SUMMER ACOUSTIC MONITORING OF BAT ACTIVITY IN CIBOLO PRESERVE

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Bats represent 25% of all mammal species worldwide (Altringham 1996) and provide pest control services in the United States (Cleveland et al. 2006; Boyles et al. 2011). Bats can be utilized as ecological indicators of environmental stress as population declines can represent decreases in insects due to pesticides and toxins (Jones et al. 2009) and the potential bioaccumulation of toxins in the food chain. Multiple threats are impacting bats that include habitat loss, pesticide use, wind turbines, climate change, and most recently white-nose syndrome (Clark et al. 1996; Clark 2001; Blehert et al. 2009; Scheel et al. 2017). White-nose syndrome has resulted in the deaths of > 5.5 million bats in the Eastern United States (Pettit & O'Keefe 2017), but such mortality has not been documented in Texas.

Most North American bats are difficult to study due to the fact that they are nocturnal, small, fast, and easily able to avoid traps by detecting them with echolocation. Standard monitoring methods utilized to survey bats include mist-netting, emergence counts, and acoustic monitoring. Each of these methods have strengths and weaknesses based on the objectives of a study (Kunz & Brock 1975; Carroll et al. 2002; Kunz et al. 2009). Acoustic monitoring is less accurate than mist-netting for species identification but more accurate than emergence counts if more than one species is present (Miller et al. 2011). Acoustic monitoring can be used to gain general knowledge of the species present in an area but cannot be used to determine population estimates.

In temperate zones, bat species richness has been hypothesized to be dependent on the availability of multiple roost sites (Humphrey 1975). Increased urbanization results in the loss of natural habitat, fragmentation, and lower biodiversity (reviewed in McKinney 2002).

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As a secondary result, increased urbanization can impact bat species richness or populations negatively. Bat species diversity was positively correlated with forested habitat and negatively correlated with urban landscapes in Indiana (Duchamp & Swihart 2008). As habitat loss is inevitable, protected preserves will likely be the last remaining sites that offer multiple roost sites required by different species of bats.

The Greater San Antonio area and Bexar County comprises one of the fastest growing populations in the USA and is rapidly expanding into Kendall County (US Census Bureau 2017). Kendall County is the second fastest-growing county in Texas as of 2015, and is home of the largest known bat colony in the world at Bracken Cave (Davis et al. 1962).

No research is known on the species of bats present and their habitat use in Cibolo Preserve. The objectives of this study were to document the species of bats present and their habitat use in Cibolo Preserve using acoustic monitors.

Methods.–Cibolo Preserve is comprised of 202 ha and is located in Kendall County, Texas (29°46'41.98"N, 98°42'21.96"). The preserve is a private non-governmental organization devoted to research. The preserve is a protected natural area located 4 km southeast of Boerne and 40 km north of San Antonio in Central Texas. Within the center of the preserve is 2.4 km of the Upper Cibolo Creek, a perennial stream that is fed by treated wastewater effluent from the City of Boerne. Habitat types in Cibolo Preserve are typical of the Texas Hill Country and include prairies, oak savannahs, and riparian areas adjacent to Cibolo Creek.

Prairie habitat was dominated by native and non-native grasses and forbs ca. 1 m in height that included little bluestem (*Schizachyrium scoparium)*, big bluestem (*Andropogon gerardi)*, switchgrass (*Panicum virgatum*), silver bluestem (*Bothriochloa laguroides*), King's Ranch bluestem (*Bothriochloa ischaemum*), prairie coneflower (*Ratibida columnifera*), and prairie verbena (*Verbena bipinnatifida*).

Oak savannah was comprised of Escarpment live oak (*Quercus fusiformis*), sugarberry (*Celtis laevigata*), post oak (*Quercus stellate*), sugarberry (*Celtis laevigata*), and Texas persimmon (*Diospyros texana*) and an understory of native grasses and forbs. Canopy height in oak savannah forest averaged 8-10 m. Stream habitat consisted of open water and a riparian corridor dominated by bald cypress (*Taxodium distichum*), Eastern cottonwood (*Populus deltoides*) and black willow (*Salix nigra*). Canopy height in the riparian corridor ranged between 12-18 m. Edge habitat in this study was defined as distinct breaks < 5 m between prairie and oak savanna habitat with changes in canopy from heights of 1 m in prairie to 8-10 m in oak savannah.

Acoustic bat detector microphones (SM3BAT, Wildlife Acoustics, Inc., Maynard, MA) were positioned 6 m above ground level facing into potential bat flyways in each habitat type, and sampling occurred from May 2 to August 22 of 2017. Detectors were set to record in WAV format and settings were based on expert recommendations and previous research experience (Britzke et al. 2013). The frequency was limited between 16 kHz and 256 kHz. The pass duration was limited to between 2 msec and 600 msec. The trigger volume was set to 18 dB, trigger window set to 1 second, and the trigger maximum was set to 5 sec. Bat passes that could not be identified to species were classified as unknown.

During each sampling period (n = 4), two acoustic detectors were placed in different habitats for 14 d. Each habitat in the preserve was sampled four times during the summer of 2017. Surveys began 20 min before sunset and concluded 20 min after sunrise. Weather measurements including average weekly temperature, wind speed, and precipitation were collected from the Cibolo Nature Center weather station located adjacent to Cibolo Preserve. All acoustic data was automatically identified using Sonobat 4.2.2 North America, region pack TX [c20170529], southeast Texas.

Descriptive statistics were calculated for all variables. Differences in bat activity and temperature was analyzed with a t-test ($P < 0.05$).

Habitat data was checked for normality and homogeneity of variance ($P > 0.05$), and analyzed using a One-Way *ANOVA* ($P < 0.05$) if data was normally distributed. If data were not normal distributed, data were analyzed with a Kruskal-Wallis test ($P < 0.05$). If differences were detected with the *ANOVA* or Kruskal-Wallis test, appropriate means separation tests (Tukey's and mean rank test, respectively) were calculated to determine differences ($P < 0.05$). Data were analyzed in Excel and SigmaPlot.

Results & Discussion.–Over the four-month study, a total of 156,021 bat detections were recorded. Identification of 44% (n = 68,949) of acoustic detections was not possible due to similar calls between species. Based on the identification of 87,072 calls, bat activity was significantly different (H = 13.47, df = 3, P = 0.04) on average each week during May ($\bar{x}$ = 9,117; SE = 1,547) compared to the average each week in June ($\bar{x}$ = 3,560; SE = 1,979), July ($\bar{x}$ = 3,067; SE = 557), and August ($\bar{x}$ = 1,973; SE = 227). Acoustic detections declined over the summer and there was a significant decrease ($P < 0.001$) in bat detections from May 2 through June 6 ($\bar{x}$ = 18,320; SE = 1,488) compared to June 6 through August 22 ($\bar{x}$ = 4,610; SE = 763) (Fig. 1). This decline in bat activity may correspond to temperature in which significant differences (t = 9.11, df = 14, $P <$ 0.0001) were detected for bat activity at temperatures ≤ 23 °C (n = 109,921) compared to > 24 °C (n = 46,100).

Seven species of bats were documented in Cibolo Preserve based on acoustic detections during the study that include the Brazilian free-tailed bat (*Tadarida brasiliensis*), eastern red bat (*Lasiurus borealis*), northern yellow bat (*Lasiurus intermedius*), evening bat (*Nycticeius humeralis*), hoary bat (*Lasiurus cinereus*), tricolored bat (*Perimyotis subflavus*), and cave myotis (*Myotis velifer*). Species richness detected at Cibolo Preserve represents 7 of the 13 bat species known from the Central Texas. The Brazilian free-tailed bat was the most frequently detected species in Cibolo Preserve and accounted for 73% of all calls. The tricolored bat was the second most common bat representing 10% of all calls recorded. The other five species were detected at ≤ 4%. Species previously documented in Kendall County

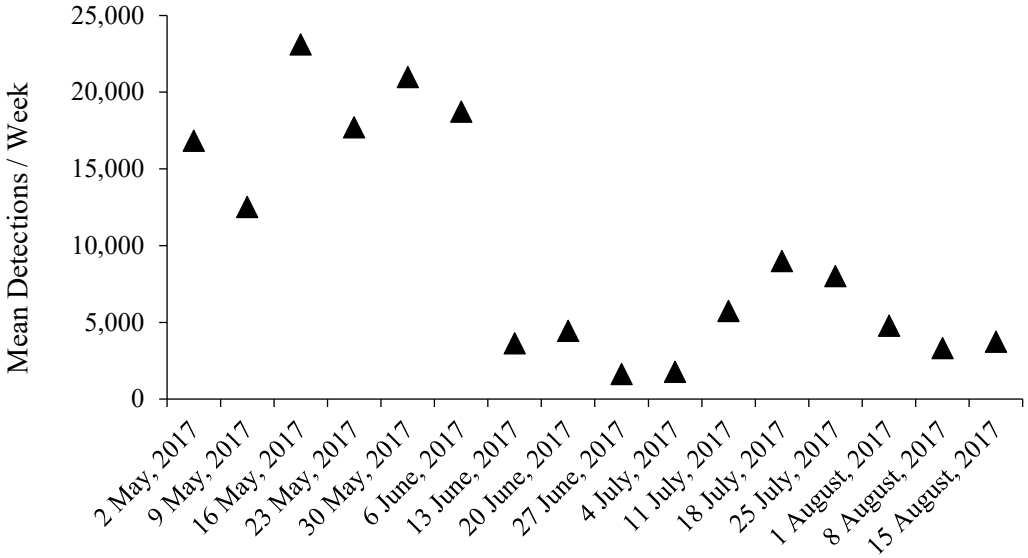

Figure 1. Mean bat detections per week for all species and habitat combined from May to August 2017. Acoustic detections dropped significantly ($P < 0.001$) between May 2 to June 6 and June 6 to Aug 15, 2017 based on t-test.

but not detected in this study include the silver-haired bat (*Lasionycteris noctivagans*), the big free-tailed bat (*Nyctinomops macrotis*), the big brown bat (*Eptesicus fuscus*), the Seminole bat (*Lasiurus seminolus*), the pallid bat (*Antrozous pallidus*), and Peter's ghost-faced bat (*Mormoops megalophylla*) (Ammerman et al. 2012).

There was no significant difference in habitat use by bats combining all acoustic calls ($H = 4.58$, $df = 3$, $P = 0.205$) (Fig. 2). The use of each habitat over the 4-month study was highly variable with more activity detected in May compared to the rest of the summer. All seven bat species were detected within each of the four habitats monitored within Cibolo Preserve but at varying percentages (Table 1). The structure and composition among the habitats in Cibolo Preserve may provide many niches for insects and bats forage opportunistically moving in and between habitats to feed (Whitaker 2004). This opportunistic foraging of bats among habitat may account for the large variation in detections as bats move to different habitats to forage.

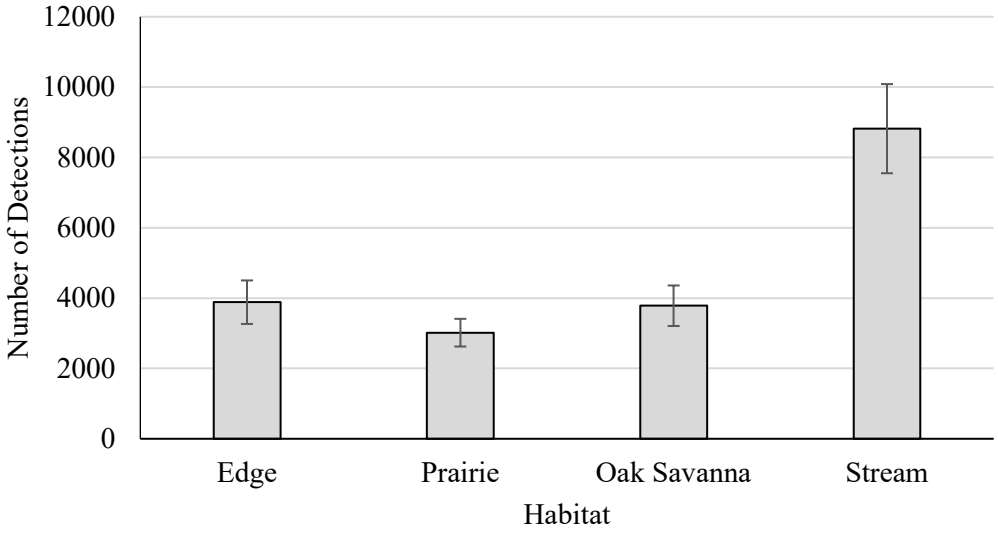

Figure 2. The mean number of acoustic detections each week from May-August, 2017 in habitats at Cibolo Preserve combining all bat species. No significant difference was detected ($P = 0.205$) and bars represent standard error.

Brazilian free-tailed bats accounted for 92, 82, 70 and 54 percent of all detections in edge, prairie, oak savannah, and stream habitat, respectively (Table 1). The tricolored bat was detected over stream habitat at a greater percentage ($P = 0.03$) than the other three habitats. No other significant differences were detected ($P > 0.05$) for the six other species of bats. With the exception of the tricolored bat, bat species used habitat in equal proportions in Cibolo Preserve. This is likely due to the small size of the preserve and ability of bats to fly long distances in search of food.

The high activity of Brazilian free-tailed bats in Cibolo Preserve is possibly due to movement of this species from multiple roosts that include Bracken Cave, Old Tunnel, Frio Cave, Camden Bridge, and other roost sites in highway underpasses close to Cibolo Preserve. Brazilian free-tailed bats are documented to travel long distance > 56 km in one night (Best and Geluso 2003) which may account for the high activity of this species in Cibolo Preserve. The tricolored and cave myotis bats should be considered for conservation efforts and more research is needed on these species in the greater San Antonio area. Both tricolored bats and cave myotis are cave-roosting species, making them susceptible white-nose syndrome.

Table 1. Habitat percent use of bat species in Cibolo Preserve from May-August 2017 (values with different letters in the same row indicate significant differences [$P < 0.05$] in habitat use by that species).

	Habitat Type Percent Use				
Species	Edge	Prairie	Oak Savanna	Stream	*P*-value
Tadarida brasiliensis	92	82	70	54	0.96
Perimyotis subflavus	1 [b]	4 [b]	4 [b]	28 [a]	0.03
Nycticeius humeralis	2	3	4	9	0.33
Myotis velifer	1	1	8	3	0.28
Lasiurus borealis	2	8	10	4	0.69
Lasiurus intermedius	1	1	2	1	0.41
Lasiurus cinereus	1	1	2	1	0.51

The limitation of this study was the inability to identify 44% of the detections and the automatic identification software is not 100% accurate (Barclay 1999). Bat species often have similar call characteristics, confounding the automatic identifier software. This study could have been improved with manual identification of bat passes. Moreover, the automatic identification software only identifies pre-determined groups of bat species for each dataset based on the region package selected, and rare species migrating through Cibolo Preserve would not be identified. However, we feel this study detected the most common bat species that utilize Cibolo Preserve for roosting and foraging during the summer.

In summary, small protected areas such as Cibolo Preserve within increasing urban areas provide a diversity of roosting and foraging habitats for bats. A wide variety of roost sites exist in Cibolo Preserve that include foliage, snags, karst features, and buildings. The seven species documented in Cibolo Preserve utilize a wide array of forging habitat and roost sites that include foliage, snags, structures, and caves. Foraging habitat in the preserve includes open prairie, oak savannah forest with varied canopy cover and gaps, Cibolo Creek and associated riparian corridors. Additional research is needed to

determine maternity roosts in Cibolo Preserve and colony size. Protections of maternity roosts is important to protect bat populations regardless of roost type. Long-term acoustic monitoring over time and space will provide species preference for foraging habitat. A winter survey of Cibolo Preserve would provide additional insight into how bat activity varies between summer and winter and a survey of adjacent urban habitat would provide insight to how bats utilize Cibolo Preserve compared to urban areas.

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