Activity and habitat use of *Myotis*, *Eptesicus*, *Lasiurus*, and *Lasionycteris* species by ultrasonic acoustic detectors at the Beaverhill Bird Observatory

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Introduction

Bats (order Chiroptera) are the most diverse group of mammals on Earth with over one thousand currently living species and are estimated to have existed on Earth in some form for over fifty million years (Kunz et al. 2011, MacSwiney et al. 2008). Bats occupy almost all terrestrial habitats and climate zones on Earth and display a rich diversity in food types including insects, fruit, nectar, fish, and even blood (Scnitzler and Kalko 2001). The diversity of food types displayed by bats is of high importance in both natural ecosystems, with nectarivorous bats aiding in the pollination of different plant species, frugivorous bats aiding in seed dispersal throughout ecosystems, and insectivorous bats aiding in the suppression of insect populations (Kunz et al. 2011). Bats also show importance in human societies with some Eastern societies considering bats to be good luck, while nectarivorous bats in North America pollinate *Agave tequilana*, the source of commercial tequila (Kunz et al. 2011). Insectivorous bats are also of great importance to humans by eating many agricultural pests, with studies showing that bats are capable of consuming over 25% of their body weight in insects each night to over 100% of their body weight when lactating (Kunz et al. 2011). Estimates show that the loss of bats in North America could lead to a loss of more than $3 billion yearly in the agricultural sector (Boyles et al. 2011). In Alberta, Canada, all 9 bat species present are insectivorous, with 6 of these species found in the Beaverhill natural area.

Bats have also been considered as possible biodiversity indicator species in assessing responses to climate change due to their global distribution, importance in natural ecosystems, and rich diversity (MacSwiney et al. 2008). Unfortunately, there are currently several threats to bats worldwide. Habitat loss as a result of logging, agricultural practices, and climate change has led to declines in some populations around the world (Dillingham et al. 2003; Mering and Chambers 2014). An increase in bat casualties due to wind turbines has also been an issue, with migratory bats especially being common casualties (Kunz et al. 2007; Frick et al. 2017). The greatest threat to North American bats, however, is White-Nose Syndrome (WNS), an infectious disease caused by the fungus *Pseudogymnoascus destructans* (Olson et al. 2011; Invasive Species Compendium [accessed 2019]). WNS is believed to have been introduced to North American bats from Europe and was first discovered in North American in 2006, in a single cave in New York (Invasive Species Compendium [accessed 2019]). Since then, it is estimated that over 5 million bats across eastern North America have died (Frick et al. 2016). WNS has spread through eastern North America to 31 US states and 5 Canadian provinces, including western locations such as Manitoba and Washington State (Invasive Species Compendium [accessed 2019]; Reeder et al. 2012). WNS affects bats during the winter months when bats have entered long states of torpor (e.g., hibernation), during which body temperatures and metabolic rates are sharply decreased to account for the lack of food availability during these cold months (Reeder et al. 2012). Unfortunately, this makes for a prime situation for infection by *P. destructans*, as this fungus thrives in cooler temperatures and the proximity of bats in wintering colonies allows for easy transmission of the disease (Frick et al. 2016).

Transmission of WNS begins in the fall when bats return to their hibernacula, and by winter the huge numbers of bats and close proximity within these hibernacula result in mass infections, especially if infected bats move between different hibernaculum sites in the fall before hibernation begins (Invasive Species Compendium [accessed 2019]). WNS-infected bats display a white growth of the fungus on their muzzles and wings and are more frequently aroused from torpor than uninfected bats (Olson et al. 2011; Frick et al. 2016). All bats show periods of arousal during torpor, with these periods typically accounting for only 1% of the total time but 80 to 90% of total energy use from increased metabolic rates and body temperatures (Reeder et al. 2012). Bats are typically able to survive these energy-costly arousal periods by building large stores of energy in the form of fat during the summer months (Reeder et al. 2012). WNS causes more frequent arousal periods resulting in higher energy usage in infected bats, causing them to deplete their fat storage quicker than uninfected bats and ultimately resulting in death (Frick et al. 2016; Reeder et al. 2012). Bat populations are extremely slow to recover from WNS due to most bat species giving birth to only one pup per year. Canada has declared three bat species endangered stemming largely from the threat of WNS to their populations, two of which (*Myotis lucifugus* and *Myotis septentrionalis*) are found in Alberta (Frick et al. 2016).

Although bats are beneficial to natural ecosystems and human economies and there are huge risks to bat populations present, there is a lack of long-term bat monitoring programs and standard survey protocols worldwide (MacSwiney et al. 2008). With the current possibility of WNS decimating North American bat populations, there is a need for standardized monitoring programs to be deployed to gather data of bat populations for future conservation efforts (Olson et al. 2011). One method for monitoring bat populations is through acoustic monitoring, as a way of inferring bat activity in different locations and by species.

Acoustic monitoring consists of analyzing echolocation calls of bat species via ultrasonic bat detectors. These detectors allow us to assess the presence and activity of bats when methods such as capturing bats are not possible and at night when bats are most active (Brigham et al. 2004). Acoustic monitoring is not a perfect method, as most (but not all) bats use echolocation, and echolocation calls of different species may overlap, but it has potential in assessing global population trends and will benefit from assembling greater call libraries of different species' echolocation calls. Echolocation is used by bats to detect and locate prey, and for spatial orientation (Schnitzler and Kalko 2001). Bats emit high-frequency sounds from their vocal cords and analyze, using specialized neuronal filters in their brains, the returning echoes off objects in their environments to determine the direction and distance of these objects relative to them (Moss 2018). Bats need to be able to differentiate between calls coming from unwanted targets (such as trees) and their prey and determine where the prey is and how it is moving through the environment, and thus the echolocation calls used are important (Fenton 1989). Although echolocation calls and signals have some specifies-specificity, typically echolocation signals fall into two categories, narrowband or long constant frequency (CF) and broadband or frequency-modulated (FM) (Schnitzler and Kalko 200; Fenton 1989; Brigham et al. 2004). CF calls are specialized for detecting weaker echoes and classifying the returning echoes, and these calls have a longer duration than FM calls and stay at a constant frequency throughout the call (Brigham et al. 2004). FM calls are better-suited to determining the exact location of targets, are of a shorter duration than CF calls, and typically start at a higher frequency and sweep down to a lower frequency during the call (Brigham et al. 2004; Schnitzler and Kalko 2001). Echolocation calls will often consist of a combination of these two types and will change based on activity. For example, when bats are searching for prey they will emit longer duration calls at lower frequencies to detect if the prey is present nearby, but calls will increase in frequency and decrease in duration when a bat has detected and is approaching its prey in order to precisely determine the prey's location (Moss 2018).

In this study, we used bat detectors to analyze the echolocation calls of *Eptesicus fuscus*, *Lasionycteris noctivagans*, *M. lucifugus*, *M. septentrionalis*, and *Lasiurus cinereus* in Tofield, Alberta, in the Beaverhill natural area. The purpose of this study is to further establish monitoring of Tofield's bat population by collecting and comparing bat activity data to previous years of study in this area. We compared bat activity between species, habitat types, and days from May through September 2019.

Methods

Study Area

This study was conducted at the Beaverhill Bird Observatory (BBO) located in the Beaverhills Natural Area, near Tofield in the dry mixed-wood natural sub-region of Alberta. The BBO is located in a predominantly forested area with mature aspen forest, grassland, wetland, stream, and lake features. There are 35 bat houses being monitored weekly. The bat houses are located across 4 different habitat types; clearing, interior, open and edge and our echolocation surveys were placed in relation to these habitats surrounding the bat houses. Most bat houses are easily accessed by or near the trail (Figure 1). The natural area is only accessible by foot; ATVs and vehicles are not permitted within the Beaverhill Bird Observatory for conservation purposes. Although two houses were not included for the majority of the study due to beaver activity and an active wasp nest, while a third house was temporarily lost due to beaver activity.

Figure 1. Map of the Beaverhill Bird Observatory bat house and habitat distribution in Tofield, Alberta.

Bat House Distribution

The bat houses within the BBO are located across 4 different habitat types: clearing (Figure 2a), interior (Figure 2b), edge (Figure 2c) and open (Figure 2d). The clearing habitat type consists of an open area with slightly-dense surrounding areas but have minimal tree coverage i.e. if the house was located in a significant open area that was surrounded by forest on all sides; open habitat consists of an open grass field with minimal shrub and no tree cover; the interior habitat is dominated by dense tree coverage i.e. bat house within the forest; and edge habitat is located along Flicker Freeway with surrounding ponds and or water.

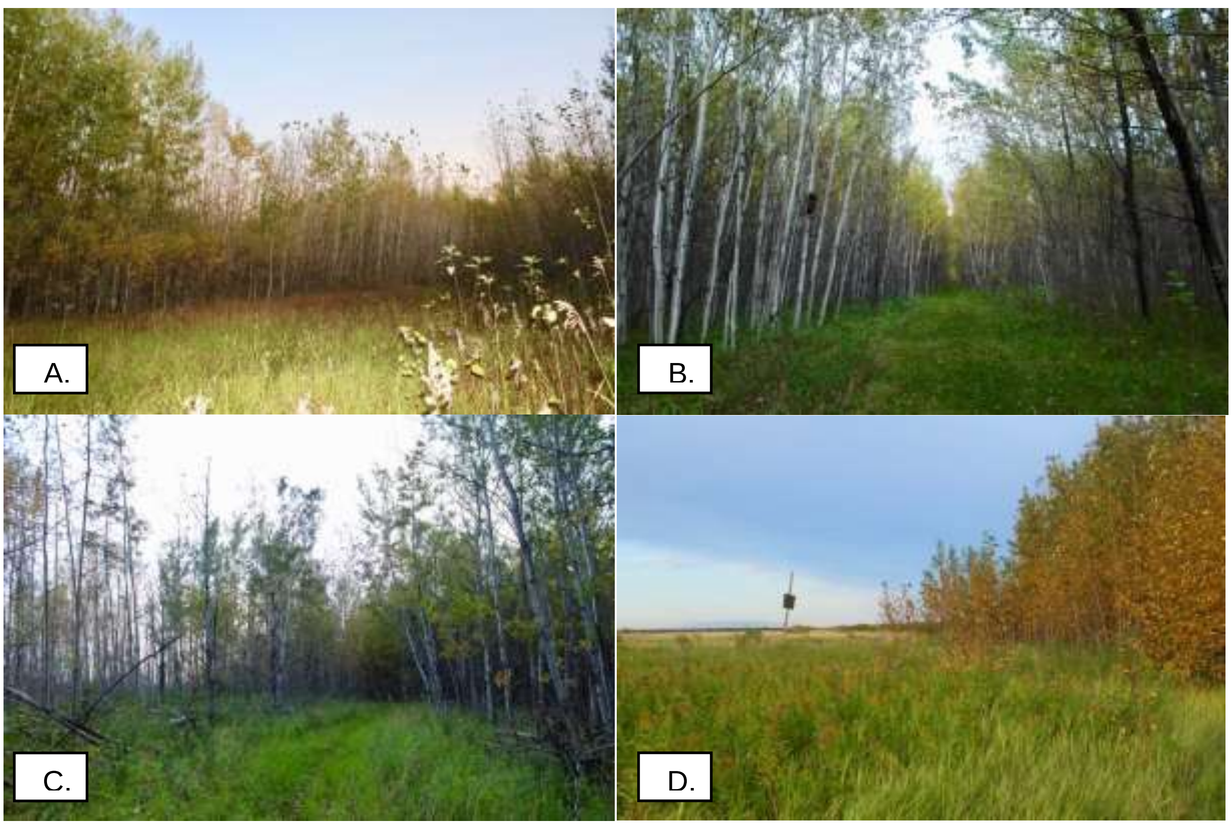

Figure 2. Four different habitat types across the Beaverhill Bird Observatory – clearing (a), interior (b), edge (c) and open (d) habitat types.

Methodology

Echolocation surveys were performed once a week between May 11 to Sept 21, 2019. Data were not used for the following weeks due to the inability to perform recordings because of weather conditions i.e. rain, thunderstorms, poor air quality or smoke; June 1, July 13, July 20, and August 02, 2019. May 17 and 25 were also not included in the analysis of calls due to no identifiable calls being found during these dates. Bat activity was surveyed by acoustic surveys using Echo Meter Touch 2 Handheld Detector connected to Amazon Kindle Fire HDX (3rd Generation) tablets. Surveys were conducted 15-45 minutes after sunset at 2 bat houses for each of the 4 habitat types. Surveys were conducted by recording using the Echo Meter for 5 minutes at each chosen bat house. Only identifiable bat calls were included in data analysis.

Other confounding variables were gathered and counted which include maximum daily temperature, percent humidity, wind speed, moon illumination and daily maximum temperatures (Appendix 4). Temperature data were obtained from the weather station at Elk Island National Park. The BBO is about 43 km southeast of Elk Island National Park and therefore assumed to be affected by similar weather conditions and temperature patterns.

Identification and Analysis

Echolocation calls were visualized as spectrograms to measure call parameters using FDA analysis on Kaleidoscope. Calls were manually identified using an echolocation call library compiled with species call parameters compiled from various sources (Appendix 1; Fenton et al. 1983; Maxell et. Al 2015) and were compared with automatic identification on the Echo Meter application. Each recording is reviewed using the Kaleidoscope program a single species at a time to be consistent and efficient. Bat activity is defined by frequency and by the presence in one sampling night. A Chi-square Goodness-of-fit test was performed to determine whether there was a difference in habitat use by bats.

Due to call overlap between species, *Myotis lucifugus* and *Myotis septentrionalis* were pooled as MYSP (*Myotis* species) and *Eptesicus fuscus* and *Lasionycteris noctivagans* were pooled as EPFU/LANO, while *Lasiurus cinereus* (LACI) was not pooled. *Lasiurus borealis* was not included as a possible species in the analysis because of the rarity of the species in this location. Echolocation call files that did not contain at least 3 identifiable bat calls were excluded from the analysis (NABat 2018).

Moon presence was identified based on whether or not the moon was present for the majority of the time during acoustic monitoring. The presence of rain was identified based on whether rain occurred during the day of sampling to account for the presence or absence of bats due to rain.

Results

Overall, 277 acoustic files showed identifiable calls from 13 of the total 20 sampling nights from May through September 2019. Some of the 277 call files showed more than one bat species present during the same period, thus resulting in 292 identifiable bat call sequences being present. No identifiable calls were found in acoustic data from May 17, May 25, June 01, July 13, July 20, August 2, and September 28, 2019. This meant that only 65% of the 20 week sampling period had identifiable calls from the acoustic data.

June had the greatest frequency of overall bat activity, and also had the greatest frequency of bat activity for all three species groups (Figure 3). May had only MYSP calls, while June and September had recorded calls from all three species groups (Figure 3). For all months except September, MYSP had the highest frequency of calls, while in September LACI had the highest frequency of calls (Figure 3). In terms of relative presence, or how many nights per month that species of bat were present in our acoustic data, MYSP bats were present the most during the sampling period, while LACI bats were present the second most, and EPFU/LANO were present the least during the sampling period (Figure 4).

In relation to habitat type, clearing habitats had the greatest number of bat files found, then interior, followed by edge, and finally open habitats had no identifiable calls found during the entire sampling period (Figure 5). MYSP calls were found in clearing, interior, and edge habitats, but were found in clearing habitats the most (Figure 5). LACI calls were also found in clearing, edge, and interior habitats, and were again found in clearing habitats the most. EPFU/LANO calls were only found in clearing habitats and were found in nearly the same amounts as LACI bats were in clearing habitats (Figure 5). Overall, MYSP species were found at a much higher frequency than the other two species groups, and clearing habitats had the greatest overall frequency of bat calls. When looking at only the presence of species, i.e. how many nights during the sampling period a species was present in each habitat type based on acoustic data, we found that MYSP were still present the most in clearing habitats, but LACI were present more times in clearing habitats than EPFU/LANO was. We also found that although MYSP showed a much great number of total call files in clearing habitats than LACI did (Figure 5), MYSP were not present in clearing habitats much more than LACI were (Figure 6), meaning that MYSP had greater activity than LACI did during the times they were present in clearing habitats. When comparing LACI and EPFU/LANO in clearing habitats, we found that although the two species groups had a very similar number of call files (Figure 5), LACI were present more than EPFU/LANO, suggesting that EPFU/LANO had a greater overall activity level in clearing habitats than LACI (Figure 6).

The echolocation activity shows a bimodal distribution for all species peaking in the months of June and July (Figure 3). MYSP peaks at the month of July, EPFU/LANO in June and LACI in June. In addition, the highest amount of bat acoustic activity with respect to their presence in each sampling night is MYSP with a total of 11 sampling nights, followed by LACI for 9 sampling nights and EPFU/LANO for 4 sampling nights (Figure 4).

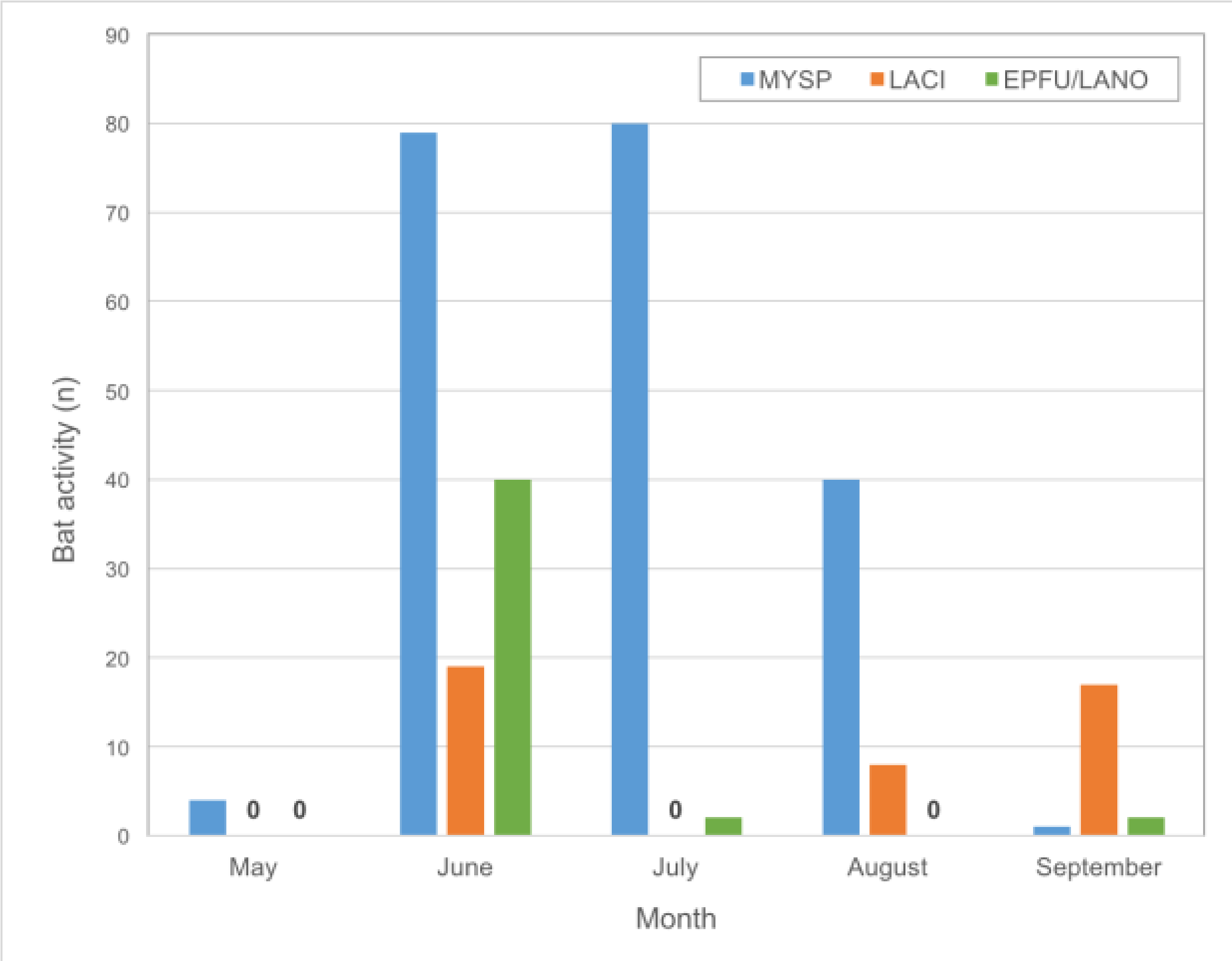

Figure 3. Total frequency of bat activity by species (n = number of identifiable calls) - MYSP (*Myotis lucifugus* and *Myotis septentrionalis*), EPFU/LANO (*Eptesicus fuscus, Lasionycteris noctivagans*), LACI (*Lasiurus cinereus*) - over the course of five months (May, June, July, August and September). Calls that were automatically identified as *Lasiurus borealis* by the Echo Meter Touch 2 were included in EPFU/LANO. Frequency indicates the number of identifiable call files for each species.

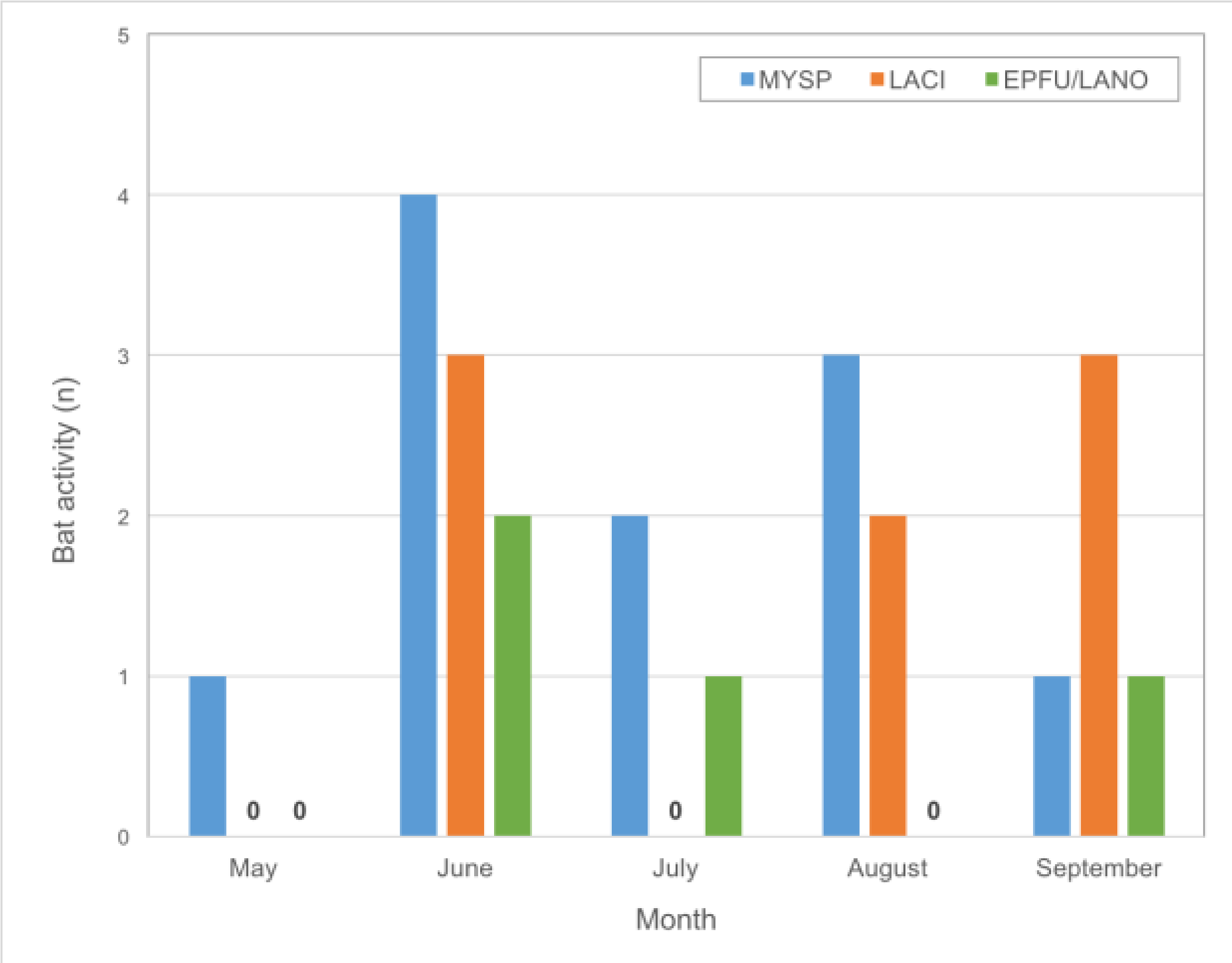

Figure 4. Total presence of species by bat activity levels (n = number of sampling nights) - MYSP (*Myotis lucifugus* and *Myotis septentrionalis*), EPFU/LANO (*Eptesicus fuscus, Lasionycteris noctivagans*), LACI (*Lasiurus cinereus*) - over the course of five months (May, June, July, August and September). Calls that were automatically identified as *Lasiurus borealis* by the Echo Meter Touch 2 were included in EPFU/LANO. Presence (Bat activity) is indicated the number of times the species was present in one of the sampling dates during each month (n).

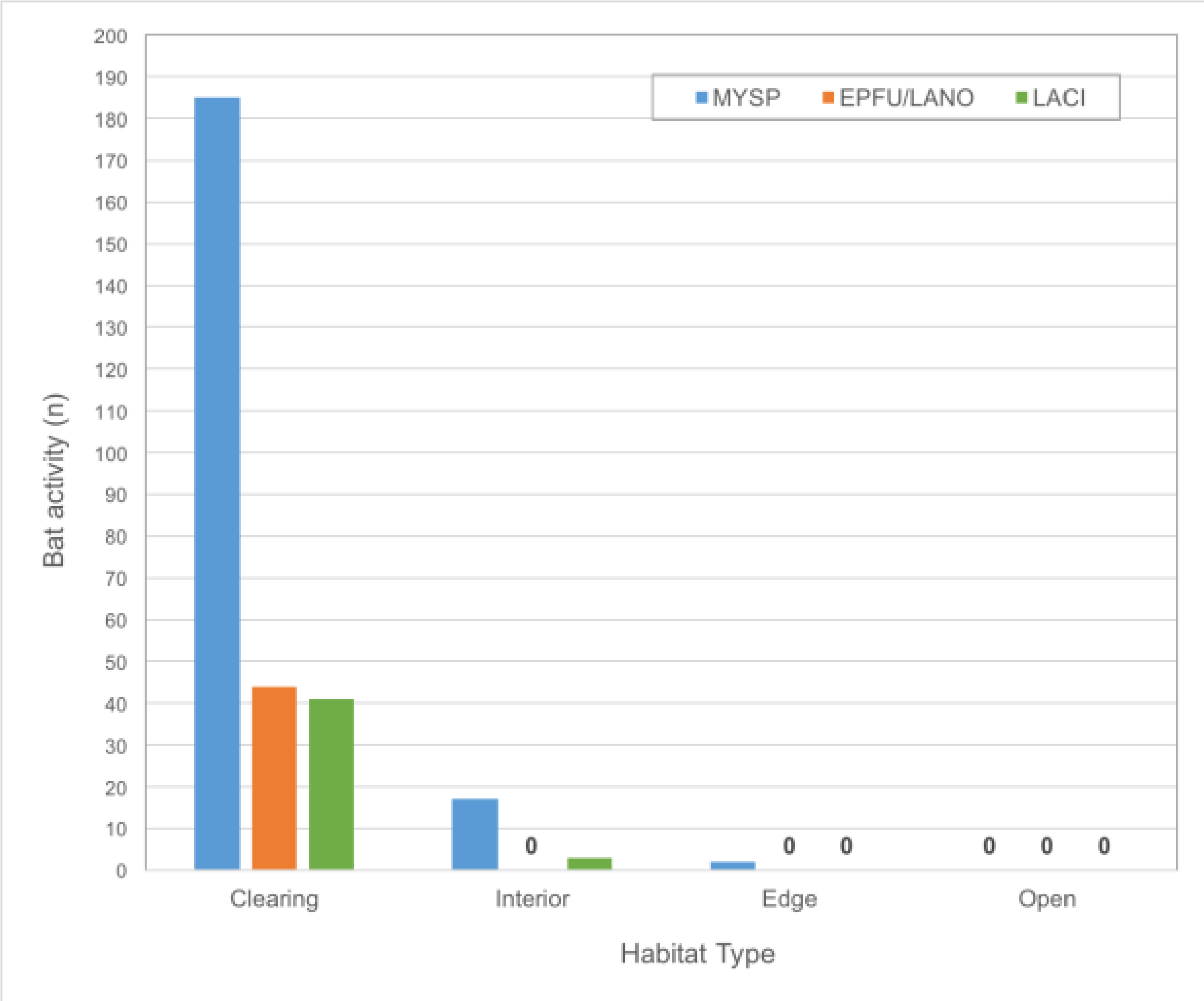

Figure 5. Bat activity as the total number of identifiable calls (n = number of call files) of the following detected species: MYSP (*Myotis lucifugus* and *Myotis septentrionalis*), EPFU/LANO (*Eptesicus fuscus, Lasionycteris noctivagans*), LACI (*Lasiurus cinereus*), over the course of five months (May, June, July, August and September) in the following habitat types; clearing, edge, open, and interior. Calls that were automatically identified as *Lasiurus borealis* by the Echo Meter Touch 2 were included in EPFU/LANO.

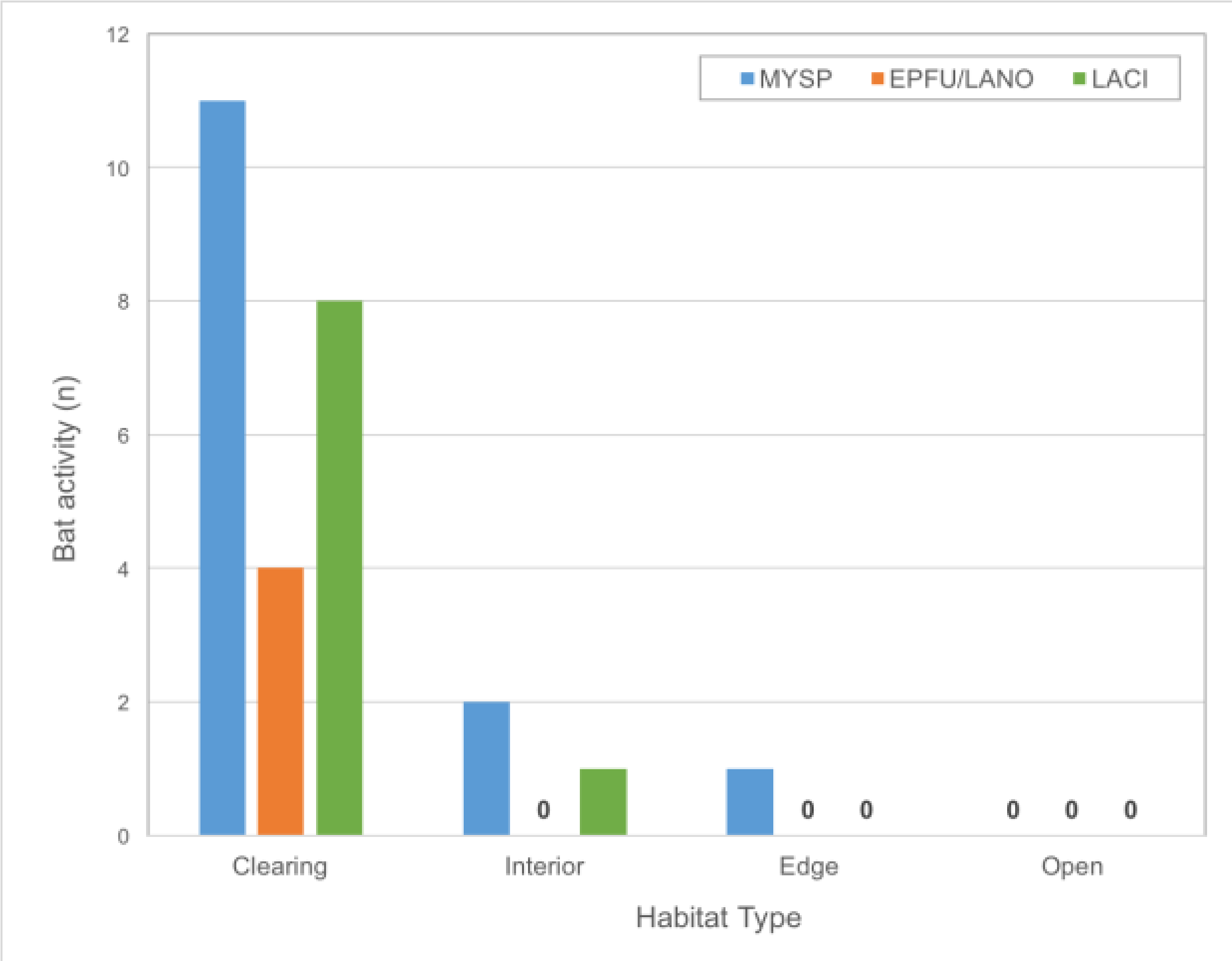

Figure 6. Bat activity as total number of occurrences (n = number of sampling nights) of the following detected species- MYSP (*Myotis lucifugus* and *Myotis septentrionalis*), EPFU/LANO (*Eptesicus fuscus, Lasionycteris noctivagans*), LACI (*Lasiurus cinereus*) - over the course of five months (May, June, July, August and September) at the following habitat types; clearing, edge, open, and interior. Calls that were automatically identified as *Lasiurus borealis* by the Echo Meter Touch 2 were included in EPFU/LANO.

A unimodal and left-skewed distribution is observed for the total number of identifiable calls with respect to habitat type (Figure 5). Over the 20-week period, a total of 292 identifiable call sequences were recorded across 4 different habitat types. The highest numbers of identifiable calls were recorded in clearing habitat (270 calls), 185 of which were MYSP, 41 calls were LACI and 44 were EPFU/LANO; followed by Interior habitat types (20 calls), 17 of which were MYSP, 3 were LACI and 0 were EPFU/LANO; followed by edge habitat (2 calls) 2 of which were identified as MYSP (Figure 6). No calls were recorded for open habitat types.

A Goodness of Fit Chi-square statistical analysis was performed to test whether there was a difference in habitat use by bats. We found that habitat preferences by all bat species in terms of total bat activity per habitat type were significantly different (Goodness of Fit Chi-Square: X^2= 712.64, df = 3, p = 0.00001, p<0.05).

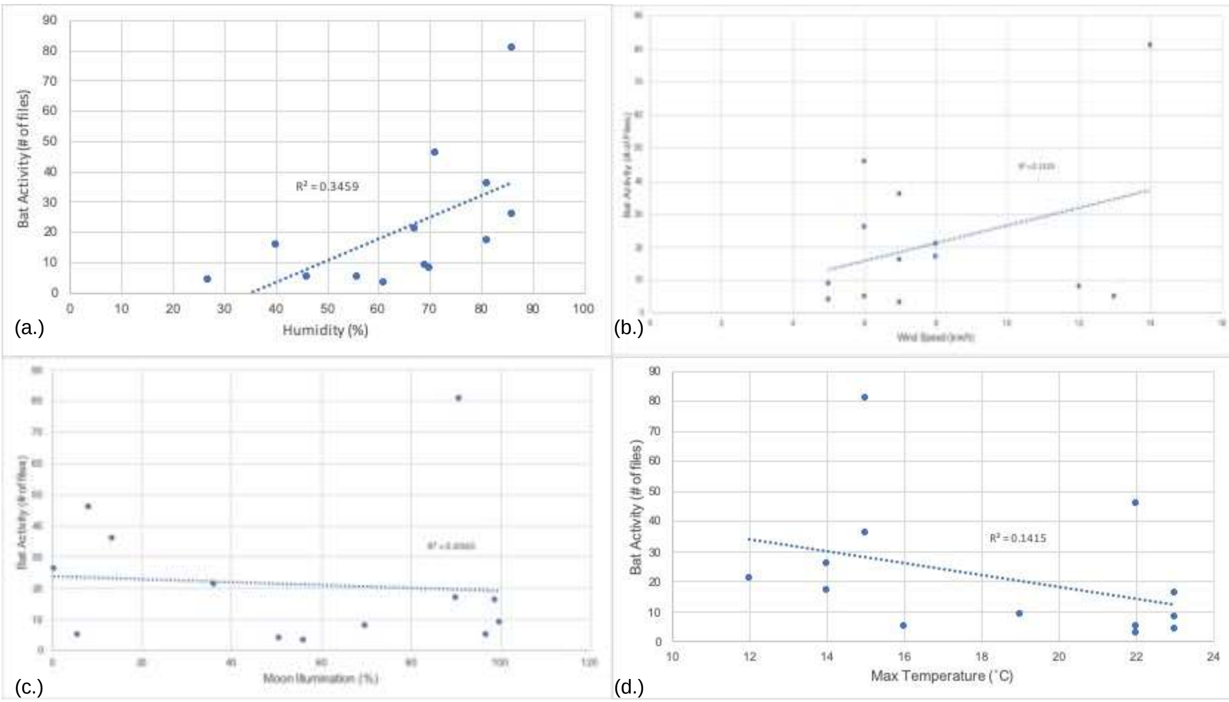

Figure 7. Total number of identifiable bat activity files plotted against percent humidity (a), wind speed (b), percent moon illumination (c) and maximum daily temperatures (d) from May 11 to September 21, 2019. R^2 indicates percent variability of total bat activity that is explained by x-axis variables.

To determine if there was a correlation between confounding variables and bat activity in terms of call files, the number of identifiable call files were plotted against each of the following variables; max daily temperature (˚C), percent humidity, percent moon illumination and wind speed. Overall, all factors show minimal to no correlation with bat activity levels. The variation in bat activity indicated by R^2 values are explained by the following; 34.6% for percent humidity (Figure 7a), 13.3% for wind speed (Figure 7b), 0.65% for moon illumination (Figure 7c) and 14.2% for daily maximum temperatures (Figure 7d).

Rain was present at some point during the day of 3 of 13 sampling nights, and these 3 nights all fell somewhere towards the middle amount of activity, suggesting that the rain may not have had an effect during these nights (Figure 8). However, there were three nights (July 13, July 20, and August 02) where acoustic sampling could not occur due to thunderstorms during the sampling period, and thus no data was recorded on these nights. The moon was present during 7 of the 13 sampling nights and was not present during the nights with the greatest and least amount of bat activity in terms of call files (Figure 4).

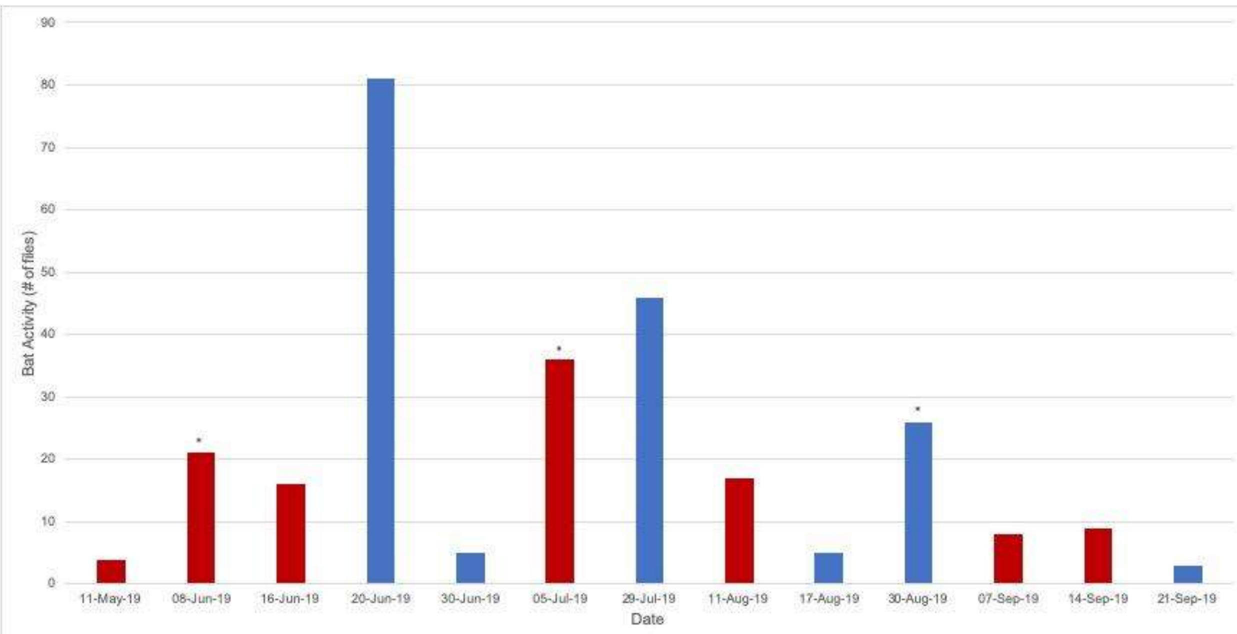

Figure 8. Total bat activity in terms of the number of acoustic files with identifiable bat calls, organized by the number of calls per date. Red columns indicate the moon was present during the sampling period, blue columns indicate the moon was not present during the sampling period. * indicates rain occurred during that sampling date.

Discussion

Our results show that MYSP were present the most out of the three species groups and displayed the greatest total number of activity while clearing habitat was the preferred habitat in relation to bat activity. Our results also suggest that the highest numbers of echolocation activity and the maximum presence of bats occur in June. The lowest number of activity occurred in May, likely due to the spring migration and the fact that not all of the bats had arrived at the BBO. Furthermore, there were smaller peaks of activity in August and September, which again coincides with the fall migration out of the BBO area and with the hibernation of any bats that do not migrate, such as the *Myotis* species. This coincides with previous studies that also found general increases in activity during June (Bayne 2012).

With regards to species, members of the MYSP group had the greatest amount of activity recorded, while EPFU/LANO and LACI had equal amounts of activity recorded. However, EPFU/LANO had the least presence overall, which the majority of their calls were recorded from a single night, June 20. LACI had similar numbers of calls as EPFU/LANO but were present more times than EPFU/LANO were with regards to sampling night. It should be noted that a low number of EPFU/LANO calls in general were detected which suggests that these species are less likely to be found at the BBO.

Lastly, in relation to habitat types, the highest numbers of all three species groups were found in clearing habitats and were not found at all in open habitats. All species were found in much lower numbers in interior habitats, and only MYSP were found in edge habitats. Therefore, there is a habitat preference with bats and that they may prefer clearing habitats because these habitats offer large numbers of trees for roosting for species that do not use bat houses such as LACI. In addition, clearing habitats provide open space for easy flying and less clutter but still has trees at the edge to help orient their position in space. Open habitats provide large areas with no clutter, but also provides the least protection from predators and the least protection from the weather. This apparent preference for somewhat but not fully open areas with some trees present were also seen in previous studies (Schnitzler and Kalko 2001; Fenton 1989). Similar studies iterate the difficulty in distinguishing between target and clutter echoes in heavily forested areas.

One of the main limitations of this study is that similar to previous studies, we also did not control for intraspecific differences in species such as sex, pregnancy, lactation, size and age (Gillmore 2018). In addition, we had a limited sample size in each habitat type. In this study, we used 2 bat houses in each habitat type with 5 minute intervals at each chosen house. Future experiments should consider more than 3 bat houses within each habitat type as the recording stations. Overall, the Echo Meter Touch 2 is advantageous as it is easily accessible due to its small and light design, as well as the fact that it can be plugged in into any Apple and Android product and comes with a free application. It allows for immediate bat species identification and allows for recordings in real time. However, there are also some disadvantages with this software. For instance, the Echo Meter automatic call identification should not be used as the sole parameter in identifying species via acoustic data. The Echo Meter would often pick up background noise and identify it as a bat or include the noise as a blank call file, or identify bat species that would be extremely rare to find in that area, such as *Lasiurus borealis* or *Myotis volans.* In addition, though calls are visualized as spectrograms immediately after each recording is done, it is challenging to assess the call sequence quality files because the viewfinder on the tablets would often squish calls altogether. Although analyzing files using Kaleidoscope would mitigate this problem. Lastly, the recordings from the Echo meter often have short durations but mobile transects must have 1-50 ms and stationary points require 2-50 ms which ensures high-quality recordings for species identification (NABat 2018). These short recordings provide insufficient number of pulses which restricts confident identification. In addition, most files that are recorded include noises, blanks or presence of destructive interference caused by echoes which limits the species identification.

One of the options included in the Echo Meter software is the Auto-identification feature. We did not use this option due to the following reasons. We believe that auto-identification overall is questionable due to reduced call quality from quality issues such as excessive noise. It is also possible that the device chose inappropriate calls (pulses) for classification (for example social calls, quiet/out of range calls, feeding buzzes), included noise as part or in place of a call, or used non-search phase or high clutter calls. Studies suggest that bat calls must be examined for quality before species analysis and only regular, search phase calls are used for species analysis, because fragmented calls are likely to result in misidentification (NABat 2018).

The use of Echo Meter Touch 2 handheld bat detectors is a novel approach to analyzing bat activity and presence at the BBO and therefore it is crucial to continue utilizing it. For instance, future experiments should involve exploring a comparative analysis of the efficacy of different echolocation sampling devices in aim to find which is the most effective method, such as comparing Song Meter Bat Recorder and Echo Meter Handheld detectors.

Conclusion

Overall, our findings suggest that MYSP are the most common type of bat found at the Beaverhill Bird Observatory based on acoustic data, and the clearing habitat type is the preferred habitat across all species in terms of echolocation activity. Future studies could include more long-term monitoring for changes in the presence of different bat species and their habitat usage.

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Appendix

Appendix 1. Compiled call parameters used for species identification and call analyses.

Common Name	Scientific	Duration (ms)	Fpeak (Fc)	Fmax	Fmin	Call Characteristics
Hoary Bat	*Lasiurus cinereus*	11 (a, b) 4-26 (a, b) 7 (a, b) 15 (a, b) > 5 © *** <30ms	20.1 (a, b) 16-32 (a, b)	20.8 (a, b) 17-49 (a, b) 25 (c)	19.7 (a, b) 16-31 (a, b) 20 © ***<20	Fairly flat, straight, low, slight u-shape, may have slight downturn @ end -rounded bottoms, may have slight upturn at end
Eastern Red Bat*** Could be found but RARE	*Lasiurus borealis*	6.8 (b) 3.2-16 (b) > 5 (c)	40.4 (b) 29-49 (b)	43.8 (b) 29-73 (b) 45 (c)	40.2 (b) 28-48 (b) 40 (c)	More rounded and higher than Hoary and silver-haired, u-shaped but upturn @ end of call
Silver Haired Bat	*Lasionycteris noctivagans*	9.2 (a) 8.9 (b) 2.3-24 (a, b) > 5 (c)	26.6 (a) 26.5 (b) 23-31 (a, b)	28.8 (a, b) 24-44 (a, b) 30 (c)	25.4 (a, b) 14-30 (a, b) 25 (c)	More rounded than Hoary Bat, higher than hoary, reverse J-shape, will not exceed 55 kHz -downward turn or no curvature @ end of call; may have abrupt change in slope midway through the call -flat calls ≥25 kHz
Big Brown Bat	*Eptesicus fuscus*	7.8 (a) 2.3-18 (a) 8.2 (b) 2.8-19 (b) 4-8 (c)	28.2 (a) 24-55 (a) 27.9 (b) 21-33 (b)	31.9 (a) 25-52 (a) 30.0 (b) 22-42 (b) 35 © ***>65 kHz	27.2 (a) 23-33 (a) 26.5 (b) 19-32 (b) 28 © ***≥20-22kHz	Slanted L/hockey stick -may go as high as 65 or above kHz -downward turn or no curvature @ end of call; may have abrupt change in slope midway through the call

Western Small-footed Bat***NO	*Myotis ciliolabrum*	3.2 (a) 1.7-5.3 (a)	44.3 (a) 38-48 (a)	49.1 (a) 40-71 (a)	40.6 (a) 31-44 (a)	Steep and curved line, clear downwards tail
Long-eared Bat***NO	*Myotis evotis*	3.7 (a) 1.1-6.5 (a) 1-2 (c)	34.3 (a) 29-43 (a)	39.1 (a) 31-71 (a)	28.1 (a) 23-43 (a)	Large straight down-slanting line
Little Brown Bat	*Myotis lucifugus*	6.0 (a) 2-9 (a) 5.8 (b) 2-7.8 (b) 2-4 (c)	40.8 (a) 35-48 (a) 39.7 (b) 34-46 (b)	44.5 (a) 36-74 (a) 43.4 (b) 38-73 (b)	44.5 (a) 28-44 (a) 36.5 (b) 27-43 (b)	Pointed L/hockey stick, angular, similar to myotis volans but longer calls, longest duration of all mytois and lowest slope
Northern Long-eared Bat***	*Myotis septentrionalis*	3.9 (b) 1.7-6.6 (b) 1-2 (c)	43.2 (b) 32-53 (b)	51.3 (b) 37-95 (b)	37.0 (b) 25-50 (b)	Smaller straight line/slash shape
Long-legged Bat***NO	*Myotis volans*	4.8 (a) 1.1-8.8 (a) 4-8 (c)	41.6 (a) 34-50 (a)	48.0 (a) 39-89 (a) 40 (c)	36.9 (a) 27-44 (a) 35 (c)	L shaped, similar shape to Little brown bat but shorter and steeper calls

A = Western Acoustic Table
B = Eastern Acoustic Table
C = Fenton 1983
*Full Article

Legend:
Yellow highlight - Found in Tofield at the Beaverhills Bird Observatory
Green highlight - Rare
No highlight - out of range, not found in Tofield at the Beaverhills Bird Observatory

Appendix 2. Raw data of echolocation activity for frequency of bat species.

Date	Habitat Type	Species	Number of Calls
11-May-19	clearing	MYSP	4
08-Jun-19	clearing	LACI	3
08-Jun-19	clearing	MYSP	18
16-Jun-19	clearing	LACI	10
16-Jun-19	clearing	MYSP	6
20-Jun-19	clearing	EPFU/LANO	38
20-Jun-19	clearing	LACI	6
20-Jun-19	clearing	MYSP	52
30-Jun-19	clearing	EPFU/LANO	2
30-Jun-19	clearing	MYSP	1
30-Jun-19	edge	MYSP	2
05-Jul-19	clearing	EPFU/LANO	2
05-Jul-19	clearing	MYSP	34
29-Jul-19	clearing	MYSP	30
29-Jul-19	interior	MYSP	16
11-Aug-19	clearing	LACI	2
11-Aug-19	clearing	MYSP	15
17-Aug-19	clearing	MYSP	4
17-Aug-19	interior	MYSP	1
30-Aug-19	clearing	LACI	6
30-Aug-19	clearing	MYSP	20
07-Sep-19	clearing	LACI	7
07-Sep-19	clearing	MYSP	1
14-Sep-19	clearing	EPFU/LANO	2
14-Sep-19	clearing	LACI	4
14-Sep-19	interior	LACI	3
21-Sep-19	clearing	LACI	3

Appendix 3. Raw data of presence of bat species in each sampling night.

Date	Habitat	Species
11-May-19	clearing	MYSP
08-Jun-19	clearing	LASC
08-Jun-19	clearing	MYSP
16-Jun-19	clearing	LASC
16-Jun-19	clearing	MYSP
20-Jun-19	clearing	EPFU/LANO
20-Jun-19	clearing	LASC
20-Jun-19	clearing	MYSP
30-Jun-19	clearing	EPFU/LANO
30-Jun-19	clearing	MYSP
30-Jun-19	edge	MYSP
05-Jul-19	clearing	EPFU/LANO
05-Jul-19	clearing	MYSP
29-Jul-19	clearing	MYSP
29-Jul-19	interior	MYSP
11-Aug-19	clearing	LASC
11-Aug-19	clearing	MYSP
17-Aug-19	clearing	MYSP
17-Aug-19	interior	MYSP
30-Aug-19	clearing	LASC
30-Aug-19	clearing	MYSP
07-Sep-19	clearing	LASC
07-Sep-19	clearing	MYSP
14-Sep-19	clearing	EPFU/LANO
14-Sep-19	clearing	LASC
14-Sep-19	interior	LASC
21-Sep-19	clearing	LASC

Appendix 4. Summary of environmental potentially confounding variables.

	May 11	May 17	May 25	June 01	June 08	June 16	June 20	June 30	July 05	July 13	July 20	July 29	Aug 2	Aug 11	Aug 17	Aug 30	Sept 07	Sept 14	Sept 21	Sept 28
Precipitation (mm)	0	0	1.2	0	2.3	0	0	0	2.5	0.6	0.6	0	1.2	0	0	2.3	0	0	0	0
High ˚C	23	16	17	26	12	23	15	22	15	22	22	22	27	14	16	14	23	19	22	3
Low ˚C	18	11	10	18	6	16	12	18	14	16	14	15	18	14	12	10	15	12	13	2
Wind (km/h)	5	21	8	12	8	7	14	13	7	5	4	6	11	8	6	6	12	5	7	7
Wind Direction	E	SE	SSW	E	WNW	S	NNE	SSW	ESE	W	E	SSE	SE	SW	N	E	S	S	NW	ENE
Sunset	21:17	21:27	21:39	21:48	21:55	22:01	22:02	22:02	21:59	21:53	21:44	21:31	21:24	21:06	20:54	20:24	20:05	19:48	19:31	19:14
Moon Presence (A)	Y	Y	N	N	Y	Y	N	N	Y	Y	N	N	Y	Y	N	N	Y	Y	N	N
Moon Presence (O)	Y	Y	N	N	Y	Y	N	N	Y	Y	N	N	Y	Y	N	Y	Y	N	N	Y
% Illumination	50.5	96.6	60.9	3.4	36.2	98.7	90.8	5.6	13.5	92.9	88.8	8.1	4.7	90.1	96.8	0.4	69.9	99.8	56.2	0.2
Humidity (%)	27	29	53	46	67	40	86	46	81	76	75	71	75	81	56	86	70	69	61	67