### Habitat use and spatial patterns of *Myotis* and large-bodied bat species assessed by the narrow-band acoustic method at the Beaverhill Bird Observatory

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#### Abstract

Temperate bats play an important role in providing essential ecosystem services to terrestrial environments world-wide. Relatively little is known about the spatial habitat-use and activity patterns of temporal bats located within the dry mixed-wood sub-region of Alberta. The objective of this study was to determine if there is an association between habitat type and presence of Myotis and EPFU/LANO taxonomic bat species at the Beaverhill Bird Observatory (BBO). This study also investigated whether there were interspecific differences apparent in spatial habitat use by taxonomic species, as indicated by the number of passes and feeding buzz echolocation calls across forest, aquatic and grassland habitat types using active narrow-band acoustic equipment. My observational data based on the detection of taxonomic species at habitat transects reported statistical insignificance for the association between habitat type and bat presence at the BBO, indicating that taxonomic species occurred independently of one another and are distributed equally across habitats (Test for Association Chi-square:  $\chi^2 = 2.08669$ , p= 0.35222, df= 2, p<0.05). Further, differential habitat use among taxonomic species was statistically insignificant, suggesting that use of spatial habitat and activity patterns (foraging, commuting, and roosting) were similar for all species irrelevant of habitat type (Goodness of Fit Chi-square:  $\chi^2$ = 2.08669, p= 0.108809, df= 2, p<0.05). Observational data found that bat activity patterns in all habitats increased from June to July regardless of habitat-use. Overall, the results from this study suggest that strong predictors of habitat use at the BBO may result from the high landscape complementation as well as the adoption of optimal foraging strategy by bat species residing in the area. Future studies could consider a multi-factorial model investigating the association of bat presence and habitat use via a synergistic effect of inter- and intra-specific differences, invertebrate abundance, and ecomorphology.

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Insectivorous bats world-wide are being threatened by the spread of emerging zoonotic viruses and habitat destruction as anthropogenic activities that threaten ecosystem integrity (Agosta, 2002). Researchers believe that these historical population declines have been attributed to habitat destruction via the disturbance and modification of roosts, spread of white-nose syndrome (WNS), bioaccumulation of toxins, introduced predators and habitat loss and fragmentation (Agosta, 2002; Pauli, Zollner, & Haulton, 2017). There are ecological and economic rationales for conservation of temperate forest bats due to their ecosystem services (i.e., arthropod suppression, seed dispersal, nitrogen dispersal, and pollination) (Agosta, 2002; Kunz, Braun de Torrez, Bauer, Lobova, & Flemming; 2011). Bats serve as a strong indicator species because they are unable to respond rapidly to disturbance and population declines due to their K-selected life history characteristics (Vaugham, Jones, & Harris, 1997).

Habitat selection by bats is more specialized for roosting habitat (tree and snag cavities or tree foliage), and in contrast, more opportunistic in their choice for foraging habitat (Ethier & Fahrig, 2011), both of which are essential to maintaining population size, fecundity, and overall fitness (Vaugham et al., 1997; Agosta, 2002; Ethier & Fahrig, 2011). Foraging rate in bats is strongly correlated with aerial insect densities and bat population size, where good quality habitats provide the highest rate of insect capture and can support larger local bat populations (Vaughan et al, 1997). Temperate bats preferentially roost in forested areas with high landscape complementation. This occurs when roosting sites are in close proximity to foraging sites and facilitate movement between different habitats while reducing energy expenditure and complementing resource needs (Ethier & Fahrig, 2011). Further, understanding species interactions with their habitat is crucial for predicting their vulnerability and responses to disturbance as anthropogenic activity expands into native habitat (Clare, Barber, Sweeney, Herbert, & Fenton, 2011)

In the past, conservation efforts have focused primarily on mitigation strategies for rare or endangered species; however, studying the most widespread and abundant species can further enhance our understanding of bat behavior and habitat preference (Pauli et al., 2017). Based on the general distribution and results from previous surveys, of Alberta's nine bat species the most likely species to occur near in the dry mixed-wood sub-region of Alberta near the Beaverhill Lake include: the small-bodied little brown bat or MYLU (*Myotis lucifugus*) and large-bodied big brown bat or EPFU

(Eptesicus fuscus) and silver haired bat or LANO (Lasionycteris noctivagans) species (Reimer, Lausen, Barclay, Vassal, & Irwin, 2014; Natural Regions and Sub-regions of Alberta, 2006, p.vi). Previous studies have reported that ecological morphology in bats influence differential habitat use (Kalcounis & Brigham, 1995). Echolocation call and wing morphology are indicative of partitioning of dietary and habitat resources, and the adoption of certain foraging strategies (Kalcounis & Brigham, 1995; Vaughan et al, 1997). MYLU forage over aquatic/riparian features and bottomland forest habitat types; while EFLA/LANO prefer to forage over open areas, along forest edges and aquatic habitats (Pauli et al., 2017). Myotis ecomorphology reflects their ability to exploit diverse and cluttered habitats as their low mass or wing loading capacity enhances their maneuverability while foraging in forest habitats, and produce a short, high frequency, wideband echolocation call (Kalcounis & Brigham, 1995; Nelson & Gillam, 2017; Wund, 2006). The EPFU/LANO optimally forages in open areas as they produce a relatively long, low frequency, narrowband call and have a high body mass and wing loading capacity making them less maneuverable (Kalcounis & Brigham, 1995; Wund, 2006). MYLU and EPFU/LANO typically roost in forest habitats, but have also been known to select roosts in man-made structures (Krauel & LeBuhn, 2016). Aquatic environments are preferentially used for foraging, drinking, and commuting for both taxonomic groups. In this study, the predictions will be based on previous knowledge that *Myotis* are a clutter-adapted bat species that prefer to forage in forest and aquatic habitats; while EPFU/LANO have a tendency to forage in open areas along forest edges and over aquatic habitats due to their morphological constraints in maneuverability (Brooks, 2009). The interspecific trends across these taxonomic bat species leads us to question whether wing loading or mass, maneuverability, call sequence and flight performance result in differential habitat use for foraging (Kalcounis & Brigham, 1995).

Although foraging activity, roost selection and habitat use for the MYLU and EPFU/LANO species have been well documented across North America, the relationship between ecomorphology and habitat use for these bat species located in the dry mixed-wood natural sub-region of Alberta is lacking. The purpose of this project was to determine if there is an association between habitat type and the presence of *Myotis* and EPFU/LANO bat species at the Beaverhill Bird Observatory (BBO) assessed by the active narrow-band acoustic method. Further, we investigated whether there were interspecific differences in spatial habitat use between the taxonomic bat species. This accomplished by manipulating habitat type and actively recording the number of passes and feeding buzzes made

*Myotis* and EPFU/LANO at recording stations along habitat associated transects. We hypothesized that *Myotis* and EPFU/LANO exhibit differential activity patterns across the forest, aquatic, and grassland habitats due to ecomorphology and echolocation call characteristics, which may restrict their use of specific habitats. We predicted that the forest and aquatic habitats support the highest frequency of *Myotis* passes and feeding buzzes at recording stations; while the open grassland and aquatic habitats support the highest frequency of EPFU/LANO.

#### Methods

#### Study area and sites

The study was conducted at the Beaverhill Bird Observatory (BBO) located near Tofield in the dry mixed-wood natural sub-region of Alberta (Natural Regions Committee 2006). The BBO is a naturalized area that is predominantly forested with a mix of mature aspen forest and grasslands, but with significant areas of wetland, stream, and lake features (Figure 1) (BBO website).

#### Acoustic detection

Bat activity was surveyed acoustically using the QMC Instruments (1983) narrow-band active bat detecting technology supplied by the BBO. Bat echolocation surveys took place once a week between June 3, 2017 and July 16, 2017 and observational acoustic data was collected for two hours beginning 45 minutes after sunset, when bat activity was greatest (Crampton & Barclay, 1998). A total of three to five recording stations were set every 60-100 m apart along each of the three 300m long walking transects (Figure 1). Each transect was assigned a distinct habitat type: forested, grassland, and near water (Figure 1). The grassland and near water habitat transects contained three recording stations set 100m apart; while the forested transect had five recording stations set every 60m apart. All transects were sampled per night with a randomized starting order to minimize any bias associated with peak bat activity. Bat species that are most likely to be encountered at the BBO include MYLU and EPFU. Since other large-bodied bat species LANO and EPFU cannot be distinguished, they will be combined to be EPFU/LANO in this study. Hoary bats (LACI) are less commonly detected in this area, therefore are not included in sampling. The QMC Instruments bat detector was set to 25 kHz (EPFU/LANO) and 40 kHz (MYLU) for two minutes per frequency at each recording station to detect and differentiate between species. The number of passes and buzz call sequences were recorded during the two minute recording time. To quantify bat activity levels, observational data recorded was based

on the number of passes and feeding buzzes per unit time per minute for the 25 kHz and 40 kHz frequencies at each recording station. A feeding buzz was identified by an acceleration in echolocation pulse, which occurs when foraging for insects; while a pass was identified by a single echolocation with one second between call sequences (Crampton & Barclay, 1998). Passes and buzzes in this study were an index of habitat use across the three transects. The date, start and end time, wind speed (Beaufort scale), percent cloud cover, and temperature at the start were recorded for each transect before starting the survey.

#### Analysis

To determine whether there was an association between habitat type and presence of MYLU and EPFU/LANO, echolocation activity of MYLU and EPFU/LANO species across the forest, near water, and grasslands habitats were compared using a contingency table. A Test for Association Chi-square statistical analysis was performed to test for an association in the distribution of the species' response to habitat type. To determine whether there was a difference in habitat use by MYLU and EPFU/LANO, we performed a Goodness of Fit Chi-square test using Yates' Correction factor. This concluded whether the observed data fit the expected pattern of frequencies for each habitat type. To illustrate the effects of habitat type on the presence of the two taxonomic bat species, I graphed: 1) the total number of call sequences for MYLU and EPFU/LANO species habitat, 2) the number MYLU buzzes and passes per habitat, 3) the number of large-bodied bat buzzes and passes per habitat (Figure 2). Further, seasonal trends in echolocation activity for the two species were graphed over the seven-week study period (Figure 2).

#### Results

Bat activity was evident for MYLU and EPFU/LANO in all habitats studied at the Beaverhill Bird Observatory at the start and end of the study overall (Figure 2). Bat echolocation activity was the highest at the end of June until mid-July for all transects (Figure 2). Passes and feeding buzzes occurred in all three habitat types, with the highest overall activity (indicated by call sequences) recorded for large-bodied species (29 calls) and the lowest activity recorded for MYLU (22 calls) across all habitats (Table 1). MYLU had the highest frequency of feeding buzzes in the forest habitat (7 buzzes), in comparison to the aquatic (3 buzzes) and grassland (3 buzzes) habitats; thus, the highest frequency of passes in the forest habitat (7 passes), and lowest in the aquatic and grassland habitats

(3 passes each) (Table 1). EPFU/LANO had the highest frequency of feeding buzzes in the aquatic (5 buzzes) and forest (4 buzzes) habitats in comparison to the grassland habitat (3 buzzes); thus, the highest frequency of passes in the forest habitat (9 passes) and lowest in the aquatic (5 passes) and grassland (3 passes) habitats (Table 1).

Both MYLU and EPFU/LANO taxonomic species occurred at similar rates across habitat categories, indicating independence between species and habitat type (Test for Association Chi-square:  $\chi^2$ = 2.08669, p= 0.35222, *df*= 2, p>0.05) (Figure 3). The observed echolocation activity (total call sequences, passes, and buzzes) for MYLU and EPFU/LANO in each habitat type was statistically insignificant (Goodness of Fit Chi-square:  $\chi^2$ = 2.08669, p= 0.108809, *df*= 2, p>0.05). This indicates that habitat use for EPFU/LANO and MYLU were equal in the habitat categories and fit the expected pattern of frequencies for each habitat type (Figure 4).

#### Discussion

This study provided the first active narrow-band acoustic data of bats at the Beaverhill Bird Observatory and documented the association between habitat type and bat presence, spatial activity patterns, and habitat use. The presence of clutter-adapted MYLU (40 kHz) and open-adapted EPFU/LANO bat species (25 kHz) was confirmed at the BBO in the forest, aquatic, and grassland habitat types in the natural area over the study period, which coincides with findings from Audet (2015) and Reimer et al. (2014). Higher occurrence and acoustic activity across habitat types was recorded for EPFU/LANO than MYLU species overall, providing further evidence of their common occurrence and widespread distribution of taxonomic group (Ethier & Fahrig, 2011).

In the scientific community, it is well known that species morphology is strongly correlated to the structure of bat communities and habitat use (Brooks & Ford, 2005). EPFU/LANO species are considered habitat generalists, as their ecomorphology and call characteristics suggest these taxonomic species lack maneuverability, therefore restricting the use of closed-forest habitats (Brooks & Ford, 2005; Jung et al., 1999; Kalcounis & Brigham, 1995). In contrast, *Myotis* species are clutter-adapted specialists, as their low body mass and wing loading, and short, high frequency, wideband echolocation call morphology enhance navigation and foraging skills in forest habitats (Kalcounis & Brigham, 1995; Wund, 2006). At the scale sampled in this survey, we fail to reject our null hypothesis and conclude based on acoustic data that there is no statistical association between habitat type and

bat presence at the BBO during the study period (Test for Association Chi-square:  $\chi^2 = 2.08669$ , p= 0.35222, *df*= 2, p>0.05). This indicates that MYLU and EPFU bat species responded similarly to each habitat type in terms of spatial distribution/LANO and occur independently of one another (Figure 3). This data coincides with the findings of Brooks (2009) which recorded an abundance of all species, especially the large-bodied bats, at open habitats in general, partially-closed canopy forests, and aquatic habitats. Aquatic habitats are indicative of species diversity and abundance as they attract both open-adapted EPFU/LANO and clutter-adapted Myotis taxonomic species and have been identified as a primary means for foraging, drinking, and commuting behaviors (Brooks, 2009). A study conducted by Brooks and Ford (2005) reported that MYLU equally used open-canopy, openaquatic, and closed-meadow habitats. Although there is no apparent statistical significance, observational data suggests that echolocation activity for MYLU was centralized to the forest habitat, while open-adapted larger-bodied bats showed similar patterns of activity in both the forest and aquatic habitats (Figure 2). Temporal and spatial variation in activity levels among bat species in forest and aquatic habitats suggests that both sites may have been used as a commuting route between roosts and foraging sites (Brooks, 2009). In particular, open-stream sites are used for preliminary foraging for MYLU and later commuting to cluttered forest habitats for the second round of foraging activity (Brooks, 2009). Our study was limited by the inability to distinguish bat species present at the BBO other than using the 25 kHz and 40kHz narrow-band acoustic equipment. Knowing the activity of specific bat species would allow us to account for the inter- and intra-specific differences in MYLU and EFLA in terms of habitat use to avoid generalizations across morphologically similar species. Although *Myotis* are cluttered-adapted species, MYLU are considered to be habitat generalists in comparison to the northern long-eared bat (Myotis septentrionalis), which is a forest specialist. Based on the location and habitat features, it is likely that MYLU are the only Myotis species present in this study. This suggests that species identification is important to further understand habitat use as niche space among sympatric species may be partitioned based on differential habitat use and foraging activities.

Ecomorphology and echolocation call characteristics are often predictive of spatial habitat-use in temperate bat species. The interspecific differences in habitat-use measured by echolocation activity for EPFU/LANO and *Myotis* taxonomic bat species across habitat categories were statistically insignificant and did not support my predictions (Goodness of Fit Chi-square:  $\chi^2$ = 2.08669, p=

0.108809, df=2, p>0.05). Habitat-use characterized by foraging, roosting, and commuting activities for Myotis and EPFU/LANO were equal in the forest, aquatic, and grassland habitats (Figure 4). Although the statistical analysis of the data does not support our hypothesis, we can infer from the observational data that *Myotis* had the highest number of feeding buzzes and passes in the cluttered forest habitat; while EPFU/LANO foraged similarly in both open and cluttered habitat types but passed through the forest habitat more frequently (Figure 3). Further, high commuting activity (passes) in the forested and edge habitats indicate its potential use as a travel corridor between roosting and foraging sites for both taxonomic groups as mentioned previously. Since community bat populations are largely influenced by the availability of roosting sites and prey abundance, lack of differentiation in habitat use in this study could indicate that all habitats equally provide similar levels of prey abundance and dietary richness for MYLU and EPFU/LANO species, coinciding with previous studies (Clare et al., 2014; Ethier and Fahrig, 2011). Further, foraging site selection in bats is often influenced by the optimal foraging theory, or the trade-offs between the energetic costs of travelling from the roosting site to foraging site, predation risk, and energy gains. Bats will commute a larger distance to foraging sites that are more diverse and energy rich in prey items (Clare et al., 2014). Based on our results, MYLU and EPFU/LANO species at the BBO may select habitats based on habitat continuity, where the roost (forest habitat) and foraging sites (forested and aquatic habitats) are in close proximity and the landscape structure facilitates easy access to resources while eliminating energy expended via commuting and reduced risk of predation (Ethier & Fahrig, 2011).

In this study we found that there was an increase in echolocation activity in general from the beginning of June until the end of July, close to pre-fledging time (Figure 1). Habitat use and selection of foraging sites may be more complex as gender, age, and pregnancy/lactation may also have an effect on habitat use, which was not accounted for in this study. Optimal foraging is theoretically more important for pregnant and lactating females than males, as the high energy costs for reproduction result in energetically driven dietary switches (Ethier & Fahrig, 2011; Clare et al., 2011). The consequences of not foraging optimally can have detrimental effects on reproductive success, survival, and population size. It could be likely that our results were also influenced by temporal variation in insect availability across the three habitats at the BBO, as bat populations are known to be more attracted to high insect concentrations than habitat type (Veselka, McGuire, Dzal, Hooton, & Fenton, 2013).

Overall, in this study we observed that there is no association between habitat type and bat presence at the BBO. Further, we recorded that habitat-use indicated by passes and feeding buzzes among Myotis and large-bodied taxonomic bat species was equal across forest, aquatic, and grassland habitat types. The results from this study indicates that habitat use may be more complex, where factors affecting bat presence may be influenced by factors other than ecomorphology and theoretically known foraging strategies. We did not control for fluctuations in invertebrate populations nor intraspecific differences in species, including sex, pregnancy, lactation, size, and age, which could arguably be strong predictors of spatial habitat use in *Myotis* and EPFU/LANO bats in central Alberta. Another lesser-known predictor of habitat use and selection based on habitat features is landscape complementation (Ethier & Fahrig, 2011). Based on this study, it remains unknown whether the presence of bats at the BBO can be attributed to habitat type and use alone. An improvement of this study could consider using a combination of wide-band acoustic technology and mist netting to distinguish inter- and intra-specific differences among bat species at the BBO. In addition, it would be advantageous to conduct bat surveys more frequently and for a longer duration to obtain a larger sample size, which was a strong limiting factor in this study. Future studies could consider a multifactorial model for investigating the association of bat presence and habitat use, thus determining a synergist effect of all factors together and independently on habitat selection and use.

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#### Appendix

**Table 1. Summary of echolocation activity for the little brown bat (MYLU) and big brown bat (EFLA) species in the forest, near water, and grassland transects.** The total number of passes (sum of passes and buzzes), and the total number and percent of feeding buzzes for each taxonomic species was recorded over the seven week survey period. Observational values were compared to determine whether there was a statistical significant difference in the frequency of passes and buzzes for each species among transects.

		Forest	Near Water	Grassland
MYLU	Total Passes	14	4	4
	Feeding buzzes	7 (50%)	3 (75%)	3 (75%)
EFLA	Total Passes	13	10	6
	Feeding buzzes	4 (31%)	5 (50%)	3 (50%)



**Figure 1. Beaverhill Bird Observatory trail map.** The forest transect was located on Warbler Way, the grassland transect was located ~100m north of the forest edge along Short-eared Owl Street, and the near water transect was located adjacent to the weir on the east side of the Beaverhill lake running north to south.

HABITAT USE AND SPATIAL PATTERNS OF *MYOTIS* AND LARGE-BODIED BAT SPECIES ASSESSED BY THE NARROW-BAND ACOUSTIC METHOD AT THE BEAVERHILL BIRD OBSERVATORY



Figure 2. Seasonal trends in echolocation activity (total passes and buzzes) for the little brown bat (MYLU) (blue circle) and the big brown bat (EFLA) (red circle) at recording stations for the forest, near water, and grassland habitat transects from June 3, 2017 to July 16, 2017. Highest number of passes/buzzes were recorded along the forest habitat transect (8 passes/buzzes).



Figure 3. Distribution of total call sequences for EFLA (red) and MYLU (blue) species in the forest, near water, and grassland habitat transects.



Figure 4. Distribution of buzzes (red) and passes (blue) for MYLU (graph on the left) and EFLA (graph on the right) bat species across the forest, near water, and grassland habitat transects.