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Relationship between bats and prairie-dog (Cynomys ludovicianus) colonies in Western Kansas

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RELATIONSHIP BETWEEN BATS AND BLACK-TAILED PRAIRIE DOG (*CYNOMYS LUDOVICIANUS*) COLONIES IN WESTERN KANSAS

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GRADUATE COMMITTEE APPROVAL

The graduate committee of Mario N. Rodriguez approves this thesis as meeting partial fulfillment of the requirements for the Degree of Master of Science.

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ABSTRACT

Although it is known that prairie dog colonies can increase biodiversity in the areas surrounding them, there is extraordinarily little known about the relationship between bats and black-tailed prairie dogs (*Cynomys ludovicianus*). In a recent study from the Front Range of Colorado, several bat species were found to be consistently flying around prairie dog colonies, presumably foraging due to increased insect diversity and abundance. Despite the importance of prairie dogs to the ecosystem, prairie dog colonies are usually eradicated due to the perception that they adversely impact cattle grazing. If insectivorous bats are attracted to prairie dog colonies in Kansas, it would add to the list of ecosystem services provided by prairie dogs. We conducted acoustic bat surveys, attempted to confirm acoustic detections with mist netting, and sampled insects within and adjacent to prairie dog colonies. We also used new radio tracking technologies to closely monitor bat use of prairie dog colonies. We anticipated that bats, including Western small-footed myotis, would be more active over prairie dog colonies compared to the surrounding grasslands without prairie dog colonies. Our findings support part of this prediction. Significantly more bat calls were recorded over prairie dog colonies compared to non-prairie dog colonies. However, there was no significant difference in Western-small footed myotis calls on or off prairie dog colonies. There was no significant difference between the insect biomass on prairie dog colonies and non-prairie dog areas. Since there is not enough data to support the hypothesis that prairie dog colonies have more insect biomass than non-prairie dog areas, it leaves a gap in knowledge and creates potential areas for further research to be conducted. This study highlights the important ecological relationship between prairie dogs and bats and suggests that higher presence of bats on prairie dog colonies could lead to economic benefits to the agricultural industry through the predation of agricultural pests.

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INTRODUCTION

Bats provide important services to multiple ecosystems. In temperate regions, one of these ecosystem services is insect control, including agricultural pests, which is beneficial to humans. For example, in New Jersey, USA, bats consumed 160 known species of agricultural insect pests or disease vectors (Maslo et al., 2022). The same study found that aquatic pests, such as mosquitoes, were eaten by the Little brown bat (*Myotis lucifugus*) at a much higher rate compared to other bats. Mosquitoes are one of the most dangerous disease vectors among insects, therefore species that help reduce mosquito populations are a benefit to human society.

In addition, bats are especially helpful to farmers through reducing pesticide costs. For example, on a national scale insectivorous bats have been found to save US farmers an estimated $22.9 billion a year (Boyles et al. 2011). A different study focused on Texas found that the Brazilian free-tailed bat (*Tadarida brasiliensis*) saved an estimated $1.7 million in pesticides for the corn earworm alone (McCracken et al. 2012). Recent advances in genetic sequencing techniques have made fine-scale dietary analyses possible. A study done with vampire bats showed that by using DNA metabarcoding, diet analysis of predators is possible at an affordable and scalable rate (Bohmann et al, 2018). However, because of their nocturnal habits, determining where bats are foraging and how they use a landscape has proven more difficult. In addition, there is little to no literature on previous research on the ecology of bats in the Great Plains compared to other biomes.

Another mammal important for Great Plains ecosystems is the black-tailed prairie dog (*Cynomys ludovicianus*; abbreviated hereafter as BTPD or prairie dogs). A keystone species is a species on which other organisms in the ecosystem largely depend. Prairie dogs are often identified as a keystone species as they were found to influence the structure and function of surrounding plant communities due to their foraging and burrowing behaviors (Martinez-Estevez et al., 2013). They serve as a food source for carnivores, and provide shelter for several species of mammals and insects (Bangert et al, 2006). There are a large number of species that are affected by prairie dogs. The Ferruginous Hawk (*Buteo regalis*) is listed as a Species in Need of Conservation (SINC) and are known to prey on prairie dogs. In a study done in Western Kansas, most nests were found to be within 2 miles of prairie dog colonies, which are the most likely food source for these hawks (Clark, 2021). Prairie dogs are also a food source for other carnivores such as coyotes (*Canis latrans*), badgers (*Taxidea taxus*), swift foxes (*Vulpes velox*), and black-footed ferrets (*Mustela nigripes*) (Martínez-Estévez et al, 2013). Their vacant burrows serve as dens for black-footed ferrets (*Mustela nigripes*), burrowing owls (*Athene cunicularia*), and prairie rattlesnakes (*Crotalus viridis*) (Kotliar et al, 1999).

Prairie dogs also affect vegetation within their colonies through an increased uptake of nitrogen because of increased grazer selection, which then increases the amount of crude protein available in the vegetation (Whicker and Detling 1988). Prior to the near extinction of bison (*Bison bison*), prairie dogs were known to have coexisted with approximately 60 million bison who preferentially grazed on prairie dog colonies due to better forge nutrition (Miller et al. 1994). Despite these ecosystem services, BTPD have been persecuted by local governments because they are perceived as an agricultural pest that competes against livestock for forage and have little ecological value (Lybecker et al, 2002).

The eradication of BTPD colonies has been sanctioned by federal, state, and local governments and citizens for decades; combined with the uncontrolled spread of plague among prairie dog populations, there has been a reduction of prairie dog populations by 98% of their historical size (Smith, 1967). Beginning in the early 1900's, federal and state governments began prairie dog control programs, poisoning millions of acres in the hope of benefiting the U.S beef industry (Miller et al. 1994). In 1903, it was estimated that prairie dogs inhabited two and a half million acres in Kansas. In 1957 it was estimated that prairie dogs only inhabited 24,000 ha in Kansas (Smith, pg. 5, 1967). More recently, it is estimated that prairie dogs inhabit 52,600 ha in Kansas (Charles et al., 2019). Even in 1994, the Environmental Protection Agency estimated that around 80,000 ha are poisoned annually (Miller et al. 1994).

Prairie dog eradication is not only the result of human persecution however; there are several additional reasons for their decline. For example, the plague, caused by the bacteria *Yersinia pestis*, has ravaged prairie dog populations since the 1940's (Cully et al, 2010). Another reason for the decline of prairie dogs is the over-hunting of bison (*Bison bison*) in the late 1800's and early 1900's. The prairie dog relies on short, sparse vegetation that the buffalo helped maintain (Smith, pg. 6, 1967). Rigorous studies investigating the effect prairie dog colonies have on habitat across all rangelands may help change public perception of prairie dogs, facilitating their protection or minimizing persecution. Prairie dogs have shown direct competition with grazers; however, it was found to have a small negative affect on steer mass gain (Augustine and Derner, 2021). It should be noted that prairie dog control programs are needed to protect rangeland and help minimize the amount of direct competition to cattle, however due to the substantial ecosystem services that prairie dogs provide, studies are investigating what proportion of rangeland prairie dog colonies should be allowed to inhabit (Olson et al, 2016). Another study found that prairie dogs can help stop the encroachment of shrubs, a growing issue in the Midwest that threatens rangelands (Ponce-Guevara, Eduardo, et al. 2016). Specifically, in the Great Plains grasslands are being transformed into juniper woodlands, which leads to a decrease in grassland biodiversity and lower livestock production (Twidwell et al, 2013). For example, woody encroachment is the leading cause of decline in the lesser prairie chicken (*Tymanuchus pallidicintus*; Fuhlendorf et al. 2002).

Until recently, no one had investigated the association between bats and BTPD colonies. A study conducted on the Front Range of Colorado found that several species of bats, including the Western small-footed myotis (*Myotis ciliolabrum*) were more active over BTPD colonies than over adjacent grasslands without prairie dog colonies (Adams, 2021). This is inconsistent with what is known about the foraging habits of *M. ciliolabrum* which is presumed to be a forest-edge specialist (Adams, 2003). The consistent foraging of *M. ciliolabrum* (a Tier 2 Species of Greatest Conservation Need in Kansas) and other bat species over prairie dog colonies suggests that some characteristics of these colonies, such as increased availability of crude proteins in vegetation or increased selection by grazing mammals, is attracting more diverse insects compared to the surrounding prairie (Adams, 2021; Bangert et al. 2006; Smith, pg. 6, 1967). Adams (2021) hypothesized that insect richness and abundance was what attracted more bat activity over prairie dog colonies.

It is not yet known whether similar patterns of bat activity occur over BTPD colonies in Kansas or whether the phenomenon observed in Colorado is indeed due to greater richness and abundance of insects as has been hypothesized. Extraordinarily little is known about the diet of most bat species, including *M. ciliolabrum*; however, they have been found to consume arthropods in the orders Coleoptera (family Scarabaeidae), Hymenoptera, Hemiptera, Lepidoptera, and Diptera (Valdez et al. 2021). This study examined fecal pellets of bats under a dissecting microscope to identify the diet of bats to Family, but lacked finer taxonomic resolution. Big brown bats (*Eptesicus fuscus*) in Fort Collins, CO were normally found to be beetle specialists, but alter their diet seasonally, switching to moths in late September (Valdez et al. 2014). Prairie dog colonies have been found to increase arthropod diversity (Bangert et al. 2006) which may attract insectivorous bats. However, this matter should be investigated in greater detail. No one has ever examined the direct relationships among bats, prairie dog colonies, and insects. This is the knowledge gap addressed by this project.

Verifying that bats are indeed spending more time over prairie dog colonies could be facilitated by tracking tagged bats. Traditionally, tracking bats with VHF radio tags has involved scientists having to search areas for signals with antennas or portable receivers. GPS technology can be used for large fruit bats, but the batteries are too heavy to be safely attached to the small bats found in North America. Because of these technological barriers, we still know little about fine-scale habitat use by individual bats. MOTUS wildlife tracking system is a collaborative network of researchers that use radio telemetry to track a wide variety of species, especially small species that can travel great distances such as birds and bats (Taylor et al, 2017). This system, along with CTT nodes, an innovation that can automatically localize tag signals and upload data to Motus towers allows for near GPS-level movement tracking of species too small and vagile for large GPS tags and hand-held receivers. Tags suitable for the network and small enough for bats are battery powered with life spans ranging from one week to one year depending on tag and battery size. The tags can weigh less than a gram and can be adhered with most skin-safe adhesives. Using this new technology, researchers are able to attach transmitters to bats and collect fine-scale movement data constantly for the expected lifetime of a particular tag, typically 7-30 days (Taylor et al. 2017).

The first aim of this study was to determine if bats are more active over prairie dog colonies compared to surrounding grasslands without prairie dog colonies. To do so, we replicated and extended the methods of Adams (2021) by deploying paired acoustic detectors along transects on The Nature Conservancy's Smoky Valley Ranch and a nearby privately-owned ranch, both part of the Chalk Bluff Ecological Focus Area in Logan County, KS. We also attempted to increase the capability of the existing MOTUS network at Smoky Valley Ranch by installing "nodes" that repeat the signals from radio tagged animals back to the tower, allowing signal triangulation that rivals GPS, thereby providing fine-scale movement information for animals too small to GPS collar.

The second aim of the study was to determine if insects were more abundant over prairie dog colonies compared to surrounding grasslands not inhabited by prairie dogs. To do this, we surveyed the nocturnal insect biomass on prairie dog colonies and the adjacent grasslands without prairie dog colonies to test Adams' hypothesis.

METHODS

Study area: Smoky Valley Ranch and Haverfield Ranch

Smoky Valley Ranch is owned by The Nature Conservancy and located in Logan County in western Kansas. This is an 18,600-acre ranch whose vegetation consists of mixed-grass and shortgrass prairie. Acoustic transects on grasslands within this property were in close proximity to forest edges and river systems that could have an effect on bat activity in the area. Smoky Valley Ranch manages a diversity of habitats and for species including Black-tailed prairie dogs and Lesser-prairie chickens. These habitat goals are achieved through managed grazing of cattle and bison and prescribed fire. There are Black-tailed prairie dog colonies that have been monitored and managed since 2000. Several bat species have been recorded to be present in Logan County, the Brazilian free-tailed bat (*Tadarida brasiliensis*), big brown bat (*Eptesicus fuscus*), and the Western small-footed myotis (*Myotis ciliolabrum*), a species of bat that has been identified to have a consistent presence over BTPD colonies (Adams, 2021). It should be noted that Smoky Valley Ranch went through a historical drought during the time data was being collected. Droughts have large impacts on all levels of an ecosystem including loss in plant growth, which affects grazers and insect population levels, in turn affecting prey avaliability for insectivores such as bats.

Haverfield Ranch is a privately owned 600+ acre pasture about 7 miles northwest of Smoky Valley Ranch. This pasture was grazed by a small number of cattle for a limited time when bat detectors were deployed. It is also one of the few properties in Logan County that allows for prairie dog colonies to be present and does not eradicate the population. State law (K.S.A. 80-1202, 80-1208) requires that any landowners that allow prairie dogs to be present must include exclusion areas to keep cattle from grazing on forage on the edge of the pastures to deter colonies from spreading into neighboring properties that do not want prairie dogs on their land. These exclusion areas were important to this study because they provided an area where bat detectors could be deployed in the absence of prairie dogs.

Aim 1: Determine if bats are more active and foraging over prairie dog colonies.
Acoustic detector transects

Data collection

There were 3 acoustic sites on Smoky Valley Ranch and two acoustic sites on the Haverfield property. Each site consisted of two parallel transects running north to south (with the exception of one transect on the neighboring Haverfield Ranch which ran south to north due to the limitations with property boundaries). Each transect had one bat detector (SM4BAT, Wildlife Acoustics, Maynard, MA). One bat detector was located 25m within a prairie dog colony, while the other echolocation detector was placed 25 meters outside the edge of the colony in the surrounding prairie, keeping a 50m distance between the detectors. Surveys were conducted at a total of five sites. Acoustic detectors were protected by cattle panel enclosures during deployments (Figure 1). These enclosures were made by placing cattle paneling around detectors, and tied to two metal T-posts on either side of the detectors. Figure 2 shows a diagram of the sampling design. Site names and starting and ending transect coordinates are provided in Table 1. Each bat detector recorded from sunset to sunrise for 10-14 days, after which the detectors were moved 50 meters along the transect line following techniques from a previous study (Figure 2; Adams, 2021). The detectors were deployed July 12 through October 31, 2022. (Figure 3 represents the aerial pictures of the 5 sites. Sampling started late into the field season due to supply chain issues that lead to equipment being back ordered and arriving at sites well into the field season.

Data analysis

Kaleidoscope Pro 5 (Wildlife Acoustics) was used to automatically identify (auto ID) bat calls. The auto IDs were manually vetted to verify species identities. The protocol steps were as follows: 1. Removed calls with fewer than 5 pulses. 2. We assumed the auto ID was correct if the computed confidence was over 80%. 3. We removed calls whose computed confidence was less than 20%. 4. That list of IDs was then cross referenced with the Kansas Mammal Atlas (Schmidt et al, 2021) to verify that the ID was realistic. Those that were not realistic were manually inspected and usually removed. For example, all calls auto ID'd as *Nyctinomops macrotis*, a species potentially found in Kansas, were found to be insect noise upon visual inspection. 5. Because calls can be very similar, the software provides alternative IDs. When the presence of a species from the alternative ID was more likely to occur in Logan County than the original ID, then the alternative ID was the species used to represent that call. For example, the Gray Myotis (*Myotis grisescens*) is an endangered species and has never been found in Logan County or in much of Kansas for that matter. The other species that we removed were the Pallid Bat (*Antrozous pallidus*), Gray Myotis (*Myotis grisescens*), Northern Myotis (*Myotis septentrionalis*), Little Brown Myotis (*Myotis lucifugus*), Tri-colored Bat (*Perimyotis subflavus*), Evening Bat (*Nycticeius humeralis*), Cave Myotis (*Myotis velifer*), and the Big Free-tailed Bat (*Nyctinomops macrotis*). Any calls that were "ambiguous", or between 20%-80% confidence of correct primary identification were only used in calculations for total number of bat passes.

The echolocation detectors are triggered by ultrasonic sounds - usually a bat passing overhead - and record these sounds until the sound stops. Each recording from the echolocation detector is automatically stored as one row of data. In order to summarize the number of bat echolocation recordings captured each night, we summed the number of rows of data for a given night, calling this metric "number of bat passes".

Mann-Whitney U tests were performed to determine if there were differences in the median number of bat passes recorded over prairie dog colonies and non-prairie dog areas. For these analyses, all calls identified as "bat" were used. Mann-Whitney U tests were also used to determine whether there were differences in the number of bat passes for each unambiguously identified bat species between prairie dog and non-prairie dog areas. Finally, we graphed the number of bat passes against time to better understand daily variation in bat activity. All statistical analyses were performed using R (R Core Team, 2021) and RStudio (RStudio team, 2020).

Mist netting and capturing bats in roosts

Data collection

Acoustic detection methods are not perfect, so capturing bats to confirm species identities is recommended. Capturing bats also allowed for radio tagging and would have aided in dietary analyses. All field protocols were approved by FHSU IACUC #22-0006 and performed under Kansas permit #SC-091-2022. Mist netting techniques were implemented on five nights, July-October, from sunset to 4 hours after sunset to capture and identify bats as described in Winhold and Kurta (2008). Mist netting occurred over water sources near prairie dog colonies. No bats were captured in mist nets for this project.

Two roosts in human-made structures exist on SVR. On 01 October, 2022, we entered these roosts to collect guano and caught two Western small-footed myotis by hand. The bats were weighed, sexed, wing-punched, and transmitters were adhered following accepted protocols (Carter, 2009). The wing-punches and guano were stored in desiccant for future genetic analyses. Several different sizes of transmitters were purchased to accommodate the different species and sizes of bats. The transmitters that were attached to the two Western small-footed myotis weighed 0.35g and transmitted at a frequency of 434 MHz.

Radio tracking bats

Data collection

Due to supply chain issues, the supplies and equipment for radio-tracking bats were not delivered until late August, causing this part of the project to become exploratory.

Nodes were placed in a grid-like fashion with approximately 250m between each node (Figure 4). All nodes had an exclusion cage placed around them to protect from livestock. These cages were set up with the same method mentioned in the deployment of acoustic detectors. A total of 20 nodes were deployed in an attempt to develop a protocol for future research. Because of the exploratory nature of this field season, the calibration steps necessary for accurate triangulation were not performed (Paxton et al, 2022).

Data analysis

Radio telemetry data were analyzed using the MOTUS package (Brzustowski and Lepage, 2022) in RStudio (RStudio Team, 2020). The data were filtered to remove invalid runs and runs less than 3 as suggested by MOTUS (Brzustowski and Lepage, 2022). We assumed that any pings after 08 October 2022 and in states outside the known range of Western small-footed myotis were the result of two unique tags pinging a tower simultaneously, resulting in a signature similar to a third tag (Brzustowski and Lepage, 2022). Several readings from Pennsylvania in December 2022 were removed for this reason. The filtered data were then visualized to determine which nodes detected the tagged bats at what times.

Aim 2: Determine if insects are more diverse and abundant in prairie dog colonies

Data collection:

Insect light traps were placed both within and outside of prairie dog colonies to sample insect diversity on 5 nights. These consist of simple funnel traps that had a battery powered LED light source and container with ethanol to collect the insects attracted to the light (Valdez et al. 2021). Insects were collected and preserved in ethanol filled collection jars at the base of the funnels.

Data analysis:

The protocol used to measure biomass was replicated from a previously published protocol (Hallman et al. 2017). A coffee filter was used to prevent small insects from getting trapped in the sieve, which would have affected the total biomass measurement. Samples were then poured onto the coffee filter located at the base of the angled stainless-steel sieve (10cm diameter) of 0.8 mm mesh width. The sieve was placed at 30 degrees over a plastic container. The placement of the sieve in this manner accelerated ethanol runoff and the measuring process. As runoff continued and dripping occurred, a stopwatch was used to measure the times between drops. When the time between two drops reached 10 seconds for the first time and the coffee filter was dry, the weighing process was performed with a laboratory scale. The coffee filter and weigh boat weights were then subtracted from the overall measured weight. Due to light traps being deployed for differing lengths of time, biomass measurements were corrected by dividing biomass by the number of hours each light trap was deployed. The Kruskall-Wallis and Mann-Whitney U statistical tests were used to analyze the insect biomass data. All statistical analyses was completed by using the software R (R Core Team, 2021).

RESULTS

Aim 1: Determine if bats are more active and foraging over prairie dog colonies

Bat activity patterns using acoustic data

Six bat species were recorded in the study area: big brown bat, *Eptesicus fuscus* (abbreviated EPTFUS); Eastern red bat, *Lasiurus borealis* (LASBOR); hoary bat, *Lasiurus cinereus* (LASCIN); silver-haired bat, *Lasionycteris noctivagans* (LASNOC); Western small-footed myotis, *Myotis ciliolabrum* (MYOCIL); and Brazilian free-tailed bat, *Tadarida brasiliensis* (TADBRA). Silver-haired bats were recorded on only a few nights, so were not analyzed further. The number of bat passes recorded for each species and ambiguous bat calls are reported in Table 2.

There were significantly more bat passes per night over prairie dog colonies compared to non-prairie dog colonies (Figure 5; W=57263, p-value = 0.001). Daily bat activity varied substantially over the course of the study, with most bat passes occurring over prairie dog colonies and activity peaking in late July through early August and mid-September (Figure 6). All unambiguously identified species were significantly more active over prairie dog colonies except for Western small-footed myotis (Table 3). As a whole, significantly more bats were detected over prairie dog colonies and were more active in August and September with activity dropping into October (Figure 6). There was substantial daily variation in the number of bat passes between species (Figure 7). Big brown bats had a sharp increase in activity for several days in the beginning of August (Table 3; Figure 7). Eastern red bats exhibited higher activity around the same time as the big brown bat (Table 3; Figure 7). Hoary bats shared a peak of activity with the previous species mentioned above, as well as a drop off in activity in mid-October (Table 3; Figure 7). Western small-footed myotis were not significantly more active over prairie dog colonies (Table 3; Figure 7), there was peak activity on the non-prairie dog transects in the end of August and beginning of September. Brazilian free-tailed bats had 4 separate nights where the number of bat passes surpassed 20, which was more often than any other species detected (Table 3; Figure 7).

Only two sites - West and Far West - had significantly more bat passes on prairie dog colonies compared to off (Table 4); the number of bat passes was not significantly different between prairie dog and non-prairie dog transects for the other sites. Activity at these other sites was consistently low (Figure 8). Daily bat activity varied substantially at each site (Figure 8). The bat activity over the Long pasture was not significantly higher over prairie dog colonies than areas that did not have prairie dogs (Table 4); this site had the least amount of activity when compared to the other two sites on Smoky Valley Ranch (Figure 8). The bat activity over the West pasture was significantly higher over prairie dog colonies (Table 4); bat activity over prairie dog colonies for this site dropped off in early October (Figure 8). The bat activity over the Far West pasture was significantly higher over prairie dog colonies (Table 4), although there was a 2-week period where activity over the prairie dog colony dropped to 0, because the detector batteries died during this time period (Figure 8). Haverfield East's bat activity was not significantly greater over prairie dog colonies (Table 4), the Western small-footed myotis had peak levels of activity in early August on this site (Figure 8). Haverfield West's bat activity was not significantly different between transect type (Table 4); the prairie dog colony transect had no bat activity from late August until the end of the deployments. We retain our alternate hypothesis that overall bat activity was higher over prairie dog colonies when compared to non-prairie dog grassland.

Bat activity patterns using radio tracking

The two Western small-footed myotis fitted with radio tags pinged the node grid system for 7 days from October 1st through October 7th. During the development of this protocol, multiple nodes pinged tags simultaneously, which is necessary to triangulate specific locations of tagged bats during the time the ping was received. In Figure 9, detection time stamps were graphed against node station ID. This data shows that tagged bats were flying over our grid system every day.

Aim 2: Determine if insects are more abundant in prairie dog colonies

Insect traps were deployed for 5 nights, resulting in a total of 11 samples. There were 4 samples from prairie dog colonies and 7 samples from non-prairie dog areas. Non-prairie dog samples were from shortgrass and mixed-grasslands and sand sagebrush dominated pastures. There was no significant difference in the corrected biomass between prairie dog and non-prairie dog colonies (Figure 10) for any statistical test (t-test: $t = 0.381$, $df = 3.972$, $p = 0.723$; Kruskall-Wallis test: chi-squared = 0.0357, $df = 1$, $p = 0.85$; Mann-Whitney U test: $W = 13$, $p = 0.927$). Biomass varied substantially with collection date (Figure 11). We reject our alternate hypothesis; insect biomass was not significantly greater on prairie dog colonies when compared to non-prairie dog areas.

DISCUSSION

Overall, there were significantly more bat passes over prairie dog colonies when compared to non-prairie dog areas, which confirms our prediction. Of the six bat species acoustically detected at the study sites, only one species, the Western small-footed myotis, was found to not differ in activity between prairie dog and non-prairie dog colonies. There was also no significant difference between insect biomass on prairie dog colonies and non-prairie dog areas.

Adams (2021) and we hypothesized that bats were more prevalent over prairie dog colonies due to an increase in insect abundance on prairie dog colonies compared to off colonies. However, the statistical analysis of the insect samples we collected did not support this hypothesis. Additional sampling are needed before drawing conclusions due to small sample size. We suggest this because there was a great deal of variation in the biomass of insects captured on a given night, which could disguise detectable differences. It is also possible that some other aspect of insect ecology could be driving the increased activity of bats over prairie dog colonies. For example, there might be greater insect species richness, meaning that bats could be more active over prairie dog colonies because of the increased number of insect species. The insects themselves could be more nutritious for foraging bats, since prairie dog colonies have been shown to increase the amount of crude protein (nitrogen) due to increased grazer selection (Whicker and Detling, 1988). More sampling would be required to make further inferences on the relationship between prairie dogs and insects.

Although we cannot definitively say why bats were more active over prairie dog colonies, we can say that in general, bats are significantly more active over colonies than non-prairie dog areas. This knowledge adds to the growing list of ecosystem services prairie dogs provide. Another service prairie dogs provide is increased grazer selection which leads to an uptake in crude nitrogen available to grazers in the forbs on prairie dog colonies (Whicker and Detling, 1988). Essentially, prairie dogs have affects on the nitrogen cycle within an ecosystem, as well as affects on food webs and habitat availability for a wide variety of species. This study, with Adams' (2021) findings adds an additional ecosystem service: increased activity of bats, a mammal that consumes agricultural pests and vectors of human and animal diseases (Maslo et al, 2022). This strengthens the evidence that prairie dogs provide essential ecosystem services and are a keystone species.

The work in the Front Range of Colorado that we modeled the present study after, found that all detected species were more active over prairie dog colonies compared to nearby grasslands (Adams 2021). Our data did not support the hypothesis that the Western small-footed myotis would be more active over prairie dog colonies as had been found in the Front Range of Colorado (Adams, 2021). However, like the previous study, every other species was more active over prairie dog colonies. This is interesting because in the Front Range of Colorado the Western small-footed myotis was the most prevalent species over prairie dog colonies for every site (Adams, 2021). One possibility as to why there was no significant difference in Western small-footed myotis (*Myotis ciliolabrum*) in Kansas compared to Colorado could be because these bats were foraging for specific species of insects that were only on prairie dog colonies in Colorado but could be found on other habitat types in Kansas.

Another possible explanation is roost location. The two Western small-footed myotis that transmitters were adhered to roost in a human-made structure to the southeast of the West pasture where both the preliminary node setup and two of the acoustic transects were located.

Due to the location of the roost, as well as water sources between the roost and the West pasture site, the lack of significant difference in western small-footed myotis activity could be attributed to bats having to fly over the non-prairie dog transect in the West Pasture in order to get to the prairie dog colony in the West Pasture. In order to know for sure future research with radio telemetry and the tagging of bats would be needed but can possibly be highlighted with further work being implemented with the CTT nodes.

Roost use may have influenced the activity of the other bat species in the study area. Eastern red bats (*Lasiurus borealis*) are known to roost in trees (Mager and Nelson, 2001); big brown bats (*Eptesicus fuscus*) roost in trees, rock crevices, and human made structures (Klüg-Baerwald et al, 2017); Brazilian free-tailed bats (*Tadarida brasiliensis*) roost in caves or crevices of human-made concrete bridges (Allen et al, 2011); and hoary bats (*Lasiurus cinereus*) roost in the foliage of trees, to have easy access to open areas to forage (Klug et al, 2012). Nearly all of these roost types are available on Smoky Valley Ranch in close proximity to the West and Far West pastures with the exception of caves. This could explain the greater overall bat activity for these two sites when compared to the other three sites. It should be noted that during the summer that this study was conducted, invasive trees were removed at Smoky Valley Ranch near the West and Far West study sites. We have no direct evidence that this disturbance impacted roost availability for bats in our study area, but it is a possibility.

Daily bat activity was highly variable across sites, and we suspect that one or more factors may have contributed to this variation. One factor could be that multiple known roost locations as well as water sources were located closer to sites that experienced more activity, such as the West and Far West pastures. Another is the severe drought that occurred across Western Kansas in 2022. During July 2022, Logan County was classified as a D3 Extreme

Drought, which is described as a time where emergency grazing is open, river levels are low, and emergency water supplies are needed ("Logan County Conditions"). By October 2022, the drought progressed into a D4 Exceptional Drought. A D4 Exceptional Drought is described as all crops are severely impacted, all aquatic species and food chains are affected, and wildfires and dust storms are likely to occur ("Logan County Conditions").

There were several limitations to this study. The first was not successfully capturing bats using mist netting techniques to confirm species identifications from the acoustic calls. In order to increase the likelihood of catching bats using mist netting techniques, mist netting should occur on more nights and with more equipment. Another limitation were the ambiguous calls that we know are for sure were bat calls, but could not confidently identify to species. Whereas ambiguous calls let us know that a bat was present, it does not allow us to confidently identify what species was present. The 80% confidence threshold was arbitrarily selected by this study. Further literature that is done over the automatic identification of bat acoustics could lead to a different threshold being selected. This can significantly affect results, for example there could be western small-footed myotis calls that were not kept because the confidence level was below 80%. Additionally, this study started later in the season than is typically advisable, due to supply chain issues we encountered when ordering acoustic detectors, insect-traps, and CTT tags. These issues prevented us from starting acoustic and insect surveys until well into the summer field season. Even with half the field season missed, bats were found significantly more active over prairie dog colonies. Additional sampling time is expected to only bolster our findings.

There are many ways to extend this study and strengthen the evidence that an ecological relationship exists between prairie dogs and bats. First, the acoustic surveys should start earlier in the season, as early as May. This will be easy to accomplish as the equipment is already purchased and in the possession of Fort Hays State University. Another is to analyze acoustic data with a software called "Sonobat", a software that can auto identify if there are more "buzz calls" over prairie dog colonies. These types of calls are hallmarks of bats feeding and would indicate if bats are actually foraging more over prairie dog colonies when compared to non-prairie dog areas. Gathering data in a non-drought field season will also be preferable, but this may require several field seasons to be completed until the precipitation levels are closer to the seasonal average in Logan County.

In conjunction with acoustic surveys, insect biomass should also be sampled monthly starting in May and attempt to increase the sample size of both prairie dog samples and non-prairie dog samples. In addition, identifying the insect taxa captured at both site types would address whether insect species richness is greater over prairie dog colonies. Finally, nutritional analyses of insects from both site types may reveal if site type influences the nutritional value of prey items captured by bats over prairie dog colonies.

One of the most promising directions this future research can take is radio telemetry. From the preliminary protocol developed in this study, we know that the two tagged Western small-footed myotis individuals returned to the West pasture every night for a week. This was fortuitous because we did not know whether the tagged bats would be detected by the MOTUS system at all after they were released. A large study of vampire bats tagged fifty individuals and deployed a large sensor array only to find that the bats preferred a pasture adjacent to the array (Ripperger and Carter. 2021).

Several variables can be introduced to this study to improve the protocol, such as changing the specific radio backpack to increase the battery life or changing the distance between nodes to reduce the overlap of pings; this would need to be done on a small scale because three nodes are needed to ping a tag in order to triangulate specific locations of tagged bats. One possibility would be moving the node grid to the Far West pasture because the habitat is similar to the West pasture (trees, prairie dog colonies, surrounding grassland, nearby chalk bluffs with cliff crevices, etc.). The Far West pasture had the most bat activity of any site, potentially making it an ideal telemetry site. It should be noted that the current configuration of the MOTUS would need to be changed, this would be done by manually pointing the antennas on the MOTUS station so that the line of sight of the tower covers the Far West pasture.

An additional future direction is determining which insects the bats are consuming by collecting bat guano and sequencing the insect DNA it contains. DNA metabarcoding is a novel way to assess biodiversity in ecosystems, with the CO1 segment being used to identify animals. To do this, future researchers would extract a small segment of mitochondrial CO1 from guano samples, then amplify and sequence that DNA. The sequences would be compared to existing genetic databases to determine the insect species ingested by the bat that left the guano deposit. Combined with insect species richness data from the light traps, these data could be used to determine if bats were choosing to forage over prairie dog colonies because of the insect species found there.

In conclusion, there was significantly more bat activity over prairie dog colonies when compared to non-prairie dog areas. All species recorded were significantly more active over prairie dog colonies when compared to non-prairie dog colonies with the exception of the Western small-footed myotis. Insect biomass did not significantly increase on prairie dog colonies. With the establishment of a preliminary grid system using the nodes and CTT tags further research can attempt to determine what habitat bats are selecting. This study did encounter limitations, the exciting factor in this is that by increasing sample size and the potential of sampling occurring in non-drought years will allow future research to amend and adjust these limitations and highlight the essential relationship between two important organisms in Western Kansas, prairie dogs and bats.

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TABLES

Table 1. Acoustic detector deployment site names and GPS coordinates.

	Smoky Valley Ranch	Haverfield Ranch	Prairie Dog Transect: Starting GPS Point (Decimal Degrees)	Prairie Dog Transect: Ending GPS Point (Decimal Degrees)	Non-Prairie Dog Transect: Starting GPS Point (Decimal Degrees)	Non-Prairie Dog Transect: Ending GPS Point (Decimal Degrees)
West Pasture	✓		(38.85838, -101.00277)	(38.85527, -101.00071)	(38.85835, -101.00336)	(38.8553, -101.00124)
Far West Pasture	✓		(38.86969, -101.0099)	(38.86796, -101.00964)	(38.86995, -101.01039)	(38.86774, -101.01012)
Long Pasture	✓		(38.86033, -100.96355)	(38.85728, -100.96176)	(38.86008, -100.96402)	(38.85732, -100.96243)
Haverfield East		✓	(38.94536, -101.07083)	(38.93767, -101.06584)	(38.94541, -101.01029)	(38.94227, -101.06942)
Haverfield West		✓	(38.9325, -101.09177)	(38.93567, -101.09173)	(38.93251, -101.09234)	(38.93567, -101.09229)

Table 2. Summary table representing the number of bat passes by species and site type in Logan County, Kansas from July 12, 2022 - October 31, 2022.

Species	Prairie Dog	Non-Prairie Dog	Total
Eptesicus fuscus (Big brown bat)	129	98	227
Lasiurus borealis (Eastern red bat)	233	185	418
Lasiurus cinereus (Hoary bat)	131	108	239
Lasionycteris noctivagans (Silver-haired bat)	27	17	44
Myotis ciliolabrum (Western small-footed myotis)	248	235	483
Tadarida brasiliensis (Brazilian free-tailed bat)	132	124	256
Ambiguous bat	617	544	1161
Total Bat Passes	1517	1311	2828

Table 3. Summary of statistical values for bat passes between prairie dog and non-prairie dog transects for each species.

Species	W	p-values	Prairie Dog	Non-Prairie Dog
			Mean	
***Eptesicus fuscus* (Big brown bat)**	**5512**	**0.05085**	**2.076**	**1.408**
***Lasiurus borealis* (Eastern red bat)**	**18954**	**0.008986**	**1.966**	**1.373**
***Lasiurus cinereus* (Hoary bat)**	**4765.5**	**3.607E-06**	**3.183**	**1.787**
***Myotis ciliolabrum* (Western small-footed myotis)**	**29597**	**0.6768**	**1.262**	**1.34**
***Tadarida brasiliensis* (Brazilian free-tailed bat)**	**5003.5**	**2.012E-08**	**4.78**	**1.839**

Table 4. Summary of statistical values for bat passes between prairie dog and non-prairie dog transects for each site.

			Prairie Dog	Non-Prairie Dog
Site	**W**	**p-values**	**Mean**	
Far West	**53908**	**2.277E-13**	**3.748**	**1.793**
West	**166238**	**0.01603**	**1.488**	**1.669**
Long	**12355**	**0.9266**	**1.212**	**1.208**
Haverfield West	**2295**	**0.676**	**1.207**	**1.248**
Haverfield East	**13210**	**0.567**	**1.22**	**1.238**

FIGURES

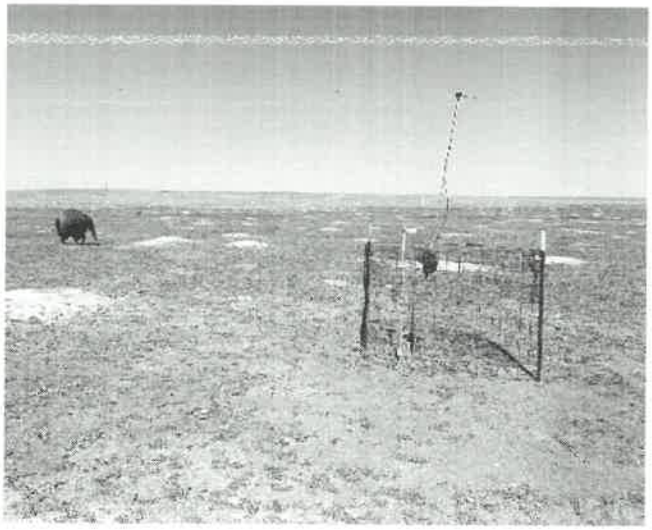


Figure 1: Detector deployment on Smoky Valley Ranch, Logan County, KS. Note the protective structure surrounding the detector.

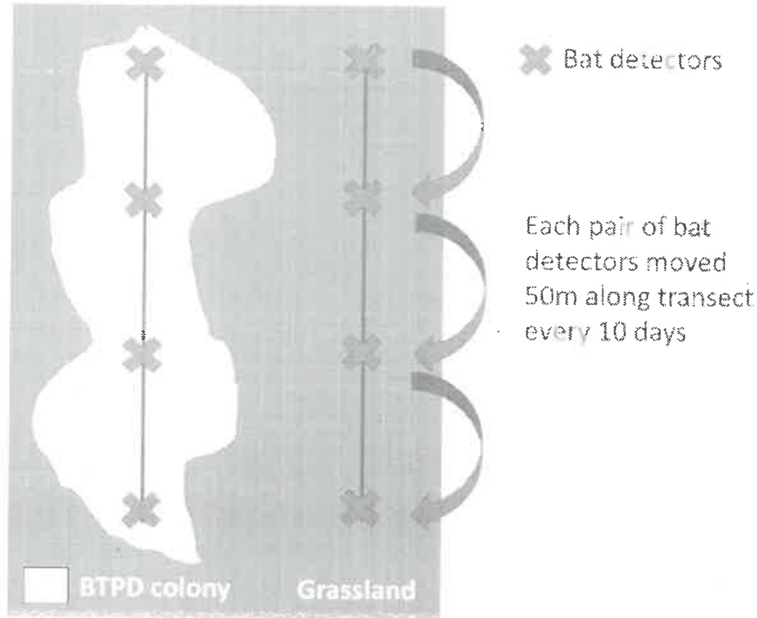
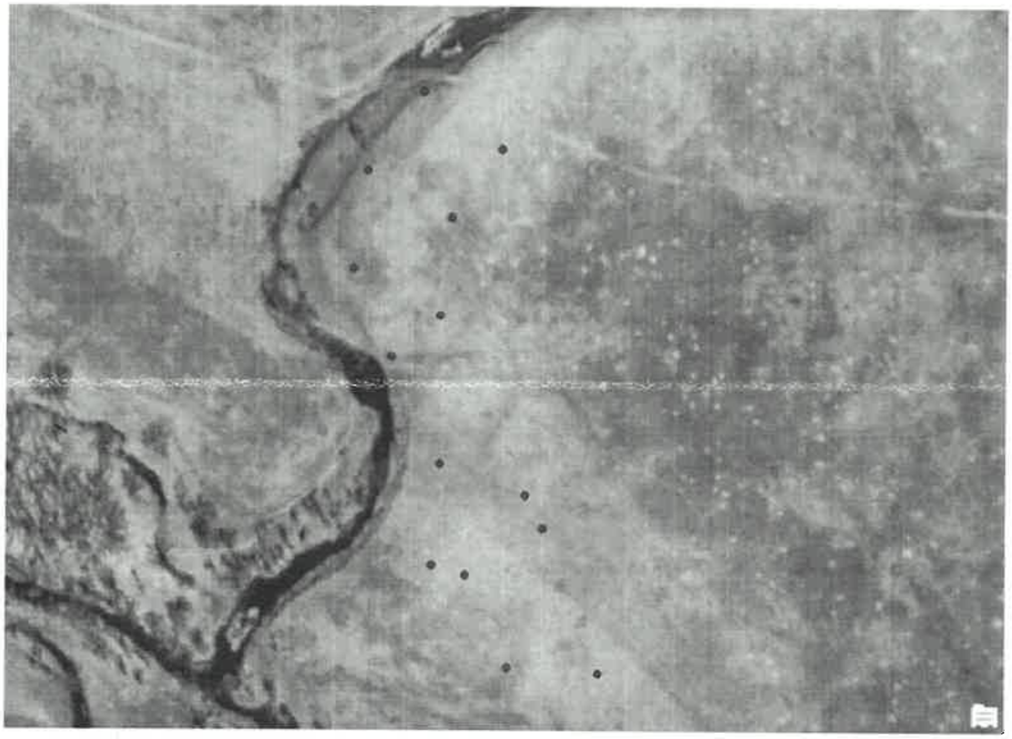
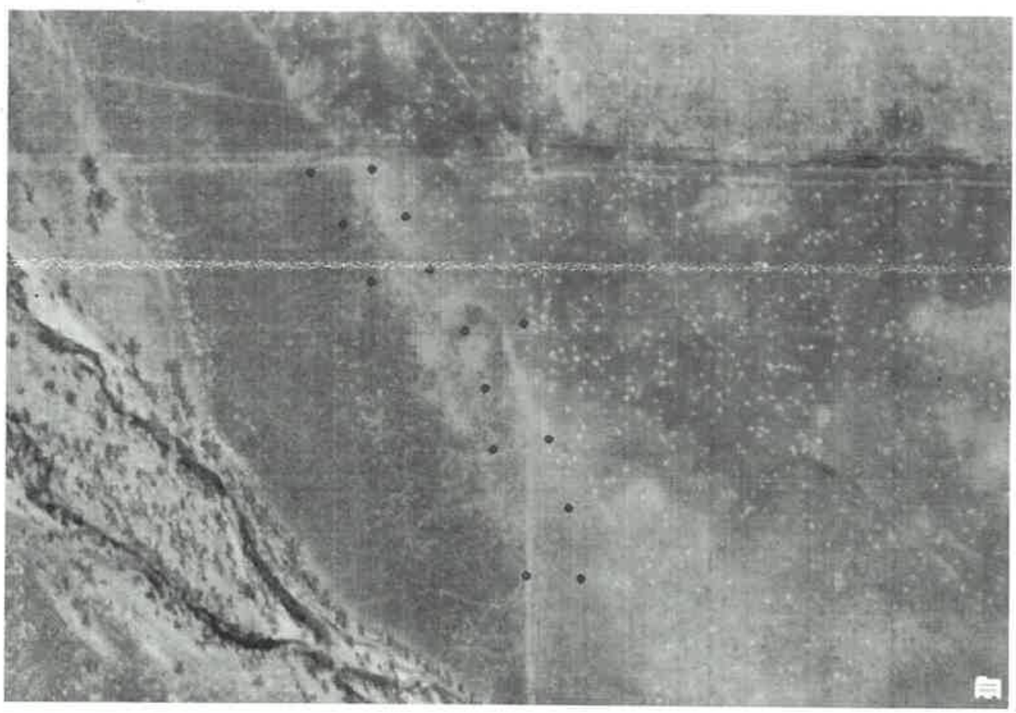


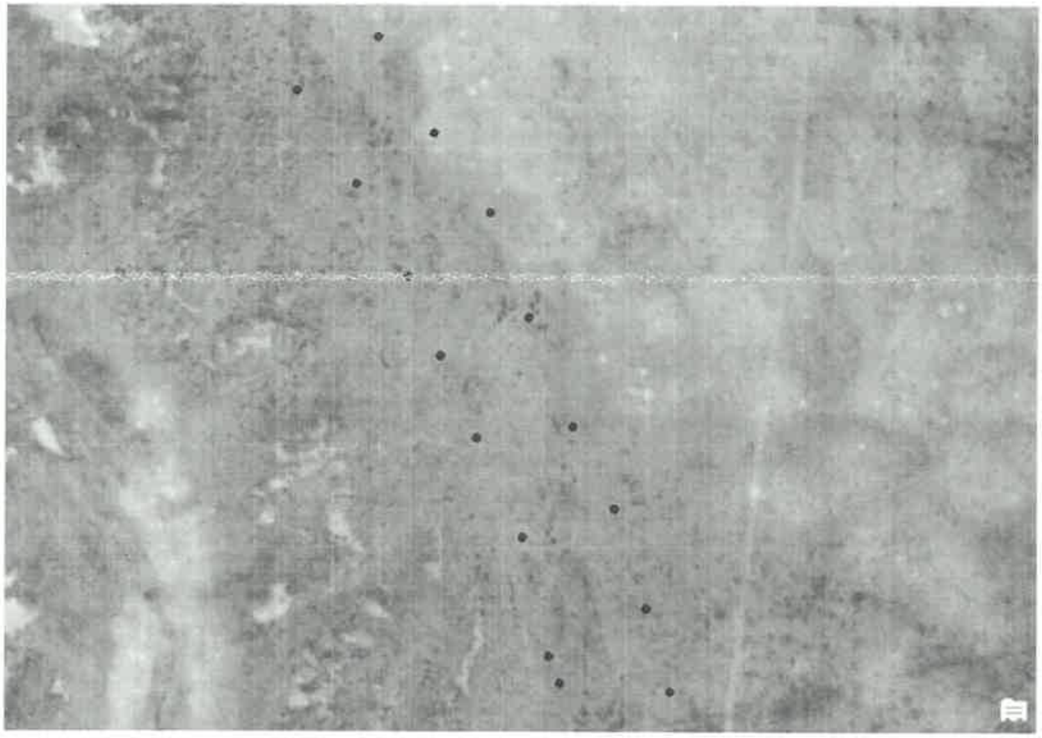
Figure 2: Schematic of the acoustic survey methods. Acoustic detectors were moved in parallel 50ms down transect every 10-14 days.



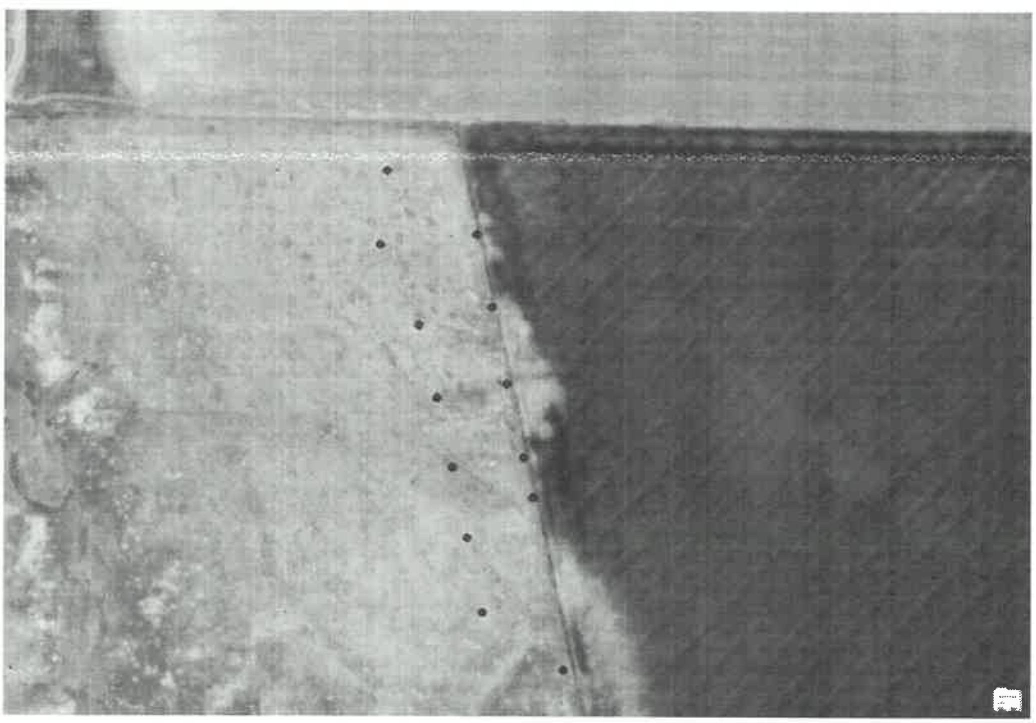
Far West pasture



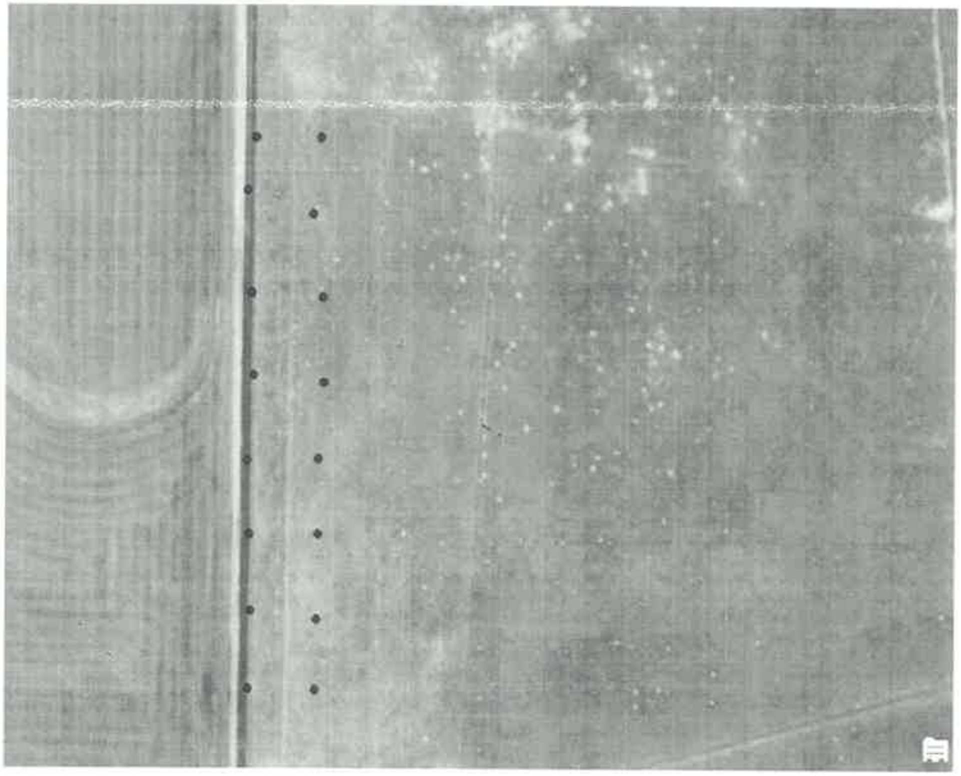
West pasture



Long Pasture



Haverfield East



Haverfield West

Figure 3: Aerial images of acoustic deployment sites. These images depict the habitat at and placement of acoustic detectors at each of the 5 sites, the two Haverfield sites were located on the same property around 7 miles northwest of Smoky Valley Ranch where the other 3 sites were located.

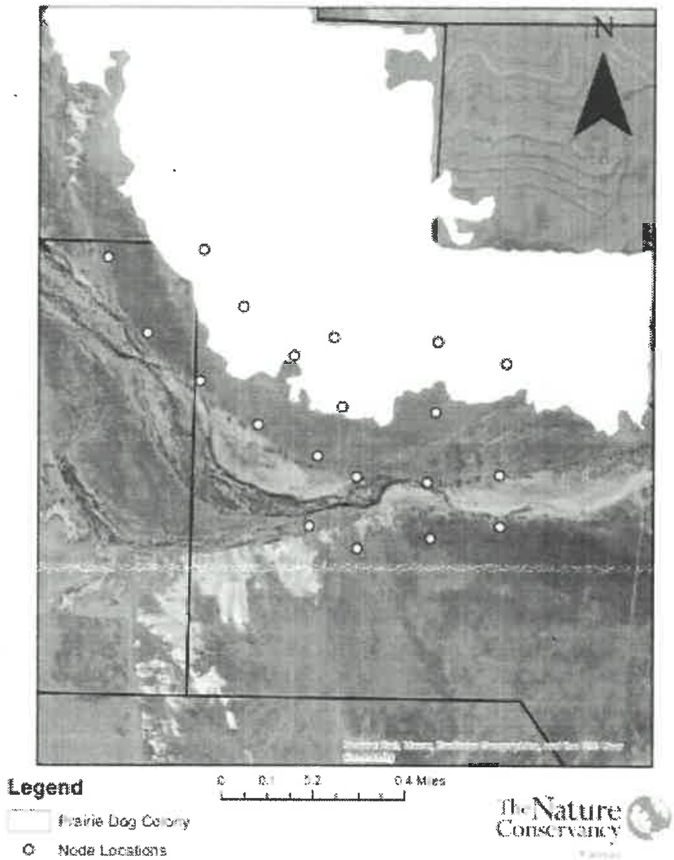


Figure 4: Image of setup of grid with nodes. The green shaded area is a known prairie dog colony.

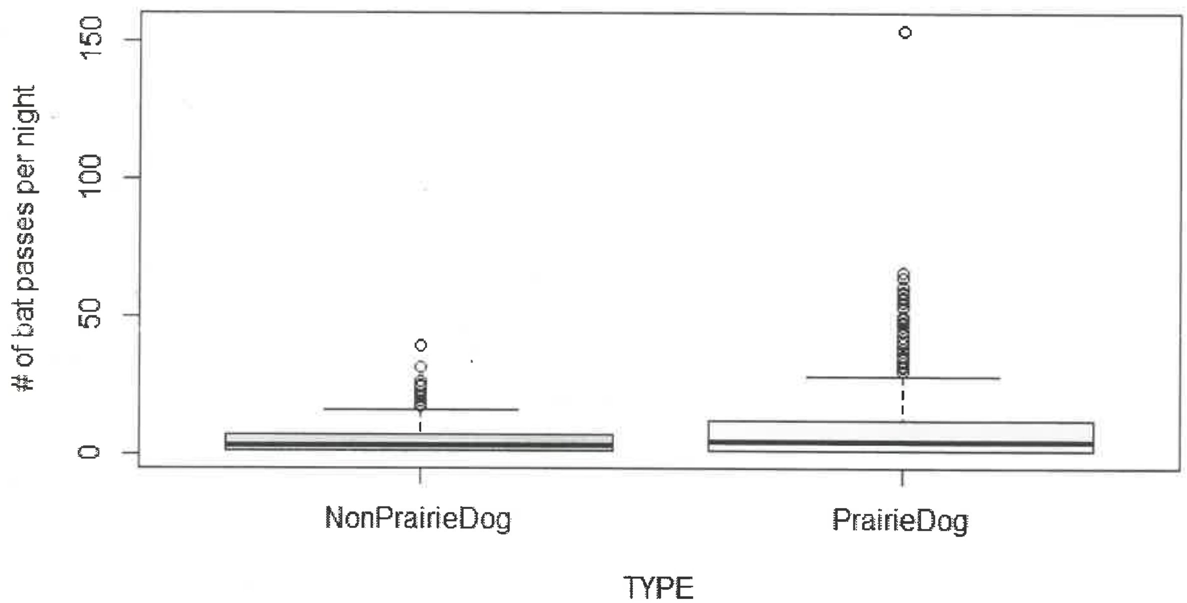


Figure 5: Total bat passes per night for both prairie dog colonies and non prairie dog areas. There were significantly more calls over prairie dog colonies. ($t = 5.977$, $df = 408.06$, p-value < 0.001). The boxplot shows that the averages for both sites are close to one another, the prairie dog sites had larger outliers than the non-prairie dog areas. Outliers are representative of data more than 1.5 times greater than the upper quartile. The whiskers are representative of the data in between the upper quartiles and the maximum.

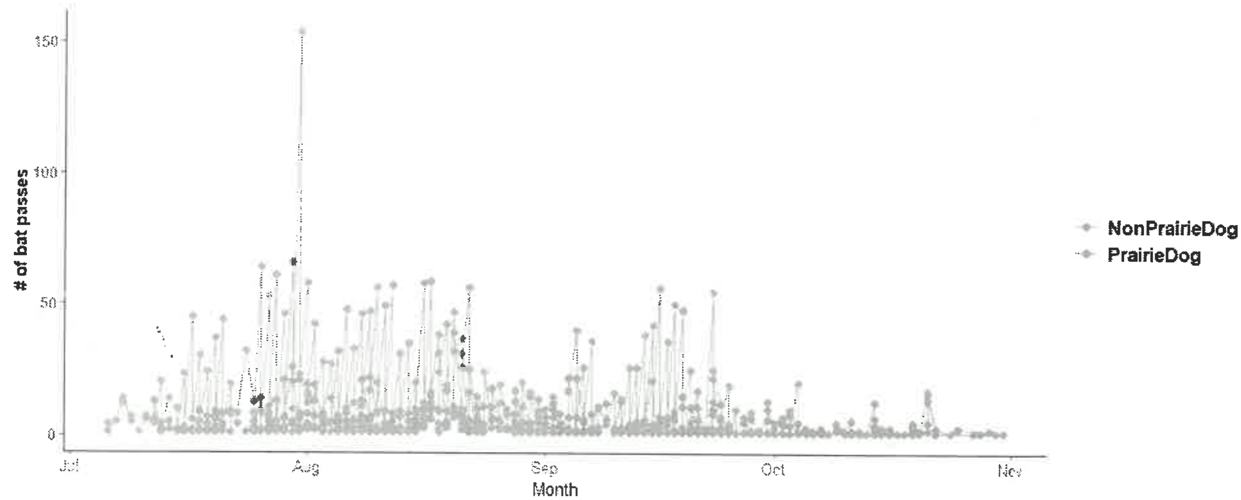


Figure 6: Daily total bat activity for both non-prairie dog (green) and prairie dog (yellow). Each point is the number of bat passes recorded in one day.

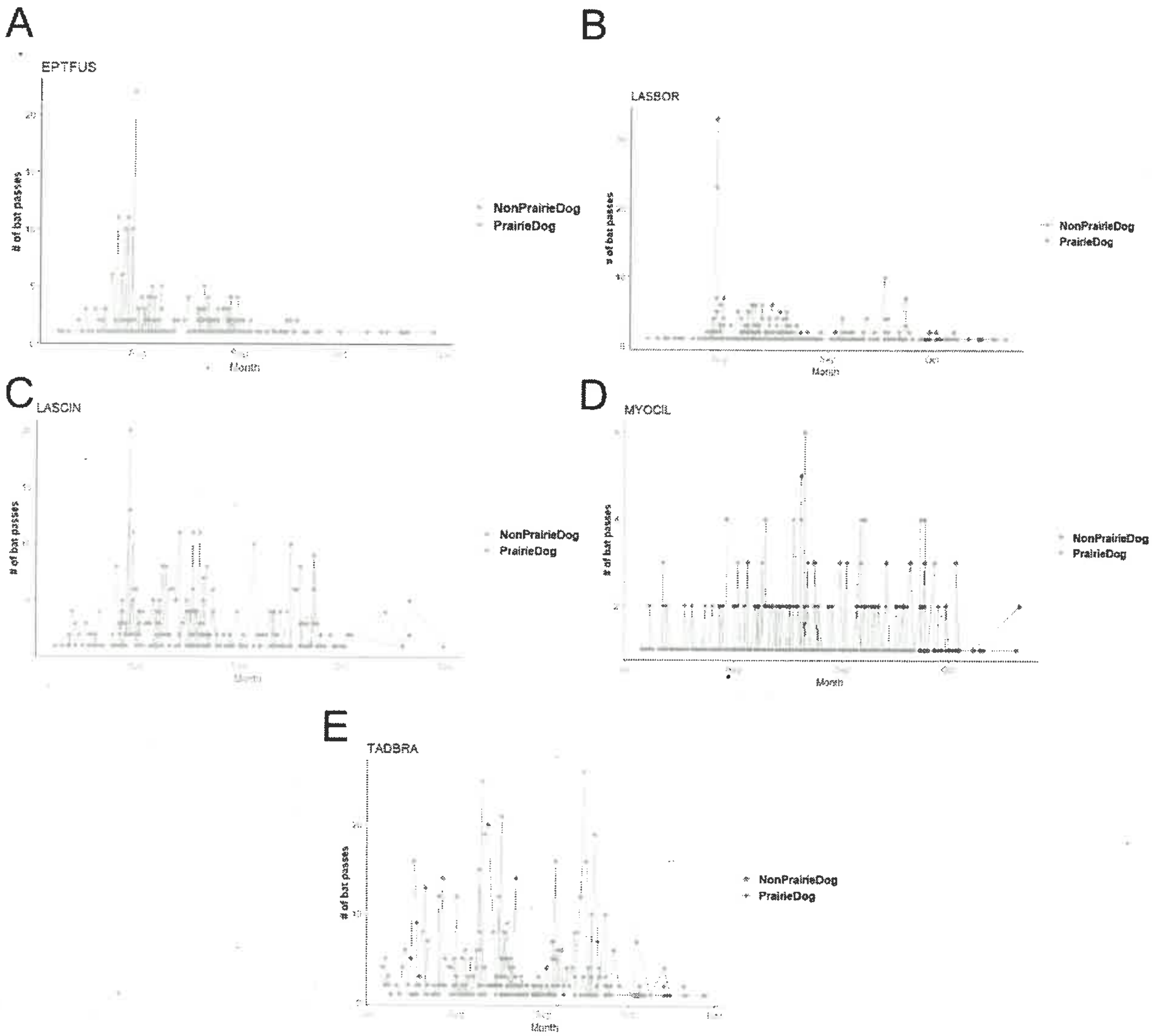


Figure 7: These graphs represent bat activity for both prairie dog colonies and non-prairie dog areas by species. A. Daily activity for Big Brown Bats. B. Daily activity for Eastern Red Bats. C. Daily activity for Hoary Bats. D. Daily activity for Western small-footed myotis. E. Daily activity for Brazilian free-tailed bats. The y-axes are not equal for every graph.

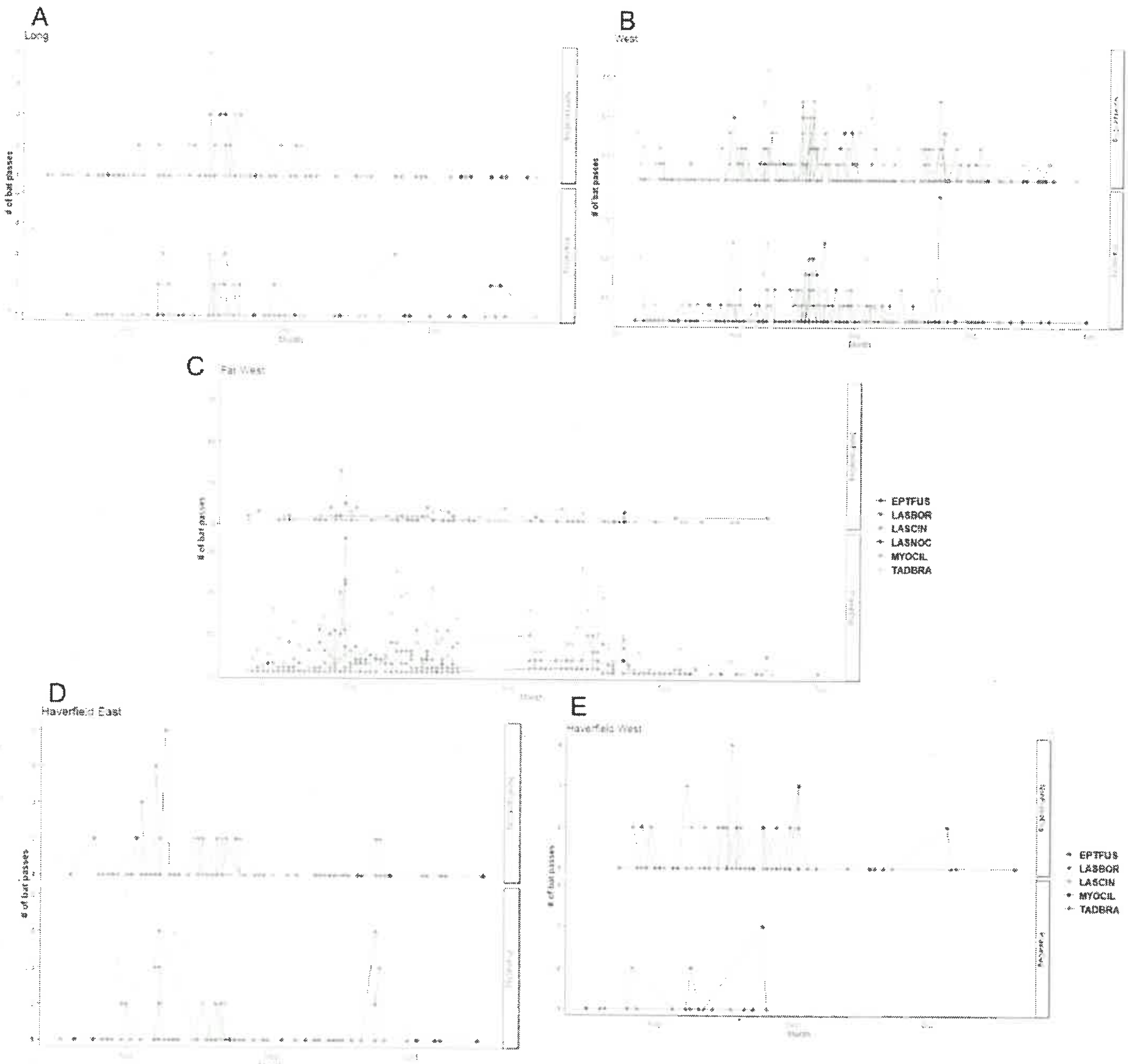


Figure 8: Graphs represent bat activity for all species by site. Each panel has calls detected in non-prairie dog areas on top and calls detected over prairie dog colonies on the bottom. A. Daily bat activity over Long pasture. B. Daily bat activity over West pasture. C. Daily bat activity over the Far West. D. Daily bat activity over Haverfield East. E. Daily bat activity over Haverfield West. The y-axes are not equal for these graphs.

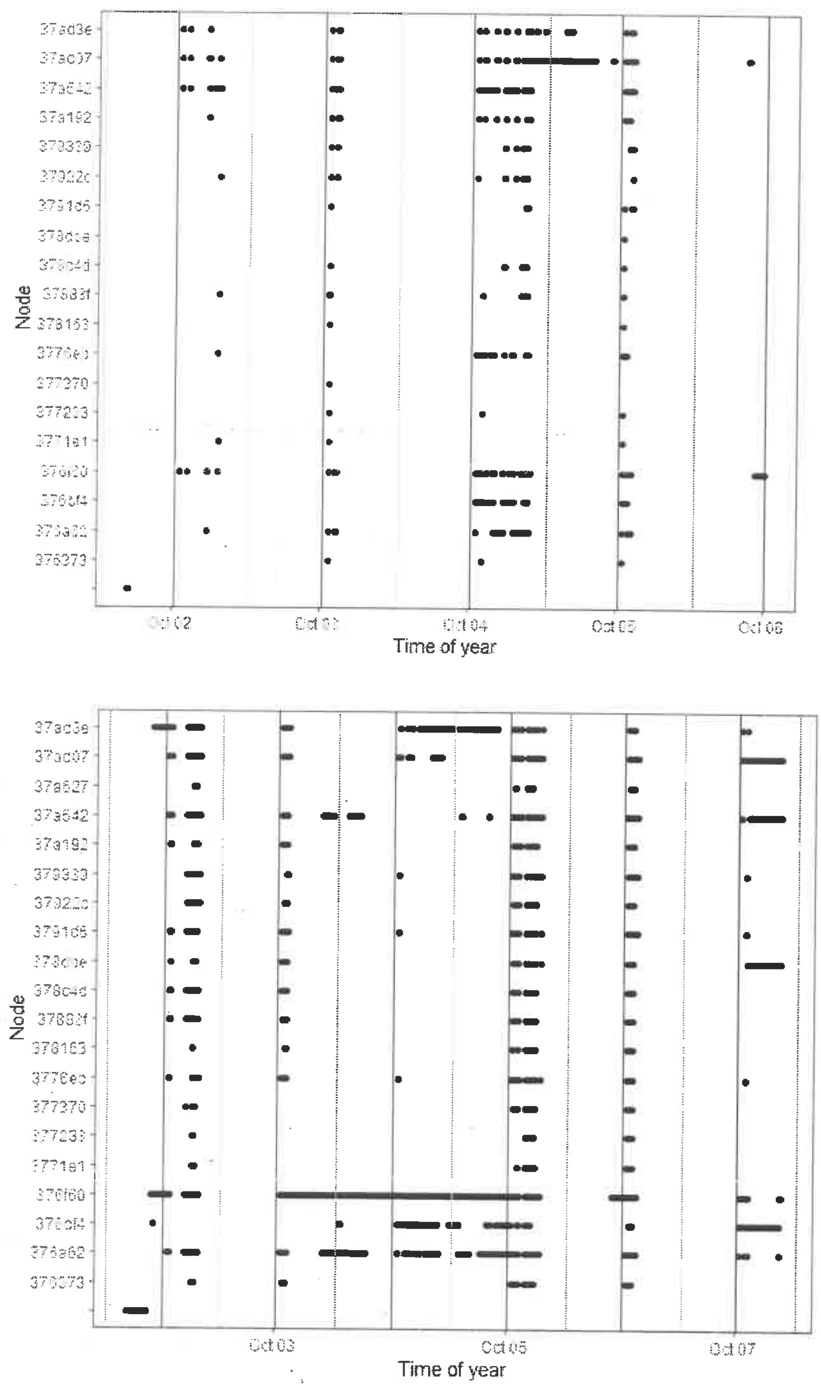


Figure 9: Motus A: Represents the node pings from Tag ID#69869. Motus B: Represents the node pings from tag ID#70806. Pings recorded by tagged bats per node over the week the tags were active. Each black circle indicates a reading from a node. Yellow vertical lines indicate sunrise and blue vertical lines indicate sunset.

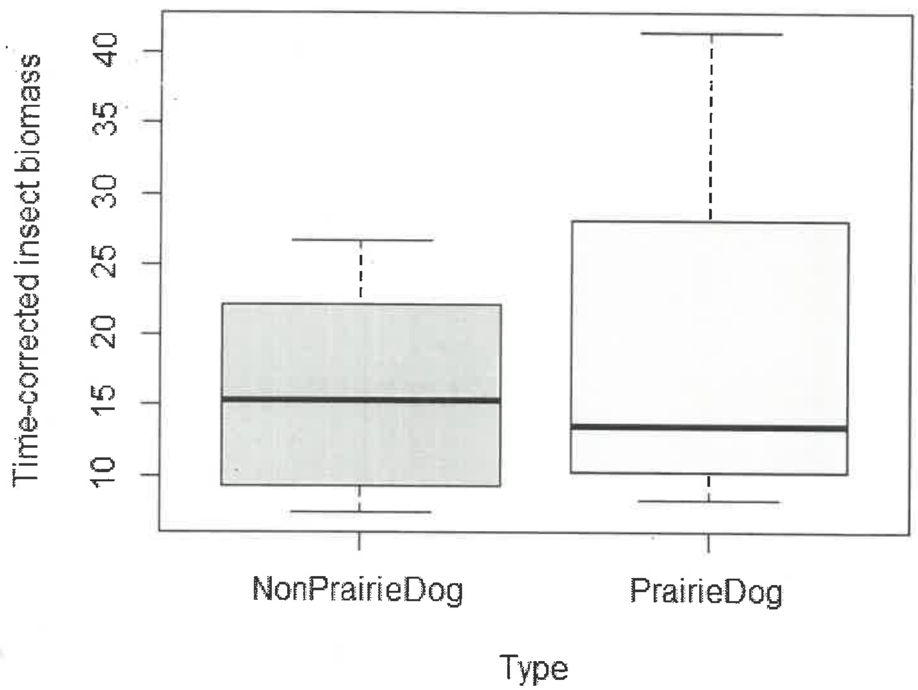


Figure 10: Total insect biomass for both non-prairie dog areas and prairie dog colonies.

The green represents non-prairie dog areas and yellow indicates prairie dog colonies.

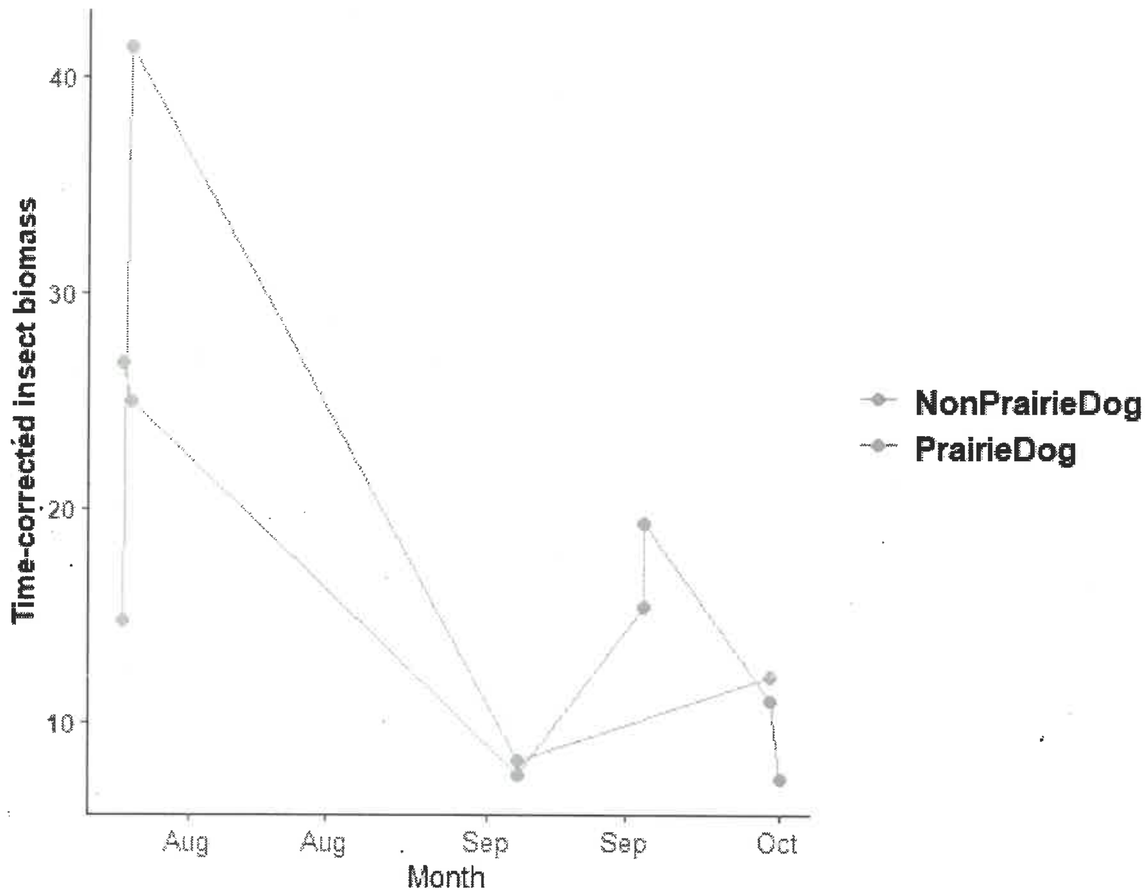


Figure 11: Insect biomass at each collection time point. Green indicates non-prairie dog collection sites and yellow indicates prairie dog colony collection sites.

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