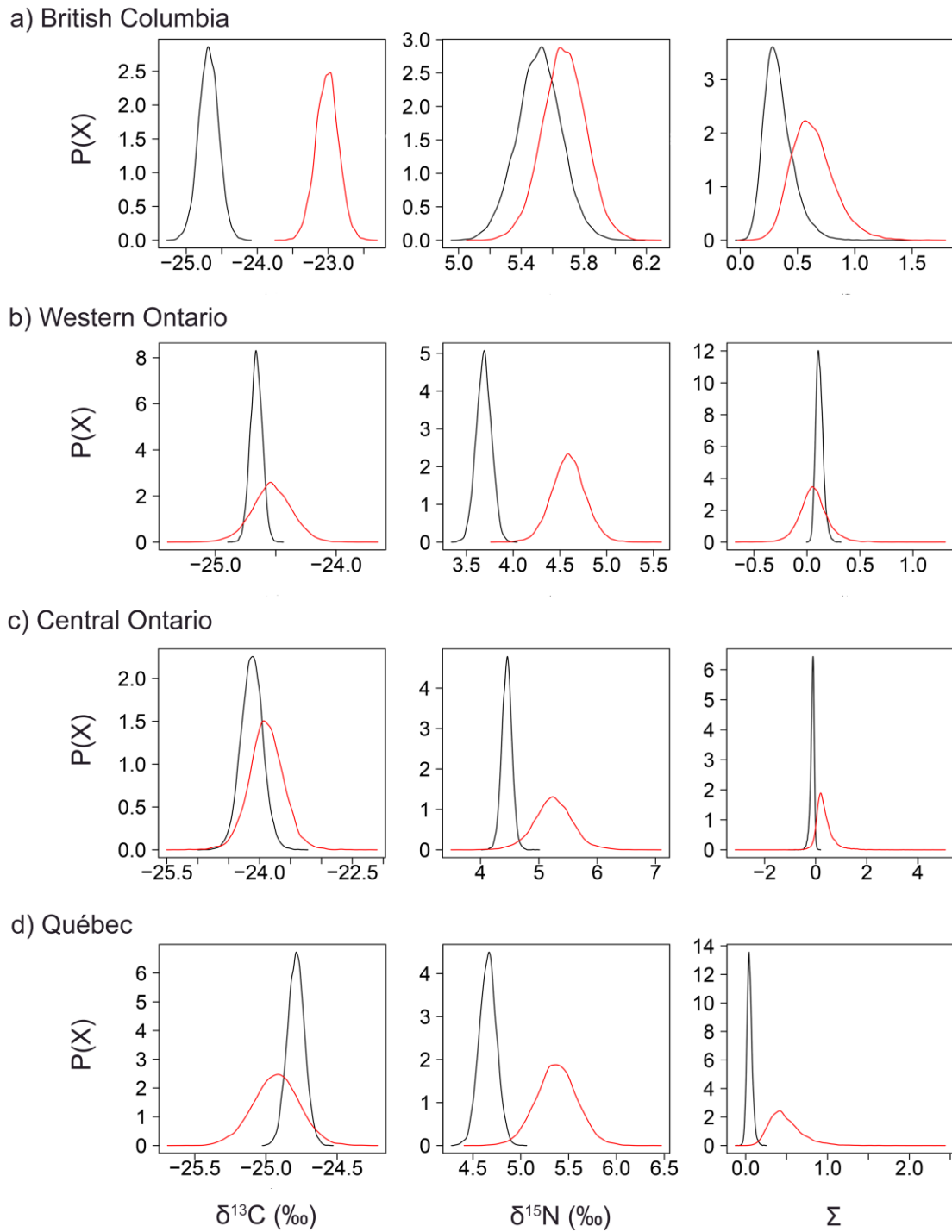


**Figure 3.1** Stable isotope ratios of lynx (black points) and bobcat (red points) in each of our study regions: a) southeastern British Columbia, b) western Ontario, c) central Ontario, and d) Québec. Ellipses are the 95% probability ellipse.

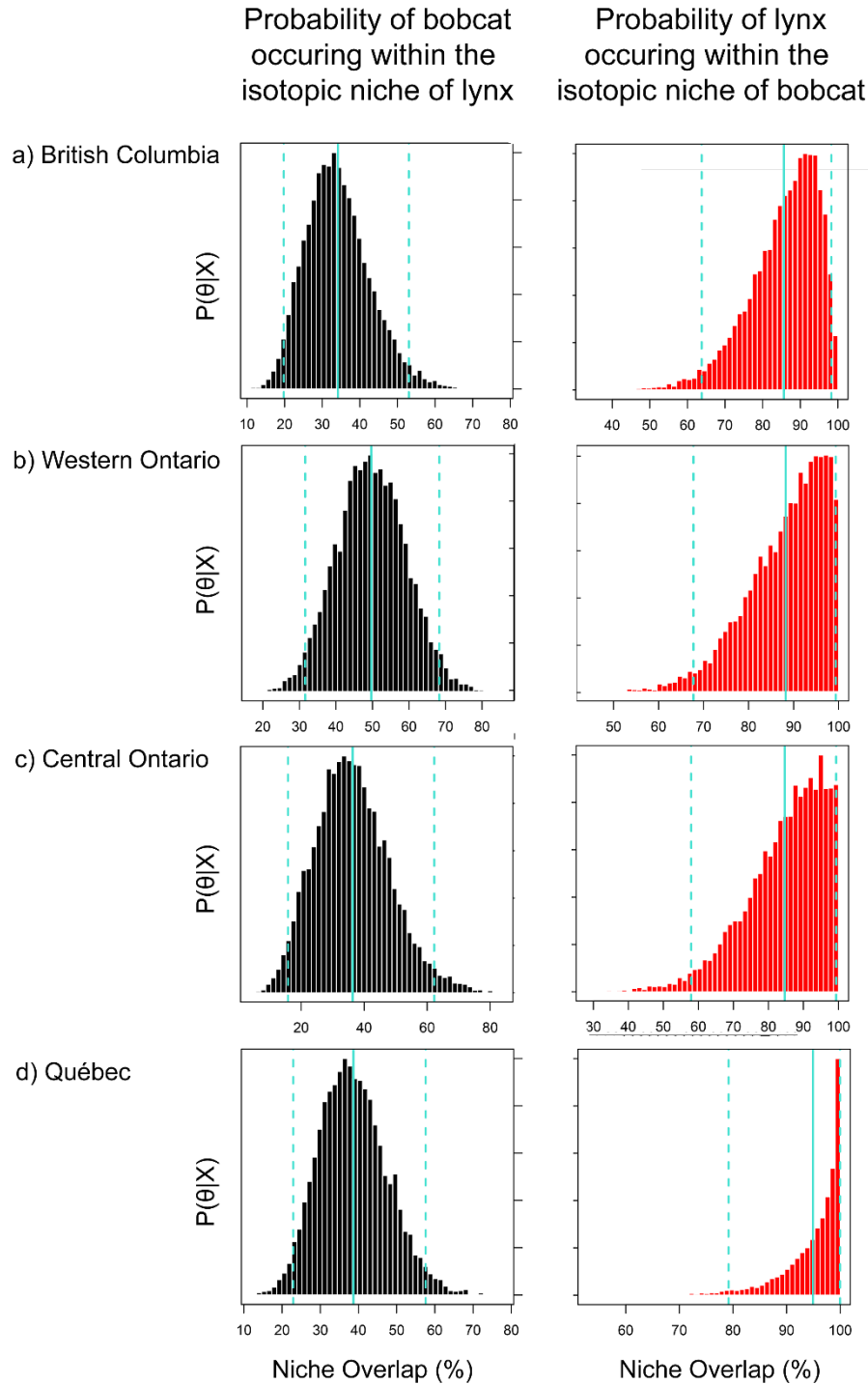


**Figure 3.2** Posterior distributions of dietary niche breadth estimates ( $SEAB$ ) of lynx and bobcat in all study regions. Central tendency is the mode of posterior draws, and boxes indicate 50%, 75%, and 95% credible intervals.





**Figure 3.3** Probabilistic 95% niche region (posterior density distributions) of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and variance of both ( $\Sigma$ ) in stable isotope ratios of lynx (black) and bobcat (red) in a) British Columbia, b) western Ontario, c) central Ontario, and d) Québec.



**Figure 3.4** Posterior distributions of the probability that an individual of one species falls within the 95% niche region of the competitor species in each study region. Blue lines indicate the mean (solid) and 95% credible intervals (dashed) of the posterior distribution.

## **Chapter 4: Does facultative use of alternative prey mitigate chronic stress in specialist consumers?**

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C. M. Burstahler and C. V. Terwissen designed the project and completed sample preparation for stable isotope analysis (Burstahler) and cortisol analysis (Terwissen). C. M. Burstahler was responsible for analysis and interpretation of the data, and writing the manuscript. G. F. Mastromonaco and J. D. Roth contributed to critical revision of the work, including interpretation of the data and editing the manuscript.

### **Abstract**

Coping mechanisms to deal with chronic stressors are moderated by the neuroendocrine system, but we do not yet understand how species' responses feed back into stress axis regulation in free-living organisms. Canada lynx (*Lynx canadensis*) are specialist consumers of snowshoe hare (*Lepus americanus*) that increase use of alternative prey when hare densities are low. As food limitation is physiologically stressful, we hypothesized that facultative use of alternative prey could mitigate escalation to a chronic stress response. We compared diet specialization of lynx across their distribution with concurrent cortisol concentrations measured in hair to identify

whether use of alternative prey reduced chronic stress. We measured cortisol concentrations and stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in autumn-grown lynx hair, which reflects a 6-8 week period heading into winter ( $n = 117$ ). We used multiple regression to determine the influence of lynx diet specialization on cortisol concentrations, while controlling for harvest geolocation. We found no evidence for a relationship between diet specialization and cortisol, suggesting that use of alternative prey does not confer a measurable reduction in long-term stress axis activation. However, lynx cortisol concentrations increased significantly towards the northwestern region of lynx distribution, suggesting that some other ecological variables present important stressors for lynx in autumn. Our research represents one of the first efforts to combine hormone and diet analyses to test the effect of prey choice on physiology of free-living animals and suggests that dietary plasticity does not alter an individual's experience of chronic stressors, despite important implications to population- and community-dynamics.

## **Introduction**

Phenotypic plasticity may enable species persistence through environmental stressors at the population- and community- level (Miner et al. 2005), but whether these responses feed back into stress axis regulation at the individual level is unclear. Environmental perturbations activate the hypothalamic-pituitary-adrenal (HPA) axis, which modulates facultative physiological and behavioural changes such as mobilization of energy stores, increase in foraging behaviour, and changes in habitat use (Wingfield et al. 1998). Prolonged activation of the stress axis (chronic stress) leads to allostatic overload and development of pathologies considered harmful to the individual (McEwen and Wingfield 2003), although not necessarily to fitness (Bonier et al. 2009; see also Boonstra et al. 2013). While facultative responses may diminish the rate of population

decline (Abrams 1999; Englund et al. 2008), an outstanding question remains whether these responses feed back into stress axis regulation to mitigate chronic stress. In fact, use of alternative resources by a specialist may reflect numerous scenarios: it may be an adaptive facultative behaviour, it may indicate naiveté, or it may reflect a reduced ability to secure preferred resources due to inexperience or competition (Mery and Burns 2010; Tuomainen and Candolin 2011). By integrating behavioural outcomes with individual physiologies, we can elucidate a mechanistic understanding of how behaviour affects individual condition (Cook et al. 2014).

Glucocorticoids such as cortisol, which mobilizes energy stores and modulates the immune system during a stress response, can be measured as an indicator of stress in vertebrates (Boonstra et al. 2014). Acute activation of the HPA axis triggers a negative feedback loop between signalling from the hypothalamus and glucocorticoid release from the adrenal glands to deal with the stressor and return to allostasis (Wingfield et al. 1998). However, chronic activation of the HPA axis diminishes the feedback effect, resulting in accumulation of cortisol systemically and development of pathologies (Boonstra 2004). Animal tissues with slow turnover rates, such as hair, integrate circulating cortisol over days to weeks, providing a measure of chronic stress experienced by the individual in most mammals (Sheriff et al. 2011, but see also Salaberger et al. 2016). With adequate validation of methods, integrated tissues may provide a window into the chronic stress physiology of free-living animals (Dantzer et al. 2014).

Canada lynx (*Lynx canadensis*) are facultative specialists of snowshoe hare (*Lepus americanus*) that expand their dietary niche to include alternative prey when snowshoe hare densities decline (O'Donoghue et al. 1998; Burstahler et al. 2016). However, recent evidence suggests that dietary niche expansion is not universal among lynx: young animals are

predominantly responsible for population niche expansion when snowshoe hare abundance declines in northern regions (Burstahler et al. 2016), whereas all individuals use alternative prey towards southern regions despite fluctuations in hare abundance (Burstahler et al., *in prep*). In southern Canada, snowshoe hare densities are considerably lower and not always cyclic (Hodges 2000; Murray 2000), characterizing an environment where uncertain access to preferred food in each season is significantly greater than that for northern conspecifics. Hence, ubiquitous use of alternative prey by lynx in southern populations may reflect the success of niche expansion in mitigating preferred prey limitation.

Our objective was to compare diet specialization of lynx across their distribution with concurrent cortisol concentrations in hair to identify whether use of alternative prey affects chronic stress. If use of alternative prey alleviates stress axis activation associated with snowshoe hare scarcity, then we would expect low hair cortisol concentrations when alternative prey is consumed. Otherwise, if use of alternative prey is a maladaptive behaviour in response to the stress of prey limitation, we would expect cortisol to increase with use of alternative prey. Finally, a lack of relationship between cortisol and diet would indicate neither a benefit or disadvantage to use of alternative prey and would suggest that alternative prey equivalently replaces snowshoe hare in terms of chronic stress physiology. Combining diet specialization measures with stress hormone analysis will shed light on the utility of alternative prey to specialist consumers and how dietary plasticity relates to individual physiology.

## **Methods**

Hair is fed by a blood vessel through the follicle and incorporates systemically circulating molecules during growth, including glucocorticoids and amino acids used to form keratin and

(Russel et al. 2012; Schwertl et al. 2003). Once grown, hair is metabolically inert, preserving a record of the animal's hormones and diet during the period of hair growth (Schwertl et al. 2003). Some evidence suggests that local production of cortisol by sebaceous and sweat glands may contribute to cortisol measured in hair (Keckeis et al. 2012; Salaberger et al. 2016), however previous work has demonstrated that cortisol measured in lynx hair reflects biologically relevant shifts in systemic cortisol during the period of hair growth (Terwissen et al. 2013). Intramuscular injection of adrenocorticotropin hormone (ACTH) over a five-week period during autumn moult increased measurable cortisol concentrations in hair of captive lynx, thereby validating its use in this species (Terwissen et al. 2013). Canada lynx moult biannually over a 6-week period, in April-May and in October-November (Quinn and Parker 1987), thus the cortisol concentrations and stable isotope ratios of winter-trapped samples reflect average autumn conditions for lynx during this period.

Lynx hair was cut from the hind leg of trapped animal pelts collected at the North American Fur Auctions (Toronto, ON) and Fur Harvester Auction Inc (North Bay, ON). Pelts were accompanied by a geolocation for the trapline where the animal was harvested. From our collection, we selected lynx specimens with enough tissue to run both stable isotope analysis and hair cortisol analysis (> 1 g). We cut hair samples away from the skin, as close to the base of the hair shaft as possible. To facilitate different washing methods between hormone- and stable isotope- analysis, a small subsample was set aside for stable isotope analysis (roughly 1 mg of hair). Because the stable isotope ratios of consumers depend on those of their prey (Phillips et al. 2014), we also collected white winter guard hair from the haunch of snowshoe hare in 2013 with the assistance of government agencies, scientists, and trappers in Alaska, Yukon Territory, Manitoba, Ontario, and Québec, and we used snowshoe hare stable isotope ratios collected from

our previous work in the Northwest Territories in 2001 (Roth et al. 2007). Snowshoe hare stable isotope ratios do not vary from year to year (Roth et al. 2007), but atmospheric changes in  $\delta^{13}\text{C}$  affect comparison of stable isotope ratios across decades and so we corrected the stable isotope ratios of hares from the Northwest Territories to reflect changes in baseline  $\delta^{13}\text{C}$  since 2011 (Long et al. 2005). We tested for regional differences in hare stable isotope ratios using analysis of variance and Tukey's HSD at a 95% confidence level. Lynx and snowshoe hare hair samples were prepared for stable isotope analysis by washing with soap and water, drying in an oven (60°C), homogenizing to a powder using scissors, and wrapping 0.6 mg subsamples in tin capsules. Stable isotope ratios were measured on a continuous-flow stable isotope mass spectrometer at the University of Windsor Chemical Tracers Lab (Windsor, ON).

To prepare lynx hair for hormone analysis, samples were washed with 100% methanol for 20 seconds using a spray bottle to remove surface contaminants, then dried in a fume hood, and cut into 0.5 cm pieces. We then added 1 ml methanol per 0.01 g hair tissue and placed samples on a rotator for 24 hours to extract cortisol (RotoFlex R2000, Argos Technologies, Elgin, IL, USA). We centrifuged samples for 10 minutes at 35 000 rpm then transferred 450  $\mu\text{l}$  of the extracted solution to new tubes and dried the samples in a fume hood. We reconstituted samples in 150  $\mu\text{l}$  enzyme immunoassay buffer solution (0.1 mM sodium phosphate buffer, pH 7.0, containing 9 g of NaCl and 1 g BSA per litre) for a 3-fold concentration, then sonicated samples for 20 seconds in an Elmasonic waterbath (Elma GmbH & Co KG, Germany) prior to hormone analysis. Hair cortisol concentrations were quantified using the enzyme immunoassay procedure described in Terwissen et al. (2013).

Collection locations of our samples covered a large geographic area over a five-year period and thus must comprise of a mix of prey assemblages and availability. Because snowshoe



hare are depleted in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  compared to all other prey species and do not vary annually (Roth et al. 2007), simple Euclidean distance models can be employed to represent diet specialization (DS) on snowshoe hare. We used a simple Euclidean distance model measuring the distance in isotope space from individual lynx to the regional mean of snowshoe hare stable isotope ratios after correction for isotopic discrimination. The implicit assumption of a Euclidean distance model is that increasing proximity to the value of snowshoe hares indicates greater consumption, therefore a lynx feeding exclusively on snowshoe hare will have a diet specialization index of 0%. As carnivores derive the bulk of their energy intake from the meat of prey, snowshoe hare stable isotope ratios were first corrected for within-animal difference between hair and muscle tissue stable isotope ratios by subtracting 1.5 ‰ from  $\delta^{13}\text{C}$  and 0.6 ‰ from  $\delta^{15}\text{N}$  based on measurements from European rabbits (Neilson et al. 2005). Snowshoe hare stable isotope ratios were then corrected for diet-tissue trophic discrimination measured in Canada lynx (+ 2.4‰ to  $\delta^{13}\text{C}$  and + 3.3‰ to  $\delta^{15}\text{N}$ ; Parnig et al. 2014). A general linear model was used to compare diet specialization of lynx across geolocations.

We used multiple regression to measure the influence of diet specialization on lynx cortisol concentration, while controlling for harvest geolocation. Cortisol measurements were log-transformed to fit a normal distribution. We identified multicollinearity among predictors using Pearson correlation coefficients and variance inflation factors (*VIF*), where a *VIF* greater than 10 was considered problematic (Quinn and Keough 2002, p. 128). Latitude and longitude were highly correlated predictors (Pearson correlation coefficient,  $r = 0.95$ ). When considered in a multiple regression model of  $\log(\text{cortisol})$ , variance inflation factors exceeded our threshold for latitude (*VIF* = 11) and longitude (*VIF* = 11), while diet specialization posed no issue (*VIF* = 1.0). As such, we combined latitude and longitude using principal component regression into a

single axis, PC1, to represent lynx harvest geolocation where values ranged from northwest Alaska (PC1 = -2) to southeast Québec (PC1 = 2). PC1 explained 98% ( $\pm 1.4$  SD) of the variance in harvest geolocation. Substituting PC1 for latitude and longitude in the multiple regression of  $\log(\text{cortisol})$  showed considerable improvement to the variance inflation factor for geolocation ( $VIF = 1.0$ ). PC1 and diet specialization were minimally correlated (Pearson correlation coefficient,  $r = 0.10$ ). All predictor variables were standardized to z-scores that are normally distributed with a mean of zero and a standard deviation of one, so that all coefficients resulting from the model could be compared with equal effect strength. Once all variables were suitably transformed, we conducted a multiple linear regression of lynx diet specialization and harvest geolocation on cortisol (log-transformed). Finally, we conducted a *post hoc* power analysis of the regression to ascertain statistical power to detect a medium ( $f^2 \geq 0.15$ ) or large ( $f^2 \geq 0.35$ ) effect using Cohen's criteria at  $\alpha = 0.05$  (Cohen 1988). All analyses were conducted using R version 3.3.1 (R Development Core Team, 2013), including the “car” package for  $VIF$  calculations (Fox and Weisberg 2011), the “caret” package for principal components regression (Kuhn 2016), and the “pwr” package for power analysis (Champely 2017).

## Results

The 117 lynx samples analyzed spanned five years, from autumn 2006 through autumn 2010, and harvest locations of lynx ranged from the west coast of Alaska to southeastern Québec (Figure 4.1). Snowshoe hare stable isotope ratios varied among regions for both  $\delta^{13}\text{C}$  ( $F_{5,66} = 4.3$ ,  $P = 0.0018$ ) and  $\delta^{15}\text{N}$  ( $F_{5,66} = 7.8$ ,  $P < 0.0001$ ; Table 4.1). Diet specialization indices (DS) ranged from 0.29‰ to 3.7‰ (mean = 2.0‰, SD = 0.62), before standardization to z-scores; and, diet specialization indices were comparable across geolocations ( $F_{1,115} = 1.0$ ,  $P = 0.31$ ; Figure 4.2).

Individuals that were highly specialized are represented by negative z-scores (DS = -3) and individuals that consumed greater amounts of alternative prey are represented by positive z-scores (DS = 3). Hair cortisol concentrations ranged from 0 to 185 ng/g (Figure 4.2).

Our multiple regression model explained 38% of the variation in lynx cortisol (equation:  $\log(\text{cortisol}) = 3.0 (\pm 0.10) + 0.13 (\pm 0.10) \text{DS} - 0.61 (\pm 0.07) \text{PC1}$ ,  $F_{2,114} = 36$ ,  $P < 0.0001$ ).

Cortisol concentrations increased towards northwestern latitudes ( $t = -8.4$ ,  $P < 0.0001$ ), but were unrelated to diet specialization ( $t = 1.3$ ,  $P = 0.19$ ; Figure 4.3). *Post hoc* power analysis indicated an effect size of 0.60, or > 99% power to detect a medium or large effect of diet and geolocation on cortisol.

## Discussion

Whether individuals were highly specialized consumers of snowshoe hare or used alternative prey, a range of long-term cortisol concentrations were expressed by lynx suggesting that facultative use of alternative prey does not alleviate the chronic stress response. All regions presented a similar range of diet specialization indices, indicating that we adequately captured a range of environmental conditions prompting niche expansion in some individuals, and *post hoc* power analysis confirmed our ability to detect any ecologically relevant effect of prey types consumed. In controlling for harvest location, we identified a geographic gradient in cortisol concentrations of lynx. Individuals in southeastern Canada had significantly lower cortisol concentrations than animals north and west, which undoubtedly reflects spatial differences in some important ecological predictor of chronic stress for lynx.

We discuss three alternative hypotheses that might explain why long-term cortisol concentrations did not correlate with diet specialization of lynx in our study. First, switching

prey types may confer benefit to glucocorticoid modulation over short time frames not measured here. Acute food deprivation promotes glucocorticoid release and increased foraging activity in vertebrates (Fokidis et al. 2012; Cottin et al. 2014); and, facultative responses like use of alternative prey are spurred to cope with the acute stress of food deprivation (Wingfield 1998). Furthermore, the initial glucocorticoid response to food deprivation sees a return to baseline glucocorticoid concentrations within a short time frame in captivity, even if food availability is not immediately restored (Lynn et al. 2003). Second, repeated exposure to a stressor can alter an organism's perception of the stimulus and result in downregulation of HPA activation (Rich and Romero 2005; Cyr and Romero 2007); and, as the peak densities of snowshoe hare are considerably lower towards southern latitudes (Hodges 2000; Murray 2000), southern lynx may have habituated to chronically low hare densities. Finally, emerging evidence suggests that either the proportional contribution of preferred prey consumed or the perceived limitation of preferred prey can affect the chronic stress response. For example, Bryan et al. (2014) found that increasing specialization on salmon (*Oncorhynchus* spp.) by grizzly bears (*Ursus arctos*) reduced the chronic stress response measured in hair, but for black bears (*Ursus americanus*) in the same region the availability of salmon (not the proportion of salmon in the diet) correlated with cortisol. The authors conclude that the direct relationship between prey choice and cortisol concentration in grizzly bears reflects superior nutritional benefit of specialization on salmon, whereas for black bears it is the perceived social strife of increasing competition for preferred resources that triggers the chronic stress response. Like black bears, the proportion of snowshoe hare in lynx diets did not relate to cortisol concentrations, suggesting that the types of prey consumed do not affect chronic stress experienced by lynx. We strongly recommend future investigations consider preferred prey availability as a covariate to disentangle whether

perceived scarcity of preferred prey alone, regardless of prey types consumed, influences chronic stress in lynx.

Several biotic or abiotic factors may be driving the geographic gradient in lynx cortisol concentrations observed here, including the physiology of moult, differences in climate (Boonstra 2004), prey availability (Fokidis et al. 2012), density of competitors (Ritchie and Johnson 2009), or anthropogenic influence (Dantzer et al. 2014). First, moulting is regulated through the HPA-axis which is triggered by some environmental cue (e.g. photoperiod), and thus could be expressed differently across an animal's distribution. For example, the early and rapid onset of harsh winter climates towards northern latitudes may require mammals to grow insulating winter pelage denser and faster, spurring greater upregulation of the HPA-axis and possibly resulting in higher cortisol incorporation, as measured herein. Furthermore, mobilization of energy stores to maintain body mass throughout the winter is also regulated through the HPA-axis (Boonstra 2004); thus, it is possible that timing of moult is synchronous across the distribution and the elevated cortisol concentrations of northwestern lynx reflects longer exposure to cold temperatures. In addition, lynx towards southern latitudes may have habituated to the high incidence of environmental stressors associated with the region and thus downregulation of HPA-axis activation has diminished their response to new stressors (Wingfield 2003; Rich and Romero 2005). For example, adult Magellanic penguins (*Spheniscus magellanicus*) occupying areas that were regularly visited by tourists showed diminished glucocorticoid release in response to human visitation than those that were not habituated to human visitation; yet, all penguins responded similarly to the novel stressor of capture and restraint, indicating that HPA-axis activity was not compromised in either group (Walker et al. 2006). Thus, the consistently lower cortisol concentrations measured in southeastern lynx may

reflect habituation to less favourable environmental conditions and downregulation of HPA activation due to the regularity of noxious stimuli such as low and unpredictable snowshoe hare availability, higher densities of competitors, and many anthropogenic disturbances imposed by high human densities. Indeed, the dietary niche of southern lynx populations is less specialized than northern populations and does not vary with changes in hare availability (Burstahler, Roth and Murray, *in prep.*), suggesting habituation may be the case. In contrast, lynx in the northwestern region of their distribution occupy relatively undisturbed habitat by comparison (Murray et al. 2008). Larger tracts of undisturbed forest and strong cyclicity of snowshoe hares mean that most individuals (except for a few old adults) will be accustomed to living with abundant food and fewer perturbations. Under these circumstances, stressors such as snowshoe hare decline, a severe storm, or proximity to human disturbance would undoubtedly be perceived as novel life-threatening stimuli to most individuals, as it is something they are unlikely to have encountered often.

Hair cortisol analysis is increasingly used to address ecological questions in free-living organisms, however the relative novelty of this method comes with associated unknowns and limitations. It is clear that ambiguity surrounding the physiology of moult at continental scales is a major limitation for studies using these increasingly available endocrine tools and warrants critical evaluation to facilitate application to free-living organisms. In addition, cortisol data are inherently noisy because many intrinsic factors can affect glucocorticoids including sex, reproductive condition, age, and individual differences, each of which can reduce our ability to detect physiological responses when not adequately accounted for (Dantzer et al. 2014). This study benefits from the validation of an enzyme immunoassay specific to Canada lynx and evidence that hair cortisol does not appear to vary with age or sex in lynx (Terwissen et al.

2013). However, uncertainty remains about the mechanisms of cortisol incorporation into the hair shaft. Blood supply through the follicle is thought to be the primary source of glucocorticoid incorporation (Sheriff et al. 2011), yet local production of cortisol can influence measurement in some instances (Keckeis et al. 2012; Sharpley et al. 2009). For example, mechanical stress to the skin (brushing) increased cortisol concentrations measured in sheep hair (Salaberger et al. 2016), and could bias results if an animal had a pre-existing condition causing irritation near the sampling site. Wild-harvested lynx in North America are trapped using soft-catch leg-hold restraining traps that target the forelimbs or killing traps that target the neck and shoulders, both of which must be checked daily (Fournier and Canac-Marquis 2017). Thus, our measurements from the hind leg are unlikely to be affected by the trapping process. The use of hair as an integrated measure of long-term cortisol concentrations is a recent addition to ecological studies that holds great promise for extending our understanding of how environmental changes affect animal populations, however we echo the cautionary note that its application requires careful validation of methods, consideration of covariates, and tentative discussion of implications (Busch and Hayward 2009; Dantzer et al. 2014).

Environmental stressors can both upregulate and suppress stress axis activation (Middlemis Maher et al. 2013; Dantzer et al. 2014), and effects may extend to the individual and its offspring (Dantzer et al. 2013), emphasizing the complexity of studying stress physiology in free-living organisms. That chronic cortisol concentrations of lynx were higher towards northern latitudes contradicts the general trend we expected based on broad scale patterns of human disturbance and warrants further investigation. In some cases, it is possible that prey availability is more important to the organism than anthropogenic disturbance (e.g. Ayres et al. 2012), or that anthropogenic disturbance is suppressing stress axis activation (Rich and Romero 2005). Now

that enzyme immunoassay methods have been developed and validated for Canada lynx (Terwissen et al. 2013), we can begin testing additional environmental variables to disentangle what is driving geographic differences in chronic stress.

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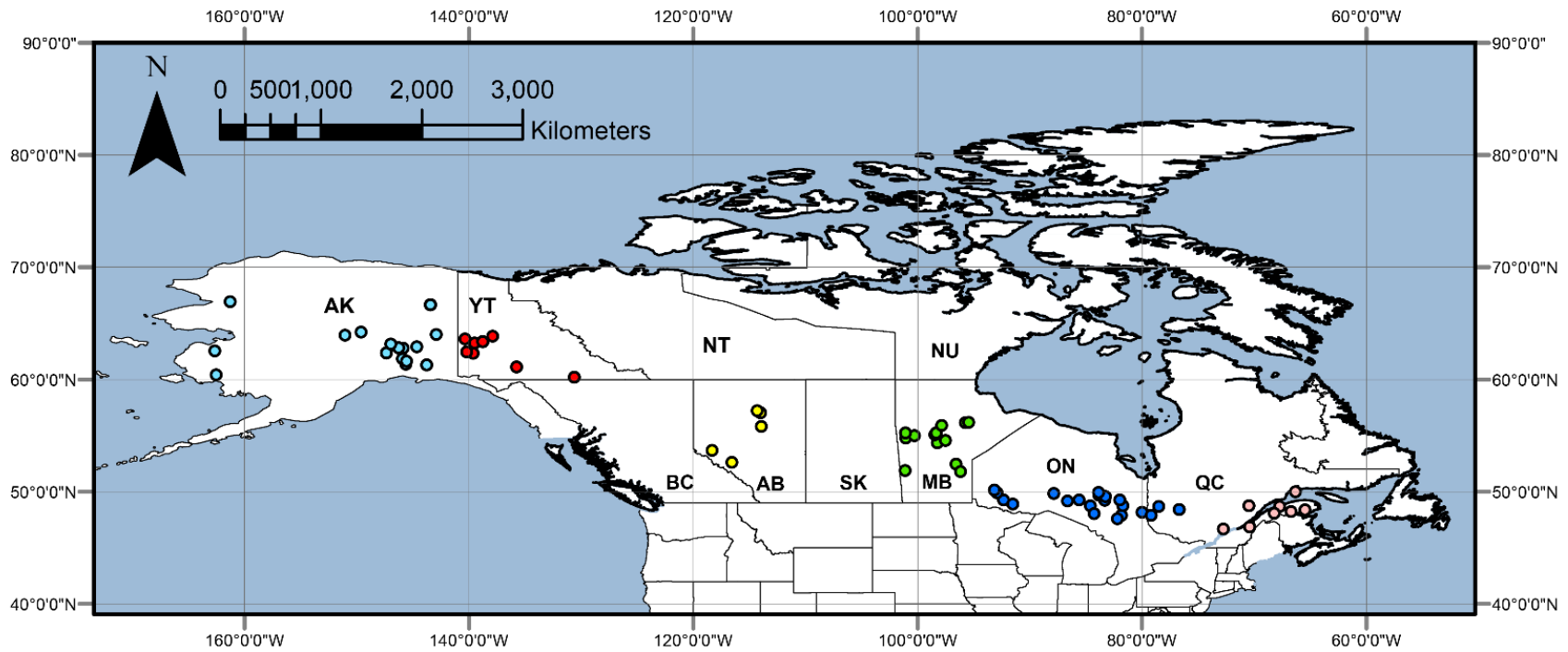
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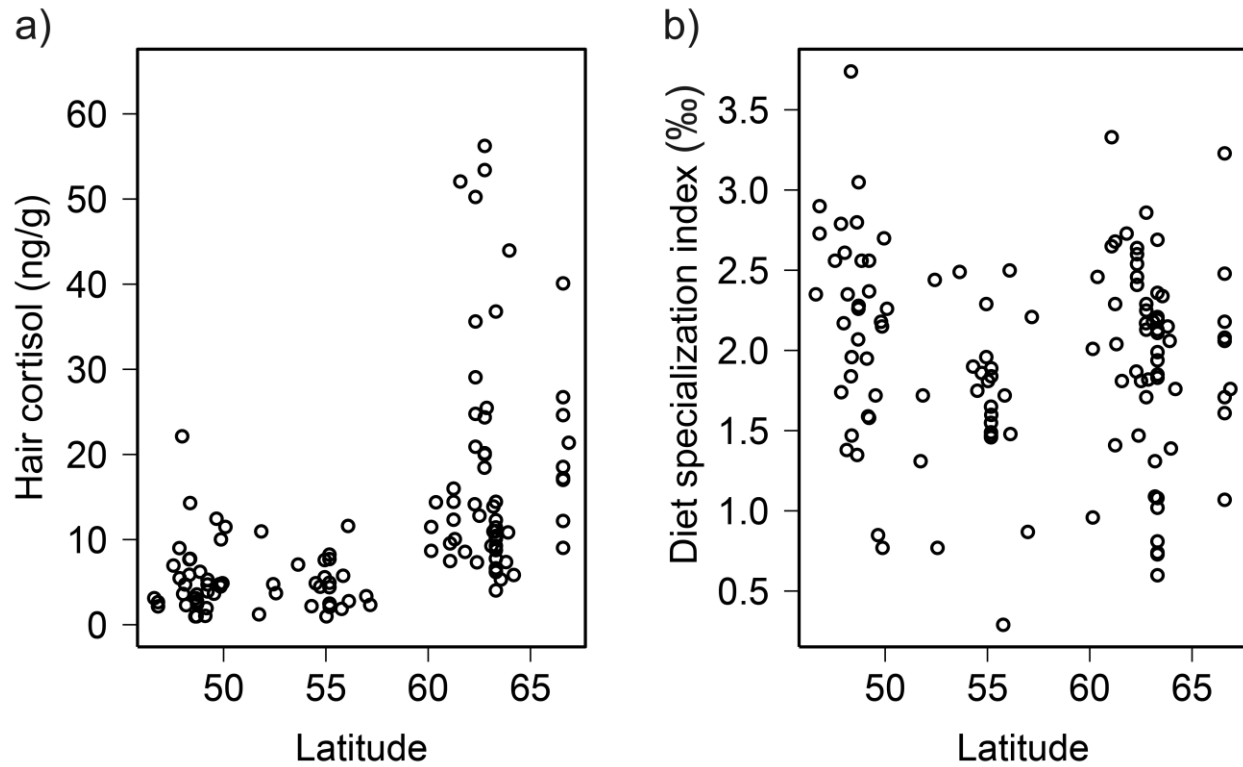
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**Table 4.1** Regional differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of snowshoe hare winter guard hair. Means with the same letter indicate no statistical difference between regions.  $\delta^{13}\text{C}$  values from the Northwest Territories were corrected for changes in atmospheric carbon since 2001 (Long et al. 2005).

Province	n	$\delta^{13}\text{C}$ (‰)	± SD	$\delta^{15}\text{N}$ (‰)	± SD
Alaska	7	-27.16 <sup>a,b</sup>	0.73	2.23 <sup>a,b,c</sup>	1.36
Yukon	18	-26.74 <sup>a</sup>	0.64	1.74 <sup>a,c</sup>	1.02
Northwest Territories	6	-26.75 <sup>a</sup>	0.93	3.25 <sup>a,b</sup>	1.44
Manitoba	10	-27.35 <sup>a,b</sup>	0.76	3.46 <sup>b</sup>	1.53
Ontario	19	-27.26 <sup>a,b</sup>	0.52	2.72 <sup>a,b</sup>	1.13
Québec	12	-27.91 <sup>b</sup>	0.94	0.78 <sup>c</sup>	0.96

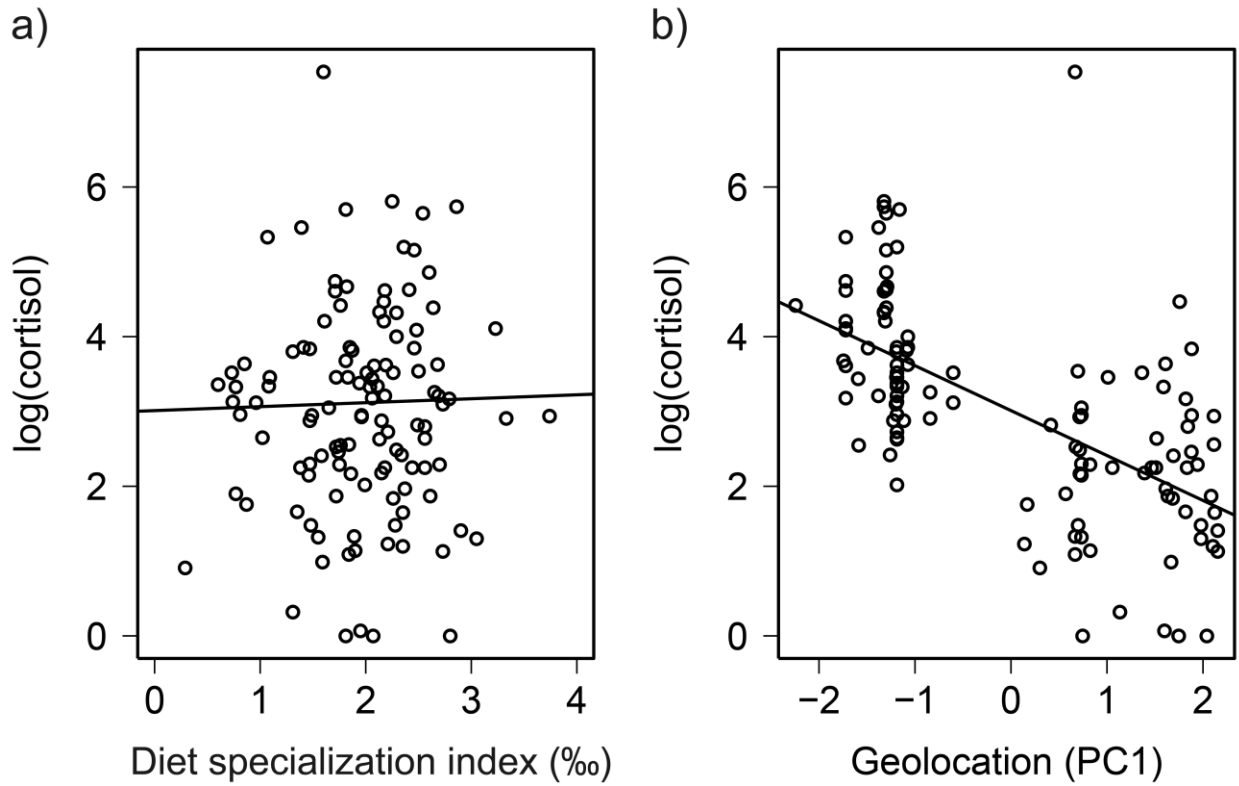


**Figure 4.1** Harvest geolocations of Canada lynx (points, n=117). Colours represent the regional snowshoe hare stable isotope ratios used to calculate lynx diet specialization index: Alaska (light blue), Yukon (red), Northwest Territories (yellow), northern Manitoba (green), southeastern Manitoba (blue), and Québec (pale red).



**Figure 4.2** Geographic summary of (a) cortisol concentrations (ng/g) and (b) diet specialization indices measured in Canada lynx. Individuals whose stable isotope ratios overlap with mean regional snowshoe hare stable isotope ratios (after correction for trophic discrimination) have values closer to zero.





**Figure 4.3** Effects of (a) diet specialization and (b) harvest geolocation on log(cortisol) in Canada lynx. Individuals whose stable isotope ratios overlap with mean, regional snowshoe hare stable isotope ratios (after correction for trophic discrimination) have values closer to zero. Harvest geolocation is presented as the principal components regression (PC1) between latitude and longitude of the trapline.

## General Conclusion

Variation in the realized ecological niche of an organism informs us of the most important selective pressures of an environment (e.g. prey availability, intraguild competition, climate gradients), and enables forecasting of species' responses to changing environmental conditions by defining the limits of the fundamental niche. However, we are often limited in our ability to accurately predict outcomes because observations typically represent the perspective of one level of biological organization, lack replication, and therefore do not inform on the fundamental niche and full potential for adaptive responses. Herein, I have used the opportunity afforded by collaboration with fur auction houses and stable isotope analysis to investigate foraging strategies of free-living organisms across spatiotemporal scales from an individual, population, and community perspective. Collectively, this thesis uses the ecological niche concept to test elements of foraging theory at an unprecedented spatial scale and provides substantive support for the niche variation hypothesis (Van Valen 1965, Chapter 1), the facultative specialist hypothesis (Glasser 1982, Chapter 2), and the generality of the specialist-generalist paradigm (Chapter 3). Furthermore, I have detailed a conceptual model for identifying adaptive foraging strategies that is well suited to stable isotope data, but also relevant to comparison of any bivariate niche axes (Chapter 2). Finally, it poses the novel question of whether niche expansion, spurred by stressful environmental conditions, feeds back into individual physiology by amplifying or ameliorating the chronic stress response (Chapter 4).

These contributions not only advance our understanding of ecological niche dynamics and foraging theory, but provide valuable insight to adaptability and vulnerability of specialist species under a range of food web scenarios. Mounting concern for “global functional homogenization” as specialist species continue to decline has spurred considerable demand to

forecast species responses to global change before they go extinct (Clavel et al. 2011). This thesis provides detailed accounts of adaptive foraging decisions observed in a dietary specialist in response to changes in abundance of preferred prey, availability of alternative prey, stability of predator-prey dynamics, and intraguild community structure. Indeed, different combinations of these environmental variables prompted different adaptive foraging strategies by lynx, suggesting that specialists may be less vulnerable to global change provided the ecological opportunity for facultative niche expansion exists.

The demonstrated plasticity of Canada lynx diets across spatiotemporal scales corroborates the feedback between food web properties and realized ecological niches in several ways. Low hare abundance in the relatively simple food web of northern boreal forests prompted niche expansion via between-individual variation suggesting that intraspecific competition was the predominant force driving foraging strategy (Chapter 1; Van Valen 1965). In contrast, ubiquitous use of alternative prey by southern lynx suggests that interspecific competition for chronically limited hares may be an important factor dictating foraging strategy in these areas (Chapter 2 and 3; Bolnick et al. 2010). Furthermore, it appears that the relative stability of predator-prey dynamics may also prompt different foraging strategies, where increasingly irruptive demographic changes force a more generalized strategy on all individuals (Chapter 2). Overall, the variability in dietary niches observed herein provide invaluable evidence for the interacting influence of food web complexity and population dynamics on facultative foraging strategies, owing in large part to the methodological advantages of stable isotope analysis.

This thesis exemplifies several ways that stable isotope analysis has progressed our ability to test foraging theory. Whereas alternative methods of diet analysis such as scat, stomach content, and DNA barcoding are limited in scope to short-term prey use and may overestimate

the contribution of trace items to animal diets, stable isotope analysis can provide estimates of long-term proportional consumption of the most important prey types (Phillips et al. 2014). Furthermore, the small amount of tissue required for stable isotope analysis facilitates sample collection at a much larger spatial scale than alternative methods of diet analysis. I have capitalized on these strengths to test the facultative specialist hypothesis at a continental scale in an upper trophic level consumer, and revealed compelling new evidence for adaptive foraging behaviour. Additionally, I present one of the first efforts to address the effect of foraging strategy on individual physiology by combining diet reconstruction using stable isotope analysis with newly developed methods for measuring cortisol concentrations from animal hair (Chapter 4). One possible limitation of stable isotope analysis is that severe food deprivation can affect stable isotope ratios of consumer tissues and impair accurate diet estimation (Doi et al. 2017), however stable isotope ratios of lynx muscle tissue were not related to body condition (Appendix A).

Fundamental studies are the basis from which we approach applied conservation problems like global declines in specialist species and, while ecology is a burgeoning scientific discipline, our understanding of how species function and interact in complex real-world scenarios is still in its nascence (Courchamp et al. 2015). This thesis responds to recent calls for clarification on the fundamental ecology of foraging strategies along the specialist-generalist spectrum (Devictor et al. 2010; Pagani-Núñez et al. 2016), identifying drivers of intrapopulation niche variation (Bolnick et al. 2010), and testing the generality of ecological patterns across populations, species, and communities (Courchamp et al. 2015). Collectively, this thesis demonstrates how prey availability and community complexity differentially affect the foraging strategies of specialists at an unprecedented spatial scale and contributes to our general understanding of how realized ecological niches can vary within a species' fundamental niche.

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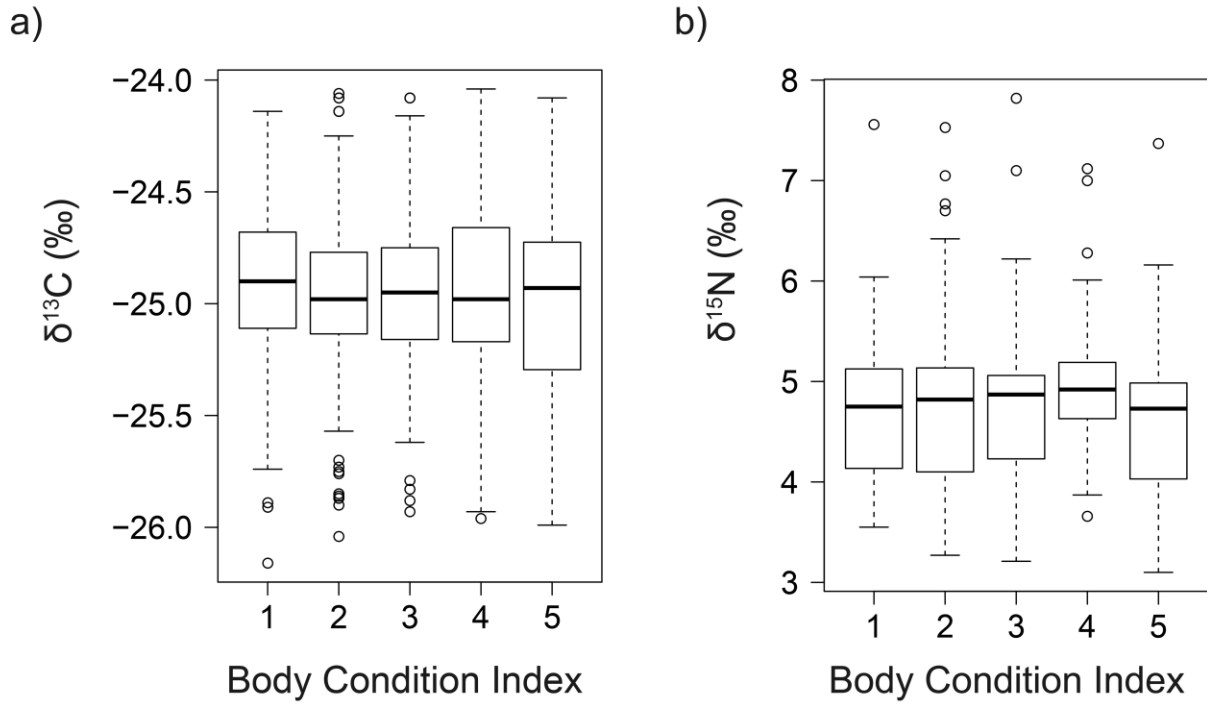
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## **Appendix A. Analysis of lynx muscle stable isotope ratios and associated body condition index**

One possible limitation of stable isotope analysis is that severe food deprivation can affect stable isotope ratios of consumer tissues and impair accurate diet estimation (e.g., Hobson et al. 1993). Meta-analysis has revealed substantial variation in the influence of fasting on different species' stable isotope ratios and, while a range of effects have been observed, most experiments detected no difference between pre- and post-starved states (Doi et al. 2017). I verified that fasting does not grossly affect lynx stable isotope ratios by comparing the values measured in muscle from Fort Providence, NT (Chapter 1) with associated body condition indices that were assessed on necropsy. Body condition was ranked from 1 (emaciated) to 5 (fat) based on subjective assessment of visceral fat conducted by R. Mulders and other Government of the Northwest Territories staff. Stable isotope ratios of emaciated individuals (body condition rank 1) were not distinct (MANOVA,  $F_{4,366} = 0.55$ ,  $P = 0.82$ ; Figure A.1), thus it is unlikely that starvation has biased the conclusions drawn herein.

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**Figure A.1** Muscle (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  of lynx harvested in the Northwest Territories and associated body condition index (n = 371). Body condition was ranked from 1 (emaciated) to 5 (fat) based on subjective assessment of visceral fat conducted by R. Mulders and other Government of the Northwest Territories staff.