Do little brown bats (*Myotis lucifugus*) use conspecific
calls and odours to find new roosts?

by

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ABSTRACT

Do little brown bats (*Myotis lucifugus*) use conspecific
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I tested the hypothesis that conspecific calls and odours would attract little brown bats (*Myotis lucifugus*) to new roosts. I predicted bats would discover and use treatment boxes before control boxes. I built six new bat boxes and placed them near pre-existing roost boxes on the Hubley property, Newfoundland, Canada. Conspecific calls were broadcasted from one box and a cloth scented with conspecific odours placed in another. The other four boxes were controls. I compared the discovery date and number of bat visits between box types. Contrary to my hypothesis, bats did not first discover or prefer treatment boxes, thus these cues did not attract them to new roosts. The highest number of bat visits were made by juveniles to one control box towards the end of the study. Perhaps juvenile bats become more exploratory at the end of the summer when they are self-sufficient and leaving their summer roosts.

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INTRODUCTION

Animal communication

There are many reasons animals communicate, such as to signal the location of food to group members (e.g. Biesmeijer & Seeley, 2005), to compete for mates (e.g. Clutton-Brock et al., 1979), to warn of predators (e.g. Kirchhof & Hammerschmidt, 2006), to defend territories (e.g. Davies, 1976), and to beg for food (e.g. Kolliker et al., 1998). For example, some species may use conspicuous colouring to warn away predators (Schuler & Hesse, 1985; Summers & Clough, 2001), while others use this mode of communication to attract mates (Milinski & Bakker, 1990; Saetre et al., 1994). Communication allows interaction among individuals, and can be important for group cohesion and functioning (Dugatkin, 2009; Healy & Rowe, 2010). The most well-conserved form of communication is chemical (i.e., pheromones), and it remains important for organisms of most vertebrate taxa (Enquist et al., 2010; Mappes & Stevens, 2010; Wilson, 1975). Some advantages of chemical communication include the ability to transmit the signal through darkness and around obstacles, a potentially long signal life compared to other signal types, and a potentially large range of transmission. The greatest drawbacks include the slow speed of transmission and fade-out of the signals (Enquist et al., 2010; Mappes & Stevens, 2010; Wilson, 1975). An example of chemical communication is that of scent-marking by canids. They mark their territories to advertise their presence to potential intruding conspecifics, communicate with pack members, and advertise themselves to potential mates or contenders (Apps et al., 2012; Peters & Mech, 1975). Another example of communication via chemicals is seen in many ant species,

where individuals lay trail pheromones to guide other workers to food sources (Attygalle & Morgan, 1984; Morgan, 2009).

Auditory communication is another important form of communication used by many animals (Enquist et al., 2010; Mappes & Stevens, 2010; Wilson, 1975). Like chemicals, acoustic signals can transmit through darkness and around obstacles. They can also travel long distances, often exceeding that of chemical signals. However, acoustic signals are more conspicuous and energetically expensive to produce than chemical signals (Enquist et al., 2010; Mappes & Stevens, 2010; Wilson, 1975). An example of auditory communication is singing in many bird species, where males sing to attract females and to keep other males out of their territory (Searcy & Andersson, 1986; Weisman & Ratcliffe, 2004). Another acoustic signal example is the context-specific suite of alarm calls used by many monkey species, such as chimpanzees (*Pan troglodytes*; Crockford & Boesch, 2003) and tamarins (*Saingunus fuscicollis* and *S. mystax*; Kirchhof & Hammerschmidt, 2006). There are several other modes of communication, including visual (Montgomerie et al., 2001; Peters & Mech, 1975), electrical (Arnegard & Carlson, 2005; Curtis & Stoddard, 2003), and tactile forms (Hertenstein, 2002; Lihoreau & Rivault, 2008). All have important functions in conveying information from the signaler to the recipient (Enquist et al., 2010; Mappes & Stevens, 2010; Wilson, 1975).

Bat biology and communication

Bats (Chiroptera) are the second largest mammalian order, and exhibit a vast range of ecological and behavioural diversity (Altringham, 2011; Dechmann & Safi, 2005; Fenton, 2001). They are also K-selected species, with most giving birth to one or

two young per year that weigh between 1/4 to 1/3 of the mother's weight. In addition,

they are long-lived, with individuals of a number of species having lived more than 30

years (Altringham, 2011; Fenton, 2001). Order Chiroptera has a variety of social systems,

with most species being social at some point during the year (Kerth, 2008; Kunz &

Lumsden, 2003). For example, proboscis bats (*Rhynchonycteris naso*) live in mixed male-

female colonies and defend their territories (Bradbury & Vehrencamp, 1976). Thomas'

fruit-eating bats (*Artibeus watsoni*) live in moderately stable harems with one male and

multiple females, but no social bonds or cooperative behaviours occur among group

members (Chaverri et al., 2007). The common vampire bat (*Desmodus rotundus*) also

lives in harems but their society is more complex and stable than *Artibeus watsoni*; social

bonds and cooperative behaviours such as allofeeding and allogrooming occur among

group members (Wilkinson, 1984, 1986). Another type of social system in bats, and

common among temperate species, is maternity colonies, where females roost and raise

their young together. Some form of social bond is common and some cooperative

behaviours, such as mutual warming, often occur among group members (Kerth, 2008;

Neuweiler, 2000). Both the little brown bat (*Myotis lucifugus*; Broders & Forbes, 2004)

and Bechstein's bat (*Myotis bechsteinii*; Kerth & König, 1999) live in maternity colonies

during the summer. Males of these species roost either solitarily or in small groups

usually separate from the females.

Communication is necessary to maintain social systems (Wilson, 1975), but we do

not yet have a complete understanding of how this is achieved in bats (Altringham &

Fenton, 2003; Dechmann & Safi, 2005). Bats rely mainly on hearing, olfaction, and

vision to perceive their environment, and likely communicate for the same multitude of

reasons as other taxa (Altringham & Fenton, 2003; Neuweiler, 2000). Much of the research on communication in bats has looked at the exchange of information about foraging sites. For example, evening bats (*Nycticeius humeralis*) transfer information by leading each other to foraging sites (Wilkinson, 1992), while greater spear-nosed bats (*Phyllostomus hastatus*) use screech calls to recruit and coordinate foraging among group members (Wilkinson & Boughman, 1998). Other studies have focused on communication about roosting sites. Bechstein's bats, which switch communal roosts regularly, transfer information about suitable roosts among colony-members (Kerth & König, 1999; Kerth & Reckardt, 2003). Information transfer about hibernacula may also be important, particularly for the swarming behaviour of bats. For example, little brown bats use conspecific calls to find the entrance to hibernacula during swarming (Avery et al., 1982; Barclay, 1982; Fenton, 1969).

Suitable roosts are a vital resource for bats, as they offer protection from weather and predators, potentially confer physiological benefits, and may be rare depending on the type of roost, such as in the case of caves or abandoned mines (Humphrey, 1975; Kunz & Lumsden, 2003). Bats roost in a variety of locations including stable roosts such as caves and rock crevices, and more ephemeral roosts such as under tree bark or leaves. Roost selection within species may also vary by sex (Humphrey, 1975; Kunz & Lumsden, 2003). In the fall and winter, little brown bats hibernate in underground openings such as caves and abandoned mines, while maternity roosts of this species are commonly found in man-made structures, such as houses and bat boxes (Fenton, 1970; Fenton & Barclay, 1980). During the summer, female little brown bats exhibit low roost fidelity, switching roosts every 3-10 days (Crampton & Barclay, 1998). Although they switch roosts, they

have a high fidelity to roost areas, with individuals switching between alternate roosts within these areas (Humphrey & Cope, 1976; Kunz & Lumsden, 2003; Norquay et al., 2013). The distance between successive roosts is typically 200-1000 metres in little brown bats (Broders et al., 2006; Crampton & Barclay, 1998). Roost switching may have several advantages, including lowering ectoparasite loads and reduced predation potential (Fenton et al., 1994; Reckardt & Kerth, 2007; Kunz & Lumsden, 2003). Weather may also be an important factor to explain roost-switching; each roost may have a slightly different microclimate and bats may choose warmer or cooler roosts depending on ambient temperature, time of year, body condition, and reproductive status, as some roosts will provide more optimal temperatures for raising young compared to others (Humphrey, 1975; Kunz, 1982; Kunz & Lumsden, 2003). Since they likely consider many features when they choose a roost, bats presumably search out and sample new roosts to determine which ones are suitable, but few research studies have focused on this subject.

It has been demonstrated that bats use memory for various spatial tasks. For example, they are able to remember the location of foraging (Clarin et al., 2013; Melber, Fleischmann, & Kerth, 2013) and roosting sites (Humphrey & Cope, 1976; Norquay et al., 2013) and can learn to associate various sensory cues with the availability of food (Page & Ryan, 2005; Page, von Merten, & Siemers, 2012). Further, bats retain these memories throughout hibernation (Ruczyński & Siemers, 2011). Microbats rely on echolocation for small-scale spatial orientation (Jensen et al., 2005), and use it in combination with spatial memory to locate previously used roosts (Schnitzler et al., 2003). Recent research demonstrates the role of echolocation in finding new roosts as

well (Ruczynski et al., 2007; 2009). However, observations imply it can be a difficult task to discern between suitable roosts and the rest of the environment using echolocation alone. The results of these experiments suggest bats may rely on additional sensory cues, such as conspecific calls and odours, to find novel roosts (Ruczynski et al., 2007; 2009).

The auditory system is an important component of the sensory system in many bat species, especially the echolocating microchiroptera. Echolocating bats emit sound waves and analyze the echoes to build a "picture" of the immediate landscape. This allows them to orient in the environment and identify the location and size of objects, such as prey or roosts, without the use of vision. As such, echolocation is critical to many bat species that are active at night (Altringham, 2011; Fenton, 2001; Griffon, 1986). The characteristics of bat echolocation calls vary based on the ecology and foraging behaviour of a species, and can be divided into three main groups. Bats that forage in cluttered areas, like a forest, generally have short, low-intensity, broadband echolocation calls ideal for locating and identifying prey in a high-clutter environment (Fenton, 1990; Norberg & Rayner, 1987). The brown long-eared bat (*Plecotus auritus*) is an example of a bat with this type of ecology and echolocation call characteristics (Waters & Jones, 1995). There are also species that forage along edges, such as above water like the little brown bat (Fenton & Bell, 1979). These individuals usually have short, high-intensity echolocation calls with both narrowband and broadband components (Fenton, 1990; Norberg & Rayner, 1987). Bats that forage in open areas, such as the common noctule (*Nyctalus noctula*; Rachwald, 1992), generally have long, high-intensity, narrowband calls of lower frequency ideal for hunting over long distances (Fenton, 1990; Norberg & Rayner, 1987).

Bats can also change some characteristics of their echolocation calls depending on the situation. For example, many species increase the rate of call production as they close in on a prey target; called a feeding buzz, this provides the bat with more precise information on the location and movement dynamics of their prey (Fenton, 2001; Griffon, 1986). Bats may also change the frequency of their calls in several other situations, such as to avoid collisions with other bats (Ulanovsky et al., 2004). Research shows little brown bats use echolocation calls for general orientation, and modified calls during near-collisions and the as newly volant young during flight (Barclay, Fenton, & Thomas, 1979). Bats also emit non-echolocation calls called social calls, which, unlike echolocation calls that are mainly ultrasonic, are often audible to humans. These calls are used in a variety of situations, such as agonistic interactions, mating, or mother-pup interactions (Barclay et al., 1979; Fenton, 2001; Pfalzer & Kusch, 2003). For example, little brown bats use social calls to defend their position within a roost, to aid in recognition of mothers and pups, and to signal a male's interest to a female during mating (Barclay et al., 1979).

A significant body of research has accumulated on our understanding of the role of vocalizations in bat communication. For example, Jones and Siemers (2011) suggested that echolocation calls may encode information on geographic location, colony, sex, body size, and age in various bat species. Research on little brown bats has shown there is significant variation in the structure of the echolocation calls of individuals from different maternity roosts (Pearl & Fenton, 1996), and differences among individual bats reveal individual identity, state of lactation, and age in some situations (Kazial et al., 2008). Some studies have looked further to determine if bats can recognize these differences in

call structure and thus discriminate between calls of different bats. Experiments with several bat species have revealed that some can identify the sex of the caller (Kazial & Masters, 2004), discriminate between calls from different caves and calls of different groups within a cave (Boughman & Wilkinson, 1998), identify familiar and unfamiliar individuals (Voigt-Heucke et al., 2010), and discriminate between specific individuals based on their echolocation calls (Yovel et al., 2009). Kazial, Kenny, and Burnett (2008) found that little brown bats can also recognize individuals based on variation in their echolocation calls. This ability to distinguish between conspecifics could have implications for the social behaviour of a species. For example, Bechstein's bats live in fission-fusion societies, where colonies regularly split and regroup, but often form regular groups within their colony (Kerth & König, 1999). The ability to distinguish one colony-member from another could be important for the formation and cohesion of these groups.

It is not only important to understand if bats can recognize differences in the calls of conspecifics, but if and how a bat will respond to these calls. Several studies have looked at the response of bats to social calls of conspecifics. For example, social calls can be important for foraging: they can be used to warn off other bats when insects are scarce (Barlow & Jones, 1997) or coordinate foraging at a feeding site (Wilkinson & Boughman, 1998). Social calls also provide information about the location of conspecifics to free-flying individuals and attract them to roosts (Furmankiewicz et al., 2011; Schöner et al., 2010). Research has also looked at how bats respond to echolocation calls of conspecifics. Dechmann et al. (2009) and Gillam (2007) have shown that bats are attracted by conspecific echolocation calls produced during prey capture. A jamming avoidance strategy has also been observed in some bat species; the big brown bat

(*Eptesicus fuscus*) may cease vocalizing when flying with conspecifics and uses conspecific calls to navigate (Chiu et al., 2008), while the European free-tailed bat (*Tadarida teniotis*) changes the frequency of its calls when flying and foraging with conspecifics to avoid collisions (Ulanovsky et al., 2004). Two experiments by Ruczynski et al. (2007, 2009) found that playback of conspecific echolocation calls decreased search time of three bat species to find novel roosts. Research with the little brown bat has shown that this species approaches conspecific social and echolocation calls while foraging and locating current roosts (Avery et al., 1982; Balcombe & Fenton, 1988; Barclay, 1982); thus these calls likely serve as cues for the location of critical resources, and could be important for facilitating the location of novel roosts.

Some research on the olfactory system of bats has been devoted to understanding the role of the vomeronasal organ and whether it signifies well-developed olfactory capabilities. This olfactory organ is found in many mammals and is known to regulate sexual behaviour through pheromones, which are a type of intraspecific signal (Neuweiler, 2000; Schmidt, 1985). Of the bat species examined, the vomeronasal organ has only been found in the Neotropical leaf-nosed bats (Phyllostomidae), Parnell's mustached bat (*Pteronotus parnellii*), and the common bent-winged bat (*Miniopterus schreibersii*) (Cooper & Bhatnagar, 1976). It has been suggested that this organ plays a role in synchronizing the reproductive cycles within colonies. However, many bat species without a vomeronasal organ manage to synchronize their reproductive cycles very precisely (Neuweiler, 2000). The other suggestion is that the function of this organ, due to its location, is connected to the examination of food in, or close to the mouth, specifically the detection of food odour and flavour (Cooper & Bhatnagar, 1976; Neuweiler, 2000).

This makes sense in the vampire bats as they feed on blood of other mammals, which thus involves the recognition of pheromones from taxonomically related species, but is less plausible in frugivorous bats as plants belong to completely different taxa than mammals. As such, the role of this organ in bat olfaction is still unclear (Neuweiler, 2000; Schmidt, 1985).

A significant portion of research on the importance of olfaction in bats has focused on fruit bats since they have obvious and well-developed primary olfactory systems (Neuweiler, 2000). Research has shown that olfactory cues are important for foraging in fruit bats, and various frugivorous species can locate fruit (Raghuram et al., 2009; Tang et al., 2007), and distinguish between ripe vs. unripe fruit based on olfactory cues alone (Hodgkison et al., 2007; Korine & Kalko, 2005; Luft et al., 2003; Thies et al., 1998). In addition, the Indian short-nosed fruit bat (*Cynopterus sphinx*) can learn to associate a non-fruit odour with the presence of food (Acharya et al., 1998; Zhang et al., 2014).

In contrast to frugivorous bats, insectivorous species have small, simple olfactory structures, which may indicate a reduced olfactory capacity (Neuweiler, 2000). However, many insectivorous species appear to use olfaction for communication (Dechmann & Safi, 2005). Odour profiles of glandular secretions are unique among individuals, and may contain information about age and sexual maturity (Caspers et al., 2011), or colony and individual identity (Safi & Kerth, 2003). Other studies have investigated whether bats can differentiate among other bats based on odour cues. Research has shown insectivorous bats can discriminate between familiar and unfamiliar conspecifics (Bloss et al., 2002; De Fanis & Jones, 1995a), and between sexes (Bouchard, 2001) based on odour

cues. In addition, mothers can distinguish their own infant's odour from other conspecific infants, and pups reciprocate this recognition (De Fanis & Jones, 1995b; Loughry & McCracken, 1991). Little brown bats often initiate muzzle to muzzle contact after landing in hibernacula during swarming. This species' pararhinal glands, a type of sebaceous gland located on the snout, are large and conspicuous during mating, and appear to produce behaviourally significant secretions (Thomas et al., 1979). Further, this species uses scent posts; mothers and volant pups visited and marked a tree along the way to a foraging site, which may have provided an orientation cue for newly volant young to locate roosts (Buchler, 1980). Recent experiments have looked at novel roost-finding behaviour in bats. Ruczynski et al. (2007, 2009) found that olfactory cues may have decreased search time for new roosts. Female Bechstein's bats were able to recognize roosts that were recently used and thus most likely parasitized by the bat fly *Basilia nana*, even if roosts were unfamiliar. The authors suggested recognition may have been assisted by olfaction of bat urine and droppings, as only recently occupied boxes were avoided, but not those from previous years (Reckardt & Kerth, 2007). More studies are needed to determine the role of olfaction in finding new roosts.

Life history of study species

At the end of summer or beginning of fall (August/September in Newfoundland), little brown bats abandon summer roosts and large congregations of bats begin to occur at the entrance of underground openings such as caves and abandoned mines where swarming occurs (Fenton, 1969; Norquay et al., 2013). During swarming, mating is promiscuous and indiscriminate (Thomas et al., 1979). Females store the sperm from

swarming during hibernation and fertilization occurs in the spring (Fenton & Barclay, 1980; Thomas et al., 1979). When hibernation ends, bats disperse to their summering sites, with females presumably heading to the same maternity roost-sites each year (Fenton & Barclay, 1980; Norquay et al., 2013). During summer, adult females forage during the evening and may have multiple bouts of feeding with periods of roosting in between (Anthony et al., 1981; Fenton & Barclay, 1980). Females may use night-roosts during these periods instead of returning to their day (maternity) roosts, which allows them to travel further to foraging sites (Henry et al., 2002). When temperature is low, this species will forage for shorter periods and roost longer during the night (Anthony et al., 1981; Kunz, 1982). During the day, they roost together in day-roosts and often go into torpor. If daytime temperatures are high, individuals may arouse from torpor more frequently and move to cooler areas of the roost (Anthony et al., 1981; Kunz, 1982). Grooming is also common during arousal from torpor (Fenton, 1969; Kunz, 1982).

Gestation lasts approximately two months in little brown bats and birth is staggered, usually occurring over a three-week period mid-summer when the abundance of insects increases (Fenton & Barclay, 1980; Humphrey & Cope, 1976; O'Farrell & Studier, 1973). After giving birth, the foraging behaviour of females changes and they visit the maternity roosts more often during the night to nurse their pups. As such, the distance they travel to foraging sites may greatly decrease (Henry et al., 2002). Energy demands on the females are also greatest during lactation (Fenton & Barclay, 1980). Pups eyes open just after birth and they start to produce vocalizations almost immediately. In approximately 10 days they can thermoregulate, and by week 3 they can fly. Juveniles begin consuming insects and weaning starts during week 3 as well (Buchler, 1980; Fenton

& Barclay, 1980). The first flights of juveniles are clumsy but within several weeks their flight becomes indistinguishable from adults and juveniles become self-reliant (Buchler, 1980; O'Farrell & Studier, 1973). Their vocalizations also change over this time; at first their echolocation calls are of a lower frequency than adults but these differences disappear over the course of the first week of flight (Barclay et al., 1979; Buchler, 1980).

Hypothesis and predictions

The goal of my experiment was to investigate the communicative potential of bat calls and odours. Specifically, I tested the hypothesis that conspecific calls and odours would attract female little brown bats to new roosts, and that this species could locate new roosts more easily when these cues were present. I predicted that bats would discover and use new roost boxes (i.e., bat boxes) with conspecific calls or odours earlier and in greater numbers than control roost boxes. Specifically, I predicted that the cumulative number of bat visits per day (CBV) at bat boxes would be greater in those boxes with conspecific calls and odours than control boxes. I also predicted bats would discover the conspecific call box first and the CBV would be higher in this box compared to the conspecific odour box.

METHODS

Study site

This experiment was conducted from May 16 to August 16 2015 on private property belonging to the Hubley family in Newfoundland, Canada (coordinates:

47°15'29.8"N 53°17'26.6"W) adjacent to Salmonier Nature Park, Newfoundland. Salmonier is a wildlife rehabilitation park that has a maternity colony of >1000 wild little brown bats that roost in 11 bat boxes during the day. This experiment focused on three of these boxes (boxes 109, 110, and 111) and the bats roosting in them. These three boxes are situated one kilometre south along the road (route 90 - Salmonier Line) from the main park entrance. Box 111 is located on the side of a barn, while boxes 109 and 110 are located back to back on a pole approximately 3 m above ground level and 1.5 m from the barn. Box 111 was built by the owner's family and placed on the property many years ago (exact date unknown) while boxes 109 and 110 were built and placed on the Hubley property by Salmonier staff between 2006 and 2008. I chose the Hubley property to conduct my experiment because it is an isolated site very close to Salmonier and has been part of our research efforts (i.e., trapping and tagging) in Newfoundland since 2011.

Experimental set-up

Six new wooden, single-chamber bat boxes were built and erected on wooden poles approximately 2 m above ground level at the Hubley property on May 16, 2015. Bats were first present in the area at the end of April. Boxes were spaced 8 m apart from each other and the pre-existing boxes on the property (Figure 1). Box locations and spacing were chosen based on the size and geography of the site. In addition, all box locations were chosen with a clear line-of-sight to the pre-existing boxes to prevent any obstacles (such as the house or barn) interfering with the conspecific calls being heard at the pre-existing boxes. Each box location was assigned a number and the location for the

treatment boxes were chosen randomly by pulling numbers from a hat. All new boxes faced the same direction, towards the pre-existing bat boxes on the property (Figure 1).

Each box had a high-definition infrared video-camera (Birdhouse Spy Cam, West Linn, USA) inside at the top connected to a DVR (FLIR Systems Inc., Boston USA) to record any bats entering and exploring the box. Boxes measured 81.3 cm high, 40.6 cm wide, 14.6 cm deep at the top, and 10.1 cm deep at the bottom (Figure 2). The tapered profile provided a better view of the entire box from the camera. The materials used to construct the boxes were 3/8 inch plywood for the front, back and top, and 2 x 6 inch sides ripped to the tapered profile. Boxes were painted a dark brown to match the pre-existing bat boxes at the site. The boxes also had a 40.6 x 19.0 cm vertical landing pad at the bottom to allow bats to land below the box then crawl inside (Figure 2). The inside of the box and front of the landing pad were covered in white plastic mesh to enable bats to cling to the surface. A sheet of acrylic glass was placed inside of the box near the top separating the camera from the rest of the box to prevent bats crowding near the camera and blocking its view of the box. Cameras recorded video during the night from 20:00 to 06:00 to include sunrise and sunset. Video files were downloaded from the DVR onto an external hard-drive during the day.

Conspecific calls were broadcasted from box 3 and a cloth scented with conspecific odours was placed in box 2. The other four boxes (boxes 1, 4, 5, 6) were controls with no additional sensory cue. A PIT-antenna was also placed with subsequent PIT-reader (Dorset Identification, Aalten, Netherlands) at the entrance of the conspecific call and odour boxes, as well as control box 6 to identify any PIT-tagged individuals. At the beginning of the summer, approximately 1350 animals had been tagged at Salmonier

and Hubley's property since 2011. Another 262 bats were PIT-tagged at Salmonier and Hubley's over the summer in 2015.

Conspecific calls of little brown bats, which range from 40-90 kHz, were recorded on May 14, 2015 at a sampling frequency of 500 kHz from bat boxes 109 and 111 using an ultrasound microphone (M500, Pettersson Elektronik, Uppsala, Sweden) connected to a laptop (HP EliteBook 745, Hewlett-Packard Co., Mississauga, Canada). Intensity of the bat calls ranged from 50-90 dB. Recordings were taken at 20:00, when bats were in the roosts and vocalizing intensively. Before recording the bat calls, the microphone was turned on, and the settings adjusted to those we would use for recording calls of little brown bats (detecting frequencies between 40-90 kHz). We then observed the output to see if any background noise was picked up by the microphone. No background noise was detected, so we did not filter the bat calls after they were recorded. Calls were re-sampled to a sampling frequency of 192 kHz using Praat (Boersma & Weenink, 2014). Fourteen separate files were recorded, each approximately one minute long, and combined into a single ten-minute file which was played using Windows Media Player (Windows Media Player 12, Microsoft, 2016) on the laptop connected to a unidirectional ultrasound speaker with a built-in amplifier (L400, Pettersson Elektronik, Uppsala, Sweden). The speaker was placed inside a square wooden box (22.9 x 33.0 x 10.1 cm; Figure 2) to protect it from rain. This box was attached directly below the landing pad of bat box 3, and the speaker inside broadcasted the calls continuously from this location toward the pre-existing boxes. Calls were played as loudly as those heard from the pre-existing bat boxes and were audible from all experimental and pre-existing bat boxes at the site. A box with the same dimensions as the speaker box was placed below the other bat boxes as

controls. Calls were recorded from pre-existing roosts instead of recording from a bat in one of the new boxes to prevent any bias about the new boxes from that bat which may influence the decisions of other individuals through information transfer. In addition, the calls were recorded from the bat boxes on the Hubley property and not Salmonier Nature Park in case the bats from these two locations did not actively mix, which could result in bats only responding to other individuals from the same location.

I cut a square piece of cotton cloth (5 x 5 inches) and placed it in bat box 109 on May 8 and left it until May 16, 2015 to allow it to become scented with odours from the bats (urine/feces/glandular secretions, etc.). Then, this cloth was placed in bat box 2 and a second cotton cloth of the same size was placed in box 109 and left for one week. At the end of every week, these two cloths were switched to provide a fresh supply of conspecific odours in bat box 2. A cloth of the same material and size was placed in each of the other five boxes as controls. Scent was used from the bats at the Hubley property and not Salmonier Nature Park for the same reasons as the calls.

Data analysis

All video recordings were viewed and analyzed weekly using VLC Media Player (VideoLAN, Paris, France). Any conspicuous behaviours occurring around and within the boxes were noted and divided into four categories: 1. landing - refers to bats landing near the entrance of a box but not entering; 2. entering - refers to bats coming inside a box and investigating or grooming; 3. night-roosting - refers to bats that spent at least two consecutive minutes hanging upside down in one spot and often grooming during the night; 4. day-roosting - refers to bats that spent an entire day in a box. The first time a box

was entered was recorded and the date of entry was compared among the three box types. The time a bat arrived and departed was also noted to determine the number of bats within a box each day. In addition, the cumulative number of bat visits per day (CBV) for each box was calculated by adding the total number of bat visits to a box per day over seven days. Chi-Square tests were performed to look at the distribution of CBV's among all six boxes. A Fisher's Exact test was performed to determine if the probability of a bat visiting a treatment box was different from that of visiting a control box (presence/absence in control vs. treatment boxes; Sokal & Rohlf, 1995). Minitab random sample generator (Minitab 17, Minitab Inc., 2016) was used to select two control boxes to compare to the two treatment boxes. All tests are two-tailed and were performed using QuickCalcs (GraphPad Software Inc., 2016). Results were considered statistically significant when $P < 0.05$.

Management for white-nose syndrome

To help prevent the spread of white-nose syndrome, we took the following precautions: 1. all audio and video equipment that needed to be tested before use in the field was stored in a separate building from the bat lab at Saint Mary's University to prevent contamination with other bat equipment - all other items were purchased in Newfoundland; 2. researchers did not bring any equipment/clothing that was used in the field in Nova Scotia - only clean/new items were taken; 3. when handling bats, researchers wore clean Tyvek suits and nitrile gloves.

RESULTS

A total of 1,087 bat visits were recorded on the video cameras. Of these, 28 were in the conspecific call box, 12 in the conspecific odour box, and 1,047 among the four control boxes. Of the bat visits to the control boxes, the majority (95.32%) were to box 6. The probability of bats visiting a treatment box was not significantly different from those visiting a control box (presence/absence per week; Fisher's Exact Test: $p = 0.55$). Each of the four behaviours were observed (Table 1), and landing, entering, and night-roosting occurred throughout the night. Some control boxes appeared to be selected earlier than treatment boxes (Table 2) but there is no way to statistically test for differences in the Julian Day of first entry into a box. The first control box was selected on July 6 which was 52 days from its original placement. Female little brown bats were not observed in the treatment boxes until July 13 (59 days from first placement; conspecific odour box) and July 16 (62 days from first placement; conspecific call box). Of all the bats that investigated the odour box, 77.78% (7 of 9 visits) also investigated the scented cloth. In comparison, very few visits (0.02%; 8 of 507 visits) to other boxes resulted in investigation of the unscented cloths. The first box used as a day-roost was control box 4 on August 3, with one bat spending the day in the box. The only other box used as a day-roost was control box 6 on August 15, with 7 bats spending the day in the box. The treatment boxes were never used as day roosts.

A total of 62 PIT reads from 20 different bats were recorded at the new boxes, all of which were adult females and were recorded from control box 6. Four of these bats entered the new boxes, while the other sixteen only landed below the new boxes on the landing pad but never went inside. In addition, ten of these bats roosted in pre-existing

boxes at both Salmonier Nature Park (boxes 101-108) and the Hubley property (boxes 109-111), while the other ten bats visited only those boxes on the Hubley property during the summer in 2015. In comparison, a total of 5,233 PIT reads from 241 different bats were read in the pre-existing boxes on the Hubley property over the course of the summer in 2015.

The average weekly CBV's (mean $\pm$ SE) for the new boxes ranged from 1.29 $\pm$ 0.61 to 75.14 $\pm$ 48.11 over the course of the experiment (Figure 3). From weeks 8-12, the CBV's in all six boxes were similar (Figure A1). In the last four days of the experiment (end of week 13/beginning of week 14) the CBV in box 6 dramatically increased from 3.20 $\pm$ 2.71 to 255.00 $\pm$ 57.00 (Figure A2). Thus, the distribution of total bat visits among all six boxes was not similar ($\chi^2 = 2243.53$, df = 5, $p < 0.0001$). However, if box 6 (a control) is excluded, then the distribution of total bat visits is similar among the five boxes (two treatments and three controls; $\chi^2 = 3.645$, df = 4, $p = 0.46$).

DISCUSSION

It took little brown bats two months to visibly discover the new bat boxes, and at least another month to use them as roosts. The first box entered was a control box and two of the control boxes appeared to have been selected before either treatment box, although all six experimental boxes were entered for the first time within ten days of each other. There was no difference in the frequency of landing, entering, or night-roosting behaviours among the six boxes. Only two of the boxes (both controls) were used as day-roosts, but for just one day each until the end of the study period; the two treatment boxes

were never used as day-roosts. The number of bat visits to each box were similar for the

majority of the experiment. However, control box 6 received almost 1000 bat visits in the

last four days of the experiment.

Contrary to my hypothesis, there was no evidence that conspecific calls and

odours attracted female little brown bats to new roosts more than control boxes, and this

species did not appear to locate new roosts more easily when these cues were present.

Previous research has shown that a variety of bat species recognize and respond to social

and echolocation calls of conspecifics (e.g. Furmankiewicz et al., 2011), including little

brown bats (Barclay, 1982). Yet, during this experiment, and contrary to my prediction,

little brown bats did not locate or use the bat box with conspecific calls more than control

boxes. The calls we recorded were of bats in a pre-existing roost, so it is possible a bat

investigating a new roost would give different calls than those we recorded. Research

shows bats use different calls in different situations (e.g. Barclay, Fenton, & Thomas,

1979) and bat calls can encode various types of information, such as colony and sex

(Jones & Siemers, 2011). Lastly, it is possible this species is not attracted to conspecific

calls at new roosts. To extend this research, one could use previously recorded calls from

the bat boxes at Salmonier Nature Park to determine if there are any differences in

response to those recorded from Hubley's. Ideally, calls of bats in a new box would be

recorded to determine if this could explain the lack of response to the calls. Perhaps a new

bat box could be built and a bat placed inside to record calls, but that specific box not be

used for the field experiment. Alternatively, perhaps the calls we recorded made it sound

like the new box was fully occupied, since the calls were taken from a box with a large

number of bats in it. Since bat boxes would have a limited amount of space, bats may not

be attracted to them if they seem fully occupied. To test this, it would be ideal to record calls from one or a few bats, instead of an entire roost.

Research shows little brown bats may use scent posts for orientation (Buchler, 1980), and this species often initiates muzzle to muzzle contact after landing in hibernacula during swarming (Thomas et al., 1979). During this experiment, a large percentage of bats exploring the odour box also investigated the scented cloth, and many bats appeared to initiate muzzle to muzzle contact inside the new boxes. These results suggest this species may recognize the odour of conspecifics. However, this species did not locate or use the bat box with conspecific odours more than control boxes. It appears this cue, while recognized, is not enough to attract this species to new roosts, or is not an expected cue in this situation and is thus ignored. To extend this research one could conduct some preliminary experiments on olfaction in little brown bats, such as placing them in a maze with olfactory cues, to determine if they respond to this cue in a novel environment. Maze experiments using conspecific odours have been performed with several insectivorous bat species, including the big brown bat (*Eptesicus fuscus*; Bloss et al., 2002), and the common pipistrelle (*Pipistrellus pipistrellus*; De Fanis & Jones, 1995a).

It took little brown bats approximately three months to discover and use the new bat boxes as roosts. Previous studies on Bechstein's bats have found this species takes, on average, less than one month to discover a new roost in the summer, and another two months to use it as a roost (Kerth, Ebert, & Schmidtke, 2006; Kerth & Reckardt, 2003). Thus, both species took approximately the same amount of time to discover and use new roosts. Bechstein's bats and little brown bats have similar roosting behaviour; they are

temperate species that return to the same summering sites each year (Kerth, Safi, & König, 2002; Norquay et al., 2013), but maintain multiple roosts within these areas and switch roosts every couple days (Crampton & Barclay, 1998; Kerth & König, 1999). However, it appears that temperate species with low roost fidelity still require at least several months to locate and use novel roosts. It is possible once a bat colony has a sufficient number of suitable roosts, those individuals will not actively search for new roosts, as they are not in need of them. In this experiment, four of the new bat boxes, including both treatment boxes, were never used as day-roosts. The number of bat visits was also very low until the last four days of the experiment. It appears our experimental period may have not been long enough to allow bats sufficient time to discover and use the new boxes. Based on these results, it would seem important to conduct this experiment over multiple summers, instead of one. It would also be important to observe if bats used the new boxes earlier and if the number of visits was higher in the second summer, or if bats still only used the older boxes. One could also note if bats responded differently to the conspecific odours or calls in the second summer.

Only a small number of PIT reads were recorded in the new bat boxes, all of which were recorded in control box six and none in the treatment boxes. When trapping bats at Salmonier Nature Park in 2015 (for a separate study), approximately 1/4 of bats captured (77/339) were PIT-tagged (Broders, unpublished data). In comparison, only 1/20 bat visits to control box six were by PIT-tagged bats. This number seems much lower than expected compared to the number of individuals tagged in the colony. This suggests that many of the bats visiting the new boxes were untagged juveniles. In addition, most of the bat visits occurred near the end of the experiment, in mid-August. A study by Menzies

et al. (2013) found older juvenile little brown bats (caught end of August) were more exploratory than younger juveniles (caught beginning of August). A study by Patriquin et al. (2010) found juvenile northern long-eared bats (*Myotis septentrionalis*) were more exploratory than adults. Perhaps juvenile little brown bats become exploratory at the end of the summer when they are more self-sufficient and start leaving their summer roosts. It is also possible that the higher number of juveniles in the new bat boxes was because these individuals got confused when trying to locate their normal roost and ended up in the new boxes instead. This species does rely on memory to find pre-existing roosts (Humphrey & Cope, 1976; Norquay et al., 2013), but it seems logical it would take juveniles time to learn the specific location of their roosts.

Control box 6 was the most popular box during the experiment, yet it was not the first box discovered, nor did it have a larger number of bat visits than the other boxes for the majority of the experiment's length. In addition, all new boxes were situated close to each other, so there was no obvious difference in location. Lastly, the number of bat visits in this box stayed relatively low until the last four days of the experiment. It appears there was some behavioural change that occurred in this species at the end of summer but the reason is not obvious. Little brown bats usually leave the maternity roosts at Salmonier Nature Park (including the Hubley property) between August 12-15 (Broders, unpublished data). We do not know where they go at this point. It is possible they are roosting nearby, but no longer require the bat boxes. Perhaps this behavioural changed is correlated with the maturation of the juveniles; at the end of the summer, juveniles become self-sufficient and no longer rely on the adult females (Fenton, 1969; Menzies et al., 2013). During the summer, adult females roost together in maternity roosts and select

warmer roosts optimal for raising young (Humphrey, 1975; Kunz, 1982). There are some costs associated with this roosting behaviour though, such as potentially higher ectoparasite loads (Kunz, 1982; Kunz & Lumsden, 2003). Once juveniles are self-sufficient, adult females no longer rely on warm roosts to raise their young (Kunz, 1982; Kunz & Lumsden, 2003) and both juveniles and adults may choose to roost elsewhere (Fenton & Barclay, 1980).

In addition, as previously discussed, juvenile little brown bats may become more exploratory at the end of the summer, which may explain the significant increase in the number of bat visits to box 6 in mid-August at the end of the experiment. However, it is unclear why the bats chose box 6 over the other boxes. It is possible that after the first few bats visited box 6, other bats could have learned of its location and suitability through information transfer from those individuals. For example, in an experiment by Kerth and Reckardt (2003), female Bechstein's bats followed other individuals to suitable bat boxes significantly more often than to unsuitable bat boxes even though they had never visited the new boxes themselves. One could further examine this question by conducting a similar experiment using the same box locations but new bat boxes to determine if the bats would select the box in location 6 a second time.

In North America, bats receive little attention from the public and in research, yet are extremely important (Weller, Cryan, & O'Shea, 2009) as they are avid predators of crop and forest insect pests and provide pest suppression services to the agricultural industry worth billions of dollars (Boyles, Cryan, McCracken, & Kunz, 2011; Kunz, Braun de Torrez, Bauer, Lobova, & Fleming, 2011). An emerging fungal disease, called white-nose syndrome, has been decimating bat populations in eastern North America

since it was first observed in New York in 2006. At least six million bats have died from

this disease (U.S. Fish and Wildlife Service, 2012), and these deaths could result in

substantial economic losses (Boyles et al., 2011; Kunz et al., 2011). The little brown bat,

a species once common throughout the continent, has been devastated by white-nose

syndrome, and local extirpation and extinction is expected in many areas (Frick et al.,

2010). It is vital we understand as much about the biology of these animals as we can to

protect this species in areas where it still occurs. Bats are also important for their use as

models for studies in ecology and evolution. For example, studies of how bats process

sound has informed our basic understanding of how mammal's brains works (Fenton,

2001). Bats are important models for a wide range of other topics including food webs,

biogeography, and emerging diseases (Kunz & Fenton, 2003). In addition, the ability of

bats to both echolocate and fly makes them unique among mammals, which allows

researchers to address questions that cannot be studied in other taxa (Dechmann & Safi,

2005).

CONCLUSION

The results of my research further our understanding of some aspects of roosting

behaviour and communication in bats. Understanding these characteristics of bat biology

and behaviour could have important implications for land management and conservation

of little brown bats, which is becoming increasingly important as population numbers

decline due to white-nose syndrome. The results also highlight some of the complications

involved with trying to attract bats to novel roosts or areas, particularly in the field. These

complications include what calls to record and how to record them, and the time it takes to attract bats to new roosts. In addition, although little brown bats appear to recognize conspecific calls and odours, as has been shown by previous studies (e.g. Buchler, 1980; Kazial et al., 2008), they may not respond to them in all situations as shown by this study.

This type of research could potentially be used to attract threatened bat populations to protected areas as a conservation strategy. If employed to attract bat species such as the little brown bat, which is listed federally as "endangered" under the Species at Risk Act (Species at Risk Public Registry, 2016), using conspecific calls and odours seems promising as several other experiments have shown (Reckardt & Kerth, 2007; Ruczyński et al., 2007, 2009), but the specific calls and odours used and their source must be carefully investigated to ensure the highest chance of success. However, the results of this experiment, in combination with previous studies by Ruczyński et al. (2007, 2009), the high fidelity many bat species have to their roosting sites (e.g. Norquay et al., 2013), and the time it takes for them to locate new roosts (e.g. Kerth & Reckardt, 2003), suggest that protection of areas with established bat roosts should have a higher priority than those without.

The results of this experiment also provide insights into how the behaviour of little brown bats changes at the end of summer. Few studies have focused on this transitional period and how bats behave during this time. These results may indicate that juvenile bats do not necessarily leave their summer sites, but may choose to roost elsewhere at the end of summer instead of staying in the maternity roosts. In addition, they may become more exploratory and investigate new roosts more readily at this time. A better understanding of the roosting behaviour of little brown bats can contribute to our

understanding of roosting behaviour in other bat species as well, which as noted previously, is important for conservation efforts. In addition, this kind of research could inform our understanding of communication in other bat species and potentially animal communication as a whole.

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Table 1: The types of exploratory/roosting behaviours observed and the number of bat visits within each category from May 16 to August 16, 2015 in the new bat boxes on the Hubley property, NL.

Box Type (Number)	Behaviour				
	Landing	Entering	Night Roosting	Day Roosting	Total
Odour (2)	3	9	0	0	12
Call (3)	11	17	2	0	28
Control (1)	4	8	1	0	13
Control (4)	1	12	1	1	13
Control (5)	7	16	1	0	23
Control (6)	472	526	202	7	998
Total	498	588	207	8	1,087

Table 2: First date of entry and subsequent Julian Day in the new bat boxes on the Hubley property, NL.

Box Type (Number)	First Date of Entry	Julian Day
Control (5)	6-Jul-15	1
Control (6)	10-Jul-15	5
Control (1)	13-Jul-15	8
Odour (2)	13-Jul-15	8
Call (3)	16-Jul-15	11
Control (4)	16-Jul-15	11
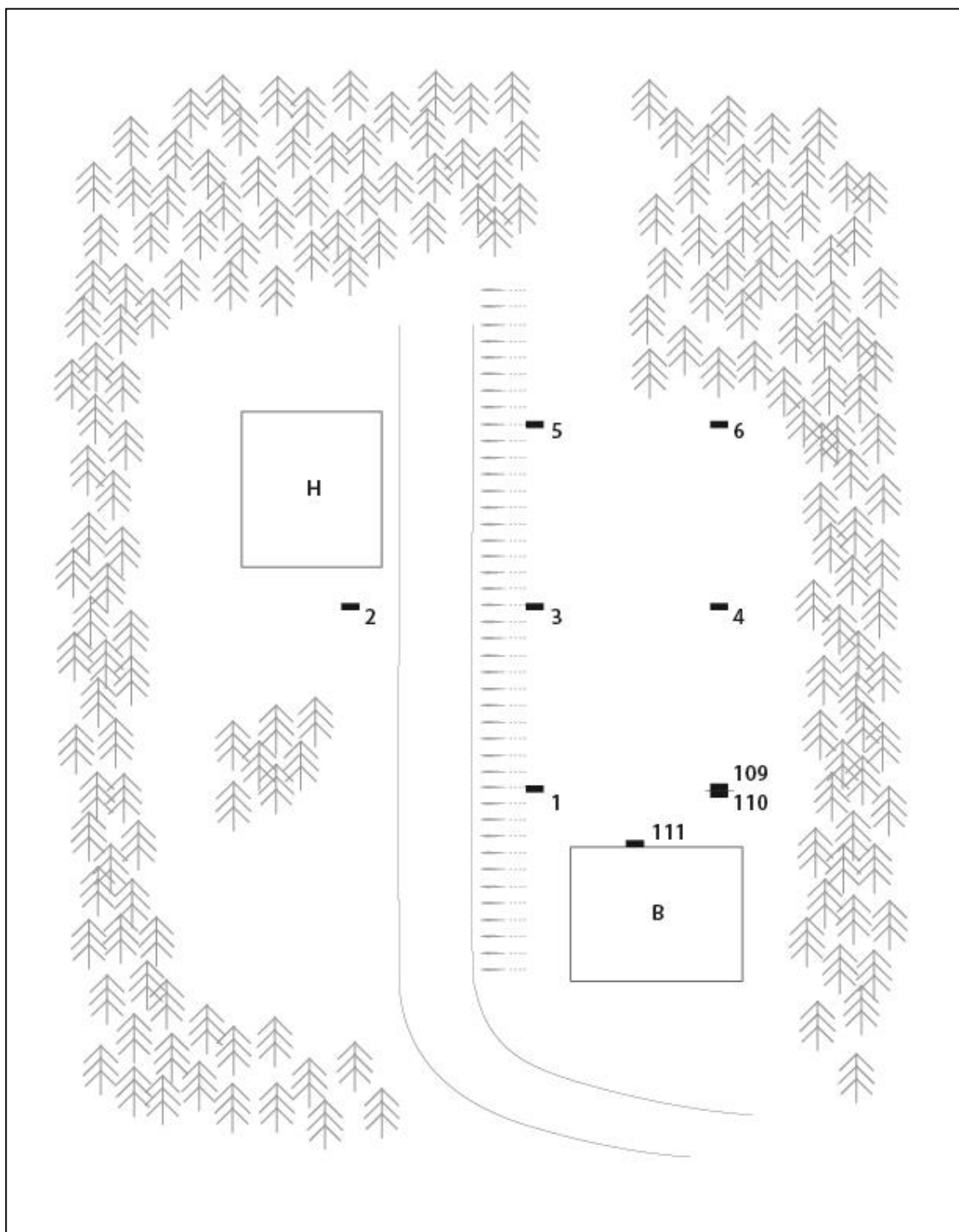
Figure 1: Aerial drawing of the experimental set-up on the Hubley property, NL. (Box types: 2 - odour, 3 - call, 1, 4, 5, 6 - control; H - house, B - barn; drawn by M. Harrish, 2015).
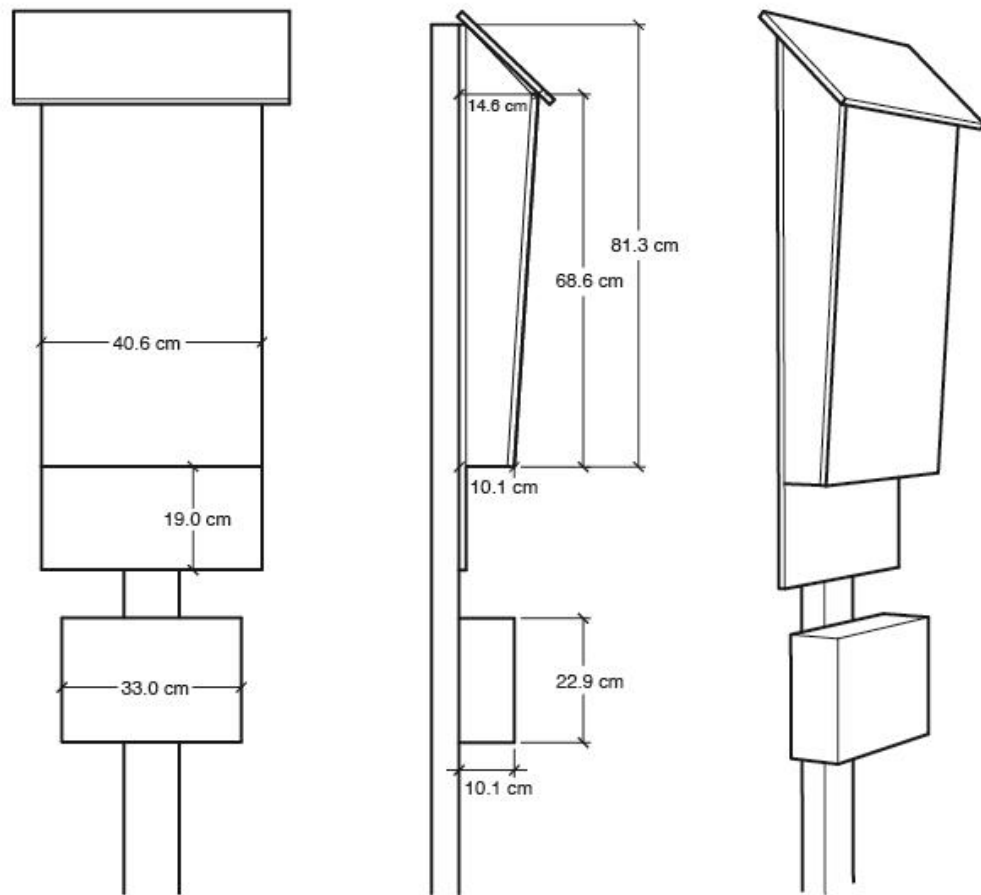
Figure 2: Drawing of an experimental bat box with landing pad and speaker box, and their dimensions (inches; drawn by M. Harrish, 2016).
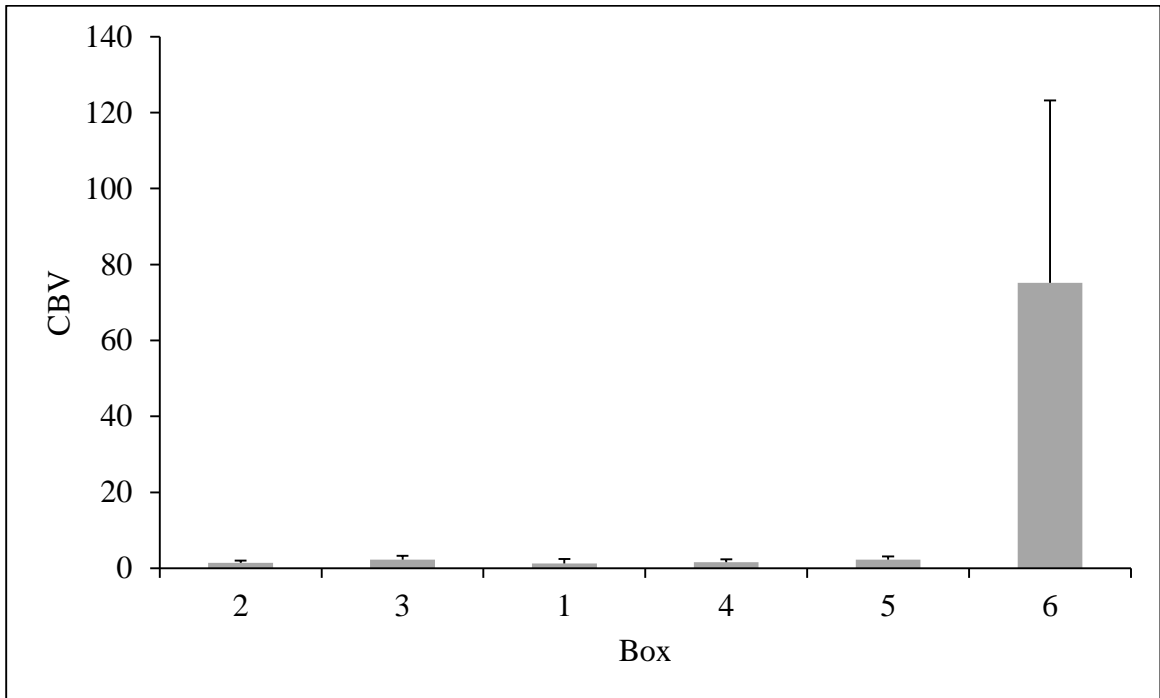
Figure 3: Average weekly cumulative number (mean and standard error) of bat visits (CBV) for weeks 8 to 14 (July 4 to August 16, 2015) for the new bat boxes on the Hubley property, NL. (Box types: 2 - odour; 3 - call; 1, 4, 5, 6 - control).
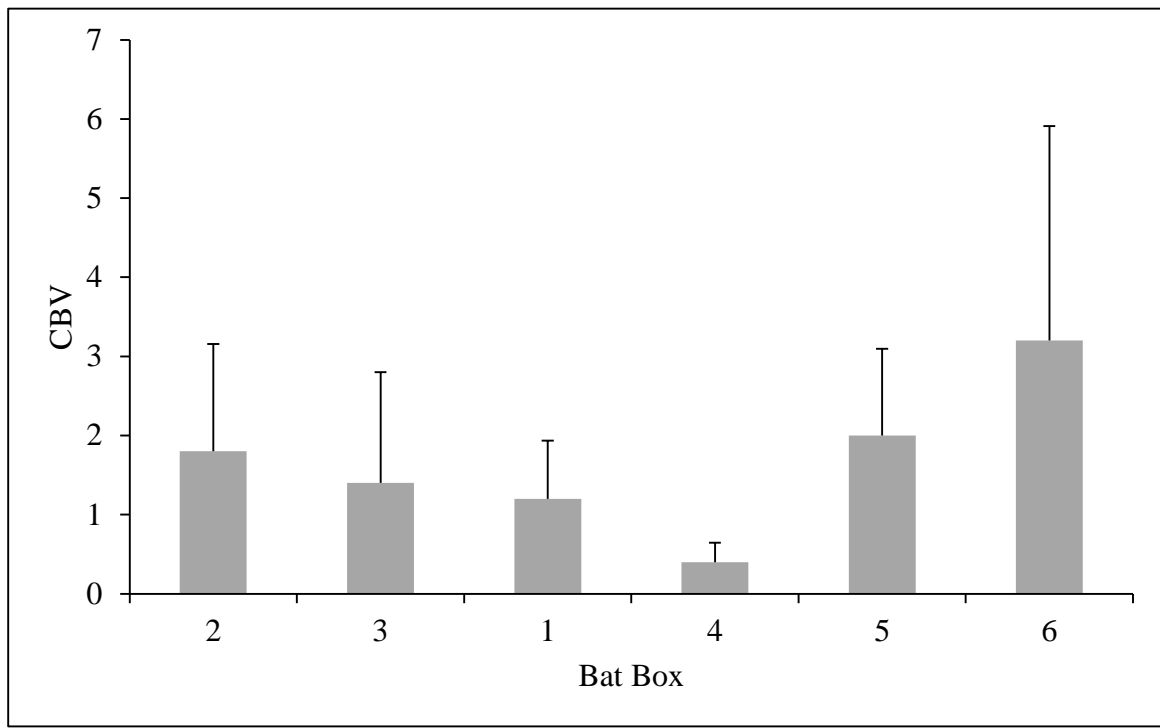
APPENDIX

Figure A1: Average weekly cumulative number (mean and standard error) of bat visits (CBV) for weeks 8 to 12 (July 4 to August 7, 2015) for the new bat boxes on the Hubley property, NL. (Box types: 2 - odour; 3 - call; 1, 4, 5, 6 - control).
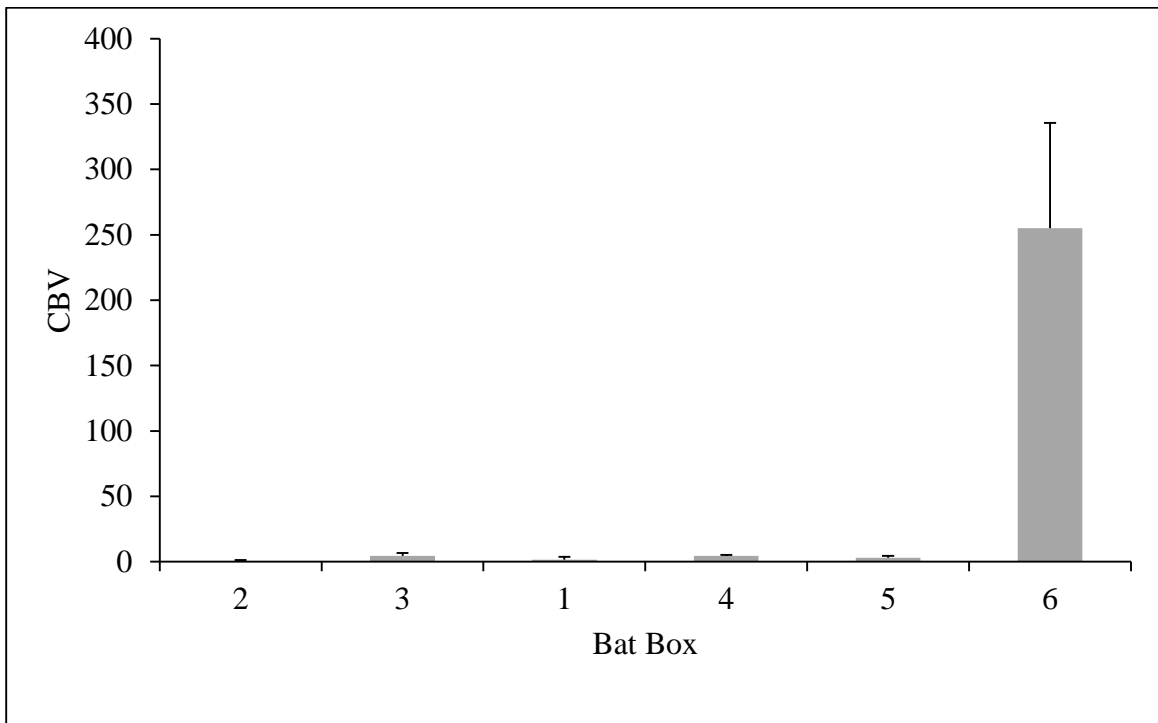
Figure A2: Average weekly cumulative number (mean and standard error) of bat visits (CBV) for weeks 13 and 14 (August 8 to 16, 2015) for the new bat boxes on the Hubley property, NL. (Box types: 2 - odour; 3 - call; 1, 4, 5, 6 - control).