# Forest Roost Use by Little Brown Bats (*Myotis lucifugus*) in Ontario

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# ABSTRACT

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Roosts offer bats protection from predators, shelter from external environmental conditions, and a space where sociality, mating, and the rearing of young can occur. However, knowledge gaps still remain for many forest roosting species, such as the little brown bat (*Myotis lucifugus*) surrounding what roosts are selected, and what variables are influential at differing scales of selection. As a species-at-risk, identifying and predicting roost habitat selection may aid conservation and management. I identified forest roosts in a previously unexamined area of this species' range using radio-telemetry, and measured tree-scale characteristics of located roosts. I then used a logistic model selection process with stand-scale variables to predict roost presence across forest stands. Height of trees in a given stand was the best predictor of roost presence - which may be linked to solar exposure and other thermal benefits. I then examined roost-level variables effecting the abundance of roosting bats in an artificial roosting environment (bat boxes). I found that temperature and social effects were both significant predictors of bat abundance, with warmer minimum temperatures in the box having a positive effect. These results suggest maternal bats may select roosts with higher minimum temperatures, likely due to the energetic benefits that may be gained over the course of reproduction.

**Keywords:** Little brown bat, *Myotis lucifugus,* habitat selection, roost choice, stand selection, bat boxes, radio-telemetry, forest management

# PREFACE

I am the sole author of Chapters One and Four (General Introduction and Discussion), while Chapters Two and Three have additional co-authors. For Chapter Two, Jeff Bowman assisted in the study design and both Jeff Bowman and Christina Davy provided meaningful comments on the manuscript. For Chapter Three, Meghan Murrant assisted with data collection, Christina Davy was instrumental in the study design, and both Jeff Bowman and Christina Davy provided meaningful comments on the manuscript.

# ACKNOWLEDGEMENTS

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## 1. General Introduction

Understanding the habitat requirements of a species is essential to its conservation. For bats, roosting habitat is vital. Roosts can be broadly defined as the spaces bats inhabit while not in flight, and include features that are both natural (e.g. vegetation, caves, rock formations), and artificial (e.g. buildings, bridges, mines, tunnels etc.) (Fenton and Barclay 1980; Russo and Ancillotto 2015). Most bats spend a majority of their lives roosting (Kunz 1982), and roosts offer bats protection from predators, shelter from external environmental conditions, and a space where sociality, mating, and the rearing of young can occur (Kunz and Lumsden, 2003; O'Shea and Bogan 2003). As a result, the availability of suitable roost habitat is important if individual bats are to survive and reproduce; loss of such habitat in a landscape may lead to population declines (Brigham and Fenton 1986). In North America, many bat species use forest trees for roosting: taking residence under exfoliating bark, in hollow cavities, or in exposed foliage (Hayes 2003; Miller et al. 2003; Barclay and Kurta 2007). In bark- and cavity-roosting species, these sites often share common features such as placement in stands with more open canopies, a higher density of snags, and a closer proximity to water (Kalcounis-Rüppell et al. 2005). Water is likely important for several reasons; insect abundance is greater near and over water (Warren et al. 2000; Fukui et al. 2006; Broders et al. 2006), and water bodies provide a drinking source, particularly during pregnancy and lactation (Kurta et al. 1989b; Korine et al. 2016). Many forest-dwelling bat species demonstrate high roost site fidelity, returning consecutively to the same roosts they have used previously, within and across years (Lewis 1995; Willis et al. 2003; Veilleux and Veilleux 2004). As obligate insectivores, many forest bats also play a central role in supressing arthropod populations in forest ecosystems (Kunz et al. 2011). The capacity of bats to use and adapt to changes in their surrounding environment likely varies by landscape and species (Davy et al.

2007). At the same time, lack of knowledge regarding roost requirements limits informed conservation (Marcot 1996). The full range of roost tree species, their condition, and other microhabitat requirements are still not known for many forest-dwelling bat species.

The motivations for why bats choose particular roost trees has long been a topic of research. Roost choice is thought to occur at the stand level (Crampton and Barclay 1998; Jung et al. 1999) and reflects sex and age-based groupings that may, in part, be driven by thermoregulatory differences among males, nonreproductive females, and reproductive females (Broders and Forbes 2004; Hamilton and Barclay 1994; Johnson and Lacki 2014; Mattson et al. 1996; Russo et al. 2017; Veilleux et al. 2004; Wilkinson and Barclay 1997). Bats have a high surface-area-to-volume ratio that makes maintenance of normal body temperature (normothermia) energetically costly (Barclay and Harder 2003; Dzal and Brigham 2013). Bats also face strict energetic limitations due to the costs of flight (Kurta et al. 1989a), which is exacerbated in temperate bats by a seasonally restricted breeding period (Chruszcz and Barclay 2002). These factors create a strong selective pressure for roosting behaviours that alleviate energetic costs.

Torpor, a deliberate physiological process where bats reduce their body temperature to near ambient and slow their metabolic rate (Wang and Wolowyk 1988), can conserve large amounts of energy (Stawski et al. 2014; Studier 1981). However, if reproductive females enter torpor, gestation and milk production are also slowed (Racey 1973; Racey and Swift 1981; Wilde 1995; Wilde et al. 1999). Similarly, postnatal development is also more rapid at normothermy (Tuttle and Stevenson 1982), as ambient roost temperatures near normothermy likely aid digestion, nutrient assimilation, and growth (Hoying and Kunz 1998; Kunz 1973; Kunz 1974; Tuttle 1975).

Due to this trade-off between torpor and reproductive success, a bat's use of torpor is likely to differ significantly depending on reproductive status (Dzal and Brigham 2013). Consequently, ideal roost characteristics, such as temperature, are likely to differ across reproductive status (Solick and Barclay 2006; Ruczyński 2006). Thermoregulation through torpor may also be a factor in roost switching, a common behaviour in many temperate bat species (Sedgely 2001; Chruszcz and Barclay 2002; Lausen and Barclay 2003; Kerth et al. 2001; Kurta et al. 2002; Willis 2003; Patriquin and Ratcliffe 2016; Patriquin et al. 2016). In little brown bats (*Myotis lucifugus*), for example, individuals have been observed switching roosts at a mean of 3.67 days (Barclay and Kurta 2007; Crampton and Barclay 1998). An individual bat's energetics may, therefore, be greatly influenced by roost characteristics (Encarnação et al. 2012; Patriquin and Ratcliffe 2016; Jarolimek and Vierling 2019), and individual bats are likely to benefit from using roosts which have microclimates that best suit their thermoregulatory needs (Boyles 2007; Patriquin et al. 2016).

Animal societies, including our own, are composed of social interactions between individuals. These interactions typically revolve around reproduction, cooperation, or competition (Silk 2007). Collectively, these non-random interactions between individuals form social structures that are defined by system-level properties – i.e., local interactions translate to measurable group-level properties at broader scales (Lusseau 2003; Garroway et al. 2008; Farine and Whitehead 2015). In many cases however, interactions between individuals are not directly observable. This can be overcome by using association in place of, and as a proximate indicator of interaction – pairs of individuals are said to be in association if they are in a circumstance where interactions may take place (Whitehead & Dufault 1999; Whitehead 2004). When correlated with other individual attributes (e.g. age, sex, and reproductive state), measurements of dyadic association can give us insights into the underlying aspects that govern the formation, maintenance,

and dissolution of interactions and their resulting social structure (Whitehead 2008). Social structure has a substantial effect within populations, as it is often tied to resource use, gene flow, fitness, and the spatial and temporal distribution of organisms (Wilson 1975; Garroway and Broders 2007). Particular social structures may evolve from a balancing of the costs and benefits of an interaction, with these costs and benefits mediated largely by resource (e.g. food and shelter) distribution in the environment (Lehmann et al. 2007; Angell et al. 2013; Patriquin et al. 2016). As dispersal, reproduction, and survival are all affected by social interaction, selection promotes the evolution of non-random social structures that have adaptive benefits, and these structures may, in turn, influence the evolution of other traits (Bradbury and Vehrencamp 1998; Lindenfors et al. 2002; Whitehead 2008). Societies can be contrasted with aggregations in this respect. In aggregating species, pairwise associations are random – and while individuals may group around limited resources or environmental conditions, any fitness benefits resulting from aggregation are a passive by-product, and not a result of active interaction between specific individuals (Wilkinson, 1985).

Group dynamics are central to social structure. Fission-fusion societies are defined by individuals merging to form groups, which are in constant flux over time as some individuals break away to form subgroup units while others rejoin with the parent group at frequent intervals (Conradt and Roper 2005). This dynamic typically results in fluctuating subgroup composition, size, and spatial distribution (Aureli et al. 2008; Willis and Brigham 2004; Patriquin et al. 2010; Patriquin and Ratcliffe 2016). In such cases, individuals may selectively interact with particular members while avoiding others (Barclay and Kurta 2007). Yet despite the fluctuating and fragmenting nature of associations in such societies (e.g., individuals may cycle across a set of different roosts), evidence suggests these systems are able to maintain highly social and highly

structured interactions (McCracken et al. 2006). Fission-fusion societies have been documented in mammals with complex sociality, such as cetaceans (Christal et al. 1998; Connor et al. 2000) and primates (Kappeler and van Schaik 2002; Lehmann et al. 2007; Asensio et al. 2009), and it has been suggested that the challenge of maintaining interactions in fission-fusion societies may create greater selective pressures for cognitive and communicative abilities (Barrett et al. 2003; Aureli et al. 2008). Fission-fusion societies can also be further categorized based on the nature of group formation, which may occur within closed or open systems (Connor et al. 2000), and the nature of fission-fusion, which may occur within or across hierarchically nested tiers of social actors (Wittemyer et al. 2005; Smith et al. 2007; Whitehead 2008). Broadly, there are two alternatives to a non-random fission-fusion society. The first is a society in which interactions between individual members remain constant or are slow to change over time, with relatively static pairwise associations between individuals (Mitani et al 1991; Whitehead 2008). The second is an aggregating "non-society" (as mentioned previously), in which pairwise associations between individuals are random and are not based on a social, but more often an environmental component (Mitani et al. 1991).

In bats, patterns of association have key implications (Waag et al. 2021). A large proportion of bat species are social – this may be driven, in part, by a combination of ecological roost limitation, physiological demands alleviated by social thermoregulation, and traits of longevity and philopatry (Kerth 2008). For bats, fission-fusion dynamics are also especially pronounced, as the high mobility of flight permits groups to dissolve and reform on a nightly basis. This allows bats to better mediate the costs and benefits of group living (Aureli et al. 2008; Patriquin 2010; Patriquin and Ratcliffe 2016; Zeus et al. 2018), and how they maintain social ties throughout this process is an active and interesting area of research. While roosting together, many bat species

may gain fitness benefits via different cooperative behaviours: communicating roosting and foraging locations, removing parasites through allogrooming, sharing food, allonursing, and social thermoregulation (Wilkinson 1984, Wilkinson 1986; Wilkinson 1992ab; Wilkinson and Boughman 1998; Kerth and Reckardt 2003; Kerth et al. 2003; Willis and Brigham 2007; Carter and Wilkinson 2013; Webber and Willis 2018). In cases where such behaviors are present, observed societies are typically highly social and structured, with strong nonrandom associations between individuals (Emlen 1994; Kerth 2008).

Due to pronounced seasonal changes, temperate bats face constraints that influence their group dynamics and social structure. A restricted reproductive period creates energetic limitations, and are bats are forced to enter extended bouts of torpor during the cold winter months. Sperm storage enables up to 7 months between copulations during the fall and spring fertilization (Racey 1979; Thomas et al. 1979). Because of these factors, temperate bats typically produce fewer litters with more rapid (and costly) gestation and development, with this being especially pronounced in vespertilionids (Barclay and Harder 2003). During the summer period, many temperate bat species separate by sex, with females gathering at maternity roosts to birth and rear young, and males roosting elsewhere (Fenton and Barclay 1980; Foster and Kurta 1999; Lacki and Schwierjohann 2001; Kunz and Lumsden 2003; Broders and Forbes 2004). It has been suggested that this segregation may be due to the high costs of female reproduction; with females being energetically restricted to insect-abundant foraging sites at low elevations, while males are forced to compete for proximity to these sites – most being forced into more marginal upland areas (Encarnação et al. 2005; Senior et al. 2005; Angell et al. 2013). Fission-fusion dynamics at maternity colonies have been documented in several temperate bat species, as evidenced by frequent roost-switching and shifting group composition (Kerth and König 1999; O'Donnell 2000; Kurta et al. 2002; Willis

and Brigham 2004; Garroway and Broders 2007; Popa-Lisseanu et al. 2008; Patriquin et al. 2010; Olson and Barclay 2013; Patriquin et al. 2016). Several hypotheses have sought to explain non-random fission-fusion associations in maternal temperate bats, all of which involve potential fitness benefits. The social thermoregulation hypothesis states that a reduced energetic cost due to social thermoregulation is the primary driver behind the formation of maternity colonies in temperate climates – as this moderates the high cost of reproduction (Willis and Brigham 2007). However, maternity colonies are also observed in some tropical species, where benefits from social thermoregulation may be marginal (Neuweiler 2000). This suggests, potentially, that social thermoregulation may not be the primary (or at least sole) reason behind non-random association in temperate bats. Other behaviors, such as allonursing (e.g. Wilkinson 1992b), allogrooming (e.g. Kerth et al. 2003), and information sharing (e.g. Wilkinson 1992a; Kerth and Reckardt 2003) may also be drivers.

Both non-maternal females and, less frequently, adult males, have been observed associating with maternity colonies and one another (Perlmeter 1996; Senior et al. 2005; Angell et al 2013). However, it is not entirely clear if these associations are the result of passive environmental benefits (i.e. are random), or active social behaviors such as social thermoregulation or information sharing (i.e. are non-random). During swarming in late August-September, male and female temperate bats, largely *Myotis* and *Pipistrellus,* congregate in large numbers and come from great distances to gather around caves and mines which are used as winter hibernacula (Fenton 1969; Davis and Hitchcock 1965; Parsons K. et al. 2003; Veith et al. 2004; Burns and Broders 2014; Johnson et al. 2015). Swarming may allow bats to assess hibernacula condition (Davis and Hitchcock 1965; Veith et al 2004), as well as to acquaint young-of-the-year with their location (Fenton 1969), but a key function is also mating, with males and females that had been

previously segregated joining to copulate (Fenton 1969, Thomas et al. 1979; Wai-Ping and Fenton 1988).

Swarming clearly represents a moment for males to maximize reproductive fitness (Burns and Broders 2015b), however males who roost with females in late summer may also increase their reproductive success on the condition that females are sexually receptive shortly after weaning pups (Perlmeter 1996; Senior et al. 2005; Angell et al. 2013). In Europe, female Daubenton's bats (*Myotis daubentonii*) at intermediate quality roost sites are more likely to reproduce with roost-sharing males, whereas females at higher quality lowland sites (which tend to exclude males) are more likely to reproduce with males during swarming (Angell et al. 2013). One explanation for this observation is that benefits that males provide to females in marginal environments (e.g. social thermoregulation) may exceed disadvantages, with males also gaining an increase to reproductive fitness in the process (Angell et al. 2013). Early mating by males could provide a fitness advantage as sperm are the first to be stored in the oviduct (Racey & Entwistle 2000). For North American species, no mating activity has been observed outside of swarming sites to date (Ormsbee et al. 2007), although males of some species (e.g. *Myotis lucifugus*) have been observed roosting in the same structures as females and with one another (Davis and Hitchcock 1965; Broders and Forbes 2004). The apparent promiscuous and random nature of mating in such species (Thomas et al. 1979; Wai-Ping and Fenton 1988) suggests that any fitness advantage derived from early mating is marginal, however skewed reproduction provides evidence that nonrandom mating does occur (Watt and Fenton 1995), and may be caused by unobserved female choice or another form of mate selection (Wilkinson and McCracken 2003).

There may also be reasons for males to roost with one another. Males may be more likely to form cooperative associations if they prey on ephemeral insects and are adapted to feed in open habitats, as such circumstances allow for high potential benefits from information transfer – although other factors such as reduced predation-risk may also play a role (Safi and Kerth 2007). Further, multi-male association may be temporally dependent; groups may form early in the summer but disband later when females become receptive due to the increased mating competition associated with proximity to other males (Safi 2008). However, a diversity in male mating strategies may exist in temperate bats, even within a single species (Jahelková and Horáček 2011). If net benefits to male cooperation outweigh costs, then multi-male associations will likely be maintained into the swarming period. Non-random associations between males during swarming, have, in fact, been observed in several species of temperate bats (*Myotis lucifugus*, *Myotis nattereri*) which may indicate the presence of cooperative male coalitions to gain and maintain access to females (Rivers 2005; Burns. and Broders 2015a). Interestingly, male mating coalitions have been observed in cetacean (Connor et al. 1992) and primate species (Watts 1998; Pandit and van Schaik 2003) which also demonstrate fission-fusion dynamics (Connor 2000; Lehmann et al. 2007). Clearly, more work needs to be done in order to understand under what circumstances, and for what reasons, multi-male association occurs in temperate bats, particularly in North America.

Understanding the conditions influencing bat roosting and association may therefore give us insights into the diversity of selective processes that may influence behavior and social structure. Such information also has concrete value for conservation and management (Sutherland 1998). In North America, bats such as *M. lucifugus* are threatened by *Pseudogymnoascus destructans,* the fungal pathogen responsible for White Nose Syndrome (Blehert et al. 2009; Minnis and Lindner 2013). A combination of carry-over effects with direct mortality may limit

fitness and the capacity for adaptive immunity to occur (Davy et al. 2017). Understanding how bats move across the landscape and interact with one another will be useful for predicting disease diffusion and colony fragility (Langwig et al. 2012; Webber et al. 2016). Additionally, linking association to information on habitat quality and availability may help refine our understanding of how habitat is used by bats, and what specific roost characteristics influence association, abundance, group dynamics, and social structure (O'Donnell and Sedgeley 2006; Johnson et al. 2012; Encarnação 2012; Angell et al. 2013; Olson and Barclay 2013).

The overall objective of my research was to resolve knowledge gaps related to roost habitat selection by little brown bats in forest environments, in the hopes of informing effective conservation and management. In Chapter Two, I evaluated characteristics of forest roost habitat here in Ontario by capturing bats, following them back to roosts using radio telemetry, and then taking tree scale-measurements once roosts were located. I predicted that decay would have a positive effect on roost presence at both the tree and stand scale. Using roost locations, I then performed a stand-scale logistic analysis to predict the relative probability of roost presence within forest stand polygons in an existing forest inventory, and determined what stand-level variables had the greatest predictive power. In Chapter Three I analyzed the effect of several variables on bat abundance at the roost scale, comparing the effects of several environmental and social variables in artificial roosting boxes here in Ontario. I used a model selection approach, based on AICc, to determine what combination of variables best predicted bat abundance. Lastly, in Chapter Four I integrated my overall results to address key findings, their management implications, and potential directions for future research.

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## 2. Roost Characterization and Stand Selection by Little Brown Bats

How physiological constraints translate to selected roost qualities remains largely unknown for many forest-dwelling bat species, including *Myotis lucifugus*. In a mixedwood forest landscape in central Ontario Canada, Jung et al. (2004) found that *Myotis* (identified as either *M. lucifugus* or *M. septentrionalis*) inhabited 4 tree species: white pine (*Pinus strobus*) most often, followed by trembling aspen (*Populus tremuloides*), jack pine (*Pinus banksiana*), and white spruce (*Picea glauca*), with a strong preference for snags of decay class 4 (recently dead, with few branches and exfoliating bark). Conversely, in the mixedwood boreal forests of Quebec Canada, *M. lucifugus* roosts were found chiefly in balsam fir (*Abies balsamea*; 77.5%), followed by white birch (*Betula papyrifera*; 12.5%), and white spruce (10%); with 36 (90%) of these roosts in snags (62.5% in decay class 6) and the remaining 4 (10%) in live white birch (Fabianek et al. 2015a). Further afield, in northern Alberta Canada, *M. lucifugus* have been found predominantly in dying and dead *Populus* spp. with heart rot – with bats showing preference for trees with large diameters at breast height (DBH; approximately 1.3m), and large DBH being positively correlated with group size (Crampton and Barclay 1998; Olson and Barclay 2013). In British Columbia Canada, *M. lucifugus* roosts have been found mostly in dead (mid-decay, 3–5) lodgepole pine (*Pinus contorta*) and in living but unhealthy trembling aspen (Psyllakis and Brigham, 2006). In New Brunswick Canada, *M. lucifugus* roosts were primarily in red spruce (*Picea rubens*) – alive and recently dead to advanced decay; 1–7 – with sites highly correlated with the number of snags in the vicinity (Broders et al. 2004). In regenerating mixedwood forests in Nova Scotia Canada, *M. lucifugus* roost have been located in 10 different tree species, predominantly conifers, with 41.6% of roosts in balsam fir and red spruce, and 20.7% in white pine and eastern hemlock (*Tsuga canadensis*); either living (i.e. 1)

or in late stage decay (i.e., 6 or higher) (Lowe 2012). These disparate findings suggest that roost use by *M. lucifugus* is variable among regions, and that bats may be using whatever suitable cavity harboring tree species most available, and/or that best suit their thermal needs (Sedgeley 2001). In a given region, certain tree species (e.g. poplars, pines, firs etc.) may have characteristics that make them more prone to decay processes that result in the formation of suitable roosting cavities (Menzel et al. 2001; Parsons et al. 2003; Broders and Forbes 2004; Lacki et al. 2013). The volume of deadwood itself has been positively linked with bat species diversity in forest environments (Tillon et al. 2016).

As a result, decay may be a key driver behind roost selection in temperate bats such as *M. lucifugus*. Softer wood that lends itself to animal excavation and internal rot, and bark which splits or peels away during decay but is retained on the tree may be important characteristics behind the creation of suitable roosts. Depending on the tree species, dead trees may be more likely to have these cavity features but living trees may demonstrate them as well if sufficiently old and damaged by disease (Crampton and Barclay 1998). Parsons et al. (2003) found 6 *M. lucifugus* maternity colonies in cavities within live trembling aspen in British Columbia that were created by the wood-decay fungus *Phellinus tremulae* and *Armillaria sinapina*, and they suggest that such pathogens require time to develop advanced decay. Even in live trees, cavities may be more prevalent in larger (DBH), older, trees with more heartwood (Parsons et al. 2003). Similarly, in New Brunswick availability of roosts for *M. lucifugus* and *M. septrionalis* may be linked to the epizootics of spruce budworm (*Choristoneura fumiferana*), a major cause of death for conifers in the region (Broders and Forbes 2004). Additionally, living and dead trees may have differing thermal qualities (Coombs et al. 2010) that are energetically favourable to certain bats. Dead trees are less insulative than live trees (Wiebe 2001) and therefore cool to a greater degree during summer nights, although

their cavities will also heat more rapidly than live trees during the day (Kalcounis and Brigham 1998). This difference is due largely to the water present in the tissues of live trees (and absent in dead trees), which has a higher thermal conductivity compared to surrounding air, and buffers both positive and negative temperature changes as a result (McComb and Noble 1981). Dead trees may provide more suitable roosts for non-reproductive bats, as cooler nightly temperatures may allow deeper torpor. Conversely, maternal bats may benefit from the higher and more stable overnight temperature afforded by live trees (Sedgeley 2001). At broader scales, a high proportion of dead trees within a stand may signal good roost availability to bats (Lacki et al. 2010; Johnson et al. 2010; Fabianek et al. 2015a), as bats may use several similar trees in a cluster of snags, with the potential to switch between them upon changing conditions (Johnson et al. 2012; Clement and Castleberry 2013; Fabianek et al. 2015b; Patriquin et al. 2016; Willis and Brigham 2004). Additionally, a high density of snags in an area may be correlated with lower canopy cover due to exfoliation and top-breakage, and this could result in warmer cavities due to a lack of shading (McComb and Noble 1981; Kalcounis and Brigham 1998).

Tree trunk diameter, together with decay, may also determine roost use. Tree diameter is a proximate indicator of the potential size of cavities within that tree, and cavity volume may have a variety of consequences for gregarious bats. Larger cavities permit larger groups of bats to congregate, which may be important if bats receive benefits from cooperative behaviours such as social thermoregulation (Olson and Barclay 2013). Tree size also has direct thermal consequences. Thermal insulation of wood and bark increases with tree diameter and cavity wall thickness (Derby & Gates 1966; Nicolai 1986; Jarolimek and Vierling 2019), and, similar to water in live tree tissue, thick sills and cavity walls can buffer temperature changes relative to the external environment (Vonhof, and Barclay 1997; Wiebe 2001; Sedgeley 2001; Vierling et al. 2018). Deeper tree cavities

with larger volumes may also be warmer, if entrance area remains comparatively small (Wiebe 2001; Paclík and Weidinger 2007). As a result, cavities in larger diameter trees are likely to have greater thermal stability if they are in thicker areas (Rhodes et al. 2009; Maziarz et al. 2017). Reproductive bats may also use dead trees if the diameter of those trees is sufficiently large to provide adequate insulation (Park and Broders 2012). On a given tree, microclimate may also vary with factors such as slope (Lacki et al. 2013), height (Ruczyński 2006), and direction of cavity entrance (McComb and Noble 1981; Wiebe 2001), and larger trees with a greater variety of (or wider thermal gradient within) cavities, may allow bats respond to changing conditions at low movement cost.

Roost characteristics may influence physiological responses and energetics in bats, even within individuals that are non-reproductive (Encarnação et al. 2012). However, suitable roost tree characteristics (e.g. decay state, diameter etc.) are also likely to vary across species, region, weather, and season (Kerth et al. 2001; Vonhof and Gwilliam 2007; Lacki et al. 2009; Clement and Castleberry 2013; Patriquin et al. 2016), and may be the product of a complex interaction of factors (Clement and Castleberry 2013; Vierling et al. 2018). There is a corresponding need, therefore, to identify and distinguish regional exceptions in roost habitat characteristics from general trends, in order to understand how and why roost habitat use changes across the ranges of forest dwelling species.

Here, I sought to evaluate the underlying characteristics predicting roost presence at both the tree and stand scale. As decay is often required to produce suitable cavity features and may create beneficial thermal conditions during the summer period, I hypothesized that tree decay is the primary driver of roost habitat use by *Myotis lucifugus*, both at the tree (H1), and stand level

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(H2). At the scale of individual trees, I predicted that occupied trees would be predominately in intermediate stages of decay, and that tree species prone to decay and cavity formation (e,g. *Populus* spp.) would be favoured. At the broader scale of forest stands, decay is difficult to measure directly, but stand age may be used as a proxy, on the basis that older stands are more likely to contain dead and dying trees (Harmon et al. 1986). In terms of modeling, I predicted that the stand-level variables of age (as a proxy for both decay and height), mean quadratic DBH, and percent poplar would be important predictive factors for roost use by *Myotis lucifugus*, all being positively associated with roost presence.

## Methods

**Study Area:** *Myotis lucifugus* is widely distributed across much of North America; from Alaska to southern California in the west to Labrador and Georgia in the east. This study took place in mixedwood forests in the Eastern forest-boreal transition near Chalk River, Ontario, Canada. The study area consisted of approximately 40 km$^2$ managed by Canadian Nuclear Laboratories (CNL; 286 Plant Road, Chalk River, Ontario, Canada. K0J 1J0) that encompassed several large lakes, wetlands, hills, and valleys. Since the 1940s this area has had an intense regime of fire suppression, and a lack of any sustained forest harvest. In addition to natural roosts, *M. lucifugus* inhabit bat boxes on the property that are distributed over an area of 0.8 km$^2$ (Appendix, Figure 1). All boxes (n = 16) were composed of 3/4 inch plywood and covered with a black rubber membrane ("Nursery" Bat House, Canadian Bat Houses Inc., Thunder Bay, Ontario). These 16 bat houses were deployed in May 2017, and were first used by bats in July 2018. They were mounted on 8 pine posts (2 boxes per post). Posts were approximately 4m in height.

**Capture and Transmitter Deployment:** All work was conducted under Animal Care permits from Trent University and the Ontario Ministry of Natural Resources and Forestry, and a research permit under the Canadian Species at Risk Act (SARA). Work was suspended in 2020 due to the COVID19 pandemic, and in 2021, the national guidelines for minimizing the risks to bats in the context of SARS-CoV-2 were also followed (Canadian Wildlife Health Cooperative, 2021A), in conjunction with the general Wildlife Health and SARS-CoV-2 Handling Guidelines (Canadian Wildlife Health Cooperative, 2021B)**.** To establish the locations of roost sites, I captured, radio-tagged, and followed individual bats back to roost trees and took measurements of these sites. From May to August 2019 and 2021 I captured bats at the 8 box locations using Tuttle-style harp traps (Bat Conservation and Management, Carlisle, Pennsylvania) and mist nets (Avinet Research Supplies, Portland, Maine). I routinely decontaminated equipment according to the most recent version of the Canadian National White-nose Syndrome Decontamination Protocol (Canadian Wildlife Health Cooperative, 2017), in addition to the SARS-CoV-2 safe handling and decontamination protocol in 2021 (Canadian Wildlife Health Cooperative, 2021). After capture, I weighed, sexed, measured forearm length, and determined age of bats (i.e., adult or juvenile) through transillumination of the wing bones to view the extent of closure of epiphyseal growth plates (Brunet-Rossinni and Wilkinson 2009). If female, I assessed reproductive status (i.e. pregnant, lactating, or neither). I determined advanced pregnancy by palpation of the abdomen, and lactation by the presence of enlarged nipples surrounded by a margin of furless skin. Pregnant and lactating bats were in some cases banded but never radio-tagged, and were immediately released. I banded individuals with alphanumeric aluminum lipped bands (Porzana Ltd, East Sussex, UK) using 2.9mm bat banding pliers (Bat Conservation and Management, Carlisle, Pennsylvania). For attaching transmitters, I clipped a 1x2cm patch of hair from between the bat's shoulder blades using an electric trimmer, and swabbed the area lightly with ethyl alcohol.

Depending on the weight of individuals, I used either HOLOHIL LB-2X (0.31g, Holohil Systems Ltd., Carp, Ontario) or Lotek PicoPip Ag337 (0.29g, Lotek Wireless Inc., Newmarket, Ontario) transmitters. I solder-activated these transmitters at capture sites – they had a battery capacity of 12–21 days. I affixed transmitters using Perma-Type surgical cement (Perma-Type Company Inc., Plainville, Connecticut), gluing the transmitter between and slightly below the bat's scapulae. In each case, transmitters and glue constituted 5% or less of the bat's weight.  Bats were then held to ensure adequate tack time (~5 minutes) and baby powder was applied to the area to speed drying time and prevent inadvertent self-adhesion.

**Telemetry:** After release, I tracked bats during daylight hours using an R-1000 telemetry receiver (Communications Specialists Inc., Orange, California) and a 5-element Yagi–Uda antenna (Advanced Telemetry Systems Inc., Isanti, Minnesota). I conducted tracking daily over the summers of 2019 and 2021, recording movement of individuals to and from boxes and roost sites.

**Roost Tree Measurements:** When roost trees were identified, I recorded tree species, GPS location, height, DBH (measured at 1.3m), and decay class. I calculated height using a Vertex IV ultrasound instrument system (Haglof Inc., Madison, Mississippi) and categorized decay class using the Maser Scale (Maser et al. 1979). This scale ranges from 1 (a live, healthy tree) to 9 (a decayed stump) (Maser et al. 1979).  GPS locations were used to inform subsequent stand-level analysis.

**Data Analysis:** I conducted all analyses in R programming language (v. 4.1.2, R Core Team 2021). I calculated arithmetic means for morphological measurements of roost trees, mean and mode of decay class, and the percentages of given species in the sample of roost trees. Roost

locations were then used in conjunction with CNL's forest inventory database to obtain information on the surrounding forest stand polygons, and to identify what stand variables (e.g. average tree height, species composition etc.) best predicted bat roost occurrence. The dependent variable in this case was the presence or absence of a roost in a forest stand. Variables within the forest inventory database were populated using single photon lidar (Leica SPL100) flights of the entire study area and classified according to Ontario's Forest Resources Inventory Technical Specifications. Variables examined were stand age (AGE), quadratic mean DBH of all trees in a stand (qDBH), percent poplar composition in a stand (%Pop), primary layer crown closure (PCC), veteran (i.e. super canopy) layer crown closure (VCC), overstory height (OHT), the height splitting the veteran layer from overstory, defined as the 90th percentile of lidar height returns from a stand (VSpHT), and trees per hectare (TPH). Several variables were highly correlated and were not included together in the same models. These were OHT and VSpHT ( $r = 0.98$, $p < .001$), OHT and qDBH ( $r = 0.63$, $p < .001$), VSpHT and qDBH ( $r = 0.69$, $p < .001$),  TPH and qDBH ( $r = -0.76$, $p < .001$), and TPH and PCC ( $r = 0.78$, $p < .001$). The only other variables with moderate correlation were %Pop and OHT ( $r = 0.42$, $p < .001$), %Pop and VSpHT ( $r = 0.43$, $p < .001$), and qDBH and AGE ( $r = 0.43$, $p < .001$), and these were included together in models.

Competing logistic regressions were generated using the Generalized Linear Mixed Models Template Model Builder package (Brooks et al. 2017), each with a different combination of predictive variables. Other Model performance was then ranked using corrected Akaike's Information Criterion (AICc) – which is a statistical estimator of prediction error, and by extension, the relative quality of a given statistical model (Burnham and Anderson, 2002). After the top model was identified, I mapped the fitted values that were calculated from the top model for each of the forest stand polygons within our study area. Area of analysis was defined by a

minimum convex polygon encompassing all located roost trees, approximately 4.82 km$^2$ in size. Each fitted probability value that was calculated for a given stand is an indication of how likely a roost is to be found in that stand – and is thus an estimate of the relative roosting habitat quality in a given forest stand based on the predictive variables in the model. I then mapped these values to give an indication of the relative quality of habitat across the study area with the aim of aiding in the formulation of a management strategy. In conjunction with an understanding of what stand variables influence bat roost presence, effective forest management can ensure high quality habitat is maintained in the long term. The results of this model were then used to generate a Habitat Suitability Index for the forest stands on site, using an empirical approach taken from Brennan et al. (1986).

## Results

Across the summers of 2019 and 2021, I captured 92 little brown bats (20 male, 72 female). I affixed radio tags to 65 bats (15 to males, 50 to females) and I located 37 roost trees using telemetry. At the tree scale (Figure 1), several characteristics emerged. Roost trees were predominately dead (n =27 of 37), and decay class ranged from living at class 1, to late-stage decay at class 7 (mean = 3.35, SD± 1.53; mode = 3). Roost composition was dominated by *Populus* spp. (n = 31), with the remaining trees (n = 6) consisting of 2 red maples, 1 balsam fir, 1 paper birch, 1 black ash, and 1 white pine. Anecdotally, roost trees were often found within small forest clearings, with low canopy closure. Diameter at breast height for roost trees ranged from 21.2 to 63.4 cm, with a mean value of 40.5, SD ± 10.51 cm (Figure 1). Roost tree height averaged at 18.7, SD± 9.43 m, with the shortest tree measuring 3.5 m, and the tallest reaching 43 m (Figure 1). Bats were found roosting together in the same roost trees on multiple occasions, with one roost having 9 different radio-tagged individuals (all female) occupying it

over a 7-day period. Over the study period tracked bats were observed roosting together in 8 roost trees, including six observations of pairs of tracked bats, one group of three tracked bats, and the aforementioned group of nine. A full count of un-tagged individuals within these roosts was not possible due to height and difficulty of access.
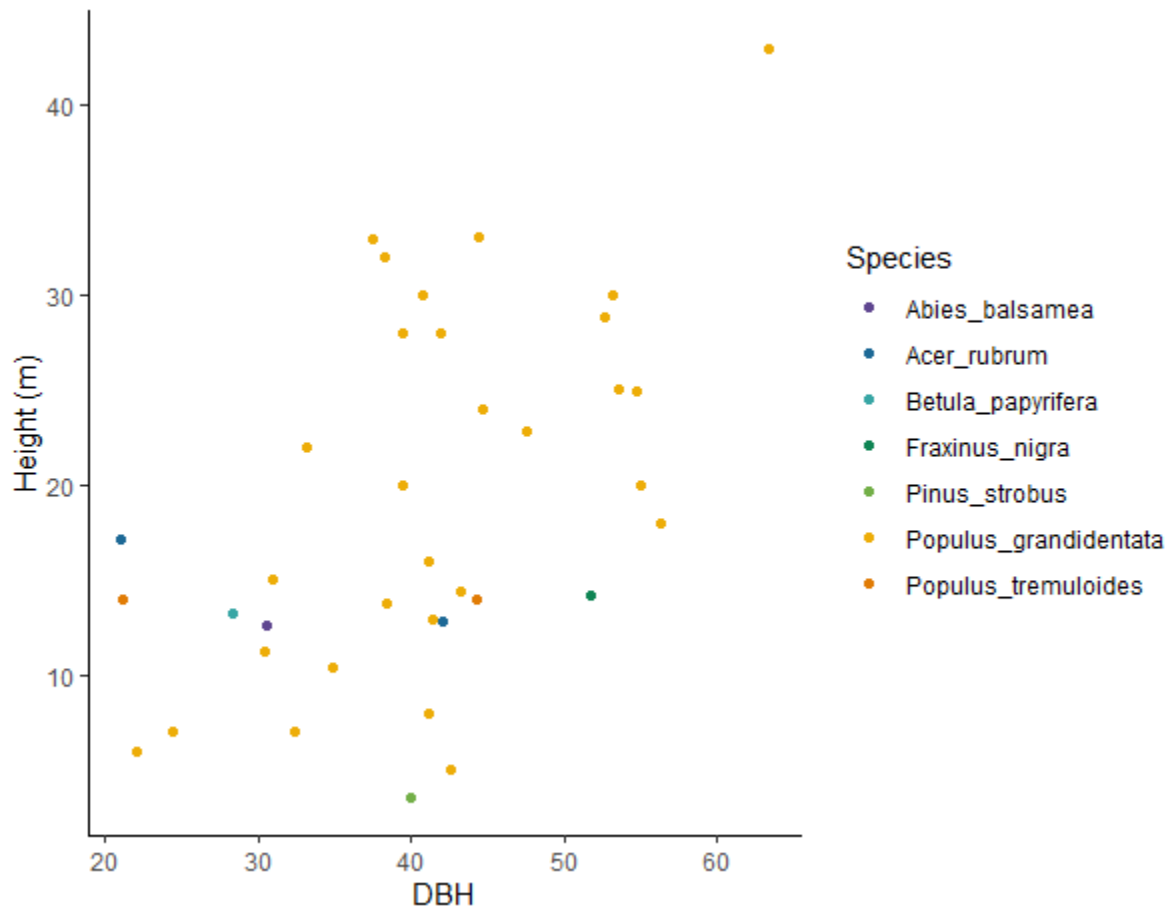


**Figure 1 –** Morphological characteristics of observed *Myotis lucifugus* roost trees in Chalk River, Ontario, Canada, collected in 2019 and 2021. Diameter at breast height (DBH) in cm, height in meters, colour denotes species.

At the stand scale, I used logistic regression to generate 16 competing models predicting roost presence (Table 1), and selected 2 based on similar performance (within ~2 Δ AICc) to the top model. These 2 models collectively accounted for 69% of the Akaike model weight among all models – weight being interpretable as the conditional probability that a given model

is the best model given the data set and performance of competing models. Tree height across

a stand was important for roost presence; the two top models had either the 90th percentile of

height returns from a stand (VSpHT), or stand overstory height (OHT) as significant predictors

**Table 1** – Model selection for models predicting *Myotis lucifugus* roost presence based on forest stand-scale characteristics in Ontario, Canada. Models are sorted in order of ascending Akaike's Information Criterion with a correction for small sample sizes (AICc; an estimator of model quality based on prediction error). VSpHT = the 90th percentile of height returns from a stand, OHT = overstory height, AGE = stand age, qDBH = quadratic mean diameter at breast height of all trees in a stand, PCC = primary layer crown closure, VCC = veteran layer crown closure, %Pop = estimated percent of the stand dominated by poplar species, TPH = trees per hectare. df = degrees of freedom, logLik = log likelihood, Δ= change in AICc from top model, weight = Akaike weights for the model.

| Model | df | logLik | AICc | Δ | weight |
|-------|----|--------|------|---|--------|
| ~ VSpHT + AGE + VCC | 4 | -76.822 | 161.788 | 0 | 0.466 |
| ~ OHT + AGE + VCC | 4 | -77.543 | 163.23 | 1.442 | 0.227 |
| ~ VSpHT + VCC | 3 | -79.276 | 164.639 | 2.851 | 0.112 |
| ~ OHT + VCC | 3 | -79.491 | 165.068 | 3.28 | 0.090 |
| ~ VSpHT + AGE + %Pop | 4 | -79.252 | 166.648 | 4.86 | 0.041 |
| ~ OHT + AGE + %Pop | 4 | 79.673 | 167.491 | 5.703 | 0.027 |
| ~ VSpHT + AGE + PCC | 4 | -80.023 | 168.189 | 6.401 | 0.019 |
| ~ OHT + AGE + PCC | 4 | -81.045 | 170.233 | 8.445 | 0.007 |
| ~ qDBH + %Pop + VCC | 4 | -81.878 | 171.9 | 10.112 | 0.003 |
| ~ AGE + %Pop +VCC | 4 | -82.172 | 172.488 | 10.7 | 0.002 |
| ~ %Pop + TPH + VCC | 4 | -82.173 | 172.49 | 10.702 | 0.002 |
| ~ qDBH + %Pop + PCC | 4 | -82.694 | 173.533 | 11.745 | 0.001 |
| ~ AGE + qDBH + %Pop | 4 | -82.712 | 173.569 | 11.781 | 0.001 |
| ~ AGE + %Pop + TPH | 4 | -83.25 | 174.644 | 12.856 | 0.001 |
| ~ AGE + %Pop + PCC | 4 | 83.282 | 174.708 | 12.92 | 0.001 |
| ~ | 1 | -89.108 | 180.231 | 18.443 | 0.000 |

of roost presence (Table 2). Veteran layer crown closure in the forest stand (VCC) was also

significant in both most supported models, although the effect on roost presence was less

significant (Table 2). Stand age appeared in two of the top models with a significant, though

negative effect on roost presence (Table 2). Percent poplar (%P) appeared in none of the most

supported models, and neither did Quadratic mean diameter at breast height (qDBH), primary

layer crown closure (PCC), or trees per hectare (TPH) (Table 1; Appendix 2). The predictors

of the most supported model (based on AICc) were the 90th percentile of height returns from a

stand, crown closure in the veteran layer, and stand age (Table 2). The 90th percentile of height

returns from a stand was a pronounced predictor, with the probability of a roost being in a stand

increasing by 21%, on average (Odds Ratio (OR) = 1.214, CI95= 1.103, 1.352), with every 1

metre increase in height. In contrast, the effect of increasing crown closure (OR= 1.068, CI95=

1.016, 1.125) and age (OR= 0.970, CI95 = 0.945, 0.996) were less pronounced, with probability

increases of 6.8% per 1% closure and decreases of 3% per 1 year, respectively. Results from

the second most supported model (Δ = 1.442) were very similar.

**Table 2** – Estimated effects of predictors included in the two most supported bat roost presence models. Significance and p-values (in parentheses). VSpHT = the 90th percentile of height returns from a stand, OHT = overstory height, AGE = stand age, VCC = veteran layer crown closure. Cond = the model intercept.

| Model | Cond | VSpHT | OHT | AGE | VCC |
|---|---|---|---|---|---|
| ~ VSpHT + AGE + VCC | -5.830*** (<0.001) | 0.194*** (<0.001) | — | -0.030 * (0.023) | 0.066* (0.010) |
| ~ OHT + AGE + VCC | -6.863*** (<0.001) | — | 0.194*** (<0.001) | -0.026* (0.041) | 0.068** (0.008) |
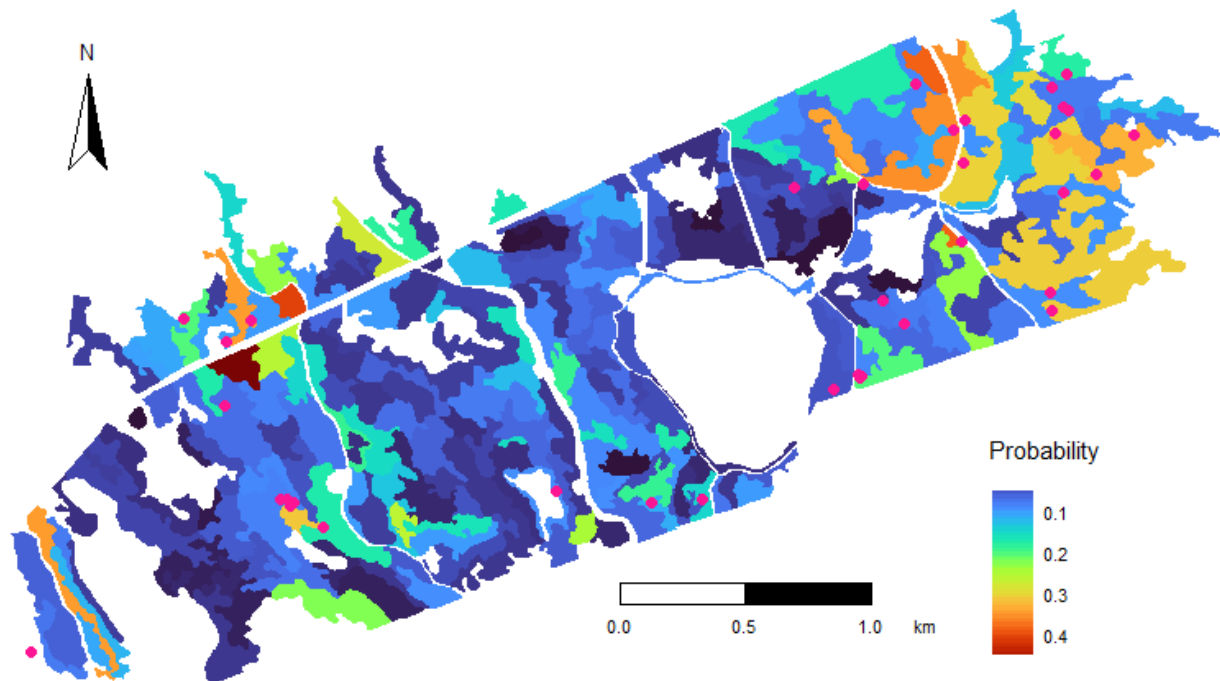
**Figure 2** – From the most supported model; predicted probability (0 to 1) of little brown bat roost by forest stand polygon in Ontario, Canada. Recorded roost locations denoted in pink. Lakes, roads, and other infrastructure polygons have been excluded from analysis.

## Discussion

At the focal-tree scale, the hypothesis that tree decay is the driving force behind used for roosting by little brown bats (H1) was tentatively supported. I predicted that roost trees would be in intermediate stages of decay, and that certain tree species prone to decay and cavity formation (e,g. *Populus* spp.) would be favoured. In general, this is what I observed. The majority of roost trees (n =27 of 37) were dead and in mid stages of decay, while also being composed primarily of *Populus* spp. (n = 31). However, the hypothesis that tree decay is the driving force behind roost presence at the stand scale (H2) was not supported. The prediction that stand age (as a proxy tree decay), together with mean DBH, and percent poplar would be the primary drivers of roost use at the stand level was not demonstrated.

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That stand age was not positively, but rather negatively, associated with roost presence is an interesting result. It may be the case that the relationship between stand age and the creation of trees with suitable stages of decay may fluctuate over time (Brassard and Chen 2008), and there may be an ideal intermediate point, after which the availability of roost trees in suitable stages of decay is reduced. Stand age may, therefore, be a poor proxy for the ideal stages of decay in this case, with the numbers of tall mature trees (measured by VetSpHT and OHT) being a more direct measurement of the roosting suitability of trees in the stand. This is supported by the fact that the 90th percentile of height returns was not strongly correlated with stand age despite correlation being significant; $r = 0.20$, $p = <0.001$), nor was overstory height; $r = 0.16$, $p = 0.006$. As *Populus* are early successional species, and the vast majority of roost trees found were within *Populus* spp. (31 of 37), ideal roosting conditions may be created once these early succession trees are mature and in decay, but before they are superseded completely by late successional species.

One explanation may be gleaned from the importance of both the 90th percentile of height returns and, alternatively, overstory height in both of our top models. The upper threshold of tree height across a given stand may again be a more important indicator of how many suitable trees are available rather than simply the overall species composition of that stand. Previous work on several *Myotis* spp. in British Columbia found a negative relationship between stand canopy height and bat roost (n =17) presence in forest stands, with *M. lucifugus* found roosting in trees that were on average 3.92 m taller than the canopy (Psyllakis and Brigham 2006). In an examination of roost (n=40) preference by male *M. lucifugus* in Quebec, taller trees were 1.50 times more likely to be selected than randomly available trees (Fabianek et al. 2015a). For many forest-roosting bats,

observed roost trees are typically taller than available trees – tree height was found to have the greatest mean estimated effect size of all variables measured, 1.52, in a power analysis review of studies of tree roosting bats (Lacki and Baker 2003). These results were echoed in another meta-analysis that found that roost trees for forest-dwelling bats were taller than random trees ($n_\text{data sets}$= 15, $n_\text{roost}$=454, $n_\text{random}$=948, effect size=0.85, $P < 0.001$), with the strongest estimated effect size as well (Kalcounis-Rüppell et al. 2005). Tree height is likely important for several reasons. Increased solar exposure that such trees receive once they surpass the canopy layer may have thermoregulatory benefits, and the greater height of trees is also likely to aid in predator avoidance and the length of potential cavities. However, the influence of height at the scale of stand selection is not typically measured. That the height of the 90$^\text{th}$ percentile of returns, and overstory height were the strongest predictors in our model selection process may be explained by a general preference for overstory trees speculated to be less exposed to predators and with higher solar exposure.

Also interesting is our result that crown closure of the veteran layer of a stand is positively associated with bat roost presence at the stand scale. This is the opposite of what would be expected given previous published literature showing preference for roost trees in locations with less canopy cover for both other forest-roosting bats and *M lucifugus* (Lacki and Baker 2003; Kalcounis-Rüppell et al. 2005; Psyllakis and Brigham 2006). However, crown closure in the veteran layer across a stand likely differs from crown closure within the primary layer of trees – evidenced by the fact these two variables are only weakly correlated; $r = 0.17$, $p = 0.005$. It may be that canopy closure in the veteran layer is another proxy for the number of overstory trees present in a stand – perhaps bats are selecting stands that have high crown closure in the veteran layer, despite selecting roost trees within those stands that are in relatively open areas. That quadratic mean DBH of a

forest stand was not a significant variable in any of the top models generated is also a surprising result (Appendix 2).  In a meta-analysis of *Myotis* spp. roosting characteristics across different areas of their range Lacki (2018) found that *Myotis lucifugu*s in western Canada inhabited larger diameter trees and formed larger colonies in trees than their eastern counterparts, and suggested this may either be due to a genuine difference in preference, or, a constraint in choice caused by a shortage of large diameter trees in eastern forests. Again however, trends at the tree scale may not directly translate to the stand scale and vice versa – the quadratic mean DBH of a stand may simply not be a good indication of what trees are actually available to roost within.

Few studies have looked at tree scale roost selection in this area of this species' range. One such study by Jung et al. (2004) found that in a mixedwood forest landscape in central Ontario, bats most often chose large snags, in open canopies, of intermediate stages of decay – citing *Populus* spp. as important. This bears similarity to my results at the roost tree scale. A key difference between the present study to note, is that the vast majority of *Myotis lucifugus* we captured were female (72 female, 20 male), in contrast to the near entire male sample captured by Jung et al. (2004; 1 female, 73 male). Another key difference is evident in the relative species composition of observed roost trees. Jung et al. (2004) found that *Pinus strobus* roosts were used most frequently, but in our case, bats were observed using *Pinus strobus* as a roost only once. It may be that this is due to a difference in roost preference between sexes (our sample was predominately female, while theirs was male), or a difference in availability of these tree species between the study areas (Chalk River, Ontario vs. Rushbrook Lake, Ontario).  In western regions of Canada, female *Myotis lucifugus* has been found roosting predominantly in *Populus* species. In Northern Alberta 134 of 135 roost trees were found to be in *Populus* spp. – 61 located within *Populus tremuloides* and 73 in *Populus balsamifera* (Olson and Barclay 2013). DBH for these

trees was 50.5 ± 16.0 cm; larger but with an overlapping standard deviation to our values of 40.5 ± 10.51 cm. Tree height in their study was also similar, with an observed value of 20.3 ± 6.8 m, compared to my value of 18.7 ± 9.43 m (Olson and Barclay 2013). In Norway, female *Myotis daubentonii* have been found roosting predominantly in crevices of *Populus tremula* (30 of 36 roosts)*,* with bats using *P. tremula* significantly more than would be expected from the distribution of available hollow trees; Fisher exact test, df = 1, *p* = .03 (Michaelsen, 2016). When compared with birch, aspens had significantly higher internal temperatures, with warmer external microclimates – due in part to the less dense woodland which the aspen groves created, increasing solar exposure (Michaelsen, 2016). Observed aspen cavities were also significantly smaller and higher than those of other roost trees, excluding some potential predators (Michaelsen, 2016). While these variables were not directly measured in my study, if the characteristics of *Populus* roosts in Ontario are similar to those in Norway, this may explain the apparent preference seen here for *Populus* spp. at the tree scale as well. *Populus* may be an important species at northern latitudes due to the thermal benefits it provides relative to other trees. As such, measuring the effect of these variables in Ontario would be an interesting and important direction of future research.

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### 3. Predictors of Bat Abundance Within Artificial Roosts

A key environmental variable linked to animal behavior is temperature. Many organisms rely on thermoregulation to adapt to daily or seasonal shifts in ambient temperatures, to ensure their internal body temperature remains within tolerable physiological limits. Body temperature is also strongly linked to metabolism through its influence on the rate of biochemical reactions involved in energy production; reactions driven by the kinetic energy of cellular molecules (Clarke and Fraser, 2004). This relationship between temperature and energetics is often more obvious in ectothermic organisms, though it is also observed in the energetic costs endotherms face in maintaining an optimal body temperature though numerous means (e.g. panting, shivering etc.).

Thermoregulation is critical to many species' energetics and survival, as evidenced by the numerous strategies that have evolved to mitigate the metabolic costs of fluctuating thermal conditions (Wilsterman et al. 2020). Aestivation, hibernation, and torpor are all such strategies that involve entering a state of depressed metabolic rate and reduced body temperature, often in response to resource scarcity (Storey and Storey 1990; Geiser 2004). Temperate bats are one group of endotherms that make use of several thermoregulatory strategies to mitigate energetic costs and persist in their environment. Bats have a high surface-area-to-volume ratio that makes maintenance of normal body temperature energetically costly (Barclay and Harder 2003; Dzal and Brigham 2013). Bats also face strict energetic limitations due to the costs of flight (Kurta et al. 1989), which are exacerbated in temperate bats by a seasonally restricted active period (Chruszcz and Barclay 2002). Torpor, a deliberate physiological process where bats reduce their body temperature to near ambient and slow their metabolic rate (Wang and Wolowyk 1988), can conserve large amounts of

energy (Stawski et al. 2014; Studier 1981). These factors create a strong selective pressure for roosting behaviours that alleviate energetic costs.

However, even organisms that deploy these strategies have critical thermal limits beyond which they experience thermal stress and death. In endotherms, understanding the relationship between ambient temperature, internal temperature, and how they both interact to set a critical thermal maximum is an important part of understanding the range of suitable habitat for a given species. The thermoneutral zone for *Myotis lucifugus* – the range within which minimum metabolic regulation is needed to maintain – is 32–37°C (Studier and O'Farrel 1976), with bats in the genus *Myotis* unable to tolerate ambient temperatures exceeding 45°C for more than 30-minute periods (O'Farrell and Studier 1970). An ambient temperature of 2°C is most energy efficient for little brown bats (*Myotis lucifugus*) in cases of extended torpor, but at temperatures below 2°C individuals expend more energy to maintain torpor, eventually awakening to re-warm. Therefore, most individuals would not survive winter hibernation at ambient temperatures less than 0°C (Humphries et al. 2005). At ambient temperatures above 40°C, behavioural thermoregulation (i.e. panting, moving to cooler areas) likely becomes necessary to avoid hyperthermia and death (Stones and Wiebers 1967; Flaquer et al. 2014)

Roost switching is a common behaviour in many bat species (Chruszcz and Barclay 2002; Lausen and Barclay 2003; Kerth et al. 2001; Kurta et al. 2002; Willis 2003) that may be driven in part by changing temperatures within and across roosts. In *M. lucifugus*, individuals switch roosts at a mean of 3.67 days (Barclay and Kurta 2007; Crampton and Barclay 1998). Within the critical range of ambient temperatures bats can tolerate, there likely exists several subranges that are energetically favourable to individual bats, and this may determine the necessity of aggregating in

roosts with other individuals. For example, at temperatures of 15-30°C clustered pallid bats (Antrozous pallidus) consume less oxygen and lose less body mass compared to isolated individuals (Trune and Slobodchikov 1976). Similarly, at temperatures of 10°C and 20°C *M. lucifugus* pups can maintain their body temperature much longer when together than when roosting alone (Fujita 1986). Because of the influence of roost microclimate, there may be considerable energetic benefits to switch roosts in response to both changing ambient temperatures and changing physiological needs (such as those which occur over the course of reproduction). There has recently been debate as to whether bat boxes (artificial roosting environments) may act as ecological traps, creating ambient thermal environments that lead to hyperthermia and mortality in the bats occupying them (Flaquer et al. 2014; López-Baucells et al. 2017; Bideguren et al. 2019; Crawford and O'Keefe 2021; Fontaine et al. 2021). In consideration of the conservation situation which many temperate bat species find themselves, and in the context of climate change and rising global temperatures, this question deserves full and thorough investigation.

Here I examine the influences of roost temperature, and prior bat presence (as a proxy for benefits related to social roosting), on abundance of bats within bat boxes in Ontario. One goal is to gain a better understanding of how these variables influence roost selection by *M. lucifugus* in temperate climates. My first hypothesis , H1, is that social thermoregulation is a driver of bat aggregation in summer roosts. If H1 is true, then I predict that the number of bats within a roost box on the day previous will be a predictor of bat abundance within a roost box. The second hypothesis, H2, is that that summer aggregations are also driven by environmental variables such as internal box temperature. If H2 is true, then environmental variables such as roost box temperature will be predictors of bat abundance within roost boxes. A third related hypothesis in this case, H3, is that higher minimum roost temperatures facilitate greater thermoregulatory

benefits than maximum roost temperatures at higher latitudes. If H3 is true, we predict abundance of bats within roost boxes to increase more strongly with increasing minimum temperatures than with any trend involving maximum temperatures.

**Methods**

I collected data from bat boxes around Perch Lake in Chalk River, Ontario, Canada in 2021. Boxes were distributed around the lake over an area of 0.8 km$^2$ (Appendix 1). All boxes (n = 16) were composed of 3/4-inch plywood and covered with a black rubber membrane ("Nursery" Bat House, Canadian Bat Houses Inc., Thunder Bay, Ontario). These 16 bat boxes were deployed by CNL in May 2017, and were first used by bats in July 2018. They were mounted on 8 pine posts (2 boxes per post). Posts were approximately 4m in height. These boxes were used exclusively by *M. lucifugus*, as confirmed by capture surveys. Temperature inside the boxes was measured from May-September, 2021. Two WeePits (ALPHA MACH INC, Sainte-Julie, Quebec) were placed at the bottom and top of each box (32 loggers total) and recorded temperature every 10 minutes. Ambient temperature in the study area was also recorded every 10 minutes at several environmental monitoring stations belonging to Canadian Nuclear Laboratories. Bat abundance in the boxes was counted daily from May to September 2021 (each afternoon), by shining a flashlight into boxes and counting the numbers of bats in the box chamber while they are roosting.

I performed all statistical analyses in R (v. 4.1.2, R Core Team 2021). I used a model selection process to evaluate the effect of box variables on bat abundance (number of bats/box). I treated box as a random effect to account for unmeasured variables. The global model for bat abundance was a Poisson generalized linear mixed model with the formula: Number of Bats ~ Number of bats at the previous interval + Daily difference between maximum box

temperature and maximum external temperature, ΔMax + Daily difference between minimum box temperature and minimum external temperature, ΔMin + Average daily humidity + Distance to water + (1 | Box), generated in the glmmTMB package (Brooks et al. 2017). ΔMax and ΔMin were calculated by taking the difference between the inside of boxes and the external environment at the coldest and warmest periods of a day, respectively – representing the amount of temperature change from ambient caused by the box environment. A single zero-inflation parameter applying to all observations (ziformula~1) was used in the global model to account for the presence of structural zeros in the data. Competing models were generated, and were evaluated based on model weight and Akaike's Information Criterion with a correction for small sample sizes (AICc) - an estimator of model quality based on prediction error that has a correction for small sample sizes (Burnham and Anderson, 2002).

## **Results**

Box temperature differed from ambient conditions, with a mean difference in temperature of 0.76 °C at the coldest period of a day (SD= 0.99, range=-2.72 to 5.23), and 11.34 °C at the hottest period of a day (SD=5.14, range=-0.18 to 27.54). I generated 12 competing models to predict bat abundance within roost boxes from the global model, and two were selected based on proximity in performance (within ~2 Δ AICc) to the top model (Table 3). All variables, with exception of distance to water, were significant predictors of the number of bats within roost boxes. The global model performed best, followed closely by a model that contained all global predictors with the exception of distance to water (Table 4). These two models accounted for 98% of the weight among all models generated (Table 3). The strongest predictor of bat abundance was ΔMin, the daily difference between minimal box temperature and minimal external temperature.

**Table 3 –** Model selection for models predicting *Myotis lucifugus* abundance within roost boxes in Ontario, Canada, from May-September 2021. Models sorted in order of ascending Akaike's Information Criterion with a correction for small sample sizes (AICc; an estimator of model quality based on prediction error. BatsPre = # of bats at previous sample interval, ΔMax = daily difference between maximum box temperature and maximum external temperature, ΔMin = daily difference between minimal box temperature and minimal external temperature, $\bar{H}$ = average daily humidity, W= distance to water. df = degrees of freedom, logLik = log likelihood, Δ= change in AICc from top model, weight = Akaike weights for the model.

| Model | df | logLik | AICc | Δ | Weight |
|-------|-----|--------|------|-----|--------|
| ~ BatsPre + ΔMax + ΔMin + $\bar{H}$ + W | 8 | -1420.8 | 2857.831 | 0 | 0.530 |
| ~ BatsPre + ΔMax + ΔMin + $\bar{H}$ | 7 | -1421.98 | 2858.142 | 0.311 | 0.454 |
| ~ BatsPre + ΔMax + $\bar{H}$ | 6 | -1430.05 | 2872.23 | 14.399 | 0.000 |
| ~ BatsPre + ΔMax + ΔMin | 6 | -1430.26 | 2872.649 | 14.818 | 0.000 |
| ~ BatsPre + ΔMax | 5 | -1436.95 | 2883.997 | 26.166 | 0.000 |
| ~ BatsPre + ΔMin | 5 | -1442.08 | 2894.251 | 36.420 | 0.000 |
| ~ BatsPre | 4 | -1449.2 | 2906.469 | 48.638 | 0.000 |
| ~ ΔMin | 4 | -1670.12 | 3348.307 | 490.477 | 0.000 |
| ~ ΔMax | 4 | -1671.02 | 3350.11 | 492.280 | 0.000 |
| ~ $\bar{H}$ | 4 | -1678.95 | 3365.959 | 508.129 | 0.000 |
| ~ DistW | 4 | -1679.65 | 3367.364 | 509.534 | 0.000 |
| ~ | 3 | -1680.7 | 3367.438 | 509.608 | 0.000 |

The number of bats is predicted to increase by 0.087 with every 1 °C difference between minimum daily temperature within the box and minimum daily temperature outside of it. This was followed in coefficient size by the number of bats at previous sample interval, and ΔMax, the daily difference between maximum box temperature and maximum external temperature. Average daily ambient humidity had a significant, although weak, positive relationship with bat abundance in all top models, while distance to water was not significant although appearing in the top model (Table 4).

**Table 4** – Estimated effects of predictors included in the two most supported models predicting the number of *Myotis lucifugus* within a roost box. Significance and p-values (in parentheses). BatsPre = # of bats at previous sample interval, ΔMax = daily difference between maximum box temperature and maximum external temperature, ΔMin = daily difference between minimal box temperature and minimal external temperature, $\bar{H}$ = average daily humidity, W= distance to water. Cond = the conditional model intercept, Zi = the zero-inflated intercept

| Model | Cond | Zi | BatsPre | ΔMax | ΔMin | $\bar{H}$ | W |
|---|---|---|---|---|---|---|---|
| ~ BatsPre + ΔMax + ΔMin + $\bar{H}$ + W + (1 \| Box) | -0.053 (0.930) | -0.996*** (<0.001) | 0.056*** (<0.001) | 0.037*** (<0.001) | 0.087*** (<0.001) | 0.010*** (<0.001) | -0.003 (0.106) |
| ~ BatsPre + ΔMax + ΔMin + $\bar{H}$ + (1 \| Box") | -0.669 (0.200) | -0.996*** (<0.001) | 0.056*** (<0.001) | 0.037*** (<0.001) | 0.087*** (<0.001) | 0.010*** (<0.001) | — |

## **Discussion**

Our results support the hypothesis that social thermoregulation is a key driver of bat aggregation in summer roosts (H1), as the number of bats within the box on the previous day was a significant predictor of bat abundance. The second hypothesis (H2) which postulates that environmental variables, such as temperature, drive summer aggregations is also supported. Environmental variables had a significant influence on bat abundance within roost boxes. This brings us to the third hypothesis, H3, that higher minimum box temperatures facilitate greater thermoregulatory benefits relative to maximum box temperatures at higher latitudes. As my prediction that bat numbers would increase in roost boxes most strongly with increasing minimum temperatures (as opposed to any trend with maximum temperatures) was observed, this hypothesis is supported.

These results add to a body of evidence pointing to the importance of minimum roost temperatures during roost selection by maternal temperate bats. Bats use behavioural thermoregulation, including roost selection, to keep within an optimal temperature range (i.e. a "Goldilocks" zone) to buffer ambient conditions for energetic benefits. For maternal temperate bats, roosting in a microclimate that maintains higher minimum daily temperatures is likely just as, or perhaps more important, than a microclimate which has lower daily maximum temperatures. In maternal *Myotis lucifugus*, ambient temperatures < 20 °C during gestation, and < 22 °C during lactation have been shown to prompt the use of torpor 50–70% of the time – in turn slowing juvenile development (Henry 2001). There may be selective pressure for maternal temperate bats to locate roosts which have minimum daily temperatures above this range, or that minimize the need to enter torpor for this reason. In Quebec, Fontaine et al. (2021) measured energy expenditure of female little brown bats and found it to be higher at cooler box roosts relative to warmer roosts, and were able to reduce this energy expenditure ($\bar{x}$ =8%) by altering box design to be 4.5 °C warmer at night and in early morning – demonstrating further support for the importance of minimum temperatures in this case. This is echoed in work with other temperate bats such as long-eared myotis (*M. evotis*), showing they prefer roosts with higher minimum temperatures and lower maximum temperatures compared to the external environment (Chruszcz and Barclay 2002). Similarly, mean cavity temperature during the night (2200-0400), and minimum daily temperature were crucial predictors for roost occupancy by noctule bats (*Nyctalus noctula*) and Leisler's (*Nyctalus leisleri*) bats in the temperate latitudes of Bialowieza Forest, Poland (52°43'N, 23°54'E), with roosts having higher mean, minimum, and maximum temperatures than unused cavities (Ruczyński 2006).

Artificial roosting habitats such as bat boxes can support recovering populations of endangered bats in areas where natural roosts may be limited or may not have optimal thermal profiles. However, bat boxes are not a one-stop solution and need to be carefully designed and matched to their surrounding climate, environment, and life history of the species they aim to provide habitat for – with consideration to the future (Griffiths et al. 2017; Tillman et al. 2021; Crawford and O'Keefe 2021; Pschonny et al. 2022). Our study in a temperate environment showed that bat abundance was greater in roost boxes with higher minimum temperatures, likely due to the energetic befits these warmer boxes provide. However, bats using bat boxes in warmer regions face potential overheating, particularly as anthropogenic climate change raises global temperatures and induces a greater frequency of regional heat waves (Crawford and O'Keefe 2021). Bats may face mortality from over-heating if temperatures within roosts reach or exceed a critical maximum for a sufficient period of time, and if bats are unable to escape or otherwise mitigate the effect of ambient temperatures. Bat box temperatures are determined by several factors, including box design, box location, solar exposure, surrounding substrate albedo, geographic area, and bat density, and boxes can be designed to minimize risk of over-heating (Crawford and O'Keefe 2021; Fontaine et al. 2021). If temperatures do approach a critical maximum, adults or volant young may be able to escape (Brittingham and Williams, 2000) but non-volant young may be at risk if they are too large to be moved by maternal roost-mates. The risk of overheating may be lower at higher latitudes, though more work will need to be done to confirm this, and we acknowledge that increasing temperatures with climate change may alter the risk of overheating for temperate bat populations. Further research with long-term temperature surveillance and bat monitoring would be better able to capture individual roost-switching among artificial and natural roosts, aiding further exploration of the behavioural and environmental drivers of roost selection within roost networks.

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## 4. General Discussion

Understanding the characteristics that define habitat of forest roosting bats at multiple scales is vital to their conservation and management. We still lack fundamental information on what roosts many forest-dwelling bats choose, why they choose the roosts that they do, and what characteristics influence their choice at multiple scales. Due to plasticity in roosting characteristics across the range of some species such as *Myotis lucifugus*, work will need to be done to separate regional differences from general trends. The central goal of my research was to identify these characteristics in forest roosting *Myotis lucifugus*, in order to predict patterns of habitat use in this and other regions of their range, as well as determine what underlying social and environmental variables influence roost choice and abundance of bats within roosts.

In Chapter Two, I examined characteristics of 37 forest roost trees located by radio-tracking 92 individuals captured at bat boxes, and found that bats roosted predominately in poplar species in intermediates stages of decay. Mean roost tree DBH was found to be 40.5 cm and roost tree height averaged 18.7 m – similar to results for this species in other regions. A key limitation of this analysis was that I was unable to examine if, and how, located roost trees differed from available trees within the same stand, a limitation due largely to lack of time and issues surrounding site accessibility. In the future, examining used vs. available trees would be a valuable direction for additional research. Nevertheless, valuable comparisons can still be made between measured characteristics for the roost trees I located, and reported characteristics in other regions. I also analyzed the effect of numerous forest variables on roost presence at the stand scale, and found that tree height within the stand (represented by the 90th percentile of lidar height returns and by overstory height) was significant factor positively predicting bat roost presence. Canopy closure

in the veteran layer was of secondary positive importance, with stand age being a weaker negative predictor.

Overall, results suggest that while decay is influential at the focal tree level, it is less-so at the stand scale, with tree height being a more direct indicator of stand suitability – the importance of tree height also being evidenced by previous results in the literature (Lacki and Baker 2003; Kalcounis-Rüppell et al. 2005). Older stands appear less likely to contain roosts, and, interpreted in conjunction with the prevalence of poplar roosts at the tree scale and importance of tree height at the stand scale, may indicate a preference for stands in which early successional dominants (such as poplar species) are at their greatest maturity. However, more work will need to be conducted to confirm this suspicion, and if true, to fully reveal why this preference exists – i.e. what specific benefits do *Populus* species provide to roosting bats, and at what age / forest successional stage are these benefits most pronounced? Previous research provides hints that these benefits may be related to forest structure and its influence on thermal energetics (Michaelsen, 2016), but this would need to be confirmed in our study area. That habitat selection in forest roosting bats is thought to occur at multiple scales makes it difficult to disentangle these interactions. A framework that simultaneously examines used versus available habitat at both the scale of roost trees and surrounding forest stands will likely be necessary, and measurement of additional variables (elevation, tree temperature, solar exposure) and their interaction effects in conjunction with forest variables will be valuable to this end.

In Chapter Three I evaluated the effects of social thermoregulation and environmental variables such as temperature and humidity on abundance of bats within constructed bat boxes. By recording box variables in conjunction with bat abundance I determined that temperature,

specifically higher minimum temperature inside the box relative to the external environment, was the strongest positive predictor of bat box abundance – stronger than the positive effect of bat abundance at the previous sampling period (Table 4). This would suggest passive environmental variables are more influential on bat abundance than social thermoregulation in this case, although caution should be taken in this interpretation as I was unable to fully disentangle the relationship between minimum daily temperature and bat presence, as bats' body heat may actually raise box temperature when enough bats roost together in a box.  In contrast to minimum temperatures, the difference in maximum temperature within the box versus outside was influential, but less important.  Humidity was also measured, though had the weakest relationship (+) of all the significant predictors. These results suggest that bats at this latitude may face stronger energetic limitations at the minimum rather than the maximum end of the daily temperature cycle.  This limitation is most pronounced in maternal bats, as they face a fitness trade-off between the development rate of their young and the alleviation of metabolic costs through torpor. This is an interesting result in the context of anthropogenic climate change, which may alter this dynamic – in a warmer climate maximum temperature may become a more extreme metabolic stress, threatening colonies in artificial roosts without suitable mitigation. However, my results indicate that is currently not the case, at least in the confines of this study area. The ecological importance of higher minimum temperatures on roost choice is tentatively supported by Chapter Two, which suggests bats may be selecting trees and stands which foster these thermal properties i.e. dead trees, which escape the canopy and have high solar exposure.

One key recommendation arising from my work is that identification, creation, and management of habitat for forest roosting bats should consider multiple scales, with an eye towards the long-term changes that will occur; both in terms of forest successional processes and

anthropogenic climate change. Artificial roosting habitat should be carefully designed with these considerations in mind, as the most suitable roosting environment will depend on climate, environment, and species-specific differences – and will fluctuate over time. Similarly, managing forest stands in such a way that they maintain high numbers of suitable roost trees will require not only knowledge of what characteristics bats are selecting at multiple scales, but also what conditions lead to the creation of these characteristics with an understanding of the timeline by which suitable roost trees may decay and reform with cycles of disturbance. In this respect, additional research is needed to fully reveal how habitat selection occurs in forest dwelling bats such as *Myotis lucifugus*, and this work should be conducted in other areas of these species' ranges if the forest communities they inhabit across their ranges differ greatly.

## Literature Cited

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Michaelsen, T.C. 2016. Aspen Populus tremula is a key habitat for tree-dwelling bats in boreonemoral and south boreal woodlands in Norway. Scandinavian Journal of Forest Research, 31(5): 477-483.

# Appendix
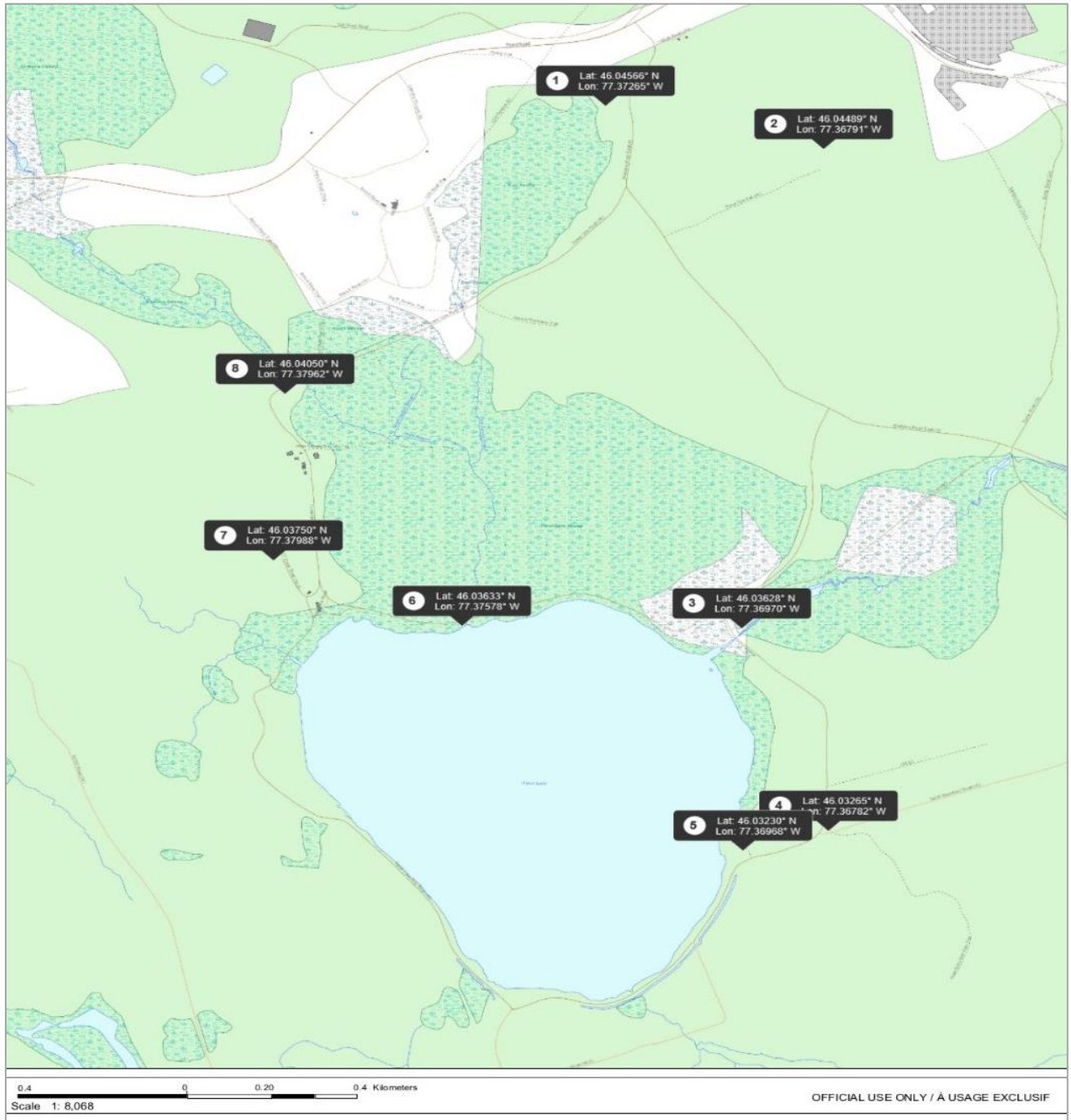


**Appendix 1.** Bat box locations in Ontario, Canada. Each site has 2 boxes mounted on a shared pole. Sites were originally established by Canadian Nuclear Labs in 2017

**Appendix 2 –** Estimated effects of predictors in all bat roost presence models. Significance denoted by * (*** =< 0.001; **=< 0.01; *=<0.05). VSpHT = the 90th percentile of height returns from a stand, OHT = overstory height, AGE = stand age, qDBH = quadratic mean diameter at breast height of all trees in a stand, PCC = primary layer crown closure, VCC = veteran layer crown closure, %Pop = estimated percent of the stand dominated by poplar species, TPH = trees per hectare.

| Model | Cond | VSpHT | OHT | AGE | %Pop | VCC | PCC | qDBH | TPH |
|---|---|---|---|---|---|---|---|---|---|
| ~ VSpHT + AGE + VCC | -5.830*** | 0.194*** | — | -0.030* | — | 0.066* | — | — | — |
| ~ OHT + AGE + VCC | -6.863*** | — | 0.194*** | -0.026* | — | 0.068* | — | — | — |
| ~ VSpHT + VCC | -6.645*** | 0.152*** | — | — | — | 0.050* | — | — | — |
| ~ OHT + VCC | -7.457*** | — | 0.161** | — | — | 0.030* | — | — | — |
| ~ VSpHT + AGE + %Pop | -5.092*** | 0.168** | — | -0.015 | 0.008 | — | — | — | — |
| ~ OHT + AGE + %Pop | -5.876*** | — | 0.160** | -0.010 | 0.010 | — | — | — | — |
| ~ VSpHT + AGE + PCC | -5.667*** | 0.206*** | — | -0.021 | — | — | 0.005 | — | — |
| ~ OHT + AGE + PCC | -6.500*** | — | 0.201*** | -0.015 | — | — | 0.003 | — | — |
| ~ qDBH + %Pop + VCC | -4.469*** | — | — | — | 0.015* | 0.034 | — | 0.057 | — |
| ~ AGE + %Pop +VCC | -3.394*** | — | — | -0.004 | 0.015* | 0.039 | — | — | — |
| ~ %Pop + TPH + VCC | -3.427*** | — | — | — | 0.016** | 0.036 | — | — | -0.000 |
| ~ qDBH + %Pop + PCC | -4.589** | — | — | — | 0.018** | — | 0.005 | 0.077 | — |
| ~ AGE + qDBH + %Pop | -3.881*** | — | — | -0.006 | 0.018*** | — | — | 0.087 | — |
| ~ AGE + %Pop + TPH | -2.805*** | — | — | 0.001 | 0.019*** | — | — | — | -0.000 |
| ~ AGE + %Pop + PCC | -3.188** | — | — | 0.001 | 0.019*** | — | 0.002 | — | — |
| ~ | -2.249*** | — | — | — | — | — | — | — | — |