Differences and similarities in exploration and risk-taking behaviours

of two *Myotis* bat species.

A Thesis Submitted to the Committee of Graduate Studies in Partial Fulfillment of the

Requirements for the Degree of Master of Science in the Faculty of Arts and

Science

TRENT UNIVERSITY

Peterborough, Ontario, Canada

Environmental and Life Sciences M.Sc. Graduate Program

September 2022

Abstract

Differences and similarities in exploration and risk-taking behaviours

of two *Myotis* bat species.

Laura Michele Scott

Behaviours that are repeatable across circumstances and time determine an individual's personality. Personality and behavioural variation are subject to selective pressures, including risks related to the use of different habitat types. I explored the ecological and evolutionary consequences of habitat selection by comparing the behaviour of two sympatric bat species, *Myotis leibii* and *M. lucifugus*. These species display overlap in roosting preferences, however, *M. leibii* tend to roost in crevices on the ground, while *M. lucifugus* tend to roost in crevices or cavities that are raised off the ground. I hypothesized that the habitat selection patterns of these two species create behavioural reaction norms at the species level. I predicted that ground roosting behaviour favours bolder personality and more exploratory and active traits when compared with bats that do not ground roost. I examined inter- and intra-specific variation in behaviour using a modified, three-dimensional open-field test and quantified the frequency and duration of behaviours such as flying, landing, and crawling. Bats were continuously video-recorded over 1-hour nocturnal and diurnal trials. I used *a priori* mixed models with combinations of individual characteristics and life-history traits to select the models that best describe each species. We found that *M. leibii* (n = 15) displayed more exploratory and bolder behaviours than *M. lucifugus* while on the ground (n = 21) and higher overall activity during the trial. I also found that *M. leibii* displayed crawling behaviours and movements consistent with foraging while on the ground which is a rare behaviour in bats and only observed in a few species (*Desmodus rotundus and Mystacina tuberculate* to my knowledge). Future research should explore biomechanical adaptations associated with ground-foraging in *M. leibii*.

Acknowledgments

Table of Contents

List of Tables

List of Figures

Chapter one

General Introduction: Habitat selection and personality

Habitat selection is the specific environmental conditions that an individual chooses. Examples of environmental conditions impacting individuals are, the risk of encountering a predator (Arndt et al., 2018), sociality (Carter et al., 2020), diet (Lino et al., 2014), and geographic range (Angert et al., 2017). Habitat selection occurs in a hierarchical order and is dependent on spatial and temporal scales (Wheatley and Johnson, 2009). The examples of environmental conditions mentioned above function on different scales both within and among each trait. Four orders of magnitude are commonly used to assess hierarchical habitat selection at the individual, population, or species level: geographic (first order), home range (second order), habitat used in the home range (third order) and choice of habitat features (fourth order) (Mayor et al. 2009). A species' use of habitat can occur on a wide range of scales; however, an individual's choice of habitat is more limited falling within one or more of the four hierarchical selection orders. Conditions such as dispersal (Travis and Dytham, 2011) or foraging behaviours (Brown and Morgan, 1995; Brown, 1999) are selected by organisms on multiple scales and it is important for researchers to take scale into consideration. For example, Hernandez (2020) found that when reviewing habitat selection literature on the northern bobwhite (*Colinus virginianus*) it is documented they have preference for woody-cover. However, estimates on the amount of woody-cover used by the northern bobwhite have wide variability ranging from 1-2% (Jackson 1969), to <20% (Lehmann 1984), to >20 but <90% (Kopp et al. 1998). This wide range of observations makes managing the landscape for the northern bobwhite challenging and ambiguous (Hernandez 2020). However, when organizing these ecological studies into a scale centred framework, Hernandez (2020) found that scale explained the variation well. Observations of northern bobwhites at the organism scale, or in an

individual's immediate environment, show a widely variable selection rate for habitat with high woody-cover estimates (ranging from 15-90%). When observing individuals at the home range scale, they selected habitat with lower and less variable woody cover estimates (26-36%). Finally, observing individuals at the study site scale indicates they selected habitats with 1-15% woody cover. When introducing a scale centred framework, Hernandez (2020) found that the amount of woody-covered used by northern bobwhites is negatively correlated with spatial extent (Hernandez 2020).

Animal personality research is of growing interest; however, the ecological and genotypic drivers impacting personality are not well understood in wild animals (Duckworth, 2010; Réale et al., 2007). Life history traits such as reproduction (Réale et al., 2009) and age (Dammhahn, 2012) influence behaviour and personality. Behaviour and personality traits appear on a gradient of two extremes, with one individual displaying only a portion of the possible behaviours on that gradient (Dingemanse et al., 2010; Avital and Jablonka, 1993). There is a wide range of possible personality traits such as shyness-boldness (Kanda et al., 2012), exploration (Wilson et al, 2010), general activity (Wilson et al., 2010), aggressiveness (Réale et al., 2009), and sociability between conspecifics (Kilgour and Brigham, 2013) which are influenced by genetics and the environment. Individuals display variability in behavioural traits depending on their environment and the context of a situation. A behavioural reaction norm is the range of behaviours that an individual displays in a given context or environmental situation (hereafter, contexts) (Via et al. 1995; Dingemanse et al., 2010). Different contexts include risk of predation, time of day, and/or maternal investment (Dingemanse et al., 2009). Individuals make repeated choices in situations that increase their fitness, and this often is dependent on an individual's life experiences (Sih et al., 2004b; Sih et al., 2004). This can lead to among and between individual correlations and/or variation in the behaviours displayed by a population or species (Dingemanse et al., 2010; Sih et al., 2004b; Sih et al., 2004; Réale et al., 2007).

Predation influences the selection of behavioural traits because it risks the removal of less advantageous traits from a population (Lima and Dill, 1990). For many taxa, the trade-off between exposure to predators and resource gain shapes the decisions individual animals make and can impact adaptation (Lima and Dill, 1990). The more exploratory or bold an animal is (e.g., risk-taking behaviours) the more resources they access; however, this also increases the risk of an interaction with a predator (Lima and Dill, 1990). Group membership influences the behavioural reaction norm of an individual through increased predator detection, increased resource, and mate acquisition. Grouping with conspecifics versus being solitary influences the reactive decisions that an individual makes, can increase the opportunity for predator detection, and decrease predator attack rates (Lima and Dill, 1990). When observing grouped animals versus non-grouped animals in the wild, grouped individuals spend less time anticipating or scouting for predators and more time foraging. Along with this, the odds of that predator attacking a larger group is lower, this could be due to faster detection or because a larger group or prey is more intimidating (Boland, 2003; Lima and Dill, 1990). Species evolve social group living when it is more beneficial than solitary living, and most social species have solitary periods of life (Lodé et al. 2021).

In this study I compare behaviour and personality of two North American *Myotis* bat species. The objectives of this study are to explore and assess exploratory and risk-taking behaviours of *Myotis leibii* and *Myotis lucifugus* in a novel environment and when faced with a potential predator, respectively. Here I explore the possible consequences of roost location on behaviours and personality of two bat species. The two species tend to select different roost locations for daytime roosting (*M. leibii* on the ground and *M. lucifugus* in high structures off the ground) with some overlap in roost preference. Choosing different roost locations exposes individuals to a different range of predators. I observe individual behaviours of two bat species in two contexts: a novel environment and when interacting with a potential predator. Anecdotal observations of *M. leibii* in the wild interacting with a novel object

and a potential predator suggest this species display more risk taking and bold behaviours in a potentially risky situation than *M. lucifugus*. I want to explore these behavioural differences through observations of individuals in a controlled but novel environment and when interacting with a potential predator. This will give further knowledge and understanding on the complex environmental factors that influence an animal's habitat selection and resulting behavioural consistencies or inconsistencies.

Chapter two

Differences and similarities in exploration and risk-taking behaviours of two Myotis bat species.

Introduction

Individual phenotypic variation influences evolution when some individuals in a population have heritable or learned traits which correlate to higher survival and reproductive rates than other individuals (Forbes and Krimmel 2010; Garnier et al., 2016). Variation occurs in many observables, heritable traits in animals (e.g., behaviour, morphology) and allows for evolutionary adaptation in a heterogenous environment (Kilgour and Brigham, 2013; Lynch and Walsh, 1998; Réale et al., 2000). However, there is a limit on the flexibility displayed in the behaviour of both individuals and groups (Mathot et al., 2012). Behavioural plasticity is when an individual displays behavioural flexibility in different environmental contexts which can lead to behavioural adaptation (Mery and Burns, 2010; Boyer, 2018). When an individual's behaviour repeatedly differs when compared to other individuals in a population, and are consistent among environmental contexts (i.e., foraging, competition, dispersal, etc.), these behaviours are considered a personality trait of that individual (Dingemanse et al., 2010; Sih et al., 2004; Réale et al., 2010; Reale et al., 2007; Dall and Mcnamara, 2004). Having two or more behavioural traits correlate with one another across one or more environmental contexts form a behavioural syndrome (Dingemanse et al.,2012; Dochtermann and Dingemanse, 2013; Sih et al. 2004a, b; Réale et al. 2007). Together, personality (repeatability of behaviours), behavioural syndromes (correlations of behaviours within and between contexts), and plasticity (variation of behaviours within and between contexts) make up the behavioural reaction norm of an individual (Dingemanse et al., 2010). In ecology a reaction norm is the different gradient of phenotypes one genotype produces in the face of variable environmental contexts (Dingemanse et al., 2010). A behavioural reaction norm is the gradient of behavioural phenotypes expressed by one individual across a variation of environmental

contexts (Dingemanse et al., 2009; Dingemanse et al., 2012). Previous research has focused on

behavioural traits such as exploration and aggression. For example, several studies examine exploratory

behaviour in great tits (*Parus major*). Verbeek et al. (1993) characterized exploratory behaviours in a

novel environment and when interacting with a novel object in a familiar environment as a personality

trait for juvenile male great tits. These behaviours corresponded to stronger feeding habits where

individuals that are faster at interacting with a novel environment were also faster and more consistent

at arriving at the feeding station (Verbeek, 1998; Verbeek et al., 1993). Subsequent studies further this

research by observing aggressive behaviours in great tits, and correlating exploration with aggression.

Researcher found that more exploratory individuals in novel contexts are also more aggressive when

interacting with conspecifics (Verbeek, 1996; Marchetti and Drent, 2000). Most recent studies on great

tits determine the importance of life-history traits (i.e., sex, age, etc.) on exploratory personality traits

(Dingemanse et al. 2002).

An individual's genotype and its immediate environment are two factors that affect the

phenotype. It is interesting to consider how common interactions with environmental factors influence

the evolutionary trajectory of a species (Réale and Dingemanse, 2010; Réale et al., 2007). Environmental

interactions between animals and their prey influence the immediate survivorship of one individual,

many individuals in a population, and/or the behavioural adaptation of an entire species (Reale et al

2007). In wild animal populations, the hunting strategy of a predator is often reflected in the risk-taking

behaviours of its prey (Heinen and Hammond, 1997; Endler, 1991; Gans and Parsons, 1966; Licht, 1986).

For example, many animals group together due to the positive influence it has on survival and

reproduction (fitness) which outweigh the costs (Grand and Dill, 1998). Three strategies that grouped

prey benefit from are the 'many eyes effect' while watching for predators (vigilance) (Rieucau and

Martin, 2008; Couchoux and Cresswell, 2011), the predator 'confusion effect' while being attacked

(Olson et al., 2013; Krakauer, 1995; Brighton et al., 2021), and the 'dilution effect', which describes the

increased odds that an alternative group member will be targeted by a predator as group size increases (Lima and Dill, 1990; Dittmann and Schausberger, 2017). Most animals live in a multi-predator and multi-contextual environment and therefore evolve a gradient of responses to various predator's dependent on their perceived odds of encounter risk. A group of ~20 vervet monkeys (*Cercopithecus aethiops*) react differently to aerial versus semi-terrestrial/arboreal predators (e.g., Crowned Eagle (*Stephanoaetus coronatus*) and Verreaux's Eagle (*Aquila verreauxii)),* versus leopard (*Panthera pardus)* and chacma baboon (*Papio cynocephalus ursinu*). The monkeys apparently perceive these predatory risks differently and adapt their behaviours accordingly. Monkeys avoided the distribution of the terrestrial/arboreal predators such as the leopard and baboon, thereby negatively impacting their own range (vertical distribution/ use of space). However, in reaction to eagles, which monkeys were highly vigilant for, individuals perceived the risk quicker and found refuge in tree cover. Individuals altered their behaviour to use a horizontal distribution and these predators did not impact range size (Williems and Hill, 2009). Grouping behaviour and vigilance appear to mediate the predation risk of eagles however monkeys must alter their habitat use to avoid or survive interactions with leopards and baboons (Williems and Hill, 2009).

Behavioural interactions between bats and their predators are not well represented in the literature due to the challenge of observing bats in the wild. Bats anti-predator or risk-taking behaviours are not well understood but, if similar to other taxa they generally should select habitat which is the most beneficial to their survival and reproductive ability. It is not common for a predator to specialize on bats (with a few exceptions), however Lima and O'Keefe (2013) suggest that a wide range of generalist predators opportunistically feed on bats. They suggest that higher predation rates might occur at roost locations with larger groups, specifically during the confusion of emergence at dusk. Individual bats in these large roosts could be benefitting from the predator dilution effect in which a higher number of individuals emerging reduces the odds of any one individual being captured (Lima and O'Keefe 2013).

Predation in or around the roost is the most commonly observed however understanding of how bats react to an attack at the roost is limited. Bats do not appear to react to scent cues from potential predators (Boyles and Storm, 2007) but seem to select roost locations perceived as safer such as with smaller roost entrances (Lima and O'Keefe, 2013). Big brown bats (Eptesicus fuscus) have been observed selecting roosts higher off the ground, with tighter and more vertical roost entrances than what was randomly available in the environment (Hamilton and Barclay, 1994) however *M. leibii* have been observed daytime roosting directly on the ground, raising young (Johnson, 2011; Bruce et al, unpublished data 2017). This suggests there could be a trade-off between potential predation and the microclimate benefits of some roosts (Lima and O'Keefe 2013). Risk-taking and escape behaviours have been observed even less than interactions with predators, but the strongest responses occur when a bat is reacting to the distress call of another individual. Bats will respond by mobbing and flying at or around the perceived predator handling the bat in distress (Lima and O'Keefe 2013). the 'dilution effect', which describes the increased odds that an alternative group member will be targeted by a predator as group size increases (Lima and Dill, 1990).

There are over 1300 species of bat globally and only a few species display ground behaviours. For example, the common vampire bat (*Desmodus rotundus*), the New Zealand short-tailed bat *(Mystacina tuberculata*) are two such species known for using the ground to forage and access food (Riskin et al., 2006). In North America, two species that have been observed actively using the ground are *Myotis leibii* and *Myotis evotis. Myotis leibii* is found along the Appalachian Mountain region in eastern North America ranging from Quebec, Canada, to Georgia, USA. *Myotis evotis* is found in western North America from Alberta, Canada to California, USA. Both species have a wide gradient of roosting microhabitat ability in that they are observed raising young in ground maternity roosts, which is not commonly observed in bats, along with more typical high structure maternity roosts (barns, house attics, bat boxes) (Bruce et al., unpublished data, 2017; Solick and Barclay, 2006). To investigate

behavioural difference between bats which tend to use the ground versus bats which tend to avoid

using the ground, we compare *M. leibii* to *M. lucifugus*. *Myotis lucifugus* is a geographically wide-ranging

species which spans all of North America from Alaska to Mexico. *Myotis lucifugus* is less often found on

the ground and to our knowledge, no maternity roost has been observed on the ground. In our study we

assume that predation risk on the ground is at minimum different between *M. leibii* and *M. lucifugus*.

Here, we evaluate the consequences of roost location on the behaviour of two sympatric,

Nearctic species of bat. We use behavioural trials to investigate how ecological differences in roost

preference manifest in their behaviours and we observed the range of behaviours that a species shows,

on average, across different contexts. *Myotis leibii* and *M. lucifugus* are two species that display overlap

in roosting preferences and the size of their maternity colonies - both can form large and small groups,

and both can roost in raised cavities (trees or buildings) as well as under rocks or in crevices. However,

M. *leibii* maternity colonies tend to be smaller and are more often found directly on the ground

(Moosman et al., 2012; Humphrey, 2017; Moosman et al., 2013; Johnson et al., 2011; Bruce et al.,

Unpublished Data, 2017). *Myotis lucifugus* individuals have been found on or near ground roost

locations (Riskin and Pybus, 1998), to our knowledge it is not documented as a maternity roost trait

preference for the species and therefore not passed onto subsequent generations through learning or

heritability. *Myotis lucifugus* colonies tend to be larger and found in raised cavities (Tuttle, 1973;

Environment Canada, 2015; Solari, 2021) which are two metres or more above the ground 98.5% of the

time (Riskin and Pybus, 1998). Maternity roost location and/or site roosts are reported to have high

fidelity for earlier female young big brown bats returning as potential mothers in future years (Lewis,

1995; Lausen and Barclay, 2002). Along with this, there have been differences in the observed

behaviours and habitat use by male and female bats due to the high energy requirements for gestation

and lactation. Female bats select roosts which are thermally beneficial to them during pregnancy and

lactation to aid with the high energy demands, however little is known about these differences in roost

choice affect an individual's response to a predator (Lintot et al., 2014). For example, non-gestating female and male *Myotis leibii* bats are observed roosting in torpor (a daily low metabolic state similar to hibernation) deep within rock crevices or fissures on cliff-faces or talus slopes (Moosman et al., 2016; Bruce et al., unpublished data 2017). However, during the maternity season, pregnant and lactating females will choose roosts more open to solar exposure which puts them at more risk of interacting with a predator (Johnson, 2011; Bruce et al., unpublished data 2017). Along with this, differences in behaviour between sexes of bats are seen when under duress and interacting with a potential predator. bats call more than females when under duress. González-Palomare et al. (2021) found that male Seba's short-tailed bat (*Carollia perspicillata)* in distress made more echolocation calls more often, louder, and harsher than females. Researchers speculate this could be attributed to hormonal differences such as higher levels of testosterone that leads to higher levels of aggression. There is vast literature indicating that in many taxa, females are less aggressive than males often attributed to hormonal differences (González-Palomare et al. 2021).

Here we compare the boldness trait through observation and examination of exploratory and risk-taking behaviours of individual bats both within- and between-species. We determine if these differences in habitat selection are represented in individuals and in different sexes within a species by observing differences in behaviour and personality. We hypothesize that the roost location selection and life history patterns of the two *Myotis* species create species-specific behavioural reaction norms. To elaborate, we think that *M. leibii*, which is rarer (Best and Jenning, 1997) and tends to roost in small or solitary maternity colonies on the ground, experience different environmental factors during diurnal roosting due to their tendency for ground roosting. In comparison *M. lucifugus*, which is phrenologically related and geographically overlapping, tends to display more high structure roosting in larger groups, and interacts less with the terrestrial environment. We speculate that these differences in habitat selection leave *M. leibii* at a higher risk of exposure to certain terrestrial diurnal predators than *M.*

lucifugus. Due to these differences in diurnal roost selection and the potentially different array of predators to interact with, we predict that *M. leibii* will show higher levels of exploratory behaviours in the context of a novel environment and higher levels of risk-taking behaviours in the context of a predator stimulus. In contrast, we predict that *M. lucifugus* would display lower exploratory and risk-taking behaviours when faced with a potential predator. *M. lucifugus* benefit from raised roost location, larger group sizes, and the associated grouping strategies. When comparing individuals within a species we hypothesize that male bats will be bolder than female bats due to higher levels of testosterone and therefore aggression. We predict that males will display more exploratory and risk-taking behaviours during behavioural trials than females.

Research has indicated that in mammals, personality and behavioural research has focused on inter-specific behavioural interactions within individuals and between individuals (Dammhahn, 2012; Michelena et al., 2009; Maiti et al., 2019; Menzies et al,. 2013) with less focus on comparing individuals among populations (Dingemanse et al., 2012) and among species (White et al., 2020; Réale et al., 2007). Our research focuses on behavioural differences between species the main purpose of our research is to observe and analyse species differences in bat exploration and risk-taking behaviour. Bat personality has been observed in individual *M. lucifugus* when exploring a novel environment however comparisons of species differences in behaviour are currently lacking. We aim to fill this knowledge gap by observing exploration and risk-taking behaviours of individual *M. leibii* and *M. lucifugus* and use this information to analyse species differences. Our results indicate that there are species differences in exploration and risk-taking behaviours between *M. leibii* and *M. lucifugus* however there are no sex differences when comparing the behaviours of individuals within a species.

Materials and Methods

Animal capture and care

We studied bats and collected data under proper Government of Ontario wildlife handling and

Trent University Animal Care Committee permits (permit numbers available upon request). We captured

M. lucifugus and *M. leibii* from 12 August – 28 September 2018 during bat swarming at five hibernacula

in Ontario, Canada. Bats were captured using a Tuttle-style harp trap (Austbat trap, Bat Conservation

and Management, Carlisle, PA, USA) set up at hibernacula entrances (Tuttle, 1974). We opened the trap

~2100h and checked the bag every 15 minutes. We identified each captured bat to species, and

recorded sex, age (young of the year vs. adult) and banded with a lipped aluminum bat band (Porzana

Limited, Norfolk, United Kingdom). We measured each forearm three times to the nearest 0.01 mm

using digital callipers (ULINE, Milton, Ontario, Canada) and weighed bats to the nearest 0.01g using a

digital scale (Smart Weigh, Nanuet, New York, USA). While we were able to confirm that some

swarming individuals were adults and others were young of the year by checking the level of ossification

in the metacarpal-phalangeal joints of the wings (Kunz and Anthony, 1982), the joints of juveniles have

often ossified by the swarming season. Therefore, we did not differentiate between adults and young of

year in our analyses. Each night, we kept the first 1-4 bats captured and placed individuals into a clean

cloth bat bag that we hung inside a ventilated, soft-sided picnic cooler to reduce noise and temperature

fluctuations between trials and during transportation (Webber and Willis, 2018). We kept individuals for

24 hours from capture time and offered them water from a syringe and live mealworms before release.

Bats were released directly at the initial capture location.

Behavioural experiments

We conducted all behavioural trials in a large, modified open-field test inside of a 4.27X3.05

metre pop-up camping tent (Ozark Trail, Dark Rest Instant Cabin Tent, Potosi, Missouri, USA). The tent

acted as an arena that provided sufficient space for aerial and gross motor behaviours, such as flight and novel roost interaction. To standardize light levels among trials, we used tape to black out the interior of the arena by sealing the windows and the trim. To investigate interspecific differences in microclimate roost selection, we installed eight modified, pine bat-boxes that were open at both ends (hereinafter 'roosts'). Each roost contained one chamber with a crevice sized either 1.3 cm small roosts(n=4), or 3 cm large roosts (n=4) following suggested measurements for the species in question (Tuttle et al., 2013). Four roosts, two of each size, were heated between 33-35°C using a 20.32X30.48 16-Watt Heat Pad reptile heating pad and thermostat (Zoo Med Laboratories, San Luis Obispo, California, USA and Inkbird Display Thermostat, Shenzhen, China). This made two sets of four different roost types which were interchangeable for randomization between trials. We mounted one set vertically on 1.5-metre cedar posts, and the remaining set laid flat on the ground at the base of the posts (Figure 1). This mimicked a variety of natural roost microclimate characteristics applicable to both species (Webber and Willis, 2018). Each roost had a hinged door for ease of access, and we lined the interior walls of the roosts with Ethylene-vinyl acetate (EVA) foam to provide insulation between the heat pad and bats. To avoid potential effects of scent between trials, we designed the roosts for ease of cleaning by attaching clear plastic shelf liners (Duck Brand Classic Easy Liner Clear, Avon, Ohio, USA) to the back wall of each roost with Velcro. We cleaned the interior arena walls and the interior and exterior of the roosts between trials with Lysol disinfecting wipes.
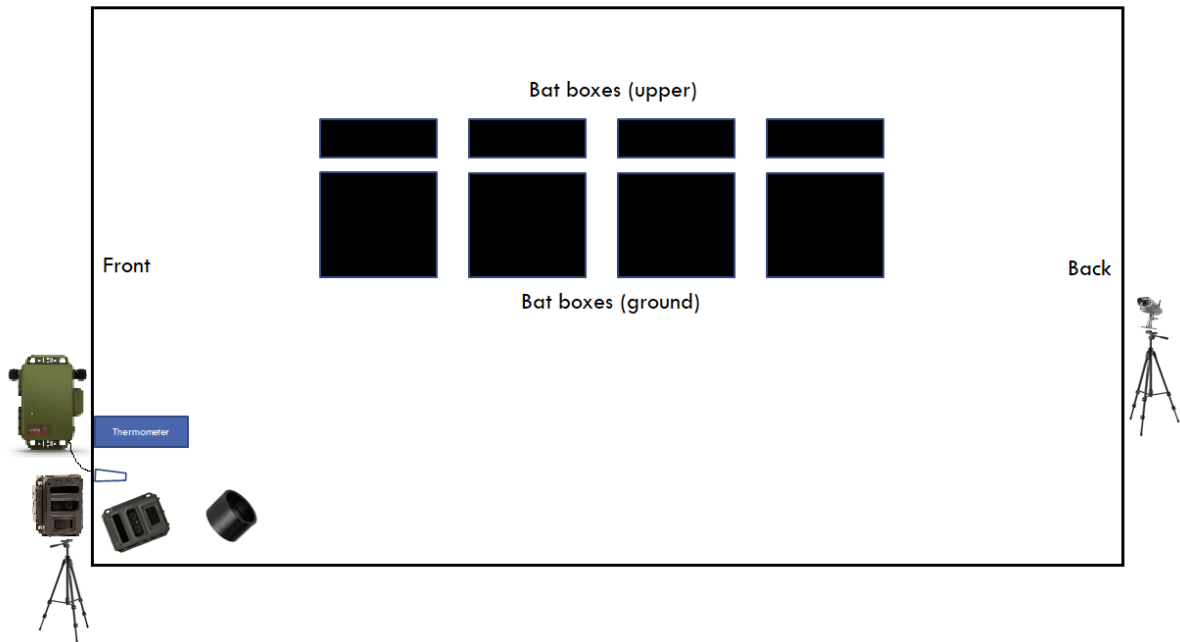
Figure 1: Schematic diagram of the layout inside the 4.27X3.05 metre pop-up camping tent (Ozark Trail, Dark Rest Instant Cabin Tent, Potosi, Missouri, USA) which acted as a testing arena.

The arena had three cameras: two Reconyx Ultrafire cameras (Holmen, Wisconsin, United States) and one SVAT GX301-C Surveillance Camera (Niagara Falls, Ontario, Canada). The two Reconyx cameras were located at the front: one on the ground and one on a tripod, each aimed towards the upper and lower roosts. The security camera was located at the back of the arena on a tripod and was aimed inwards to get a wide-angle view of the entire arena. Equipment was powered by a power inverter (Black and Decker, Towson Maryland, United States) that was connected to a 12-volt deep cycle marine battery (Marine Master, Saskatoon, Saskatchewan, Canada) outside of the arena. The trials were recorded by the cameras for subsequent viewing and scoring of behaviours.

Bats experienced three behavioural trials consisting of three contexts: two novel environment tests held in a night and day context (night trial and day trial), and one predator stimulus test (Réale et al., 2007). Bats began both night and day trials inside a cloth bat bag placed on the ground in front of a Reconyx camera and exited the bag of their own volition. Some bats exited the bag before we could

place it on the ground and the start time for trials is therefore defined as either the second the cloth bat

bag was placed on the ground, or the second the bat exited the bat bag, whichever happened first. Night

trials were held between 2130h - 0430h and occurred ~30 minutes - 4 hours after capture. Day trials

were conducted between 0830h - 1630h (~10 - 18 hours after capture). Day and night trials lasted one

hour each to maximize the accuracy of the sample of behaviours collected (Lendvai et al., 2015) by bats

in one trial and to be able to complete a repeated trial within the amount of time permitted to keep

bats according to our animal care protocol (< 24 hrs). The predator stimulus test became incorporated

into the end of the novel environment night trial when a person entered the arena to retrieve the bat

(hereby 'retriever'), stood still, and observed the bat's behaviours. This trial lasted up to five minutes if

individuals displayed a visible reaction such as flying or crawling in response to the retriever entering the

arena. If individuals made no gross motor behaviour in reaction to a person entering the arena, then the

trial was terminated after one minute. We scored gross motor behaviours of two behavioural traits

(exploration and boldness) in three behavioural contexts (night, day, and predator stimulus) within a

timeframe relevant to assessing responses to a novel environment (24 hours; Webber and Willis, 2018).

Scoring

We played the videos at a reduced speed, and they were scored by the same person for

consistency. The videos were watched twice and scored in detail on the second viewing with all three

video angles playing at once for a full view of the interior of the arena. We scored for primary

behaviours, the location in which those behaviours occurred, and secondary behaviours. Table 1

outlines the ethogram used to score behaviours in the novel environment tests (Table 1: A) and

predator stimulus test (Table 1: B). Raw data for all trials consisted of total cumulative time completing

each gross motor behaviour out of total trial time in seconds. Raw behaviours were collapsed into time

spent conducting five main corresponding behaviours: flying, crawling on the ground, landed on tent

walls or ground (not moving during this time), time spent in bat bag (or time to start the trial), and time spent exploring in and/or on roosts (Table 1).

Statistical analyses

Principal component analyses (PCA)

All analyses were conducted in R Studio version 1.2.1335. We used PCA as a reduction method of gross motor movement raw variables for all three trials following the methods of Huntingford (1976). We produced summary boxplots to visually examine the raw variables (Figure 2, 3). We ran 12 PCAs using the *prcomp* function in the R package 'stats' version 3.6.1 (Table 2). This reduced our larger number of behavioural variables to one or two main behavioural variables classified linearly by orthogonal factors. All data were standardized and if necessary were transformed with log or square root before being included in PCAs for normality. We explored PCA components visually and numerically using variable correlation plots and biplots comparing PC1 and PC2. These principal components represent new synthetic variables describing exploration behaviours in a night context, exploration behaviours in a day context, and risk-taking behaviours when interacting with a potential predator.

For within species comparisons, we pooled raw variables by species to characterize each behaviour. Using exploratory behavioural data from the night and day context, PC1 for *M. leibii* is characterized negatively by total time spent flying, crawling, and exploring roosts and positively characterized with time spent landed. This new variable represented a between-context exploration trait in *M. leibii* to determine the repeatability of exploratory behaviours between night and day contexts. For the PCA looking at boldness in a predator stimulus context, PC1 is characterized positively by time spent on the ground and time spent flying and negatively characterized by time spent landed. These new variables represented risk-taking or boldness behaviours in a predator stimulus context (Table 3).

For *M. lucifugus* we also ran four PCAs (Table 2) using the same method as *M. leibii*. For the PCA that included data from both the night and day trial, PC1 is characterized positively by time spent landed and negatively by time spent crawling, time spent exploring roosts, and time spent flying. This represented a between-context exploration trait to calculate repeatability and estimate personality for exploration between the night and day contexts. This exact pattern is also seen in PC1 of the PCA completed with only night trial data and the new variable is classified the same as above but for only night trials. PC1 of the PCA done for the day trials only is positively characterized by time spent flying, time crawling, and time exploring roosts and negatively characterized with time spent landed. Boldness in a predator stimulus context is represented negatively by time spent flying and positively by time to start a gross motor movement. These new synthetic variables were used in subsequent analyses representing linear combinations of raw behavioural variables (Table 3 and 4).

For the between species analyses we pooled all raw data from both species within a context (night, day, predator stimulus) then used a PCA to synthetically reduce the variables. PC1 was conducted using only night trial data and is characterized negatively by the behaviours time spent flying, crawling, and exploring roosts and positively characterized by time spent landed. PC1 is characterized by the same behavioural variables as the night context however with an inverse sign. These two new variables represent exploration in a day context and time to start exploring during the day. PC1 of the PCA conducted using boldness behaviours in a predator stimulus context are characterized positively by time spent flying and negatively by time to start the trial. PC1 will represent boldness in a predator stimulus context for further between species analyses (Table 5).

Within-species analyses

Adjusted/conditional repeatability

To determine if behaviour is repeatable in *Myotis leibii* and *M. lucifugus* between night and day contexts, we calculated adjusted repeatability using univariate linear mixed-effects models (LMMs) (Nakagawa and Schielzeth, 2010; Dingemanse and Dochtermann, 2013) for the exploration trait. We ran one LMM for each species, controlled for 'individual' in the models by including it as a random effect and included night or day context as a fixed effect. We used the *lmer* function in R package 'lme4' version 1.1-21 to ensure the random intercept and residual variance was estimated as these are required for repeatability estimates (Nakagawa and Schielzeth, 2010). Repeatability analyses were conducted using the *rpt.gausGLMM.multi* function in 'rtpR' version 0.9.22 and we ran one for each of the exploration traits for each species. Confidence intervals were calculated using parametric bootstrapping and significance is calculated using a randomization test (Nakagawa and Schielzeth, 2010).

For each species, we ran three separate linear models (LMs): one for exploration in the two contexts (night and day; two models) and one for a risk-taking behaviour in predator stimulus context. We did this to determine the effect of sex on exploration and boldness behavioural traits in the three contexts (night, day, predator stimulus). We ran the linear regressions using the *lm* function in the R package 'stats' version 3.6.1. Statistical significance is determined using calculated probability (p-value) with a significance of α< 0.05. All model assumptions were validated for homogeneity and normality of residuals, and we determined the linearity of the residuals using Q-Q plots, histograms, and by plotting the residuals and fitted values.

Behavioural syndrome

We quantified behavioural syndromes (behavioural correlations) within each species by comparing behaviours using intra-individual rank correlations. We compared the exploration trait in the night and day contexts with the boldness trait in three separate correlations per species. We did not

include individuals without a matching trial and removed them after the PCA. We used a Spearman's rank correlation for the analyses of both species due to the distribution of these data. If correlations were significant, we chose to use a false discovery rate (FDR) B-Y adjusted alpha to adjust the p-values which account for repeated testing of the same data.

Plasticity/stability

We calculated relative plasticity using the individual stability statistic (ISS) (Equation 1): $ISS_{t1\,t2} = 1 - ((z_{t1} - z_{t2})^2 /2)$ (Dingemanse et al., 2010; Asendorpf, 1990). This method uses z-score standardized variables in two different contexts ($z_{t1} - z_{t2}$), to determine relative stability and inconsistencies of inter-individual behaviour in a population when comparing values of two behavioural assays situated at different times for an individual. We compared the exploration trait in the night and day contexts with the boldness trait in three separate ISS analyses per species. We used PCA-created variables and did not include individuals in the ISS analyses who did not have a matching trial. Values close to 1.0 show high stability between the two contexts relative to the population whereas values that are close to 0 or negative are considered inconsistent or highly plastic between the two contexts (Asendorpf, 1990).

Between-species analyses.

To test if personality traits vary between *Myotis leibii* and *M. lucifugus* we ran three generalized linear models (GLM) with logistic regression of individual behaviour against species of bat. We used PC scores for exploration night, exploration day, and boldness in a predator stimulus context (see Table 3, 4, and 5 for all PCA combinations) as fixed effects in the three models to determine their influence on behavioural variation between species. We ran three logistic regressions, modelling exploration night, exploration day, and boldness separately (*glm*, Package 'stats' version 3.6.1). GLMs show a binomial distribution with a *logit-link* function, and results were considered statistically significant at α< 0.05. All models met the required assumptions.

Table 1: Ethogram used to score gross motor behaviours displayed by *Myotis leibii* and *M. lucifugus* during novel environment trials (both night and day contexts(A)) and predator stimulus test (B). These behaviours were collapsed into five main behaviours: flying, crawling on the ground, landed on tent walls or ground (not moving), time spent in bat bag (time to start the trial), and time spent exploring in and/or on roosts which were used for further analyses.

	Primary behaviours	Area	Secondary behaviours
A - Novel environment night and day trials – Exploration trait	In bat bag	In bat bag	In bat bag
	Flying	Flying	Flying
	Landed	Tent wall/ceiling	Grooming
	Landed	Tent wall/ceiling	Visible moving
	Landed	Tent wall/ceiling	Visible not moving
	Landed	Tent wall/ceiling	Not visible
	Landed	Ground edges	Crawling
	Landed	Ground edges	Not crawling
	Landed	Ground edges	Not visible
	Landed	Ground centre	Crawling
	Landed	Ground centre	Not crawling
	Landed	Ground centre	Not visible
	Landed	Ground	Crawling exit bat bag
	Landed	Ground	Crawling unknown
	Landed	Ground	Not visible
	Landed	Landed roost upper	Post
	Landed	Landed roost upper	In roost
	Landed	Landed roost upper	On roost
	Landed	Landed roost upper	Chosen
	Landed	Landed roost ground	In roost
	Landed	Landed roost ground	On roost
	Not visible	Not visible	Not visible
B - Predator stimulus test – Boldness trait	Flying	Flying	Flying
	Landed	Tent wall or ceiling	Moving unclear
	Landed	Tent wall or ceiling	Not moving
	Landed	Ground edges	Crawling
	Landed	Ground edges	Not moving
	Landed	Ground centre	Crawling

Landed	Ground centre	Not moving
Landed	On retriever	On retriever
Landed	Roost	In roost
No visible response	No visible response	No visible response

Table 2: Principal Component Analyses (PCA) combinations for the three behavioural trials (novel environment test night, novel environment test day, and predator stimulus test) conducted for within and between species analyses. Raw data from listed trials were put into a PCA as a data reduction method to find correlations between raw behavioural variables. These new PCA scores were used in subsequent analyses for within and between species analyses.

Myotis leibii		*Myotis lucifugus*		Between species	
PCA 1	Exploration night and day	PCA 1	Exploration night and day	PCA 1	Exploration night
PCA 2	Exploration night	PCA 2	Exploration night	PCA 2	Exploration day
PCA 3	Exploration day	PCA 3	Exploration day	PCA 3	Boldness - Predator stimulus
PCA 4	Boldness - Predator stimulus	PCA 4	Boldness - Predator stimulus		

Table 3: Principal component analyses (PCA) loadings used to create a principal component score which defined the 'exploration' trait for *Myotis leibii* and *M. lucifugus* along with the total percent variation explained in the dataset.

Species	*Myotis leibii*			*Myotis lucifugus*		
Context	Night/Day	Night	Day	Night/ Day	Night	Day
Sample size	22	15	6	37	21	16
Flying	0.53	0.50	-0.50	-0.395	-0.506	0.397
Time to start	-0.23	-0.30	0.23	-0.322	-0.074	0.257
Crawling	0.51	0.53	-0.55	-0.475	-0.395	0.477
Exploring roosts	0.26	0.15	-0.37	-0.484	-0.497	0.502
Landed	-0.58	-0.60	0.51	0.529	0.579	-0.545
Percent variation	0.55	0.51	0.55	0.6197	0.543	0.642

Table 4: Principal component analyses (PCA) loadings used to create a principal component score which defined the 'boldness' trait in a predator stimulus context for *Myotis leibii* and *Myotis lucifugus* separately, along with the total percent variation explained in the dataset. The raw variables 'time to start' and 'on retriever' had no variation (all were 0 seconds) within *Myotis leibii* and *Myotis lucifugus*

respectively, and therefore were not included in the corresponding PCA. Bolded values are the raw variables that characterize that principal component.

Species	*Myotis leibii* = 11		*Myotis lucifugus* = 17		Between species = 28	
Trait/context	Boldness 1 (PC1)	Boldness 2 (PC2)	Boldness 1 (PC1)	Boldness 2 (PC2)	Boldness 1 (PC1)	Boldness 2 (PC3)
Flying	0.60	0.48	-0.541	0.148	0.578	0.235
On retriever	0.32	-0.12	N/a	N/a	0.297	0.613
Time to start	N/a	N/a	0.690	0.050	-0.685	0.161
Ground	0.21	-0.86	-0.386	0.509	0.238	-0.72
Landed	-0.70	0.10	-0.286	-0.847	0.228	-0.159
Percent variation	0.48	0.29	52.36	0.261	0.406	0.20

Table 5: Principal component analyses (PCA) loadings used to create a principal component score which defined the 'exploration' trait for both species when comparing between species behaviours in a night and day context separately. Raw variables entered into the PCA were combined of both species to determine if species predicts behaviour.

Trial type	Night = 37		Day = 24	
Behaviours in each context	Exploration	Time to start	Exploration	Time to start
Flying	-0.527	0.184039	0.418	0.539701
Time to start	-0.031	-0.83259	0.222	-0.78293
Crawling	-0.516	0.184883	0.479	-0.28357
Exploring roosts	-0.382	0.207335	0.497	0.041342
Landed	0.556	0.442435	-0.548	-0.11669
Percent variation	0.456	0.2801	0.628	0.2287

Results

  We captured *Myotis leibii* at two sites, and *M. lucifugus* at four sites. Full trials were completed

by 37 individuals (*M. leibii*: n = 15, *M. lucifugus*: n = 21). Another 16 individuals completed a day or night

trial but had the other trial interrupted by technical issues or severe weather and these were excluded

from the final data set, therefore removing 16 trials from the dataset. Only one bat selected a roost

type, we speculate the lack of choice in most bats was due to the limited time of the trials and therefore

roost type selection was excluded from the study. We used 55 minutes of scored behaviour per bat for

the two novel environment tests and four minutes of scored behaviour for the predator stimulus test

rather than the complete one hour, and five minutes for each test, respectively. This was due to

technology errors or environmental factors causing some trials to end early and this slightly shorter

scored time was complete for most bats.

  There was a wide variation in the mean and standard deviation for all raw variables used to

summarize the exploration behaviour in the night and day contexts (Table 6a and 6b). Despite the

variation, we found some between-individual consistency when comparing exploratory behaviours

displayed during the night and day contexts in *M. leibii*. Next, during night trials individuals of *M. leibii*

spent most of the active time flying and crawling although both have a large standard error indicating

high variation between individuals. *M. leibii* started the trials quickly, on average they exited the bat bag

and began exploring the arena within the first 18s (± 20s) of the trial. During day trials, *M. leibii* showed

similar patterns of active behaviour as the night trials in that they spent the most time flying or crawling.

On average, they displayed three times the amount of cumulative time spent landed, flying, and

crawling in the night trials relative to the day trials. *M. leibii* also took longer to begin the day trials, on

average beginning the trials in the first 59s (±132s) (Figure 2). During the predator stimulus test

assessing boldness or risk-taking behaviours, *M. leibii* spent the most time flying, then landed on the

walls of the arena. On average they spent 24s±45s on the ground (crawling and/or not moving while on the ground). *Myotis leibii* individuals reacted immediately to the retriever entering the arena and began displaying strong reactive behaviours with no delay. Along with this, five individuals landed on the retriever from 1 to 5 times for an average of 7s (±15s) with one individual spending a total of 49s on the retriever (Figure 2).

Myotis lucifugus spent most of the night and day trials landed on the tent walls or ground, not displaying observable gross motor behaviours. During night trials they spent most of the active time flying, with the average time spent flying being higher during day trials but also with a higher standard error, suggesting higher variation between individuals. *Myotis lucifugus* also spent time crawling between the two contexts and on average started both trials within the first 100s however with variation 2x the time of the average (Figure 3). These patterns suggest consistency between exploration behaviours in these two contexts. During the predator stimulus trial when assessing boldness, most individuals (n = 11/17) did not make any visible gross motor behaviour in reaction to the person entering the arena and retrieving them. Two individuals flew for the full 240s, and the remaining four individuals displayed a medium level of general activity. No individuals landed on the person retrieving them from the arena (Figure 5, Table 6).
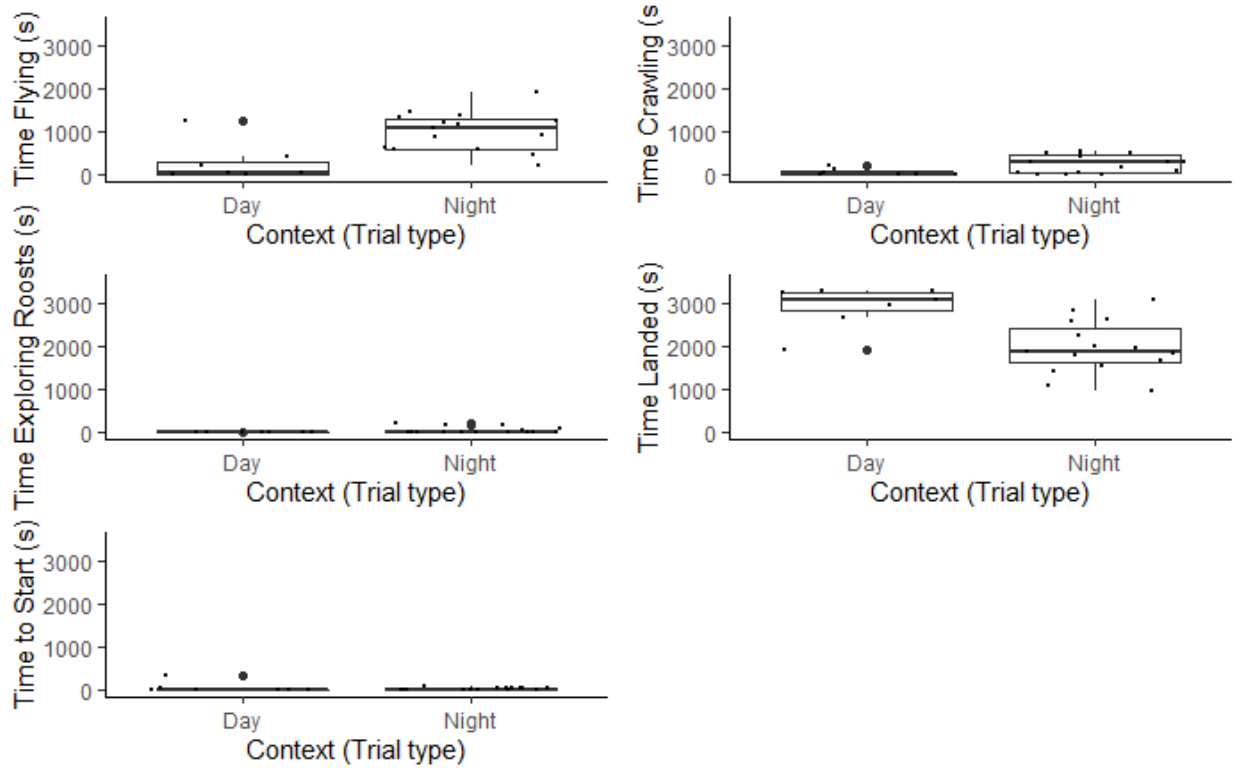
Figure 2: Boxplots showing the cumulative time spent doing each raw behaviour that was put into the principal component analyses per context for *Myotis leibii* (n=15). Data is cumulative time in seconds out of a total of 3300 seconds (55-minute trial for each context). The centre line indicates the median value of these data with the upper and lower boxes and whiskers showing the upper and lower quartiles. Data points are raw PCA variables.
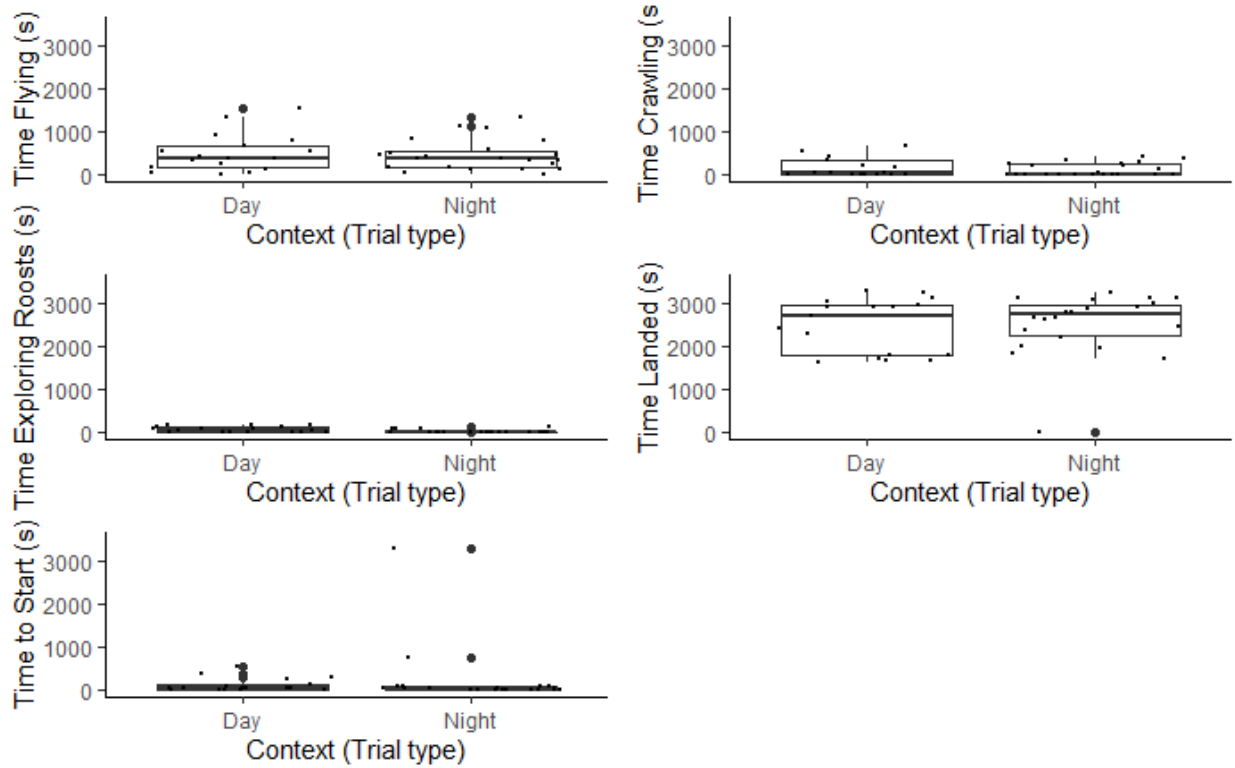
Figure 3: Boxplots showing the cumulative time individuals spent displaying each raw behavioural variable which was later analysed using a principal component analyses (PCA) for each context for *Myotis lucifugus* (n=21). Data is cumulative time in seconds out of a total of 3300 seconds (55-minute trial for each context). The centre line indicates the median value of these data with the upper and lower boxes and whiskers showing the upper and lower quartiles. Data points are jittered raw PCA variables.
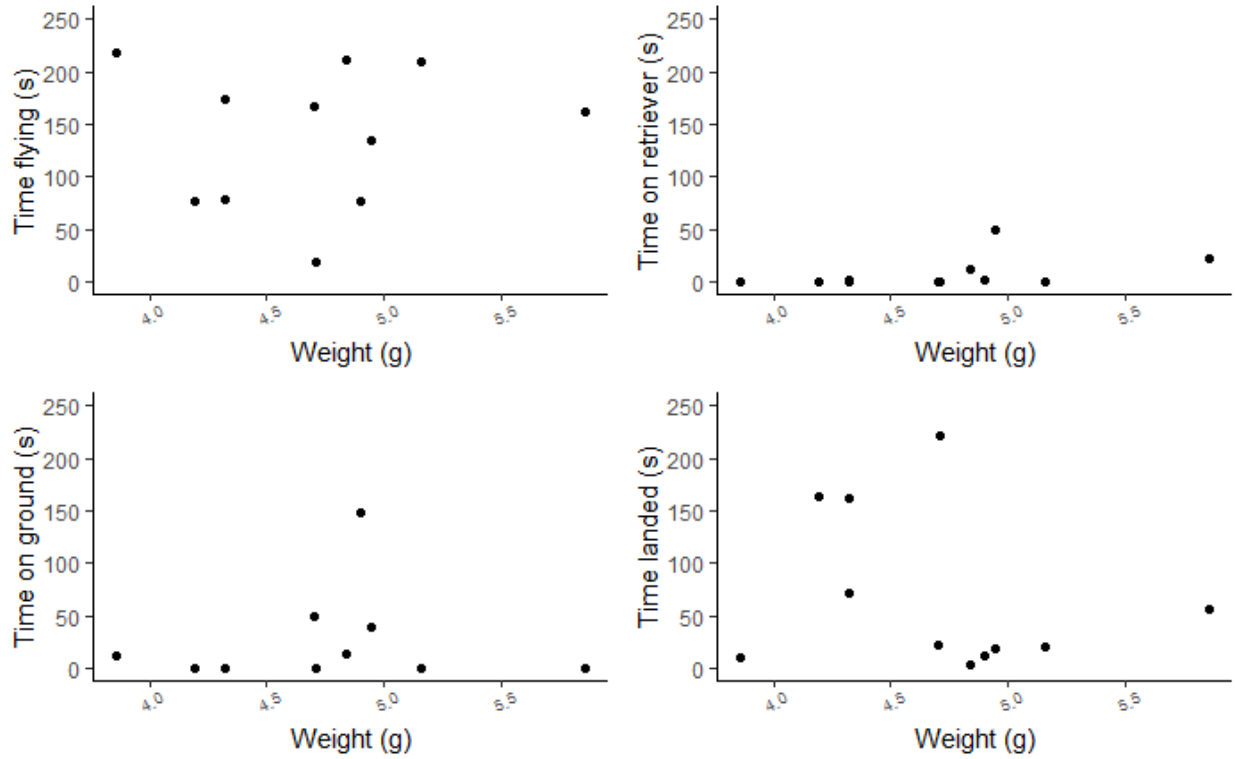
Figure 4: Scatterplot showing the cumulative time *Myotis leibii* spent displaying each behaviour in seconds (out of total trial time: 3300 seconds) on the y axis and weight in grams on the x-axis. These four behaviours were put into a principal component analysis and subsequently used to define the boldness trait.
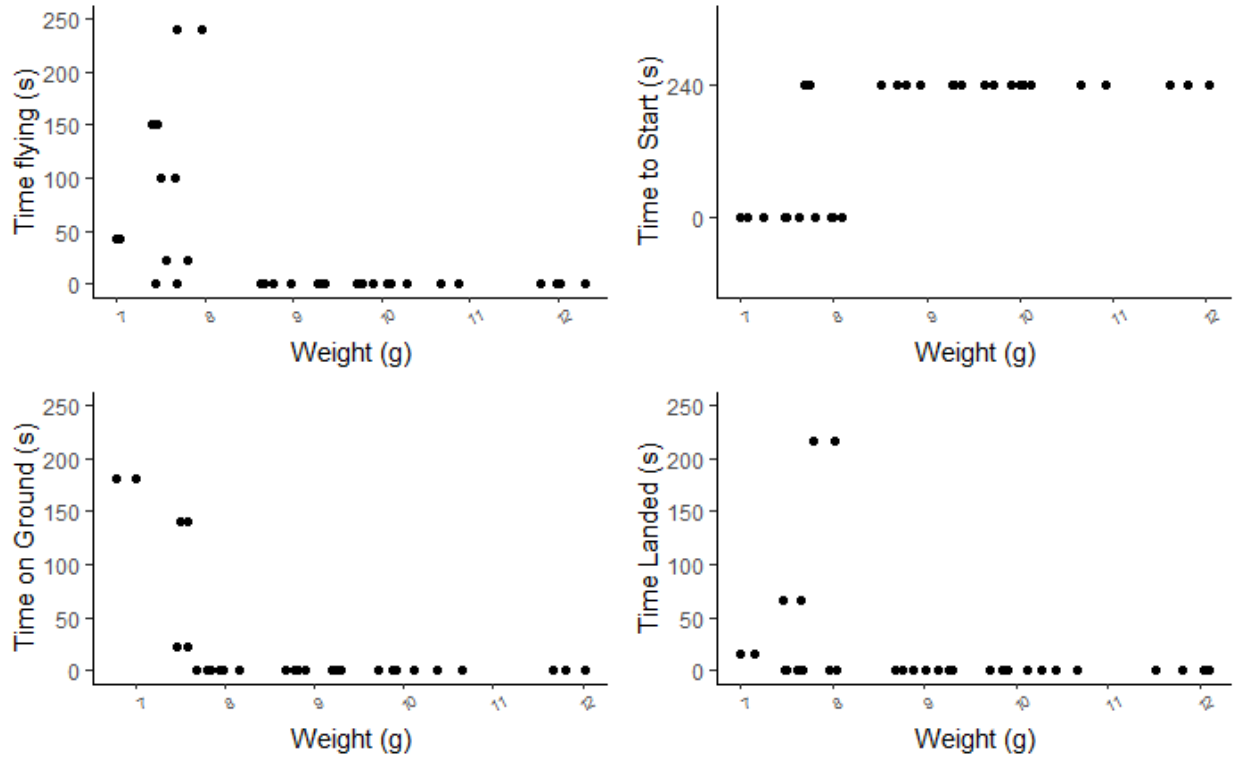
Figure 5: Scatterplot showing the cumulative time *Myotis lucifugus* spent displaying each behaviour in seconds (out of total trial time: 3300 seconds) on the y axis and weight in grams on the x-axis. These four behaviours were put into a principal component analysis and subsequently used to define the boldness trait.

Figure 6: Time in seconds (mean ± standard deviation) for the four behaviours used to calculate the boldness trait for *Myotis leibii* and *M. lucifugus.*

| | *Myotis leibii* | | | | *Myotis lucifugus* | | | |
| | Night | | Day | | Night | | Day | |
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Flying	993	453	319	484	447	365	492	442
To start	17	20	59	132	207	709	108	155
Crawling	236	207	58	87	108	142	168	220
Exploring roosts	38	67	1	2	15	33	56	59
Landed	1966	617	2863	510	2521	723	2471	636

Within-species results

Adjusted/conditional repeatability.

Context (night vs. day), is not a significant predictor of exploratory behaviour for *M. leibii*. The random intercept of the random effect 'individual' in this model is 0, indicating that the within-individual variation is high between contexts and that it is not possible to make a repeatability estimate using these data for *M. leibii* (Table 7). Context is a significant predictor of behaviour for *M. lucifugus*, with a random intercept estimate of 0.1781 ($p < 0.05$; Table 7). Individuals displayed relatively high levels of exploration during the night context and a decrease in exploration behaviours during the day context. We estimated repeatability for exploratory behaviours between night and day context and behaviours were not repeatable (r = 0.062, $p > 0.05$; Table 8).

We ran three linear regressions per species to determine the effect of sex on behaviours displayed during the three contexts (Night, day, predator stimulus). For both *M. leibii* and *M. lucifugus* all three models were insignificant indicating that sex is not a significant predictor of these three behavioural traits in a night, day, and predator stimulus context (All $p > 0.05$; Table 9).
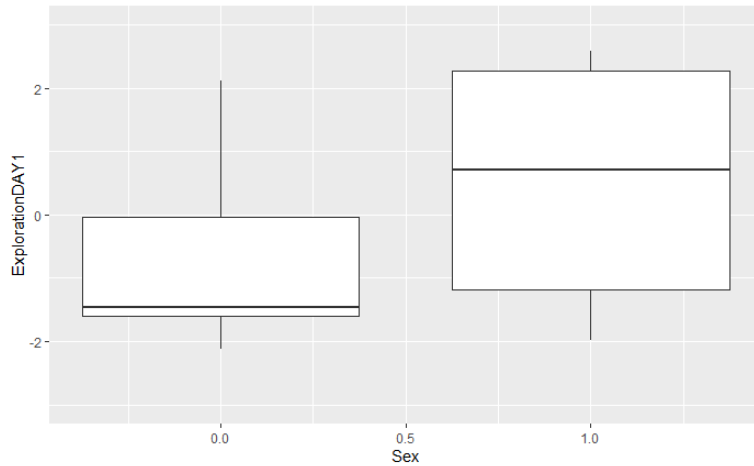
Figure 7: Bot plot showing combined principal component scores for exploration behaviours displayed during the day context on the y-axis and sex for on the x-axis for *Myotis lucifugus* with a 95% confidence interval. In this comparison, male bats were coded as 1 and female as 0.

Table 6: Linear mixed model (LMM) results for *Myotis leibii* and *M. lucifugus* to determine the effect of trial type and individual on exploratory behaviour between trials. Both models had trial type (night or day) as a fixed effect and individual as a random effect.

Model: Exploration Night/Day ~ Trial Type + (1 | Individual)	*Myotis leibii*	*Myotis lucifugus*
Trait	Exploration	Exploration
Predictor	Trial type	Trial type
Random intercept	0	0.1781±0.4221
Fixed effect estimate ± SE	0.39±0.313	-0.8019±0.5484
t-Stat	1.255	-1.461
p-value	0.36	0.0033**

Table 7: Repeatability results for *Myotis leibii* and *M. lucifugus* to determine the adjusted repeatability between night and day trials for exploration behaviour. Both models had individual as a random effect. Model = repeatability (Exploration ~ Trial type + (1 \| Individual), group name = individual, Distribution = Gaussian)

Parameter estimates	*Myotis leibii*	*Myotis lucifugus*
R	0	0.062
SE	N/a	0.173
CI	N/a	0, 0.554
p-value	1	0.393

Table 8: Linear model (LM) results for *Myotis leibii* and *M. lucifugus* to determine the effect of sex on behavioural traits within a context. * Indicates statistical significance at α = 0.05.

Species	Model	n	R^2	F-Stat	p-value
Myotis leibii	Explore Night	15	0.0014	$0.01_{1,11}$	0.9
	Explore Day	6	0.24	$1.25_{1,4}$	0.3
	Boldness Predator stimulus	11	0.004	$0.03_{1,8}$	0.9
Myotis lucifugus	Explore Night	21	0.26	$3.37_{2,19}$	0.06
	Explore Day	16	0.48	$5.94_{2,13}$	0.015*
	Boldness Predator stimulus	17	0.24	$2.02_{2,12}$	0.2

Behavioural syndrome

Myotis leibii exploration in a night and day context positively correlate (R = 0.49), but this correlation is not statistically significant (Table 10, Figure 7: a). There is also a slight but insignificant correlation between a boldness in a predatory stimulus context and exploration in a night context (R = 0.36) (p > 0.05; Table 10; 7b). There is no correlation between boldness and exploration in a day context (p > 0.05; Table 10; 7c). For *M. lucifugus* there is no correlation between any behaviour context combinations (Table 10; Figure 8a-c).

Plasticity/stability

M. leibii shows variable ISS values when comparing the output from each trial type. ISS values close to 1.0 indicate high consistency with negative values indicating low consistency between two instances of behaviour. In all three ISS analyses between contexts showed the lowest ISS value is -3.9, some individuals show perfect consistency (0.99). *M. lucifugus* display inconsistent ISS values, the highest being -3.9 for predator stimulus vs day trial, -8.1 for exploration night vs day, -9.8 for exploration night vs predator stimulus. Some individuals of *M. lucifugus* also displayed almost perfectly consistent ISS values in all analyses (Table 11; Figure 9 & 10).

Table 9: Correlation results for between-context correlations of behavioural trials for *Myotis leibii* and *M. lucifugus* using a Spearman's Rank Correlation.

Species	Behaviour/trait	N	Context	S	*p*	rho
Myotis leibii	Exploration	6	Night and day	18	0.36	0.49
	Boldness and exploration	10	Predator stimulus and night	106	0.31	0.36
	Boldness and exploration	6	Predator stimulus and day	36	1	-0.02
Myotis lucifugus	Exploration	16				
			Night and day	714	0.85	- 0.05
	Boldness and exploration	16	Predator stimulus and night	777.6	0.6	- 0.14
	Boldness and exploration	12	Predator stimulus and day	254.5	0.73	0.11

a.

b.

c.
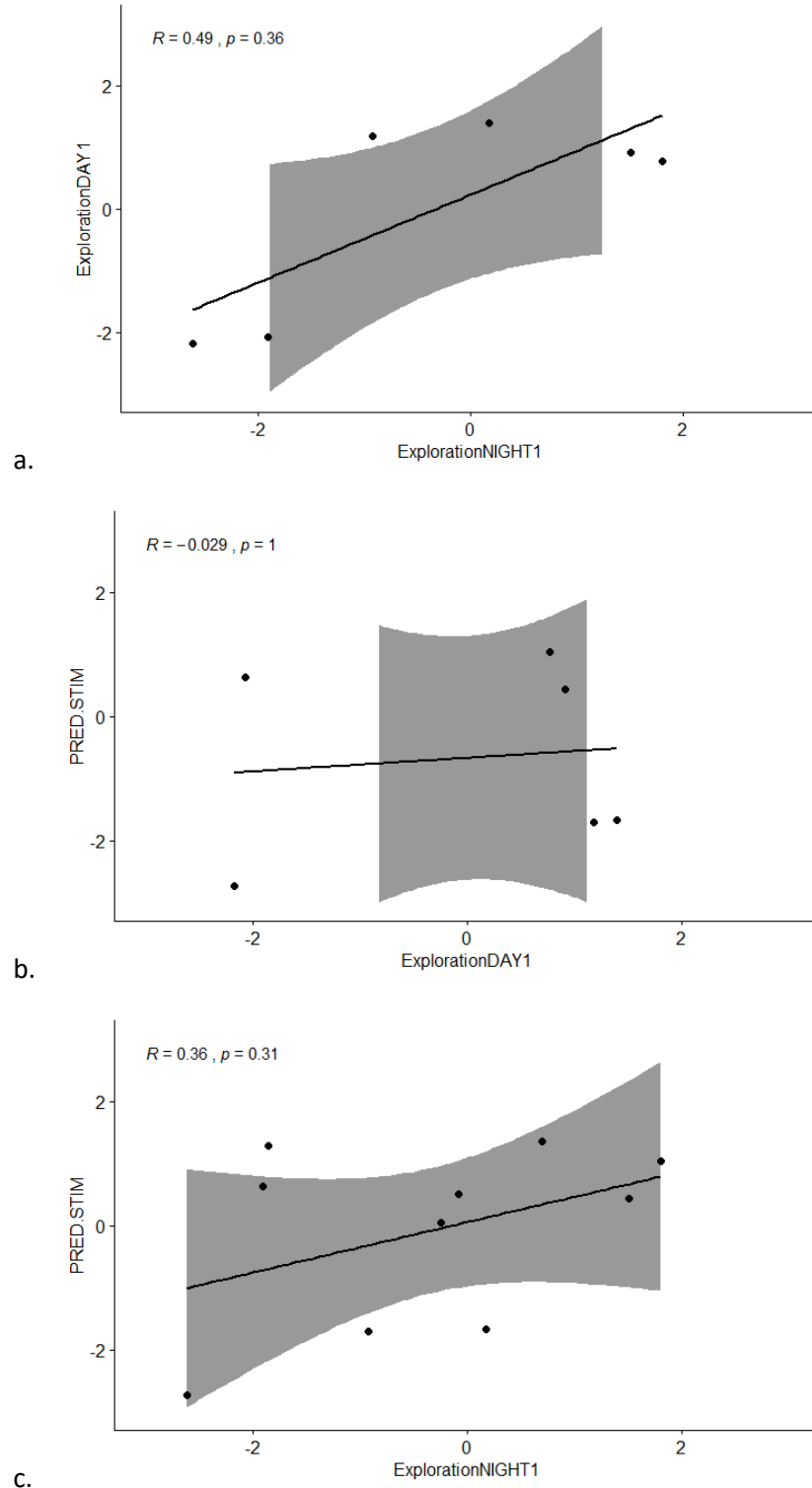
Figure 8: Correlation plots for between trait and between context correlations for *Myotis leibii* (a) is a correlation plot for between exploration night and exploration day, (b) is a correlation plot for between predator stimulus and exploration day, and (c) is a correlation plot for predator stimulus and exploration night.
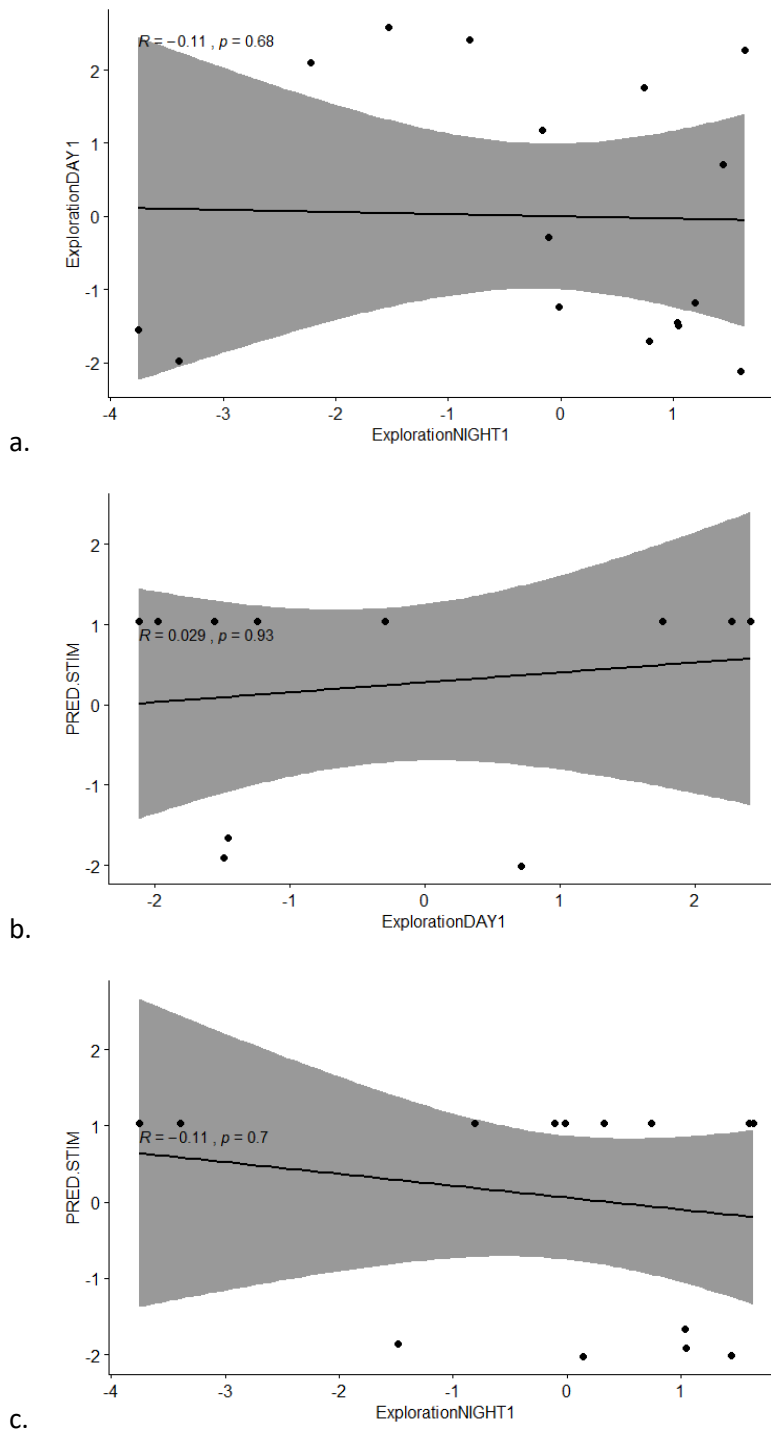
Figure 9: Correlation plots for between trait and between context correlations for *Myotis lucifugus* (a) is a correlation plot for between exploration night and exploration day, (b) is a correlation plot for between predator stimulus and exploration day, and (c) is a correlation plot for predator stimulus and exploration night.

Table 10: Results for individual stability statistic (ISS) using z-scores of behavioural variables for both *Myotis leibii* and *M. lucifugus* when comparing the variance of individuals in each context (Night & Day, Night & Predator Stimulus). *LT = Linear transformed

Bat	Band (*Myotis leibii*)	Night Mean Score	Night SD Score	Day Mean Score	Day SD Score	Z-Score Day	Z-Score Night	Z-Scores Difference (Absolute)	ISS	ISS LT*
21	200305	-0.328	1.803	1.851	1.661	0.709	-0.332	1.041	-1.213	-1.919
1	200235	-0.328	1.803	1.851	1.661	0.839	0.277	0.562	0.253	-0.141
4	200238	-0.328	1.803	1.851	1.661	0.463	1.183	-0.721	0.463	0.113
3	200236	-0.328	1.803	1.851	1.661	0.550	1.017	-0.467	0.825	0.552
41	200308	-0.328	1.803	1.851	1.661	-1.311	-1.270	-0.041	0.903	0.647
2	200234	-0.328	1.803	1.851	1.661	-1.249	-0.875	-0.374	0.986	0.748

Bat	Band (*Myotis leibii*)	Night Mean Score	Night SD Score	Predator Stimulus Mean Score	Predator Stimulus SD Score	Z-Score Predator Stimulus	Z-Score Night	Z-Scores Difference (Absolute)	ISS	ISS LT*
13	200242	-0.345	1.480	-0.080	1.434	0.950	-1.021	1.971	-3.924	-2.267
2	200234	-0.345	1.480	-0.080	1.434	0.500	-1.055	1.554	-2.233	-1.252
1	200235	-0.345	1.480	-0.080	1.434	-1.106	0.349	-1.455	-0.688	-0.325
3	200236	-0.345	1.480	-0.080	1.434	0.359	1.251	-0.891	0.428	0.344
21	200305	-0.345	1.480	-0.080	1.434	-1.132	-0.393	-0.739	0.699	0.507
4	200238	-0.345	1.480	-0.080	1.434	0.778	1.454	-0.676	0.704	0.510
11	200245	-0.345	1.480	-0.080	1.434	1.000	0.704	0.296	0.784	0.557
44	200309	-0.345	1.480	-0.080	1.434	0.414	0.180	0.234	0.824	0.582
22	200306	-0.345	1.480	-0.080	1.434	0.089	0.067	0.022	0.957	0.661
41	200308	-0.345	1.480	-0.080	1.434	-1.852	-1.536	-0.316	0.993	0.683

Bat #	Band (*Myotis leibii*)	Day Mean Scores	Day SD Scores	Predator Stimulus Mean Score	Predator Stimulus SD Score	Z-Score Predator Stimulus	Z-Score Day	Z-Scores Difference (Absolute)	ISS	ISS LT*
1	200235	1.851	1.661	-0.666	1.560	-0.641	0.839	-1.480	-3.682	-1.131
21	200305	1.851	1.661	-0.666	1.560	-0.665	0.709	-1.373	-3.148	-0.894
2	200234	1.851	1.661	-0.666	1.560	0.835	-1.249	2.084	-2.677	-0.685
41	200308	1.851	1.661	-0.666	1.560	-1.327	-1.311	-0.016	0.845	0.880
3	200236	1.851	1.661	-0.666	1.560	0.706	0.550	0.157	0.886	0.898
4	200238	1.851	1.661	-0.666	1.560	1.091	0.463	0.628	0.964	0.933

Bat #	Band (*Myotis lucifugus*)	Night Mean Score	Night SD Score	Day Mean Score	Day SD Score	Z-Score Day	Z-Score Night	Z-Scores Difference (Absolute)	ISS	ISS LT*
56	004435	-0.116	1.601	2.082	1.792	1.176	-1.275	2.452	-8.102	-2.240
52	004397	-0.116	1.601	2.082	1.792	1.439	-0.875	2.314	-7.390	-1.991
53	004406	-0.116	1.601	2.082	1.792	-1.181	0.936	-2.117	-5.124	-1.198
57	004438	-0.116	1.601	2.082	1.792	1.348	0.035	1.313	-2.064	-0.127
29	004207	-0.116	1.601	2.082	1.792	-0.956	0.535	-1.491	-2.011	-0.109
42	004288	-0.116	1.601	2.082	1.792	-0.831	0.674	-1.504	-2.005	-0.107
18	013500	-0.116	1.601	2.082	1.792	-0.811	0.664	-1.475	-1.882	-0.064
30	004197	-0.116	1.601	2.082	1.792	-0.661	0.747	-1.408	-1.564	0.047
50	004393	-0.116	1.601	2.082	1.792	-0.869	-2.179	1.310	-1.098	0.211
51	004388	-0.116	1.601	2.082	1.792	-0.691	0.088	-0.779	0.201	0.665
39	004238	-0.116	1.601	2.082	1.792	0.657	0.114	0.543	0.383	0.729
49	004356	-0.116	1.601	2.082	1.792	-1.103	-1.803	0.700	0.474	0.760
43	004293	-0.116	1.601	2.082	1.792	0.982	0.542	0.440	0.492	0.767

54	004425	-0.116	1.601	2.082	1.792	1.267	0.966	0.300	0.648	0.821
37	004216	-0.116	1.601	2.082	1.792	0.398	0.861	-0.462	0.850	0.892
48	004353	-0.116	1.601	2.082	1.792	-0.164	-0.030	-0.134	0.992	0.942

Bat #	Band (*Myotis lucifugus*)	Night Mean Score	Night SD Score	Predator Stimulus Mean Score	Predator Stimulus SD Score	Z-Score Predator Stimulus	Z-Score Night	Z-Scores Difference (Absolute)	ISS	ISS LT*
50	004393	0.050	1.481	0.119	1.405	0.652	-2.469	3.120	-9.771	-2.585
49	004356	0.050	1.481	0.119	1.405	0.652	-2.062	2.713	-7.155	-1.783
37	004216	0.050	1.481	0.119	1.405	-1.521	0.818	-2.339	-4.379	-0.931
42	004288	0.050	1.481	0.119	1.405	-1.445	0.616	-2.061	-3.130	-0.548
18	013500	0.050	1.481	0.119	1.405	-1.270	0.606	-1.876	-2.414	-0.328
40	004256	0.050	1.481	0.119	1.405	-1.530	0.053	-1.583	-1.331	0.004
48	004353	0.050	1.481	0.119	1.405	0.652	-0.145	0.796	0.281	0.498
57	004438	0.050	1.481	0.119	1.405	0.652	-0.074	0.726	0.400	0.535
51	004388	0.050	1.481	0.119	1.405	0.652	-0.017	0.668	0.490	0.563
39	004238	0.050	1.481	0.119	1.405	0.652	0.012	0.640	0.531	0.575
20	004118	0.050	1.481	0.119	1.405	-1.404	-0.667	-0.738	0.580	0.590
55	004433	0.050	1.481	0.119	1.405	0.652	0.099	0.553	0.648	0.611
54	004425	0.050	1.481	0.119	1.405	0.652	0.933	-0.281	0.922	0.695
46	004315	0.050	1.481	0.119	1.405	0.652	0.922	-0.271	0.928	0.697
53	004406	0.050	1.481	0.119	1.405	0.652	0.900	-0.248	0.940	0.701
43	004293	0.050	1.481	0.119	1.405	0.652	0.474	0.178	0.960	0.707

Bat #	Band (*Myotis lucifugus*)	Day Mean Scores	Day SD Scores	Predator Stimulus	Predator Stimulus SD Score	Z-Score Predator Stimulus	Z-Score Day	Z-Scores Difference (Absolute)	ISS	ISS LT*

| | | | | | | Mean | | | |
						Score			
53	004406	-0.149	1.718	0.310	1.314	0.552	-1.145	1.697	-3.968	-1.666
49	004356	-0.149	1.718	0.310	1.314	0.552	-1.064	1.616	-3.538	-1.437
37	004216	-0.149	1.718	0.310	1.314	-1.772	0.502	-2.274	-2.731	-1.006
50	004393	-0.149	1.718	0.310	1.314	0.552	-0.820	1.372	-2.362	-0.809
51	004388	-0.149	1.718	0.310	1.314	0.552	-0.634	1.186	-1.586	-0.394
57	004438	-0.149	1.718	0.310	1.314	0.552	1.492	-0.941	0.048	0.478
48	004353	-0.149	1.718	0.310	1.314	0.552	-0.084	0.636	0.117	0.515
54	004425	-0.149	1.718	0.310	1.314	0.552	1.408	-0.856	0.238	0.579
43	004293	-0.149	1.718	0.310	1.314	0.552	1.110	-0.559	0.738	0.846
42	004288	-0.149	1.718	0.310	1.314	-1.691	-0.780	-0.911	0.911	0.939
18	013500	-0.149	1.718	0.310	1.314	-1.503	-0.759	-0.745	0.977	0.974
39	004238	-0.149	1.718	0.310	1.314	0.552	0.772	-0.221	0.990	0.981

Between-Species Results

To determine if behavioural traits within the night context is a significant predictor of species, we ran three logistic regressions with our estimated PCA behavioural traits as a fixed effect. We found the model for night exploration to significantly predict species however the model for day exploration did not significantly predict species (Table 12; Figure 10). Boldness behavioural traits in a predator stimulus context are also a significant predictor of species (Table 12; Figure 10). In all three contexts, *M. leibii* is displaying higher exploratory and boldness behaviours than *M. lucifugus*.

Table 11: Results of three logistic regressions estimating if behaviour is a significant predictor of species within each context. The raw variables inputted into the principal component analyses (PCA) and used as a new variable were combined for both species within a context.

Context	Model	Coefficient estimate	Standard error	z score	p-value
Night	glm (Species ~ Exploration Night)	0.8273	0.2935	2.819	0.005**
Day	glm (Species ~ Exploration Day)	0.7066	0.4279	1.651	0.099
Predator stimulus	glm (Species ~ Predator stimulus)	-1.958	1.018	-1.923	0.054*

a.

b.

c.
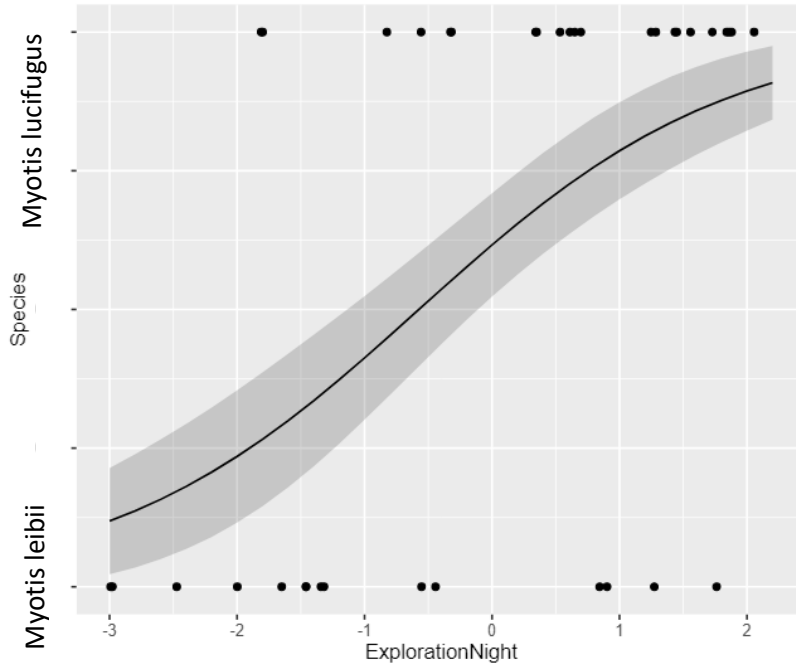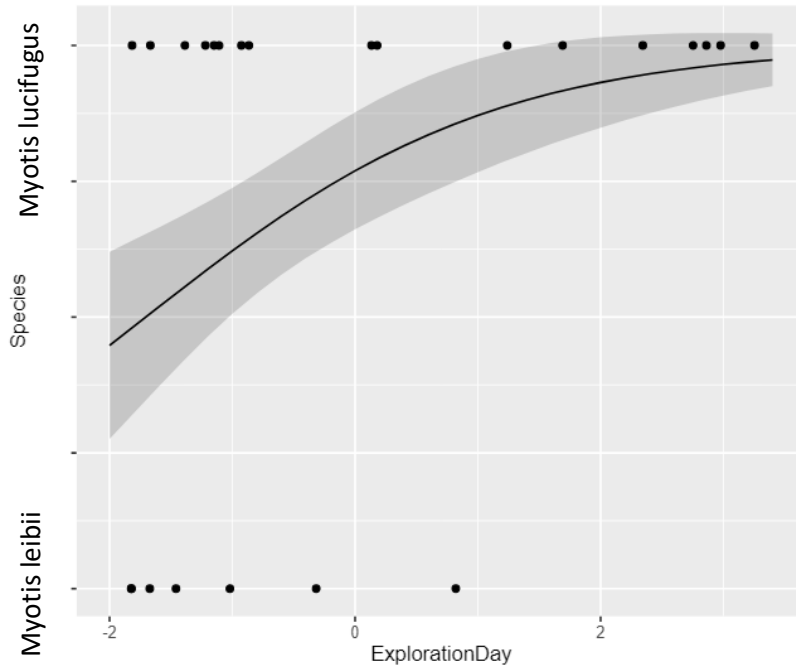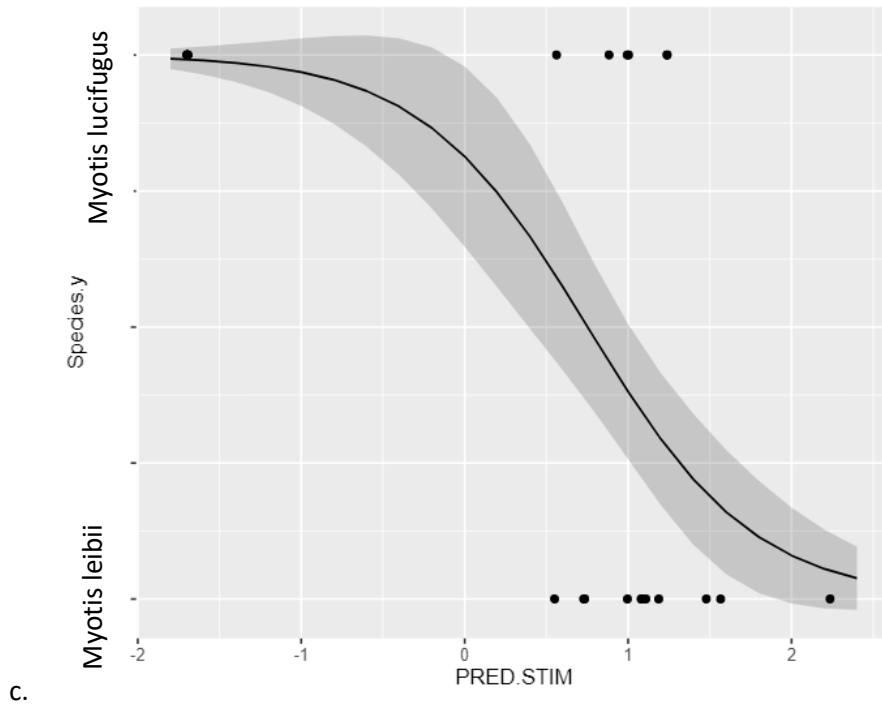
Figure 10: Scatterplot showing logistic regression results to determine if three behaviours (a) exploration in a day context, (b) exploration in a night context, and (c) boldness in a predatory stimulus context are significantly different between *M. leibii* and *Myotis lucifugus*.

Discussion

Our study provides mixed evidence for interspecific variation in exploration and risk-taking behaviours between *M. leibii* and *M. lucifugus.* We compared exploratory and risk-taking behaviours across three contexts between two species to determine if there are differences in species behaviour pertaining to roost location choices in the wild. Our results demonstrate the variation in behaviours displayed at the inter- and intra-individual levels when comparing exploration and risk-taking behaviours of bats. We did not find support for the hypothesis that patterns of roost location choice in the wild create species-specific behavioural reaction norms; however, we did find species differences in both exploration and risk-taking behaviours. We found that *M. leibii* displayed higher exploration and activity in our behavioural trials than *M. lucifugus*, most notability higher levels of exploration during the night context and higher levels of risk-taking behaviours in the presence of a potential predator. *Myotis lucifugus* was less exploratory in a novel environment and displayed relatively low levels of risk-taking behaviours while interacting with a predator.

We did not find evidence of exploration behaviour as a conditional personality trait for either *M. leibii* or *M. lucifugus* when comparing exploration behaviours between a night and day context. *Myotis leibii* showed a general consistency in exploration behaviours between a night, day, and predator stimulus context while *M. lucifugus* showed variation between behaviours when comparing night, day, and a predator stimulus context. We did not find support for the hypothesis that intraspecific evaluations of behaviour would show differences in exploratory and risk-taking behaviours when comparing sexes. There was no difference between observed behaviours when comparing male and female bats within each species, respectively.

Overall, our results did not support our hypothesis however we did observe some notable and interesting behaviours. In *M. lucifugus*, there are patterns of higher behavioural plasticity between

contexts, especially when compared with *M. leibii*. *Myotis lucifugus* as a species appear to be reacting relatively alike to one another when comparing behaviours between individuals within a context (night, day, predator stimulus), but display within-individual variation between contexts. Our results indicate that exploration behaviours within individuals are different between a night and day context and exploration is not a personality trait for *M. lucifugus*. *Myotis lucifugus* have been previously tested for exploratory personality traits using a classic rodent open-field test. Individuals displayed significantly repeatable behaviours when comparing the same context indicating that exploration behaviours within a context are a personality trait for this species (Menzies and Willis, 2010; Menzies et al., 2013). In our study, exploratory behaviours were not repeatable between a night and day context and context did predict exploratory behaviour for *M. lucifugus*. This suggests individuals are displaying different behaviours between contexts and these two contexts cannot be used to estimate repeatability.

Myotis lucifugus displayed low stability when comparing behaviours during the night context with both the day context and predator stimulus context. The lowest stability estimates for *M. lucifugus* occurred between the day context and predator stimulus context. Individuals of *M. leibii* show low plasticity and relatively consistent exploration and risk-taking behaviours when comparing contexts. Bats are nocturnal and lower activity during the day is expected because it would decrease the risk of exposure to diurnal predators (Lee and McCracken, 2001; Lima and O'Keefe, 2013). Behavioural syndromes occur in an individual or species when the evolution or ecology of the animal calls for consistent behavioural traits being displayed across contexts (Sih et al., 2004a; Couchoux and Cresswell, 2012).

We observed strong reactive and risk-taking behaviours in *M. leibii*; individuals typically chose to fly in the area closest to the potential predator (the retriever) and several individuals actively touched the potential predator more than one time during the trial. In our experiment, when *M. leibii* encounter a risk, they display more reactive and risky behaviours where they investigate the potential risk further.

When faced with a threat *M. leibii* increase their gross motor movements by increasing time spent flying which could increase their odds of escape (Lima and O'Keefe, 2013) however, this visibly puts them at a higher risk to predators. Wild animals encounter a trade-off between access to resources and risk of exposure to predators (Lima, 2008; Lima and O'Keefe, 2013; Lima and Dill, 1990). Resource gain is often observed as increased foraging success or increased thermal assistance (Lima and O'Keefe, 2013). Animals choose specific microhabitats that are resourcefully beneficial for them, especially when raising young (Hamilton and Barclay, 1994). For example, a population of big brown bats (*Eptesicus fuscus*) roosting in rock crevice locations congregate to raise young for the benefit of thermoregulation and these groups later dissipate during lactation (Lausen and Barclay, 2002). Bats can use torpor, a low metabolic state used day-to-day to assist in energy savings, however, pregnant females avoid using torpor because it can lengthen gestation (Hamilton and Barclay, 1994). During diurnal roosting in the summer months, individuals choose roost locations in rock crevices that receive direct solar exposure and are located farther off the ground than roosts randomly available in the environment (Lausen and Barclay, 2002). Researchers observed two bats depredated by bull snakes (*Pituophis melanoleucus*), confirming that roosts close to the ground are a threat to big brown bats. Individuals roost switch often, showing little within-year roost location fidelity however they show high fidelity between years between sites. Individuals selected roosts with tight entrances and a more vertical aspect. Researchers suggest these behaviours are displayed by big brown bats as both predator avoidance and thermoregulatory stabilizing strategies (Lausen and Barclay, 2002). Predatory interactions in roost locations near the ground are suggested to be riskier for big brown bats (Lausen and Barclay, 2002).

Bats will decrease thermal energy expenditure by social grouping and by solar exposure in the roost (Willis and Brigham, 2007; Johnson et al., 2011; Webber and Willis, 2018; Chruszcz and Barclay, 2002). There are various benefits of a larger group size such as increased odds of predator detection (Boland, 2003) and thermoregulatory benefits (Dzal and Brigham, 2013). Bats group together for

thermoregulatory benefits and display cooperative social behaviours with conspecifics, especially during

the maternity season (Kilgour and Brigham, 2013). Bat pups are born with no fur and need

thermoregulatory assistance which can be gained through group membership and/or directly through

biotic environmental factors (Olson and Barclay, 2013). Larger group sizes are beneficial for predator

avoidance in many mammal species; larger groups are more likely to encounter, but less likely to be

attacked by predators and the odds of a single individual surviving are increased through predator

confusion (Arndt et al., 2018; Lima, 2008; Lima and O'Keefe, 2013). Rodents benefit from larger group

sizes though increased predator detection. Solitary individuals are captured and killed by predators

more often for several species of mice and vole (Anderson, 1986). This pattern is reflected in two

species of prairie dog (*Cynomys* spp.) where larger groups detect and notify conspecifics faster than

smaller groups, increasing the odds of survival for all individuals (Hoogland, 1979; Boyles, 2007).

Individuals in larger groups of emus (*Dromaius novaehollandiaein*) spent less time being vigilant,

detected predators faster, and to save energy waited longer to escape after being detected. Overall

individuals in larger groups increased their odds of surviving a predator and expended less energy in

doing so (Boland, 2003). Non-reactive behaviours are one method of predator avoidance after the

individual is aware of a potential predator (Lima and Dill, 1990) and are reflected in the behavioural

reactions by *M. lucifugus* in our study. Many bat species benefit from group membership during roost

emergence wherein they rely on predator confusion to avoid predation (Lima and O'Keefe, 2013). In our

study, individual *M. lucifugus* reduced their levels of gross motor activity in the presence of a predator.

We suggest this is related to their reliance on grouping strategies during flying to confuse predators.

Myotis leibii increased their levels of gross motor activity during the predator stimulus context which we

suggest is also a predator avoidance strategy to escape quickly, however *M. leibii* continued to fly and

quickly appeared to become interested in the potential predator when no threatening behaviours

occurred.

Individual *M. leibii* displayed a high rate of roost switching, choosing a new roost every 1.1 days, which can be a form of predator avoidance in bats (Kunz, 1982; Lewis, 1995b; Boyles and Storm, 2007; Johnson et al., 2011) and is observed in big brown bats (Lausen and Barclay, 2002). Maternity ground roosting behaviour is a rare trait in bats however it is also observed in a maternity population of western long-eared bat (*Myotis evotis*) in Alberta (Solick and Barclay, 2006; Chruszcz and Barclay, 2002). Reproductive individuals are actively choosing ground-level roosts in rocks with high solar exposure. Non-reproductive females choose the same roost locations but position themselves deeper into the roost crevice and use torpor (Solick and Barclay, 2006). *Myotis evotis*, although not rare, are found in low numbers across a large range (Wilson and Reeder, 2005) and may not attain high enough group membership for effective social thermoregulation. Interestingly, opposite maternity roost selection is observed in a maternity population of big brown bats located in rocky, open habitats. Individuals actively choose roost openings higher off the ground than what is randomly available in the environment (Lausen and Barclay, 2003) which appears to be the predator avoidance behaviour in roost choice for big brown bats. This maternity colony is a medium-size cohesive population (34 banded adult females) which often roosts in one group. Less often, individual's roosts in smaller group sizes averaging ~eight individuals which occurred more often in the post-lactation period (Lausen and Barclay, 2003). This population (and species) appears to support large enough group membership for social thermoregulation and therefore do not need to choose ground-level, solar exposed roosts, and risk exposure to predators (Lausen and Barclay, 2003). Due to the similarities and differences between *M. leibii*, M. evotis and E. fuscus, we suspect there might be a trade off occurring between solar exposure/assisted thermoregulation and predator exposure, especially during the maternity season. Female bats in the context of raising their young are known to have higher levels of aggression towards conspecifics (González-Palomares 2021).

Our study is the first to our knowledge to deeply examine gross motor behaviours in *M. leibii*. We captured a video recording of one *M. leibii* actively foraging on the ground during the night. These bat species are displaying different behaviours from one another, which may correspond to the different pressures experienced in the roost location and life history of each species however further research is needed to investigate this relationship. *Myotis leibii* is a cryptic species and little is known about their critical roosting habitat and general habitat use; no studies to our knowledge have methodically tested the behaviour and personality of this species. Therefore, the results of this study contribute to habitat management of endangered *Myotis* bat species by contributing to knowledge about their behavioural adaptation relative to habitat use. Observation of bat behaviour helps us understand long-term behavioural adaptation to an ever-changing environment. Most bat species are at risk in Canada, recent research shows patterns of population decline and degradation of habitat in bat populations across the globe. Researchers understand little about the interactions between bats and their predators. We understand predation occurs regularly however for many predators it appears to be opportunistic predation (Arndt et al, 2002, Lima and O'Keefe, 2013). There are few studies between bats, their predators, and how interactions between the two shape risk-taking adaptations for a species. *Myotis leibii* presents an interesting opportunity for study where a unique trait (ground roosting in bats) might be causing distinct types of predatory interactions. Further research might focus on the interaction between ground use and exploration in *M. leibii* and the influence natural predators might have on individuals and groups. We hypothesize that *M. leibii* is not only using ground level-roosts for solar exposure (Johnson et al., 2011) but also to forage in the roost. Bats exploit increased activity of flying insects before sunset (Lee and McCracken, 2001; Murray and Kurta, 2004; Viele et al. 2002; Brigham and Kalcounis, 1994), including *M. leibii* (Bruce et al, unpublished data, 2017) however these individuals are increasing the risk of exposure to diurnal predators (Lee and McCracken, 2001; Arndt et al. 2018). There are anecdotal observations of *M. leibii* emerging after dusk (Best and Jennings, 1997) or being captured

nearby to known roost locations ~25 minutes after sunset (Moosman et al., 2012), but observations are otherwise scarce. We speculate that *M. leibii* may forage for insects in their crevice roost as a second form of energy gain alongside solar exposure. Many terrestrial arthropods share similar ground habitat with *M. leibii*, under rocks and in crevices along cliff-faces. We observed this behaviour in one individual for ¼ of a trial completed at an abnormal time and therefore was not included in analyses. Ambulatory ground use is rare in bats, and they are specialized in aerial locomotion (Riskin et al., 2006).

Chapter three

 General Conclusion

Risk management strategies and the behavioural evolutionary consequences of selecting

microhabitats with different fitness-related pressures are not strongly represented in the literature. Bats

are the most common taxonomic order of mammals with over 1400 species of bats extant (Zukal, 2020),

yet mechanisms underlying their habitat selection are not well understood. Bats in North America are at

risk due to loss and degradation of critical habitats. Hibernating bats in North America are experiencing

extreme population loss due to the fungal disease white-nose syndrome (WNS). Populations of common

species such as the little brown bat (*Myotis lucifugus*) have declined upwards of 95% due to WNS.

Unfortunately, we know relatively little about habitat selection by North American *Myotis* bats due to

their cryptic, nocturnal behaviour and small size. My research explores behavioural habitat section of

Myotis bat species in eastern North America concerning their maternity roost site preferences, a trait

with high fidelity in bats (Vonhof and Barclay, 1996; Lewis, 1995a).

The objectives of this study were to observe inter- and intraspecific behaviours of two bat

species with an overlapping geographic range. *Myotis leibii* and *M. lucifugus* are two species of bat

which display dissimilar roosting behaviours which could shape predator behavioural adaptation of a

species. *Myotis leibii* are less common with a range a fraction of the size of *M. lucifugus*. The former

species is observed with unique daytime roosting behaviours in the wild where they are found roosting

on the ground in the same locations, but at different times than potential predators (such as snakes and

lizards) in small groups. They have also been found in medium to large size roosts located in more

typical vesper bat roosts (high structure tree or building roosts with 10-24 individuals). *M. lucifugus* is

not found roosting on the ground and are usually found in large roosting colonies, especially while

raising young. I determined if general roost site selection impacted the risk-taking and exploratory behaviours of a ground roosting versus non-ground roosting species of bat.

Using behavioural assays, I quantified the exploration and risk-taking behaviours of *M. leibii* and *M. lucifugus* to compare within- and between-species. I first determined if exploration was a personality trait for either species by comparing exploration behaviours in two contexts: nocturnal and diurnal. We wanted to determine the consistency or lack thereof between exploratory behaviours in the two contexts. We found that exploration behaviours between night and day are not consistent within- or between-individuals and were unable to estimate repeatability. Along with this, I examined consistency and plasticity in exploration and risk-taking behaviours within each species which might indicate a behavioural syndrome occurring in a species relative to predatory interactions however we did not find significant relationships here. I also wanted to observe species differences in behaviours for exploration and risk-taking behaviours when interacting with a predator. Due to roost microhabitats of *M. leibii*, such as roost location and grouping ability of a species, these bats might react differently in novel or risky situations than *M. lucifugus* and I wanted to investigate this relationship further. I did find species differences in exploration in a novel environment during nighttime and risk-taking behaviours when interacting with a predator.

It is important to note that I originally wanted to explore differences in behaviour and microhabitat roost selection of *M. leibii* versus *M. lucifugus* using synthetic bat box roosts made to meet each species roost selection preferences in the wild. The eight bat boxes inside the arena (Figure 1) were outfitted to offer a variation in roost entrance size (large or small), temperature (heated or not heated), and location (ground or raised bat box). We expected bats to select one of these roosts during the night trial and again during the day contexts to get an estimate on roost microhabitat preference in Myotis leibii relative to Myotis lucifugus. We randomized the order of the bat boxes between the night and day trials so bats could not select the roost based on its previous known location. We also set up an

echolocation recording device to get an estimate on vocalizations made by each species. However, individuals did not select roosts and stayed active most of the trial time. Due to time constraints and technological failures, I was unable to collect these types of data from this experiment. A single *M. lucifugus* did select a large entrance, heated bat box located off the ground and again selected this same microhabitat after being randomized for the day trial. This suggests that given more time bats would have selected roosts.

Observations of bat behaviour should be a focus of future research. Depending on the research question, various methods are recommended. If future research aims to observe habitat selection rates using a synthetic microhabitat, then more time than one hour is required to observe selection in bats. I suggest testing multiple bats, either together or separate, for a longer time (~3-6 hours or more). This will ideally give bats the time they need to familiarize themselves with the novel environment and make a roost selection. If future research looks to focus on behavioural reaction norms of bats, then I suggest multiple short behavioural assays. This would allow more time for an individual bat to endure multiple behavioural trials under various contexts which would allow for the analyses of repeatability (personality), behavioural syndrome (correlations), and behavioural plasticity (individual stability statistic). This would also allow more time for researchers to observe more individuals, under multiple behavioural assays and/or contexts which would allow for a more precise estimate on individual behavioural reaction norms.

In this study I have illustrated the variation and complexity of bat behaviour and hope that future research aims to examine the behavioural selection and interactions of *Myotis leibii*. This species is unique and interested and although I did not investigate the mechanisms behind species differences in behaviour in a risky situation, it allows for a basis for future behavioural research between multiple bat species.

References

Anderson, P.K., 1986. Foraging range in mice and voles: the role of risk. Canadian Journal of Zoology, 64(12), pp.2645-2653.

Angert, A.L., Bayly, M., Sheth, S.N. and Paul, J.R., 2018. Testing range-limit hypotheses using range-wide habitat suitability and occupancy for the scarlet monkeyflower (*Erythranthe cardinalis*). The American Naturalist, 191(3), pp.E76-E89.

Arndt, R.J., O'Keefe, J.M., Mitchell, W.A., Holmes, J.B. and Lima, S.L., 2018. Do predators influence the behaviour of temperate-zone bats? An analysis of competing models of roost emergence times. Animal Behaviour, 145, pp.161-170.

Asendorpf, J., 1990. The measurement of individual consistency. Methodika. 4, 1–22.

Avital, E. and Jablonka, E., 1994. Social learning and the evolution of behaviour. Animal Behaviour, 48(5), pp.1195-1199.

Bell, A.M., 2007. Future directions in behavioural syndromes research. Proceedings of the Royal Society B: Biological Sciences, 274(1611), pp.755-761.

Best, T.L, and Jennings, J. B. 1997. *Myotis Leibii*. Mammalian Species, no. 547: 1–6. https://doi.org/10.2307/3504255.

Boland, C.R., 2003. An experimental test of predator detection rates using groups of free-living emus. Ethology, 109(3), pp.209-222.

Boyles, J.G. and Storm, J.J., 2007. Avoidance of predator chemical cues by bats: an experimental assessment. Behaviour, pp.1019-1032.

Betts, B.J., 1998. Roosts used by maternity colonies of silver-haired bats in northeastern Oregon. Journal of Mammalogy, 79(2), pp.643-650.

Brighton, C. H., Zusi, L., McGowan, K.A., Kinniry, M., Kloepper, L.N., and Taylor, G.K. 2021. Aerial attack strategies of hawks hunting bats, and the adaptive benefits of swarming. Behavioral Ecology 32, pp. 464-476.

Brown, J.S. and Morgan, R.A., 1995. Effects of foraging behavior and spatial scale on diet selectivity: a test with fox squirrels. Oikos, pp.122-136.

Brown, J.S., 1999. Vigilance, patch use and habitat selection: foraging under predation risk. Evolutionary ecology research, 1(1), pp.49-71.

Carter, G.G., Farine, D.R., Crisp, R.J., Vrtilek, J.K., Ripperger, S.P. and Page, R.A., 2020. Development of new food-sharing relationships in vampire bats. Current Biology, 30(7), pp.1275-1279.

Chruszcz, B.J. and Barclay, R.M.R., 2002. Thermoregulatory ecology of a solitary bat, Myotis evotis, roosting in rock crevices. Functional Ecology, pp.18-26.

Couchoux, C. and Cresswell, W., 2012. Personality constraints versus flexible antipredation behaviors: how important is boldness in risk management of redshanks (*Tringa totanus*) foraging in a natural system?. Behavioral Ecology, 23(2), pp.290-301.

Dall, S.R., Houston, A.I. and McNamara, J.M., 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecology letters, 7(8), pp.734-739.

Dammhahn, M., 2012. Are personality differences in a small iteroparous mammal maintained by a life-history trade-off?. Proceedings of the Royal Society B: Biological Sciences, 279(1738), pp.2645-2651.

Delm, M.M., 1990. Vigilance for predators: detection and dilution effects. Behavioral Ecology and Sociobiology, 26(5), pp.337-342.

Dingemanse, N.J., Both, C., Drent, P.J., Van Oers, K. and Van Noordwijk, A.J., 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. Animal Behaviour, 64(6), pp.929-938.

Dingemanse, N.J., Kazem, A.J., Réale, D. and Wright, J., 2010. Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology & Evolution, 25(2), pp.81-89.

Dingemanse, N.J., Dochtermann, N.A. and Nakagawa, S., 2012. Defining behavioural syndromes and the role of 'syndrome deviation' in understanding their evolution. Behavioral Ecology and Sociobiology, 66(11), pp.1543-1548.

Dingemanse, N.J. and Dochtermann, N.A., 2013. Quantifying individual variation in behaviour: mixed-effect modelling approaches. Journal of Animal Ecology, 82(1), pp.39-54.

Dingemanse, N.J., Bouwman, K.M., Van De Pol, M., van Overveld, T., Patrick, S.C., Matthysen, E. and Quinn, J.L., 2012. Variation in personality and behavioural plasticity across four populations of the

great tit Parus major. Journal of Animal Ecology, 81(1), pp.116-126.

Dochtermann, N.A., Schwab, T., Anderson Berdal, M., Dalos, J. and Royauté, R., 2019. The heritability of behavior: a meta-analysis. Journal of Heredity, 110(4), pp.403-410.

Duckworth, R.A., 2010. Evolution of personality: developmental constraints on behavioral flexibility. The Auk, 127(4), pp.752-758.

Dzal, Y.A. and Brigham, R.M., 2013. The tradeoff between torpor use and reproduction in little brown bats (*Myotis lucifugus*). Journal of Comparative Physiology B, 183(2), pp.279-288.

Environment Canada, 2015. Recovery strategy for little brown myotis (*Myotis lucifugus*), northern myotis (*Myotis septentrionalis*), and tri-colored bat (*Perimyotis subflavus*) in Canada [Proposed]. Species at Risk Act Recovery Strategy Series.

Forbes, A. A. & Krimmel, B. A. (2010) Evolution Is change in the inherited traits of a population through successive generations. Nature Education Knowledge 3(10):6

Garnier, E., Navas,M.L., and Grigulis, K., 2015. A functional approach to biological diversity. In: Plant Functional Diversity: Organism traits, community structure, and ecosystem properties. Garnier, E., Navas,M.L., and Grigulis, K.,. Published to Oxford Scholarship Online: March 2016. DOI: 10.1093/acprof:oso/9780198757368.001.0001

Garroway, C.J. and Broders, H.G., 2008. Day roost characteristics of northern long-eared bats (Myotis septentrionalis) in relation to female reproductive status. Ecoscience, 15(1), pp.89-93.

González-Palomares, E., López-Jury, L., Wetekam, J., Kiai, A., García-Rosales, F. and Hechavarria, J.C., 2021. Male Carollia perspicillata bats call more than females in a distressful context. Royal Society Open Science, 8(5), p.202336.

Hammer, M. and Arlettaz, R., 1998. A case of snake predation upon bats in northern Morocco: some implications for designing bat grilles. Journal of Zoology, 245(2), pp.211-212.

Heinen, J.T. and Hammond, G., 1997. Antipredator behaviors of newly metamorphosed green frogs (*Rana clamitans*) and leopard frogs (*R. pipiens*) in encounters with eastern garter snakes (*Thamnophis s. sirtalis*). American Midland Naturalist, pp.136-144.

Hernández, F., 2020. Ecological discord and the importance of scale in scientific inquiry. The Journal of Wildlife Management, 84(8), pp.1427-1434.

Hoogland, J.L., 1979. The effect of colony size on individual alertness of prairie dogs (Sciuridae: *Cynomys* spp.). Animal Behaviour, 27, pp.394-407.

Humphrey, C. 2017. Recovery Strategy for the Eastern Small-footed Myotis (*Myotis leibii*) in Ontario. Ontario Recovery Strategy Series. Prepared for the Ontario Ministry of Natural Resources and Forestry, Peterborough, Ontario. vii + 76 pp.

Jackson, A. S. 1969. A handbook for bobwhite quail management in the west Texas Rolling Plains. Texas Parks and Wildlife Department Bulletin No. 48, Austin, USA.

Johnson, J.B. and Gates, J.E., 2008. Spring migration and roost selection of female *Myotis leibii* in Maryland. Northeastern Naturalist, 15(3), pp.453-460.

Johnson, J.S., Kiser, J.D., Watrous, K.S. and Peterson, T.S., 2011. Day-roosts of *Myotis leibii* in the Appalachian Ridge and Valley of West Virginia. Northeastern Naturalist, 18(1), pp.95-106.

Kalcounis, M.C. and Brigham, R.M., 1994. Impact of predation risk on emergence by little brown bats, Myotis lucifugus (Chiroptera: Vespertilionidae), from a maternity colony. Ethology, 98(3-4), pp.201-209.

Kanda, L.L., Louon, L. and Straley, K., 2012. Stability in activity and boldness across time and context in captive Siberian dwarf hamsters. Ethology, 118(6), pp.518-533.

Kilgour, R.J. and Brigham, R.M., 2013. The relationships between behavioural categories and social influences in the gregarious big brown bat (*Eptesicus fuscus*). Ethology, 119(3), pp.189-198.

Kopp, S. D., F. S. Guthery, N. D. Forrester, and W. E. Cohen. 1998. Habitat selection modeling for northern bobwhites on subtropical rangeland. Journal of Wildlife Management 62:884–895.

Kunz, T.H. and Anthony, E.L., 1982. Age estimation and post-natal growth in the bat *Myotis lucifugus*. Journal of Mammalogy, 63(1), pp.23-32.

Lausen, C.L. and Barclay, R.M., 2003. Thermoregulation and roost selection by reproductive female big brown bats (*Eptesicus fuscus*) roosting in rock crevices. Journal of Zoology, 260(3), pp.235-244.

Lausen, C.L. and Barclay, R.M., 2006. Benefits of living in a building: big brown bats (*Eptesicus fuscus*) in rocks versus buildings. Journal of Mammalogy, 87(2), pp.362-370.

Lee, Y.F. and McCracken, G.F., 2001. Timing and variation in the emergence and return of Mexican free-

tailed bats, *Tadarida brasiliensis mexicana*. *Zoological Studies* 40 (4): 309–16.

Lehmann, V. W. 1984. Bobwhites in the Rio Grande Plain of Texas. Texas A&M University Press, College Station, USA.

Lehtonen, J. and Jaatinen, K., 2016. Safety in numbers: the dilution effect and other drivers of group life in the face of danger. Behavioral Ecology and Sociobiology, 70(4), pp.449-458.

Lendvai, A.Z., Akçay, Ç., Ouyang, J.Q., Dakin, R., Domalik, A.D., St John, P.S., Stanback, M., Moore, I.T. and Bonier, F., 2015. Analysis of the optimal duration of behavioral observations based on an automated continuous monitoring system in tree swallows (*Tachycineta bicolor*): is one hour good enough?. PLoS One, 10(11), p.e0141194.

Lewis, S.E., 1995. Roost fidelity of bats: a review. Journal of Mammalogy, 76(2), pp.481-496.

Lima, S.L., 1998. Nonlethal effects in the ecology of predator-prey interactions. Bioscience, 48(1), pp.25-34.

Lima, S.L. and O'Keefe, J.M., 2013. Do predators influence the behaviour of bats?. Biological Reviews, 88(3), pp.626-644.

Lino, A., Fonseca, C., Goiti, U. and Pereira, M.J.R., 2014. Prey selection by *Rhinolophus hipposideros* (Chiroptera, Rhinolophidae) in a modified forest in Southwest Europe. Acta Chiropterologica, 16(1), pp.75-83.

Lintott, P.R., Bunnefeld, N., Fuentes-Montemayor, E., Minderman, J., Mayhew, R.J., Olley, L. and Park, K.J., 2014. City life makes females fussy: sex differences in habitat use of temperate bats in urban areas. Royal Society Open Science, 1(3), p.140200.

Lodé, T., Lélias, M.L., Lemasson, A. and Blois-Heulin, C., 2021. Solitary versus group living lifestyles, social group composition and cooperation in otters. Mammal Research, 66(1), pp.13-31.

Lynch, M. and Walsh, B., 1998. Genetics and analysis of quantitative traits. *CEUR Workshop Proceedings*. Sinauer. Vol. 1. Sunderland, MA.

Maiti, U., Sadowska, E.T., ChrzĄścik, K.M. and Koteja, P., 2019. Experimental evolution of personality traits: open-field exploration in bank voles from a multidirectional selection experiment. Current Zoology, 65(4), pp.375-384.

Marchetti, C. and Drent, P.J., 2000. Individual differences in the use of social information in foraging by captive great tits. Animal Behaviour, 60(1), pp.131-140.

Mathot, K.J., Wright, J., Kempenaers, B. and Dingemanse, N.J., 2012. Adaptive strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. Oikos, 121(7), pp.1009-1020.

Mayor, S.J., Schneider, D.C., Schaefer, J.A. and Mahoney, S.P., 2009. Habitat selection at multiple scales. Ecoscience, 16(2), pp.238-247.

Menzies, A.K., 2010. Does personality correlate with energetics in little brown bats (*Myotis lucifugus*)? (Doctoral dissertation, Department of Biology, University of Winnipeg).

Menzies, A.K., Timonin, M.E., McGuire, L.P. and Willis, C.K., 2013. Personality variation in little brown bats. PLoS One, 8(11), p.e80230.

Michelena, P., Jeanson, R., Deneubourg, J.L. and Sibbald, A.M., 2010. Personality and collective decision-making in foraging herbivores. Proceedings of the Royal Society B: Biological Sciences, 277(1684), pp.1093-1099.

Moosman, P.R., Veilleux, J.P., Pelton, G.W. and Thomas, H.H., 2013. Changes in capture rates in a community of bats in New Hampshire during the progression of white-nose syndrome. Northeastern Naturalist, 20(4), pp.552-558.

Moosman Jr, P.R., Warner, D.P., Hendren, R.H. and Hosler, M.J., 2015. Potential for monitoring eastern small-footed bats on talus slopes. Northeastern Naturalist, 22(1), pp.NENHC-1.

Murray, S.W. and Kurta, A., 2004. Nocturnal activity of the endangered Indiana bat (Myotis sodalis). Journal of Zoology, 262(2), pp.197-206.

Nakagawa, S. and Schielzeth, H., 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biological Reviews, 85(4), pp.935-956.

Wilson, D. E., and D. M. Reeder (editors). 2005. Mammal species of the world: a taxonomic and geographic reference. Third edition. The Johns Hopkins University Press, Baltimore. Two volumes. 2,142 pp. Available online at: https://www.departments.bucknell.edu/biology/resources/msw3/

Olson, C.R. and Barclay, R.M., 2013. Concurrent changes in group size and roost use by reproductive female little brown bats (*Myotis lucifugus*). Canadian Journal of Zoology, 91(3), pp.149-155.

https://doi.org/10.1139/cjz-2012-0267.

Réale, D., Dingemanse, N.J., Kazem, A.J. and Wright, J., 2010. Evolutionary and ecological approaches to the study of personality. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1560), pp.3937-3946.

Réale, D., Gallant, B.Y., Leblanc, M. and Festa-Bianchet, M., 2000. Consistency of temperament in bighorn ewes and correlates with behaviour and life history. Animal Behaviour, 60(5), pp.589-597.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T. and Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. Biological Reviews, *82*(2), pp.291-318.

Riskin, D.K., Bertram, J.E. and Hermanson, J.W., 2005. Testing the hindlimb-strength hypothesis: non-aerial locomotion by Chiroptera is not constrained by the dimensions of the femur or tibia. Journal of Experimental Biology, 208(7), pp.1309-1319.

Riskin, D.K., Parsons, S., Schutt Jr, W.A., Carter, G.G. and Hermanson, J.W., 2006. Terrestrial locomotion of the New Zealand short-tailed bat *Mystacina tuberculata* and the common vampire bat *Desmodus rotundus*. Journal of Experimental Biology, 209(9), pp.1725-1736.

Sih, A., Bell, A. and Johnson, J.C., 2004a. Behavioral syndromes: an ecological and evolutionary overview. Trends in ecology & evolution, 19(7), pp.372-378.

Sih, A., Bell, A.M., Johnson, J.C. and Ziemba, R.E., 2004b. Behavioral syndromes: an integrative overview. The Quarterly Review of Biology, 79(3), pp.241-277.

Solari, S. 2018. *Myotis leibii*. The IUCN Red List of Threatened Species 2018: e.T14172A22055716. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T14172A22055716.en

Solari, S. 2021. *Myotis lucifugus* (amended version of 2018 assessment). The IUCN Red List of Threatened Species 2021: e.T14176A208031565. https://dx.doi.org/10.2305/IUCN.UK.2021-3.RLTS.T14176A208031565.en

Solick, D.I. and Barclay, R.M., 2006. Thermoregulation and roosting behaviour of reproductive and nonreproductive female western long-eared bats (*Myotis evotis*) in the Rocky Mountains of Alberta. Canadian Journal of Zoology, 84(4), pp.589-599.

Sorato, E., Gullett, P.R., Griffith, S.C. and Russell, A.F., 2012. Effects of predation risk on foraging behaviour and group size: adaptations in a social cooperative species. Animal Behaviour, 84(4),

pp.823-834.

Thomson, T., 2013. Roost ecology of eastern small-footed bats (*Myotis leibii*) in the southern Appalachian Mountains (Doctoral dissertation, Indiana State University).

Travis, J.M. and Dytham, C., 1999. Habitat persistence, habitat availability and the evolution of dispersal. Proceedings of the Royal Society of London. Series B: Biological Sciences, 266(1420), pp.723-728.

Tuttle, M.D., Kiser, M. and Kiser, S., 2005. The bat house builder's handbook. University of Texas Press.

Tuttle, M. 1973. Maternity Habits of *Myotis Leibii* in South Dakota, 73, pp. 80–83. http://www.biodiversitylibrary.org/item/106891.

Tuttle, M.D., 1974. An improved trap for bats. Journal of Mammalogy, 55(2), pp.475-477.

Verbeek, M.E., Drent, P.J. and Wiepkema, P.R., 1994. Consistent individual differences in early exploratory behaviour of male great tits. Animal Behaviour, 48(5), pp.1113-1121.

Verbeek, M.E., Boon, A. and Drent, P.J., 1996. Exploration, aggressive behaviour and dominance in pair-wise confrontations of juvenile male great tits. Behaviour, 133(11-12), pp.945-963.

Verbeek, M., 1998. Bold or cautious: behavioural characteristics and dominance in great tits. Wageningen University and Research.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S.M., Schlichting, C.D. and Van Tienderen, P.H., 1995. Adaptive phenotypic plasticity: consensus and controversy. Trends in ecology & evolution, 10(5), pp.212-217.

Viele, D.P., Kurta, A. and Kath, J.A., 2002. Timing of nightly emergence. Indiana Bat Biol Manag an endagered species, pp.199-207.

Vonhof, M.J. and Barclay, R.M., 1996. Roost-site selection and roosting ecology of forest-dwelling bats in southern British Columbia. Canadian Journal of Zoology, 74(10), pp.1797-1805.

Webber, Q.M. and Willis, C.K., 2018. An experimental test of effects of ambient temperature and roost quality on aggregation by little brown bats (*Myotis lucifugus*). Journal of Thermal Biology, 74, pp.174-180.

Wheatley, M. and Johnson, C., 2009. Factors limiting our understanding of ecological scale. Ecological complexity, 6(2), pp.150-159.

White, S.J., Pascall, D.J. and Wilson, A.J., 2020. Towards a comparative approach to the structure of animal personality variation. Behavioral Ecology, 31(2), pp.340-351.

Willems, E.P. and Hill, R.A., 2009. Predator-specific landscapes of fear and resource distribution: effects on spatial range use. *Ecology*, *90*(2), pp.546-555.

Willis, C.K. and Brigham, R.M., 2007. Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. Behavioral Ecology and Sociobiology, 62(1), pp.97-108.

Wilson, A.D., Whattam, E.M., Bennett, R., Visanuvimol, L., Lauzon, C. and Bertram, S.M., 2010. Behavioral correlations across activity, mating, exploration, aggression, and antipredator contexts in the European house cricket, Acheta domesticus. Behavioral Ecology and Sociobiology, 64(5), pp.703-715.

Zukal, J., 2020. Handbook of the Mammals of the World. Journal of Vertebrate Biology, 69(4), pp.E2003-1.