1 **Island biogeography theory and the urban landscape: stopover**

2 **site selection by the silver-haired bat (*Lasionycteris noctivagans*)**

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13   **Abstract**

14   Many migratory bats require forested sites for roosting and foraging along their migration path, but

15   increased urbanization and intensive agricultural practices may reduce the availability of stopover sites.

16   Urban forests may provide important stopover habitat, maintaining landscape connectivity in regions

17   where the majority of natural habitat has been cleared for development. Island biogeography theory can

18   be applied to urbanized temperate forest biomes where small urban forests represent islands separated

19   from the larger "mainland" forest. We used acoustic monitoring during the fall migration period to

20   investigate the use of urban forest habitat by the migratory species *Lasionycteris noctivagans* Le Conte,

21   1831. We predicted that recorded activity would have a positive relationship with forest patch area and

22   shape and a negative relationship with isolation from other forest patches, as suggested by island

23   biogeography theory. We observed greater activity at larger forest patches, and although relationships for

24   shape and isolation were not statistically supported the observed patterns were consistent with predictions.

25   Our results demonstrate the need for more in-depth research on the habitat requirements for both

26   migratory and resident bat species and the impact that ongoing urbanization has on local bat populations.

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28   **Keywords**: Migration, stopover*,* urbanization, fragmentation, *Lasionycteris noctivagans*, *Eptesicus fuscus*

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29 **Introduction**

30   Animals living in seasonal environments must cope with a spectrum of challenges throughout the

31 year. When faced with seasonal resource limitation, many species respond by migrating to more

32 favourable conditions (Dingle 2014). Consequently, migratory species occupy distinct seasonal ranges

33 and may be characterized as living in 'two worlds' (Greenberg and Marra, 2005). However, migrants also

34 transiently occupy a series of stopover habitats along their migratory route. The biology of migratory

35 species crucially depends on the different habitats and spatial distributions they occupy (Dingle, 2014;

36 Bowlin et al., 2010), and although accounting for a relatively small proportion of the annual cycle,

37 stopover habitats are crucial to the success of migratory species. Migration can represent the period of

38 greatest adult mortality (Sillett and Holmes, 2002), and successful migration may be related to the

39 availability of high-quality stopover habitats (Hutto 1998). Migration periods generally, and stopover

40 biology specifically, remain crucial knowledge gaps for many species (Bowlin et al. 2010). Therefore,

41 developing a holistic understanding of migration biology requires identifying stopover sites and

42 understanding habitat characteristics that migrants rely on (Sheehy et al., 2011). Stopover sites represent

43 important opportunities to study and observe migrants, especially for highly mobile, small-bodied species

44 that are difficult to track (Faaborg et al. 2010).

45   Some general principles apply to all migrants (Dingle 2014), but migratory requirements vary

46 among taxonomic groups (McGuire and Fraser, 2014). Temperate insectivorous bats are a group that

47 faces different constraints and tradeoffs compared to other migrants (McGuire and Guglielmo, 2009;

48 McGuire et al., 2014; Jonasson and Guglielmo, 2019; Baloun et al., 2020). Temperate insectivorous bats

49 experience a variety of challenges throughout the annual cycle and, when faced with winter as an

50 extended period of resource limitation, most species undertake migration in some form (Fleming and Eby,

51 2003). For some species, migration distances are relatively short, such as altitudinal or regional

52 migrations (McGuire and Boyle, 2013; Griffin, 1970). Other species undertake latitudinal migrations,

53 travelling hundreds or even thousands of kilometers between seasonal grounds (Fleming and Eby, 2003;

54   Cryan, 2003). Despite this, migration is a poorly understood aspect of bat behaviour (Fleming, 2019).

55   There is an abundance of research focused on the summer active season (e.g., Kurta and Teramino, 1992;

56   Fenton, 1970), and winter hibernation has been extensively studied in regional migrants (Boyles et al.,

57   2020). However, the distribution and ecology of latitudinal migrants during the winter is virtually

58   unknown and there are many outstanding questions about the ecology, behaviour, and physiology of bats

59   during migration. Most studies of bat migration are based on either the mark-recapture of banded bats

60   (Griffin, 1983) or documentation of wind turbine mortality (Baerwald and Barclay, 2009), but little is

61   known about migratory bats while *en route*. Radiotelemetry studies are beginning to elucidate migratory

62   movement patterns (Roby et al., 2019; McGuire et al., 2012), but migration movement ecology and

63   behaviours are still poorly understood.

64   Stopover sites are critical ecosystems along the migration path where bats can forage to rebuild

65   fuel stores during the night and roost during the day, sometimes stopping for multiple days before

66   continuing migration (McGuire et al., 2012; Jonasson and Guglielmo, 2019; Fleming, 2019; Moore et al.,

67   1995). Little is known about habitat requirements during stopover, and while it is likely that forested

68   habitats are important for stopover, specific roosting requirements may be more flexible during migration

69   than during breeding seasons (e.g., McGuire et al., 2012). In temperate North America, the three species

70   of latitudinal migrants (hoary bats *Lasiurus cinereus* Beauvois, 1796, eastern red bats *Lasiurus borealis*

71   Müller, 1776, and silver-haired bats *Lasionycteris noctivagans* Le Conte, 1831) all roost in trees, whether

72   in cavities or foliage, further emphasizing the importance of forested areas for stopovers. Therefore,

73   extensive land use change may be an important threat to migratory bats as large areas of forest are cleared

74   for agriculture and urban development. A global shift toward urbanization has transformed many natural

75   landscapes into a mosaic of dense urban areas, agricultural land, and fragmented patches of less-disturbed

76   habitat (McKinney, 2002; Olejniczak et al., 2018). Remaining stopover sites are often subjected to

77   anthropogenic disturbances that reduce habitat quality and species diversity (Ethier and Fahrig, 2011;

78   Moretto and Francis, 2017). Although degraded from the original habitat, remnant urban forests may be

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79   important stopover sites for migratory animals. For example, urban parks are important migration stops

80   for short-term refueling in some migratory birds (Seewagen et al., 2011) and may also represent important

81   stopover sites for migrating bats (Coleman and Barclay, 2013). Thus, it is important to understand how

82   bats use these patches to maintain suitable habitat for migratory bats and inform city planning decisions

83   when developing forested areas.

84   Island biogeography theory (MacArthur and Wilson, 1963) may be a useful framework for

85   making predictions about stopover habitat use in urban landscapes. This framework proposes that species

86   richness increases with island size and decreases with distance from mainland source population, based

87   on the probability of species reaching the island and the amount of resources and niche space available. In

88   temperate forest biomes, remnant forest patches within an urban matrix can represent islands separated

89   from contiguous forest (Olejniczak et al. 2018). In this way, island biogeography theory can be used as a

90   framework to predict the relative number of migratory bats that will stop over in an urban forest, with

91   general expectations of greater bat activity at larger and less isolated forest patches. In addition to the

92   absolute size of forest patches, the amount of edge habitat may affect the use of a patch as many species

93   of bats forage along forested edges (Walsh and Harris 1996; Jantzen and Fenton 2013). Thus, larger

94   patches may provide more roosting habitat, while patches with more edge habitat may provide more

95   foraging opportunities. We used acoustic monitoring during the fall migration period to record bat activity

96   at urban forests of varying sizes. We hypothesized that the island biogeography framework is applicable

97   to stopover site selection by migrating bats. Specifically, we predicted 1) larger forest patches (more

98   roosting habitat) would have higher bat activity than smaller forest patches, 2) forest patches with a

99   higher shape index (more edge habitat for foraging) would have greater activity than more circular

100 patches, and 3) isolated forest patches would have lower activity than those in close proximity to other

101 forests.

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103 **Materials and Methods**

104   *Study Area and Species–* We conducted our research in Waterloo region, Ontario, Canada. Waterloo

105   region has a population of approximately 617,870 (Region of Waterloo, 2019) and is experiencing an

106   increase in urbanization and agricultural output (Bucknell and Pearson, 2006). Much of the original forest

107   in the region has been cleared for agricultural use, creating a "mosaic" fragmentation pattern surrounding

108   the city. Patches of urban forest are present throughout the city in the form of municipal parks, historical

109   sites, and privately owned woodlots. We selected six forested sites with a range of areas, shapes, and

110   degrees of isolation from nearby forest patches (**Figure 1**).

111   Southern Ontario is home to eight species of bats, including sedentary species (big brown bats

112   *Eptesicus fuscus* Beauvois 1796), regional migrants (little brown bats *Myotis lucifugus* Le Conte, 1831;

113   eastern small-footed bats *Myotis leibii* Audubon and Bachman, 1842; northern long-eared bats *Myotis*

114   *septentrionalis* Trouessart, 1897; and tricolored bats *Perimyotis subflavus* Cuvier, 1832), and latitudinal

115   migrants (hoary bats *Lasiurus cinereus*, eastern red bats *Lasiurus borealis*, silver-haired bats

116   *Lasionycteris noctivagans*). All research activities were approved by the University of Waterloo Animal

117   Care Committee (Protocol #42374).

118   *Forest characterization–* To determine forest characteristics, we manually created shapefiles of each

119   study site and the surrounding forest patches within a 2 km radius using QGIS version 3.16 (Bender et al.

120   2003) Focal forest patches were defined as the contiguous forest closest to the bat detector, ending when

121   gaps between trees were >20 m (Hale et al., 2012). All surrounding forest patches were required to have a

122   core area of at least 400 m^2 to distinguish them from individual trees or sparse tree cover (**Table 1**). Trees

123   in residential areas (i.e., trees in back yards, gardens) or along roadsides were omitted from the analysis

124   (Krauel and LeBuhn, 2016). When characterizing forest patches, we included a 20 m buffer from the

125   forest edge to account for foraging habitat (**Figure 2**; Jantzen and Fenton, 2013). Characterizing specific

126   details of edges was beyond the scope of our study, but an interesting opportunity for future research

127   which may consider urban forest patches which may border on habitats ranging from grassy meadows, to

128   wetlands, to high traffic streets. We used Fragstats (version 4; McGarigal et al., 2012) to characterize

129   forest patches by converting the shapefiles of all study sites and surrounding 2 km of forest to raster

130   format and analyzing the default variables for each study site at the "patch metrics" level.

131   *Acoustic monitoring*– We used acoustic monitoring to quantify fall migration activity, recording for 85

132   nights from Aug 12 – Nov 5, 2020. We installed Songmeter SM2+ bat detectors (Wildlife Acoustics; MA,

133   USA) along the forest perimeter at the six study sites to record bat echolocation calls, as most bat species

134   have the highest detectability along the forest edge (Brigham et al, 2004). A single microphone was

135   attached to the top of a 3 m pole, >15 m from artificial light and oriented away from buildings and other

136   objects that could cause background noise or echoes. The detectors were programmed to record

137   throughout the night, from 15 min before sunset until 15 min after sunrise, and recordings (sampling rate

138   = 192 kHz) were saved as full-spectrum, timestamped WAV files.

139   *Call analysis*– All recordings were filtered by SonoBat Batch Scrubber 5.1 using the "high quality"

140   setting with a high pass filter of 5 kHz and default call quality and percent match settings (0.6 and 0.9

141   respectively). The filtering algorithm excluded recordings that did not contain bat echolocation calls,

142   however high katydid (Orthoptera) activity added background noise to the recordings that could have

143   hidden bat passes from the software. The remaining files were analyzed using SonoBat 4 Software with

144   the North America setting. Activity was quantified as the number of recordings for each species on a

145   nightly basis, allowing us to measure the relative abundance of species at different sites (Brigham et al.

146   2004). The echolocation calls of *E. fuscus* and *L. noctivagans* are very similar, but with modern

147   classification algorithms it is now possible to discriminate the two species in many cases (Lausen et al. *In*

148   *Press*).

149   *Statistical analysis*– We used R (version 3.6.1; R Core Team, 2020) for all statistical analyses. To

150   determine the seasonal period with sufficient activity for analysis (i.e., to exclude low activity period after

151   the majority of migrants had passed through the region), we considered the cumulative distribution of

152   nightly activity, keeping all dates up to and including the 95th percentile of the activity distribution, and

153   excluding nights late in the season after the main period of activity had passed; this occurred between

154   September 22 – 30 for all species.

155   We tested the effect of study site on *E. fuscus* and *L. noctivagans* activity using a likelihood ratio

156   test to compare nested generalized linear models with site as the explanatory variable. Since bat activity

157   fluctuated over the sampling period, we used a negative binomial distribution to account for

158   overdispersion of the data (glm.nb function from the MASS package; Venables and Ripley, 2002). Given

159   an overall effect of site on bat activity, we determined pairwise differences among sites using the cld

160   function from the multcompView package (Piepho 2004).

161   Fragstats provides multiple metrics to characterize each of patch area, shape, and isolation. We

162   used principal component analysis (PCA) as a data reduction technique to determine the main

163   characteristics of the forest patches at each study site (Vidal et al. 2016). Given the limited number of

164   sites relative to the number of metrics, we applied a separate PCA to the metrics within the three

165   categories of patch size (area, core area, and perimeter), shape (related circumscribing circle, shape index,

166   and fractal dimension index), and isolation (proximity index and Euclidean nearest neighbour index). We

167   performed each PCA using the prcomp function with centered and scaled data, retaining the first principal

168   component as an integrated measure of size, shape, or area (**Table 2)**.

169   To quantify the relationship between bat activity and forest characteristics, we performed linear

170   regression on the relative activity predicted by the generalized linear model and each of the three principal

171   components for each study site to determine the species-specific relationships between bat activity and

172   forest area, shape, and isolation.

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174   **Results**

175   *Site characteristics*– Forest area ranged from 11.5 Ha (Columbia Lake) to 108.1 Ha (Grey Silo), and the

176   sites encompassed a variety of shapes and levels of isolation (**Table 1; Table 2**). Open water or wetland

177   were present at all sites. The two closest sites (Bechtel Park and Woodside) were separated by 1.5 km

178   while the two most distant sites (Grey Silo and Huron Natural Area) were separated by 12.7 km. Stanley

179   Park was difficult to characterize because the park is bisected by a 20 m wide road. We conservatively

180   included the entire park when characterizing our sites, but the site area might effectively be constricted to

181   the smaller section of trees on the same side of the road as the detector. Linear regression analyses

182   (below) yielded higher R^2 values when including the smaller area for Stanley Park but did not

183   qualitatively change the interpretation of our analysis (i.e., no relationships were significant under one

184   area but not the other). Therefore, we conservatively include the larger patch area in our analysis.

185   *Acoustic monitoring*– After scrubbing for noise, we recorded 21,646 files over the 85 nights of sampling,

186   16,636 of which were identified to species (**Table 3**). Activity was recorded on 90.7% of the nights that

187   were sampled, and we detected six of the eight Ontario bat species (**Table 3**). The site with the most

188   recorded activity was Huron Natural Area (n = 7,425 passes) and the lowest activity was recorded at

189   Woodside (n = 1,447 passes). The most common species was *E. fuscus* (n = 13,416 passes) which is a

190   resident species commonly found in the region. The migratory *L. noctivagans* had the second highest

191   recorded activity (n = 2,134 passes). We detected *E. fuscus* and *L. noctivagans* at all sites for the majority

192   of the sampling period, therefore providing a comparison of resident and migratory species. Low activity

193   for the remaining four species precluded including them in further statistical analysis.

194   *Influence of site characteristics*– Activity varied among sites for both *L. noctivagans* (LR = 77.2, df = 5, p

195   < 0.0001) and *E. fuscus* (LR = 54.6, df = 5, p < 0.0001). Post-hoc analysis of *L. noctivagans* identified two

196   groups within the study sites, where the "high activity" group included the two largest sites (Huron

197   Natural Area and Grey Silo golf course), and the "low activity" group included the two smallest sites

198   (Woodside and Columbia Lake; **Figure 3**). Post-hoc analysis of *E. fuscus* identified three, less distinct

199   groups with the same three highest and lowest activity sites as *L. noctivagans*.

200   Activity increased with patch area for *L. noctivagans* (R^2 = 0.72, $F_{1,4}$ = 10.35, p = 0.03; **Figure 4**),

201   consistent with our prediction. Although the direction of the relationships between *L. noctivagans* activity

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202   and patch shape and isolation were consistent with our predictions, these relationships were not

203   statistically supported (shape $R^2 = 0.50$, $F_{1,4} = 4.01$, $p = 0.12$; isolation $R^2 = 0.35$, $F_{1,4} = 2.15$, $p = 0.23$;

204   **Figure 4**). The activity of *E. fuscus* was not related to patch area ($R^2 = 0.47$, $F_{1,4} = 3.59$, $p = 0.13$), shape

205   ($R^2 = 0.40$, $F_{1,4} = 2.66$, $p = 0.18$), or isolation ($R^2 = 0.16$, $F_{1,4} = 0.65$, $p = 0.44$), but similar to *L.*

206   *noctivagans*, the model coefficients were all in the direction predicted by island biogeography theory.

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Discussion

209   We detected six out of the eight Ontario bat species at urban woodlots in the Waterloo region, with pulses

210   of *L. noctivagans* migratory activity observed in late August and mid-September, consistent with previous

211   study in the region (McGuire et al. 2012). As only a small percentage of the historical forest cover

212   remains in the region, urban forests may represent important habitat for both resident and migratory bats.

213   As predicted, there was a positive relationship between forest area and *L. noctivagans* activity.

214   Rather than this being a selective process, we may see the most activity at forests with larger areas

215   because they act as a larger catchment for migrating bats (as predicted by island biogeography theory).

216   The weaker relationship between forest shape and activity is consistent with this interpretation. Migrating

217   bats may not select stopover sites based on characteristics such as the amount of foraging habitat

218   available, but rather the largest patches may be the most obvious on the landscape, and thus attract the

219   most bats.

220   The weak relationship between site isolation and bat activity suggests that the scale of a potential

221   isolation effect is likely to be much greater than the local scale of our study sites. Migrating bats may

222   travel hundreds of kilometers per day, therefore separate forest patches within a city are not truly isolated

223   from each other in the context of migratory movements. Bats can quickly fly between patches, requiring

224   only ~20 min to fly between our two most distant patches (assuming 9 m/s flight speed; McGuire et al.

225   2012). Stopover sites have been traditionally considered at a small scale, with the assumption that

226   migrants do not move outside of a small stopover patch until they resume migratory flight (Bächler and

227   Schaub, 2007). However, it is now clear that the scale of stopover is substantially greater, and migratory

228   birds and bats may relocate >10 km within a larger stopover landscape before continuing migration

229   (Taylor et al., 2011). Within the scale of our study, a 10 km radius nearly covers the total city area, so it is

230   not surprising that isolation was found to be an unimportant factor within the city limits.

231   Although not explicitly tested in our study, the movement of resident and migratory species

232   within the city may be influenced by different characteristics, depending on whether they prioritize

233   foraging or roosting. As a migratory species that must forage and roost in a short stopover period (< 3

234   days; McGuire et al. 2012), *L. noctivagans* may be more active where foraging areas are closer to roosting

235   sites, and a recent study suggests that access to foraging area from roosting sites might be more important

236   for *L. noctivagans* than roosting itself (Ethier and Fahrig, 2011). Activity may be more strongly related to

237   fragmentation than forest area, where landscape complementation arises when moderate amounts of

238   fragmentation reduce the distance between roosting and foraging sites (Ethier and Fahrig, 2011). As a

239   generalist species, *E. fuscus* is not expected to have a high correlation with forest fragmentation since they

240   are better able to make use of the anthropogenic landscape, often roosting in buildings instead of relying

241   on available tree roosts (Brigham 1991). Therefore, it is possible that *E. fuscus* preferentially selects areas

242   based on roost availability rather than foraging habitat. In this study we contrasted the activity patterns of

243   a migratory and non-migratory species, the two most common species at our study sites. In future studies

244   of systems with more diverse assemblages and greater evenness it may be informative to consider overall

245   assemblage activity patterns with site isolation and fragmentation.

246   According to island biogeography theory, species richness (or abundance) is determined by the

247   balance of immigration and extinction rates, where factors that make it more likely for a species to arrive

248   at the site (e.g., larger area, lower isolation) will increase abundance and vice versa. As resident species

249   that inhabit our study region year-round, *E. fuscus* may respond to resource limitation or other

250   desirable/undesirable aspects of forest patches, leading to resettling among urban forest patches. Thus *E.*

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251   *fuscus* activity at urban forest patches may be best explained by both immigration and extinction in the

252   island biogeography theory context. However, migratory *L. noctivagans* only occupy stopover sites for

253   brief periods (perhaps only 1 – 2 days; McGuire et al. 2012) and therefore island biogeography theory

254   extinction dynamics may be less relevant in this context, and occupancy is best described based on patch

255   area and immigration likelihood. Although our study focused on *L. noctivagans,* other migratory species

256   (i.e., hoary bats, eastern red bats) are expected to follow this same pattern.

257   Patch area was the most important predictor of bat activity at our study sites, but it may be

258   important to consider additional factors other than shape and isolation. High activity at Bechtel Park (a

259   medium-sized site) and low activity at Stanley Park (a large site) indicates that either habitat selection is

260   not based solely on patch area, or that bats perceive patches differently than the criteria we used to define

261   patches. We characterized sites *a priori*, but it is important to understand what types of disturbances

262   influence how bats perceive and use forest patches. "Big" forests like Stanley Park that are fragmented

263   and more heavily trafficked acted more like a small patch, and sites with smaller core areas like Bechtel

264   Park but many surrounding patches of trees may represent a larger useable habitat than the contiguous

265   forest that was measured. Additional factors such as artificial lighting, trail density, forest quality, and

266   distance to water may also influence bats, although it is unclear the extent to which these factors affect

267   migratory bats that may only stop over at the site for one or two days.

268   The complex definition of a forest is an important concept for city planning and land conservation

269   efforts. While some species are tolerant of urbanization, others require large areas of undisturbed natural

270   habitat. These are key considerations when deciding what types of forest to develop or preserve as natural

271   areas. Generalist species that are better able to use urbanized areas will not only use "natural" areas within

272   a city but the entire anthropogenic landscape as part of their functional habitat. Conservation measures

273   (e.g., habitat improvement such as providing artificial roosts) may provide greater benefit to some (often

274   more generalist) species, while others may not benefit to the same degree, emphasizing the importance of

275   understanding habitat requirements of each species (Griffiths et al., 2020). As urbanization continues, it is

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276   important to understand the role of natural areas within the urban landscape mosaic, and how this varies

277   among species that occupy these habitats, both permanently and seasonally. Acoustic monitoring is an

278   important component of monitoring programs to identify spatial and temporal variation in activity

279   patterns in urban landscapes, and provides complementary information to support studies using more

280   direct methods such as radio-telemetry, roost monitoring, and foraging ecology.

281   Our results suggest that urban forest patches, even if degraded compared to continuous forest, are

282   important for maintaining landscape connectivity for migratory and resident bat species. Effective

283   conservation and management practices must include all habitats necessary for the annual cycle, and

284   migratory stopover sites are a critical component of this cycle. Acoustic monitoring of these locations is

285   valuable for informing bat conservation efforts and understanding how both migratory and resident

286   species are impacted by ongoing urbanization at multiple scales.

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295   **Declarations**

296   *Author Contributions*- HA and LPM conceptualized the study, collected data, and analyzed the data. HA

297   drafted the manuscript while LPM contributed editorial comments on the manuscript.

298   *Competing Interests*- The authors declare there are no competing interests.

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301　*Ethics Approval* - All research activities were approved by the University of Waterloo Animal Care

302　　　Committee (Protocol #42374).

303　*Data Availability*- Data is available from the Dryad Data Repository. doi:10.5061/dryad.rr4xgxd9c.

304　　　https://datadryad.org/stash/share/OGwPRSbbNhvh8DaipkJq4b2KzP-l_XEbwIqi1K8SFag

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376   long-distance migrant silver-haired bat, *Lasionycteris noctivagans*. J. Anim. Ecol. **81**(2): 377–385.

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418　**Figure Legends**

419　**Figure 1**. Map of six study sites and detector locations within Kitchener-Waterloo, ON, acoustically

420　monitored between August 12-November 5, 2020. A = Columbia Lake (within the University of Waterloo

421　Environmental Reserve), B = Grey Silo golf course, C = Bechtel Park (municipal park), D = Woodside

422　National Historic Site, E = Stanley Park (municipal park), and F = Huron Natural Area. Blue dots mark

423　the approximate location of each microphone and grey stippling indicates major municipal parks and

424　greenspaces as provided by the base map (Stamen Design, 2014; NAD83/UTM zone 17N).

425　**Figure 2**. Example of forest shapefiles created in QGIS. The dark green polygon outlines the forest at the

426　Grey Silo study site, while light green polygons outline the surrounding forest patches and include a 20 m

427　buffer zone around the forest edge. A blue point indicates the location of the microphone (Stamen Design,

428　2014; NAD83/UTM zone 17N).

429　**Figure 3.** Relative activity of (a) *Eptesicus fuscus* and (b) *Lasionycteris noctivagans* at each study site as

430　predicted by generalized linear models. Error bars represent standard error and post-hoc groupings are

431　shown with superscript letters and bars. Note that the same sites hosted the highest and lowest activity for

432　each species.

433　**Figure 4.** Linear regression between activity and the principal components of each site for *Lasionycteris*

434　*noctivagans*, with 95% confidence interval indicated with grey bands. Higher PC scores for shape

435　represent less convoluted (more circular) shapes. Higher PC isolation values represent more isolated sites.

436　The relationship between activity and the three principal components are all in the direction predicted by

437　island biogeography.

20

Tables

Table 1. Forest patch metrics for the six study sites, grouped into the three major categories of area, shape, and isolation.

	Area				Shape		Isolation	
Site	Area (Ha)	Core area (Ha)	Perimeter (m)	Related circumscribing circle	Shape index	Fractal dimension index	Proximity index	Euclidian Nearest Neighbour index
Columbia Lake	11.5	9.5	2554	0.5	1.9	1.109	24.5	17.5
Woodside	17.7	15.7	2570	0.6	1.5	1.070	2.6	510.7
Bechtel Park	56.7	50.4	7886	0.6	2.6	1.145	12.6	182.4
Stanley Park	58.8	52.3	8168	0.8	2.7	1.148	4.7	154.7
Huron Natural Area	104.5	95.5	11492	0.7	2.8	1.149	21.7	29.6
Grey Silo	108.1	100.2	10248	0.7	2.5	1.130	108.0	18.0

Table 2: Principal components of area, shape, and isolation calculated for each study site. Each component was calculated by applying a separate principal component analysis to the metrics within the three categories of patch size (area, core area, and perimeter), shape (related circumscribing circle, shape index, and fractal dimension index), and isolation (proximity index and Euclidean nearest neighbour index).

Site	PC area	PC shape	PC isolation
Columbia lake	-2.045	1.521	0.421
Woodside	-1.861	2.330	-1.805
Bechtel park	0.018	-0.474	-0.406
Stanley park	0.118	-1.600	-0.443
Huron	1.918	-1.384	0.327
Grey silo	1.852	-0.393	1.906

Table 3. Total activity at each site, calculated as the number of files (recorded passes) for each species over the 85 day sampling period. Two Ontario species, *Myotis septentrionalis* and *Myotis leibii*, were not detected.

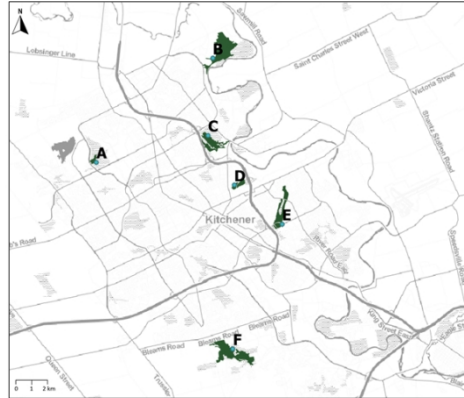| Site	Big Brown Bat *Eptesicus fuscus*	Silver-haired bat *Lasionycteris noctivagans*	Eastern red bat *Lasiurus borealis*	Hoary bat *Lasiurus cinereus*	Little brown bat *Myotis lucifugus*	Tricolored bat *Perimyotis subflavus*	No ID	Total activity
Bechtel Park	3006	640	192	167	78	37	983	5103
Columbia Lake	1260	118	227	27	22	0	834	2488
Grey Silo	1673	507	40	12	10	0	800	3042
Huron Natural Area	5522	606	68	49	25	1	1154	7425
Stanley Park	1210	185	83	10	3	1	649	2141
Woodside	745	78	6	27	1	0	590	1447
Total	13416	2134	616	292	139	39	5010	21646

Figure 1. Map of six study sites and detector locations within Kitchener-Waterloo, ON, acoustically monitored between August 12-November 5, 2020. A = Columbia Lake (within the University of Waterloo Environmental Reserve), B = Grey Silo golf course, C = Bechtel Park (municipal park), D = Woodside National Historic Site, E = Stanley Park (municipal park), and F = Huron Natural Area. Blue dots mark the approximate location of each microphone and grey stippling indicates major municipal parks and greenspaces as provided by the base map (Stamen Design, 2014; NAD83/UTM zone 17N).

338x190mm (96 x 96 DPI)

Figure 2. Example of forest shapefiles created in QGIS. The dark green polygon outlines the forest at the Grey Silo study site, while light green polygons outline the surrounding forest patches and include a 20 m buffer zone around the forest edge. A blue point indicates the location of the microphone (Stamen Design, 2014; NAD83/UTM zone 17N).
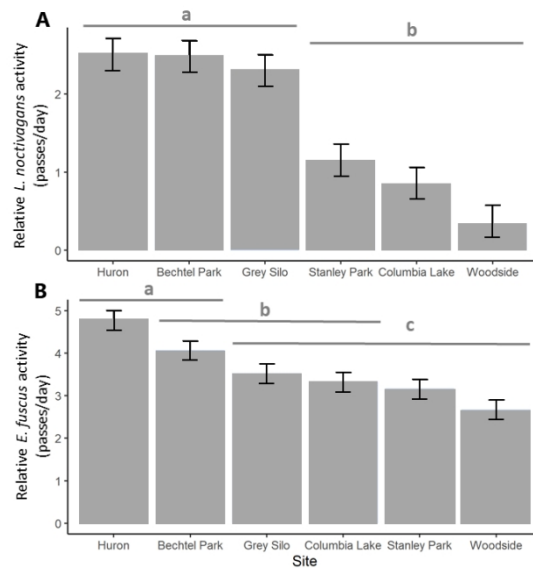
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Figure 3. Relative activity of (a) Eptesicus fuscus and (b) Lasionycteris noctivagans at each study site as predicted by generalized linear models. Error bars represent standard error and post-hoc groupings are shown with superscript letters and bars. Note that the same sites hosted the highest and lowest activity for each species.
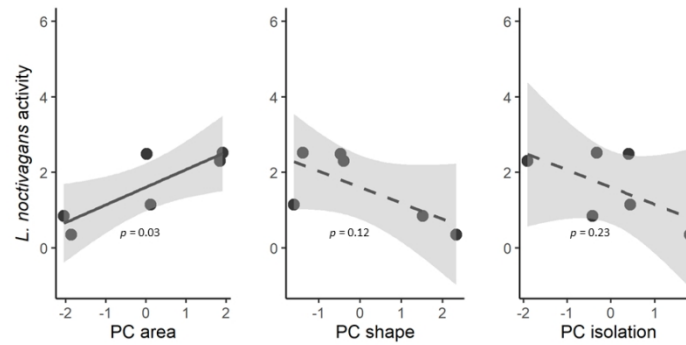
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Figure 4. Linear regression between activity and the principal components of each site for Lasionycteris noctivagans, with 95% confidence interval indicated with grey bands. Higher PC scores for shape represent less convoluted (more circular) shapes. Higher PC isolation values represent more isolated sites. The relationship between activity and the three principal components are all in the direction predicted by island biogeography.

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