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Quantifying Resource Use Among Insectivorous Bat Species with Overlapping Distributions

Shannon Whitney

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Abstract

Bats make up 20% of all mammalian species, are globally distributed, and are the only mammals capable of sustained flight. Bats have adapted to feed on insects, scorpions, aquatic arthropods, mammals, birds, reptiles, amphibians, fish, blood, carrion, fruit, flowers, nectar, pollen, and even seeds and foliage. However, the feeding ecology of these organisms is not well understood. Most bat species in North America rely on the same method of foraging and locomotion. The geographical range and habitats of these bats also commonly overlap. Bat feeding ecology studies have used fecal analysis to identify consumed prey species. Factors such as time of night, season, bat community composition, competition, habitat structure/type, and available prey likely determine feeding behaviors for bat species. This study quantified and integrated the external factors mentioned above with prey items consumed by several common species of bats in the Southeastern United States. The data collected shows a significant relationship between temperature, arthropod communities, and bat activity. This study provides data to address questions about resource use among local southeastern bat species. These data can inform bat conservation efforts and landscape management.

Keywords: resource use, feeding behavior, chiroptera, metagenomics

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Introduction

Bat Biology

Bats belong to the order Chiroptera ("cheir" [hand] and "pteron" [wing] in Ancient Greek) and are the only mammals that can sustain flight. Chiroptera is the second most diverse order of mammals, behind Rodentia, and makeup ~20% of all mammal species (Altringham, 2011). They are long-lived organisms with low fecundity and belong to higher trophic levels. Bats are also regarded as indicator species, reflecting ecosystem health (Jones et al., 2009). Previously Chiroptera was divided into two suborders based on morphology. However, genetic evidence has been used to reclassify the suborders. The current suborders are Yinpterochiroptera, which include the Pteropodidae family and the Rhinolophoidea superfamily, and the Yangochiroptera which include the rest. Molecular evidence shows a taxonomical relationship between Pteropodidae (flying foxes) and the super-family Rhinolophoidea (bats with complex noseleaves) (Teeling et al., 2005). For the purposes of this paper, Megabats refers to the Pteropodidae family.

Apart from the implied size differences between megabats and microbats, these two groups differ in many ways. Megabats inhabit the Old-World tropics. These bats primarily eat fruit, flowers, nectar, and pollen (Altringham, 2011). These bats often have dog-like faces and are referred to as flying foxes. These bats lack echolocation as a means of foraging and are heavily reliant on sight and sound (Altringham, 2011). Hence, they have complex eyes, often with a *tapetum lucidum, a* reflective layer within the eye, which helps redirect light, for an increase in visibility in low-light conditions (Ollivier et al., 2004). Fruit-consuming megabats contain a short, heavy, and strong muzzle and a strong jaw that houses simple flat teeth. The frugivore bats often crush fruits to extract the juices and discard the pulp. Nectar and pollen consuming megabats have elongated muzzles and long tongues, which help them reach their primary source of nutrients. Some species of Megabats, such as the hammerhead bat (*Hypsognathus monstrosus*) and the tube-nosed bats (*Nyctimene sp.*), use sounds for mating purposes, but not for foraging (Altringham, 2011).

Microbats are distributed worldwide. Microbats often possess smaller eyes and variable ear sizes. Many microbats rely on echolocation to forage. Echolocation consists of releasing a series of high-frequency (between 9 kHz – 200 kHz (Maryland DNR)) pulses that reflect off objects in the environment and provides the objects' locations. Microbats has adapted a wide variety of diets. These include frugivores, nectarivores, insectivores, carnivores, and even sanguinivores, which consume blood. Microbats are among the few mammals that undergo true torpor. During torpor, an animal reduces body temperature and immune function to save energy during unfavorable conditions. What makes torpor different from hibernation is that animals are not in this state for long periods. Instead, they can exit torpor when needed, such as when temperatures increase, and food is more abundant. Before inducing torpor, animals consume large amounts of food to increase body fat reserves, which get depleted over time. Since microbats share similar sizes, methods of locomotion, and methods of foraging, they often consume similar types of food. Ecologists hypothesize that differences detected in diet among bats reduce interspecific competition (Moosman et al., 2012).

Bats provide an important ecological and economic service as pollinators and insect control for human crops (Boyles et al., 2011). Over two-thirds of all bat species are insectivorous (Altringham, 2011). They serve as a natural pest control and save humans millions of dollars on pesticide applications. In the USA, bat populations reduced pesticide costs by roughly $3.7 - $55

billion per year (Kasso & Balakrishnan, 2013). Fruit-eating bats also serve as significant pollinators throughout the globe. Cash crops that bats pollinate include wild bananas, mangos, breadfruits, agave, durians, and petai. The total monetary value of these serves is estimated to be $200 billion (Kasso & Balakrishnan, 2013)

All bats naturally found in the United States are microbats, and a portion of these will be the focus of this study. A total of 47 different bat species inhabit the USA. Fourteen of these bat species have been reported in South Carolina (South Carolina Department of Natural Resources, 2019). The emergence of *Pseudogymnoascus destructans* (*Pd*), the fungal pathogen responsible for white-nose syndrome (WNS), has impacted different bat species worldwide. In the United States, 13 bat species, including two endangered and one threatened species, have been confirmed with WNS (whitenosesyndrome.org). Certain bats, such as tri-colored and little brown bats, have been drastically, negatively affected. Population sizes have decreased by up to 70% (Ingersoll et al., 2016). Before detecting *Pd*, these bat species were considered common members of the bat community; now, they are considered rare. Other populations of bat species have started recovering from WNS (Langwig et al., 2017). Bat biologists encounter these species more frequently; these include the Big Brown Bats (*Eptesicus fuscus*), Eastern Red Bats (*Lasiurus borealis*), Seminole Bats (*Lasiurus seminolus*), and South-Eastern Bats (*Myotis austroriparius*). It is common to find several species of microbats with similar diets in the same geographic area.

Knowing that bats with similar diets are commonly found together in an area begs the question of how can several bat species successfully coexist in a shared habitat? The eco-morphological paradigm states that the differences in morphology or physiology between species are responsible for the differences in ecology (Fath & Jørgensen, 2008). Many of these bats would be competing for resources when considering this paradigm, and only a few species should persist in any habitat. However, many species coexist within a single habitat. Since many bats thrive in shared environments, we suspect that these bats must be reducing or eliminating competition. One method of reducing inter-species competition is by partitioning different resources. Resource partitioning, or species packing, is a stabilizing mechanism used to explain coexistence (Chesson, 2000). Classic ecological theory suggests species diversity is fostered by differences in resource usage (Finke & Snyder, 2008). Ecologists have attempted to understand resource partitioning by creating ecological models (Chesson, 2000, McArthur 1970) and, more recently, by experimentation (Finke & Snyder, 2008).

Feeding Ecology

Behavioral ecology refers the study of how environmental pressures lead to variation in animal behavior, the behaviors that improve survivability are selected for(Davies et al., 2012). Since all organisms must eat to survive, researchers have tried to understand the underlying behavioral mechanisms of feeding. The optimal foraging theory (Emlen, 1966; MacArthur & Pianka, 1966) attempts to delineate feeding behaviors observed among different animals that use the same food resources. Optimal Foraging theory compares the energy cost associated with hunting/foraging to the energy gained from consuming resources (Schoener, 1971). Many factors influence feeding behaviors, including location/patch within an area, activity time within location, and dietary choices during foraging.

Optimal activity time is defined by the best time is for the animal to forage for food while remaining safe from predators. The optimal patch is defined by the best “hunting grounds”

within the environment where most prey is active. Finally, the optimal dietary choice is defined by which food source requires minimum effort to collect and return the highest amount of energy. If specific organisms are optimally foraging, then all these factors would be satisfied.

Researchers have developed sub-theories within the Optimal Foraging Theory, such as the Marginal Value Theorem (Charnov, 1976). This theory determines when it is ideal for an organism to move to a different patch to forage for food. This theorem suggests the optimal time to transition to a different patch is when the marginal capture rate drops to the average capture rate within a patch. The average capture rate is represented with line $g_i(T_i)$ with slope E^*n, the marginal capture rate is represented with the tangential line at the highest point within the (energy intake)/(time) line, shown in Figure 1 (Charnov, 1976).

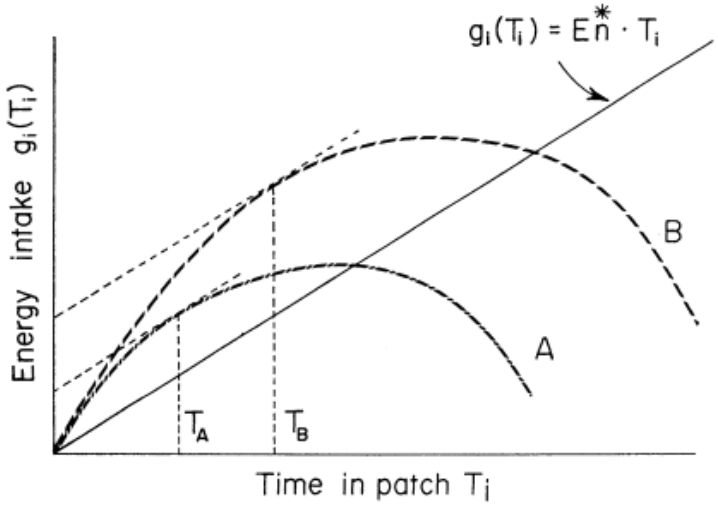

Figure 1. The general curve most organisms experience. As the time in a patch increases, the rate of energy intake increases until a certain point. This is known as the law of diminishing returns. The tangential lines (TA and TB) represent the marginal capture rates in two types of patches, A and B (Charnov, 1976).

G. H. Pyke et al, 1977 summarized previous feeding ecology investigations. They suggest that certain conditions must be met for a diet to be optimal. Each food type is associated with a value, calories or weight, and an associated handling time. Food items with the highest ratio of value/handling time are prioritized. Alongside having a handling time, each prey item also has a corresponding search time. These times are shown to be mutually exclusive and considered when looking at the total time of foraging. The optimal diet has three properties that should be quantified. The first property states that consuming a food type is independent of the abundance of that food type; this decision depends on the absolute abundance of the food type at a higher taxonomical level. In other words, animals should never specialize in a "less preferred" food type. The second property states that as the abundance of preferred food types increases, less preferred abundances will shrink, i.e., increasing preferred food abundance should lead to food specialization. The last property states, food types are either entirely included in the optimal diet or entirely excluded, in other words. Animals should not have partial preferences for a specific food.

Since insectivorous bats share the same method of locomotion and foraging, bat biologists have suspected that the success of many species inhabiting an overlapping geographic

area may be related to the specialization of diets. The anatomical structure of the jaw determines the strength possessed to masticate prey and informs biologists what the primary food type for bats may be. For example, *E. fuscus,* also known as the Big-Brown Bat, is commonly associated with foraging insects from the Coleoptera order (Moosman et al., 2012). These insects consist primarily of beetles that possess a rigid wing cover. *E. fuscus* has a strong jaw and sharp teeth, so bat biologists believe they actively hunt for beetles. Fecal analysis of prey consumed supports this idea. However, most of these studies relied on anatomical analysis of the feces and may have missed many prey items captured by the bat. Anatomical fecal studies rely on finding remains of prey items within guano samples. Bats thoroughly masticate and digest their food, so finding remnants of insects can be complicated (Zeale et al., 2011). This method of analysis is limited to the order level since remains are difficult to identify to family, genus, or species. The possibility for hard-bodied insects commonly observed in *E. fuscus* guano could be associated with rigid bodies preserving better through digestion. Advances in DNA-based techniques have provided molecular ecologists the ability to more comprehensively profile an organism's diet based on DNA barcodes down to the genus or species level.

Molecular Approaches

Using approaches from the fields of metagenomics and bioinformatics to survey an organism's diet may be more informative than fecal anatomical studies. Metagenomics is the field within genomics specializing in collecting DNA samples from environmental samples (i.e., soil or feces). DNA barcoding is a method to identify species present by using a short segment of DNA. Molecular techniques can be used for further analysis. When conducting these studies, it is common to amplify DNA specific to that of the study, for example, 16S microbial DNA, ITS fungal DNA, or Cytochrome Oxidase I (CO1) eukaryotic DNA. To specify the amplification, primers are created to select the portion of targeted DNA. These primers allow Polymerase Chain Reaction (PCR) to amplify one small portion of the DNA available.

Once DNA sequences become available, it is possible to identify the source of the DNA to the species level using bioinformatic approaches. Software such as Qiime2 or Taxonomer has made metagenomic studies more accessible (Bolyen et al., 2019; Flygare et al., 2016). By comparing DNA to a database of known DNA barcodes, it is possible to identify the organisms present in a sample. Researchers, such as Razgour et al., 2011 and Zeale et al., 2011, have used these techniques to profile bat species diets. The results have constantly showed greater species richness observed through a molecular approach compared to the anatomical method. Researchers can now determine what insect species a bat is consuming at a higher taxonomic level by using molecular approaches.

Acoustic Survey

Acoustic monitors have gained traction in bat-focused studies (Russo & Voigt, 2016). These monitors function as a passive method to detect bat activities. Since echolocation is specific to activity and be indicative of species. These monitors allow researchers to determine bat presence, activity, and estimate community structure. Bats produce various echolocation calls. The calls which consist of search phase calls, feed buzzes, and social calls (Britzke et al, 2013). Search phase calls help to navigate the environment; these are the most studied calls to date. Search phase calls are specific to species; thus these are the calls used to identify bats in a survey area.

Feed buzzes are the calls used for foraging and targeting prey items; these have a wide range of frequencies, so they are not used to determine species. Finally, social calls communicate between individuals.

Acoustic monitors provide large amounts of data that can be transformed into sonograms. These digital sonograms are further analyzed to determine species-specific variables. These sonograms are then compared to libraries of known bat calls to determine specific species. Identification of species using these sonograms can be made manually. However, neural networks automate the process. These neural networks have increased in accuracy over time, but bat biologists still advise caution because species identification is probabilistic (Britzke et al., 2013; Loeb et al., 2015).

Statement of Problem

The mechanisms behind the success of several insectivorous bat species living in a common area are not fully understood. With advances in molecular techniques, researchers have learned more about dietary preferences in different organisms. These techniques can be implemented within a niche partitioning study to assess resource use among bat species. The purpose of this experiment was to delineate the feeding ecology of bat species that are living with overlapping distribution by attempting a multifaceted approach that incorporated available food resources, prey consumed, spatial and temporal activity, and environmental components.

Integration

This study attempted to understand the success of various species of insectivorous bats within a specific area. The data collected in this study will help us better understand bat feeding behaviors to determine if resource partitioning is impactful within this system. To determine if bats specialize their diets to reduce competition, we first determined what prey is available within our study site. Insect collections and identifications methods determined the resources available within each sample site. To collect insects, we used UV light traps and CO2 traps. To identify the order of the collected insects, we used dichotomous keys. These methods draw from entomology, the branch of biology which studies insects. DNA extraction, sequencing, and identification determined what insects the bats are consuming. The DNA was then analyzed using Qiime2, a bioinformatics platform used to analyze metagenomic data. Remote data collection techniques commonly used in ecology determined the species presence/activity levels and the time which different species groups were active. Biologists have used these techniques to better understand organisms in a given study site without disturbing them. Statistics, a field within math to determine probabilities, was used to determine significance among results. This study incorporated concepts from entomology, molecular biology, bioinformatics, and statistics to help us answer a behavioral ecological question.

Objectives

The main objective of this study was to use a quantitative approach to begin to delineate the resource use in insectivorous bats with overlapping distributions. The data we collected were used to investigate multispecies coexistence within a habitat despite similar food resource needs. To quantify the resource use of each species, we studied:

- The habitat use of insectivorous bat species: Mist nets and acoustic monitors can quantify which habitats species of insectivorous bats commonly used for foraging and when they were present. Canopy coverage and foliage density were measured and are relevant environmental factors that have been related to bat species presence.
- The dietary resources available within these habitats: We collected insects within study sites and acoustically monitored over the same period. We estimated insect abundance to order for each collection time within each sample site.
- The dietary choices made by bat species: we conducted DNA barcode analysis for insects from feces collected from different bat species detected in the study site. We compared the insects detected in the feces by barcode analysis to the insects collected in the environment at each site and sample time.

Hypotheses

Based on classic ecological theory, we suspect bat activity is driven by resource availability. We hypothesized that bats would be most active during times when prey are most active as well as focus foraging efforts at energy rich sites. We predicted a positive correlation between bat activity and overall insect abundance. As the abundance of insects dropped, we predicted bats would move to a different, more energy-rich patch. Our second hypothesis was that bats will show dietary preference. We expected to see a significant difference in relative abundance of insect orders detected in the feces compared to the relative abundance detected in the environment. Based on previous studies, Razgour et al., 2011 we expected bats to specialize their diets. As seasons changed and temperatures decreased, insect abundance was also expected to decrease (Gaston & Lawton, 1988). Our third prediction was that bats would shift to a more generalized diet as temperatures and resources decreased.

Methods

Study Site

This study was done in the coastal plains of South Carolina (Beaufort County) at Palmetto Bluff. Palmetto Bluff covers 20,000 acres of land and lays between two historic rivers, the New and May Rivers. The Palmetto Bluff Conservancy maintains 320 acres, 308 in conservation easements that the North American Land Trust jointly operates.

Palmetto Bluff has a wide variety of habitats (~20 different types). Our study took place in the Maritime Forest, a forest near the shore and influenced by sea spray. South Carolina is home to several different insectivorous species of bats. The South Carolina Department of Natural Resources (SDNDR) reported 14 different species that inhabit the state. All 14 species reported are insectivorous bats. These bat species include:

- Big brown bat (*Eptesicus fuscus*) – EPTFUS
- Brazilian free-tailed bat (*Tadarida brasiliensis*) – TADBRA
- Eastern red bat (*Lasiurus borealis*) – LASBOR
- Eastern small-footed bat (*Myotis leibii*) – MYOLEI
- Evening bat (*Nycticeius humeralis*) – NYCHUM

- Hoary bat (*Lasiurus cinereus*) – LASCIN
- Little brown bat (*Myotis lucifugus*) – MYOLUC
- Northern long-eared bat (*Myotis septentrionalis*) – MYOSEP
- Northern yellow bat (*Lasiurus intermedius*) – LASINT
- Rafinesque's big-eared bat (*Corynorhinus rafinesquii*) – CORRAF
- Silver-haired bat (*Lasionycteris noctivagans*) – LASNOC
- Southeastern bat (*Myotis austroriparius*) – MYOAUS
- Seminole bat (*Lasiurus seminolus*) – LASSEM
- Tricolored bat (*Perimyotis subflavus*) – PERSUB

In Beaufort County, South Carolina, 13 of these species have been recorded. We surveyed four different sites at four seasonal times throughout the study: Summer (July), Fall (October), Winter (February), and Spring (May). These sites were selected due to previous success at capturing bats. (Figure 2). These surveys consisted of mist-netting for bats, collecting guano, collecting acoustic information, capturing insects, and measuring abiotic environmental factors.

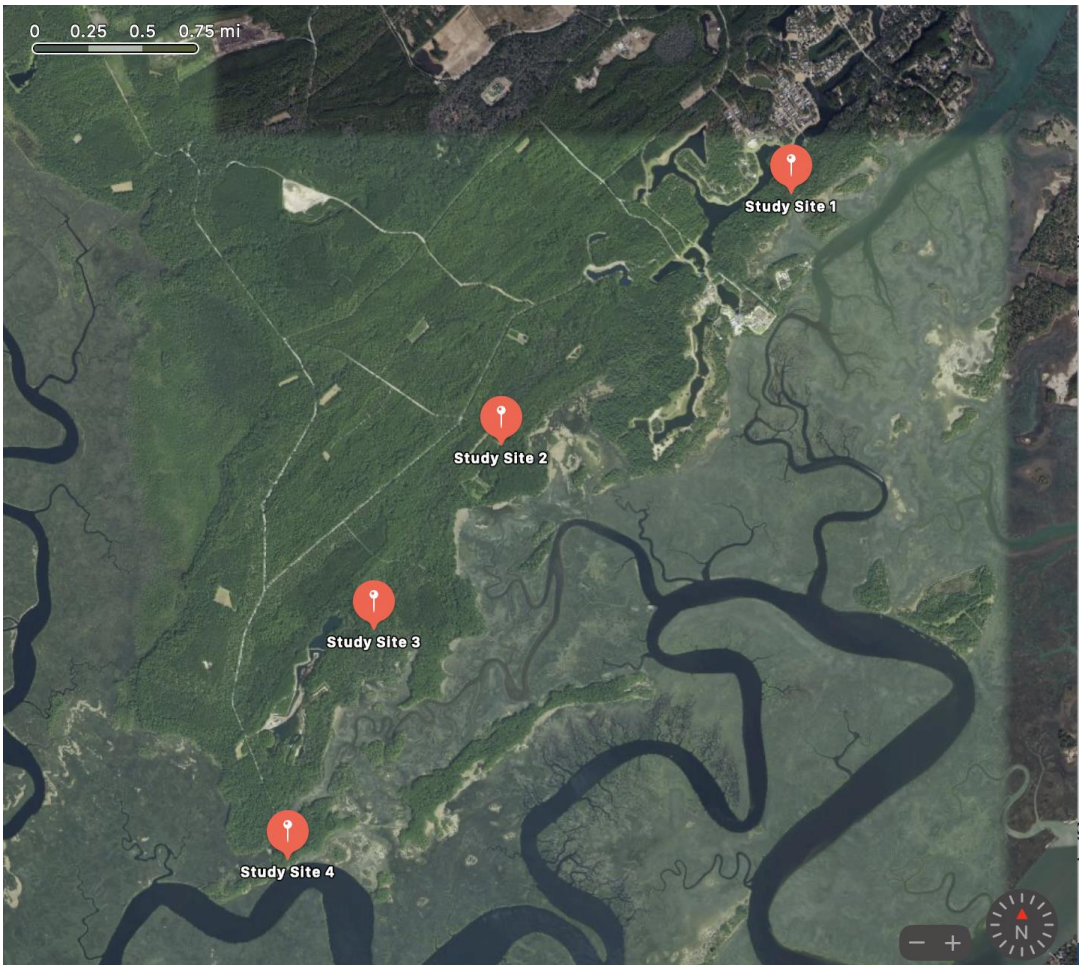

Figure 2. Map of Palmetto Bluff showing the location of the four sample sites used throughout the study. Site 1 is River Road Preserve, Site 2 is Theus Field, Site 3 is Hog Hill, and Site 4 is Big House Island. All sites are within maritime forest, however the sites varied when comparing canopy coverage, average understory height, proximity to residents, and proximity to water. Site 1 was the site closest to human homes and contained the

greatest canopy coverage. Site 2 had a mixed canopy coverage; this site was also furthest from water. Site 3 was a mixed canopy coverage; this site was second closest to water. Site 4 was the least covered site; this site was closest to water. Site 4 encountered human disturbance during the study

Canopy and Understory density

Research has shown a relationship between clutter and the activity of a bat (Loeb et al., 2015). To account for this in our study, we measured canopy and foliage within each study site. These measurements determined the degree of clutter present for each of our study sites.

To quantify canopy coverage, we used a convex spherical densitometer. We took measurements at the site of the acoustic monitor following the four cardinal directions. We then averaged the measurements to determine canopy density. We measured density at each sample site during each season.

Not all bats fly at canopy height. To determine if understory height changed bat communities, we included this height in our analysis. We used a Nikon Forestry Pro II Laser rangefinder and hypsometer (Nikon.com) to measure the height of overhead foliage. We set up two 40 meter transects, one traveling from North to South and the other traveling from East to West. Measurements were taken every 5 meters. We averaged the heights to determine the average understory height at each site. This estimated mid-range clutter between upper canopy and ground level vegetation.

Mist Netting

Mist netting followed the recommendations of the United States Fish and Wildlife Service. Net disinfection followed the standard USFWS protocol to reduce the chance of spreading fungal and viral pathogens.

Mist nets are constructed with one-ply 40 denier monofilament mesh (also known as 40/1) or two-ply 50 denier nylon (50/2). Gaps within the mesh were approximately 1.5 inches wide. Nets were placed up to 7 meters high, usually by stacking 2 or 3 nets and 20 meters wide. However, nets were adapted to selected corridors. Mist nets were placed along flight paths present in the designated study area; commonly effective corridors are wooded streams, trails, and maintained rights-of-way.

Once bats were captured, the species, sex, and life stage were noted. If the organism defecated, the guano was stored in 2mL centrifuge tubes. The fecal sample was used for molecular analysis.

Mist netting was done under the following permits:

Federal permit: TE81756A-3

SCDNR scientific collection permit: SC-59-2020

Molecular Analysis

DNA was extracted from fecal samples using a DNeasy PowerLyzer Power Soil Kit (Qiagen). We amplified extracted DNA using primers from Zeale 2011 to detect arthropod CO1 DNA. We visualized PCR products on a 3.0% agarose gel to determine if insect DNA is present. Original DNA elution concentrations were adjusted for next-generation sequencing (NGS).

DNA was sequenced in UGA Bioinformatics lab, Athens, GA with an Illumina sequencer. Sequencing can be from one end (single-end reads) or from both ends of the DNA fragments (paired-end reads). For this study, DNA sequences were paired end reads that were 250bp long.

Once sequenced, Qiime2 was used to filter reads. By following the DADA2 workflow, we were able to trim and truncate sequences to preserve quality. These sequences were multiplexed and denoised. We compared filtered reads to a custom classifier table. This classifier table comprises Barcode of Life Database (Ratnasingham & Hebert, 2007) CO1 sequences of arthropods in the United States. DNA sequences from collected samples were matched to those from the classifier table by using a BLAST (Altschul et al., 1990) style search. The minimum percentage accepted for this study was 80%. Taxa bar plots were made to show relative abundance of insects present in guano samples from Rome preliminary study.

A cluster plot was made using Bray Curtis distances. Cluster plots graph samples based on composition. Samples which are similar are graphed near each other while those that are different are further apart.

Acoustic Information

Stationary acoustic monitoring was done four times throughout 2020-2021. Monitoring was done once per season, starting in the summer of 2020, and ending in the spring of 2021. We deployed three SM3Bat and one SM4 (wildlifeacoustics.com) for two consecutive nights at each location. Microphones from the monitors stood at least three meters high. Monitors were at least 45 meters away from insect collection traps, and the monitors' microphones were faced away from the insect collection traps. All monitors were placed near previously used netting sites along airways. GPS coordinates and time settings were updated. We programmed monitors with the default sunset to sunrise program. This program turns the monitor on two hours before sunset and turns it off two hours after sunrise.

We sampled approximately three months apart from each other. Bat activity is affected by the lunar cycle (Lang et al., 2006, Rydell et al., 1996); therefore, we sampled at approximately the same lunar stage every season. The first sampling time occurred during a full moon, so future collections were done as close to a full moon as logistically possible.

Data processing:

Sonograms were analyzed using Kaleidoscope Pro 4.5.1 (wildlifeacoustics.com). Kaleidoscope filters noise files from recordings and analyses bat passes based on set parameters. Data from acoustic monitors were processed using Wildlife Acoustics Kaleidoscope Pro. The software removes low-quality calls and noise. It then auto-identifies the remaining calls. As per Reichert et al. 2018 the Kaleidoscope Pro parameters used were: call duration between 2-50 ms, the minimum number of pulses was set to 3, the maximum inter-syllable gap was set to 500 ms. Auto-ID was done through Kaleidoscope Pro, using the "Most Accurate-Conservative" option. Calls with a matching ratio < 0.5 were removed from the analysis. Six letter species codes were used for the data analysis.

Insect Collection

To estimate for the insect resources available for bat species, we collected insects at each site. Insect collections started two hours after sunset and were done every two hours until 2 AM. Arthropods were collected using BioQuip Universal Black Light Traps and BioQuip Heavy Duty EVS CO2 Mosquito Traps (bioquip.com). UV light traps collect Lepidoptera (moths), Coleoptera (beetles), and Hemiptera (true bugs), among others. UV light traps use an ultra-violet light source to attract insects and a stainless-steel cone to trap into a collection bag. This bag contained a No-Pest Strip2 (Hot Shot), which euthanized the insects collected. The CO2 traps use dry ice as a lure to attract Diptera (mosquitoes), then a battery-powered fan traps the insects into a mesh collection bag.

We transported samples in freezer gallon zip locks on dry ice. Once in the lab, the insects were weighed and stored in glassware containing 70% ethanol. Collections from both UV light traps and CO2 traps were weighed and sorted to the order, using a dichotomous key (Choate, 2010). A total of 44,156 individual insects were sorted, which represented 17 insect orders.

Statistical Analyses

To determine if data were normally distributed, we used the Shapiro test. If data was non-normal, Tukey's Ladder of Powers was used to transform data. ANOVAs were used to compare the means between arthropod abundance, arthropod weight, humidity, and canopy coverage between seasons and between sites within the same season. Tukey's post hoc test was used to determined significant differences between samples. To compare arthropod richness, temperature, and understory height; data that could not be normalized, we used a Kruskal-Wallis test to compare medians. We used Dunn's test as a post-hoc analysis for non-normal data.

Arthropod abundance and arthropod weight were visualized with box and whisker plots. Arthropod order richness between seasons was visualized using rarefaction curves. To identify changes in arthropod communities as the nights progressed, we created stacked bar plots. These stacked bar plots show relative frequencies during each collection time.

We mapped bat activity by graphing activity index (number of bat passes) per call group across the hours after sunset (Skalak et al. 2012). These graphs can help visualize patterns in bat activity and community composition. Bat vocalizations were grouped depended on the characteristic frequency of search phase calls. The groups include:

- ~ 20 kHz, bat species include LASCIN and TADBRA.
- ~ 30 kHz, bats species include EPFTUS, LASINT, and LASNOC.
- ~ 40kHZ Non-Myotis, bat species include LASBOR, LASSEM, NYCHUM, and PERSUB.
- 40-50 kHz Myotis, bat species include all Myotis species.

To determine if there was a relationship between insects found and bat activity, we correlated arthropod abundance, arthropod weight, and arthropod richness against bat activity. Correlations were performed using the Spearmen correlation index. We used R version 4.0.5 for the statistical analysis (R Core Team, 2021). The alpha value was 0.05 for all tests.

Results

Molecular Analysis

DNA sequence found in guano samples differed among species and among sample time. Taxa bar plots from Qiime2 show the relative frequency of insect DNA found in guano samples (Figure 3). Insect orders were identified in every sample. The maximum percentage of unassigned DNA in a sample was < 0.5 %. DNA belonged to 9 different orders, a total of 358 matches. Bray Curtis analysis visualized dissimilarity among samples (Figure 4). Axis are created based on sample composition; samples are then plotted to show similarity

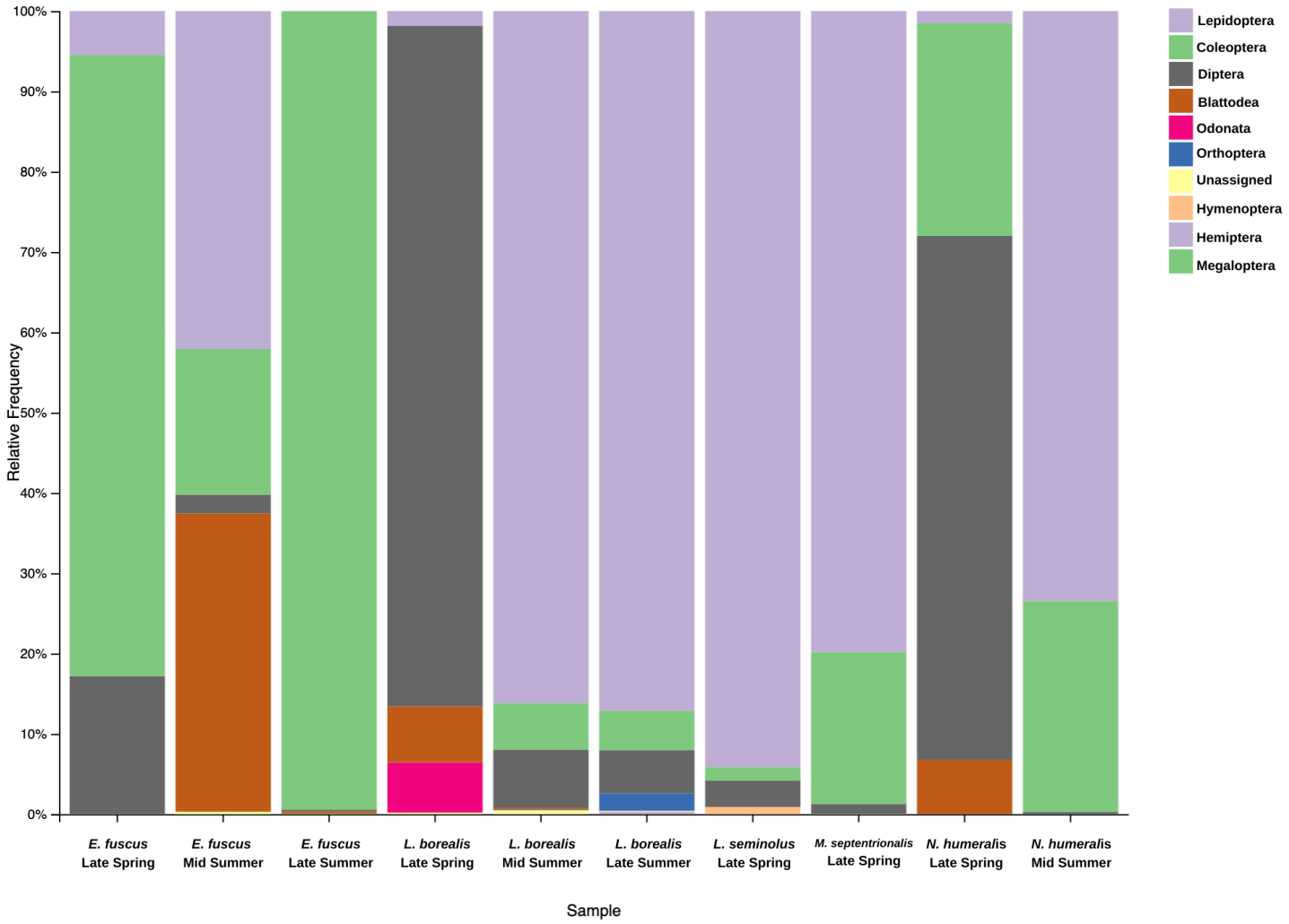

Figure 3. Taxa bar plots show the relative frequency of order DNA found in bat guano samples. Bat four-letter species codes were used. The number following the species codes identifies when in the sample was collected. Orders detected include Lepidoptera, Coleoptera, Diptera, Blattodea, Odonata, Orthoptera, Hymenoptera, Hemiptera, and Megaloptera.

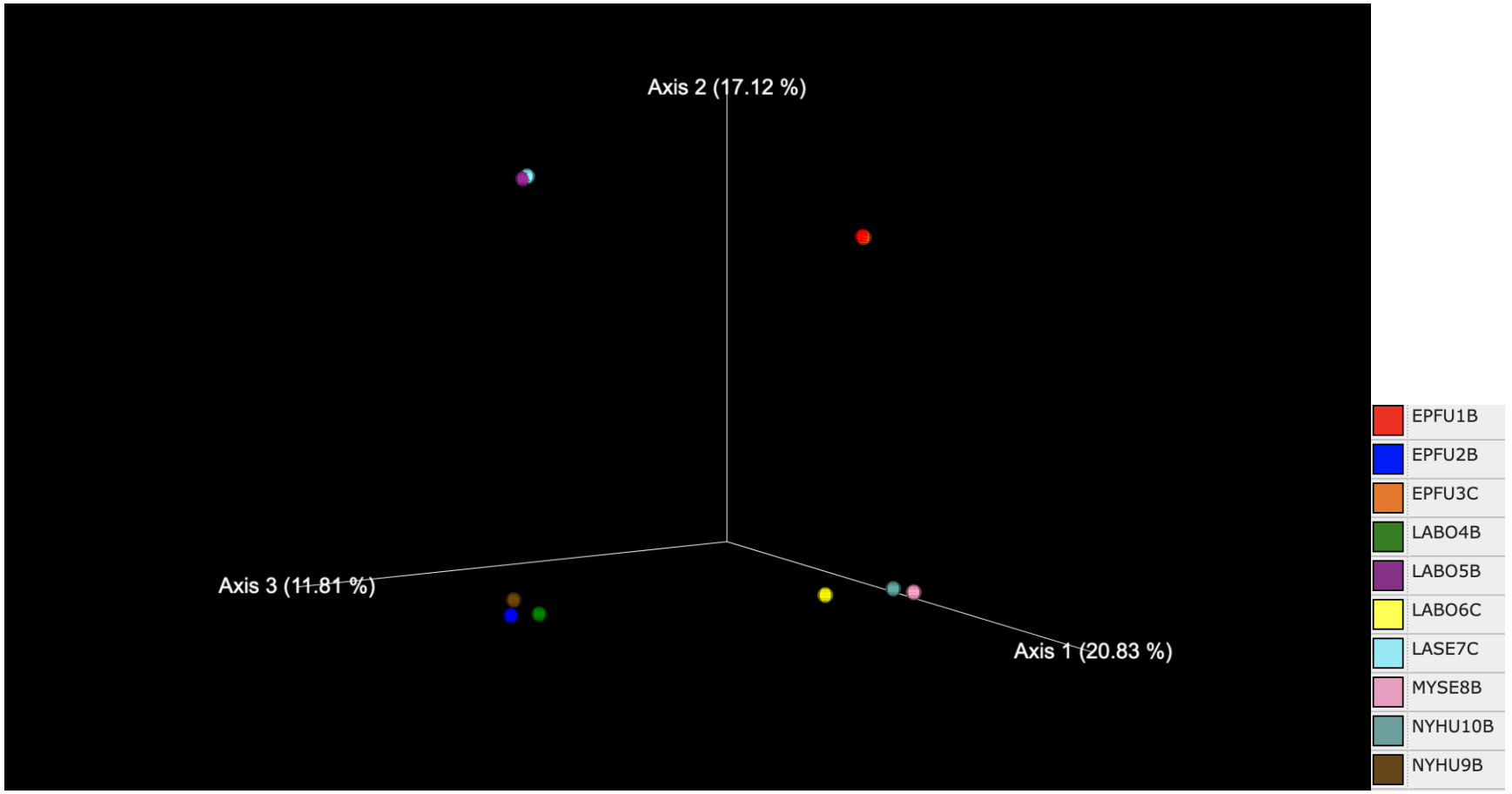

Figure 4. Bray Curtis plot for dissimilarities. Samples are placed based on composition. The more similar samples are, the closer they are within the graph. Four letter code represent species, the number that follows the species code represents the order the sample was collected (ranging from Spring, Mid-Summer, and Late Summer)

Abiotic Factors

Seasonal Analysis

As seasons progressed, mean temperature and mean humidity significantly changed (Table 1). Average temperatures between seasons were significantly different (Chi square = 39.71, p = 1.23e-08, df = 3). A post hoc analysis shows a significant difference between summer and fall (Dunn's; p = 2.02e-04) summer and winter (Dunn's; p = 1.18e-06), spring and fall (Dunn's; p = 8.09e-04), spring and winter (Dunn's; p = 5.39e-06) (Table 1). Post-hoc shows no significant differences between summer and spring (Dunn's; p = 6.89e-01) and fall and winter (Dunn's; p = 1.87e-01) (Table 1).

Humidity measurements significantly differed between seasons ($F_{3,40} = 6.219$, p = 0.001). Post-hoc analysis showed a significant difference between summer and fall (Tukey's; p = 0.02), summer and spring (Tukey's; p = 0.001) (Table 1). However, there was no significant difference between spring and fall (Tukey's; p = 0.412) fall and winter (Tukey's; p = 0.831), spring and winter (Tukey's; p = 0.104, and summer and winter (Tukey's; p = 0.153) (Table 1).

Throughout the different seasons mean canopy coverage did not significantly change ($f_{3,1}$ = 0.05, p = 0.98). Midstory heights didn't differ between sites (Chi square = 0, p = 1, df = 3).

Table 1. Averages and standard errors of abiotic factors during each sampling season. Letters represent significant differences between season after post-hoc analysis.

Variable	Summer	Fall	Winter	Spring
Temperature (C)	$21.215 \pm 0.901_a$	$17.526 \pm 0.768_b$	$17.067 \pm 0.781_b$	$20.718 \pm 0.883_a$
Humidity (%)	$90.708 \pm 1.472_a$	$83.942 \pm 1.042_b$	$85.717 \pm 1.159_{a,b}$	$82.257 \pm 2.109_b$
Canopy Coverage (%)	94.5	90.75	83.75	90.25

Site Analysis

Mean humidity and canopy coverage were compared per site within seasons using an ANOVA. Median temperature and midstory height were compared using a Kruskal Wallis test. Temperature did not significantly vary among sites during the summer (Chi square = 1.05, p = 0.79, df = 3), fall (Chi square = 1.61, p = 0.66, df = 3), winter (Chi square = 3.22, p = 0.36, df = 3), or spring (Chi square = 0.44, p = 0.93, df = 3). Mean humidity did not significantly change between sites within the same season ($F_{12,40} = 0.344$, p = 0.98). Mean canopy coverage did not differ across sites per season ($F_{12,1} = 0.17$, p = 0.97).

Mean understory height did significantly change across sites (Chi square = 15, p = 0.002, df = 3). Post hoc analysis shows a difference between sites; River Road and Theus Field (Dunn's; p = 0.029), River Road and Big House Island (Dunn's; p = 0.001), and Hog Hill and Big House Island (Dunn's; p = 0.043).

Arthropod Analysis

Seasonal Analysis

The average arthropod abundance per hour was significantly different between seasons ($F_{2,24} = 32.97$, p = 1.3e-07, Figure 5). Tukey's posthoc analysis showed a significance between summer and fall (Tukey's; p = 2.0e-5) and summer and winter (Tukey's; p = 1.0e-07); however, there was no significant difference between fall and winter (Tukey's; p = 0.06) (Figure 5).

The weight (g) of arthropods per hour significantly differed across seasons ($F_{2,24} = 79.85$, p = 2.47e-11). Tukey's post hoc analysis showed a significant difference between summer and fall (Tukey's; p = 0), summer and winter (Tukey's; p = 4.00e-07),and fall and winter (Tukey's; p = 2.33e-05) (Figure 5).

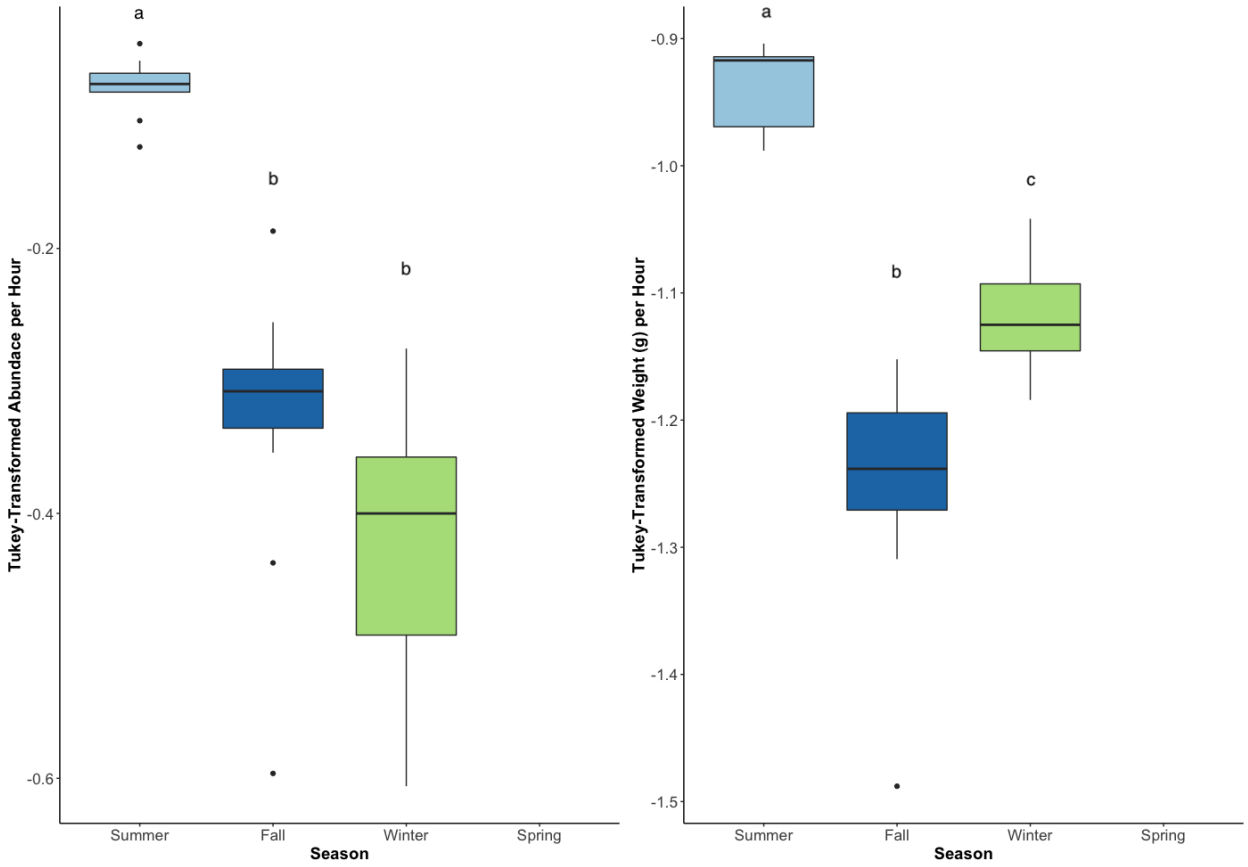

Figure 5. Averages for arthropod abundances per hour (A), arthropod weights per hour (B) per season. Ggplot removes outliers for clarity. Letters show significant differences based on Tukey's posthoc test.

The arthropod order richness per site did significantly differ between seasons (Chi square = 22.332, p = 1.415e-05, df = 2). A post hoc analysis shows significant difference between summer and winter (Dunn's; p = 1.1e-05) and fall and winter (Dunn's; p = 4.3e-03), however no significant differences between summer and fall (Dunn's; p = 0.06). Total richness drastically changed with a max of 17 different orders during the summer and a low of 5 orders during the winter (Figure 6). Rarefaction curves show an extrapolated max richness of 10 orders during the fall and a total richness of 5 during the winter (Figure 6) with increased sampling efforts.

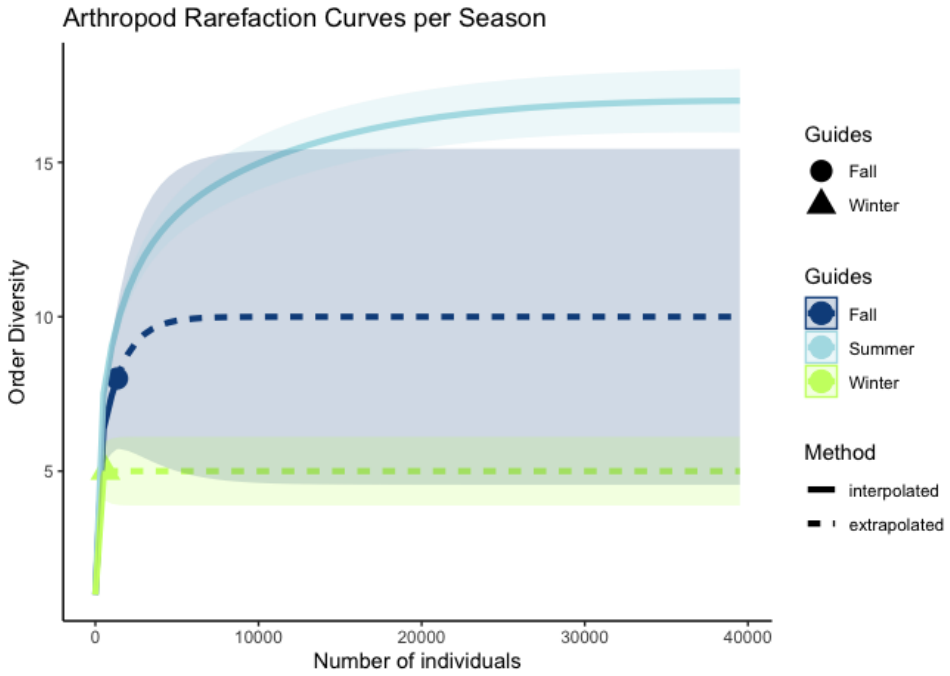

Figure 6. Rarefaction curves representing order richness throughout different seasons. Dotted lines represent extrapolated richness. Maximum richness during the summer=17, fall=8, and winter=5. With increased efforts predicted richness for fall=10 and winter=5.

Table 2. Averages and standard errors of arthropod factors during each sampling season.

Arthropod Variables	Summer	Fall	Winter	Spring
Average Arthropod Abundance per Hour	502.9405±347.8530	15.4048±6.9287	7.3214±3.2452	
Average Arthropod Weight per Hour	2.2015±1.2984	0.0625±0.0299	0.2212±0.1033	
Max Arthropod Richness	6	4	3	

Arthropod composition changed as the night progressed during all seasons sampled (Figure 7). Coleoptera relative abundance decreased after the first collection time while Diptera abundance increased. Lepidoptera relative abundance was inconsistent; during the summer, abundance was greatest in the middle of the night, during the fall, abundance was greatest early in the night, and during the winter, there was almost no change.

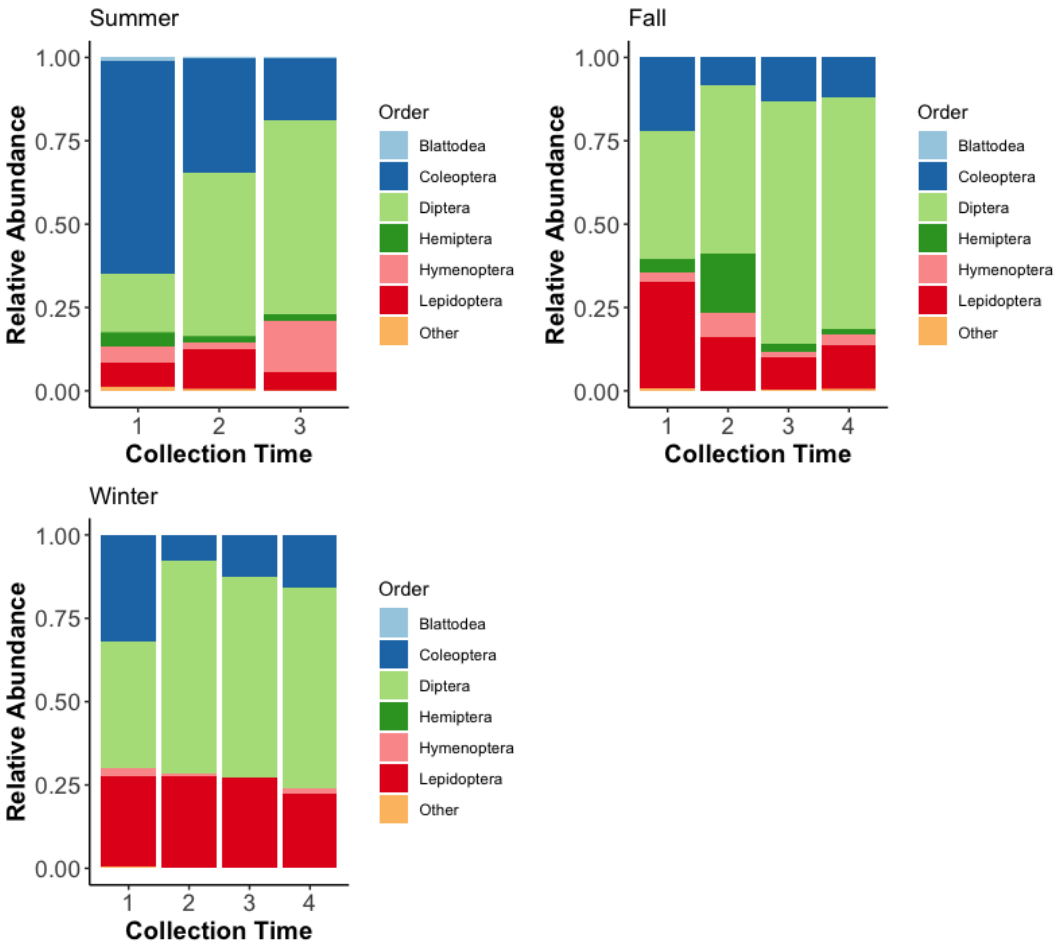

Figure 7. Relative abundance of arthropods collected throughout the night during different seasons. Only orders with a total abundance of over 100 are shown for clarity purposes. Since nights are longer during the fall and winter, there was an additional sample time.

Site Analysis

An ANOVA shows no significant differences regarding arthropod abundance per hour between sites within the same season ($F_{4,24} = 0.154$, $p = 0.959$). An ANOVA indicates no significant differences regarding arthropod weight per hour among different sites within the same season ($F_{4,24} = 2.111$, $p = 0.111$) (Figure 8). A Kruskal Wallis test shows no significant differences regarding arthropod order richness across sites within the same season (Summer; Chi square = 0.6, $p = 0.74$, $df = 2$), (Fall; Chi square = 1.45, $p = 0.48$, $df = 2$), and (Winter; Chi square = 1.63, $p = 0.44$, $df = 2$).

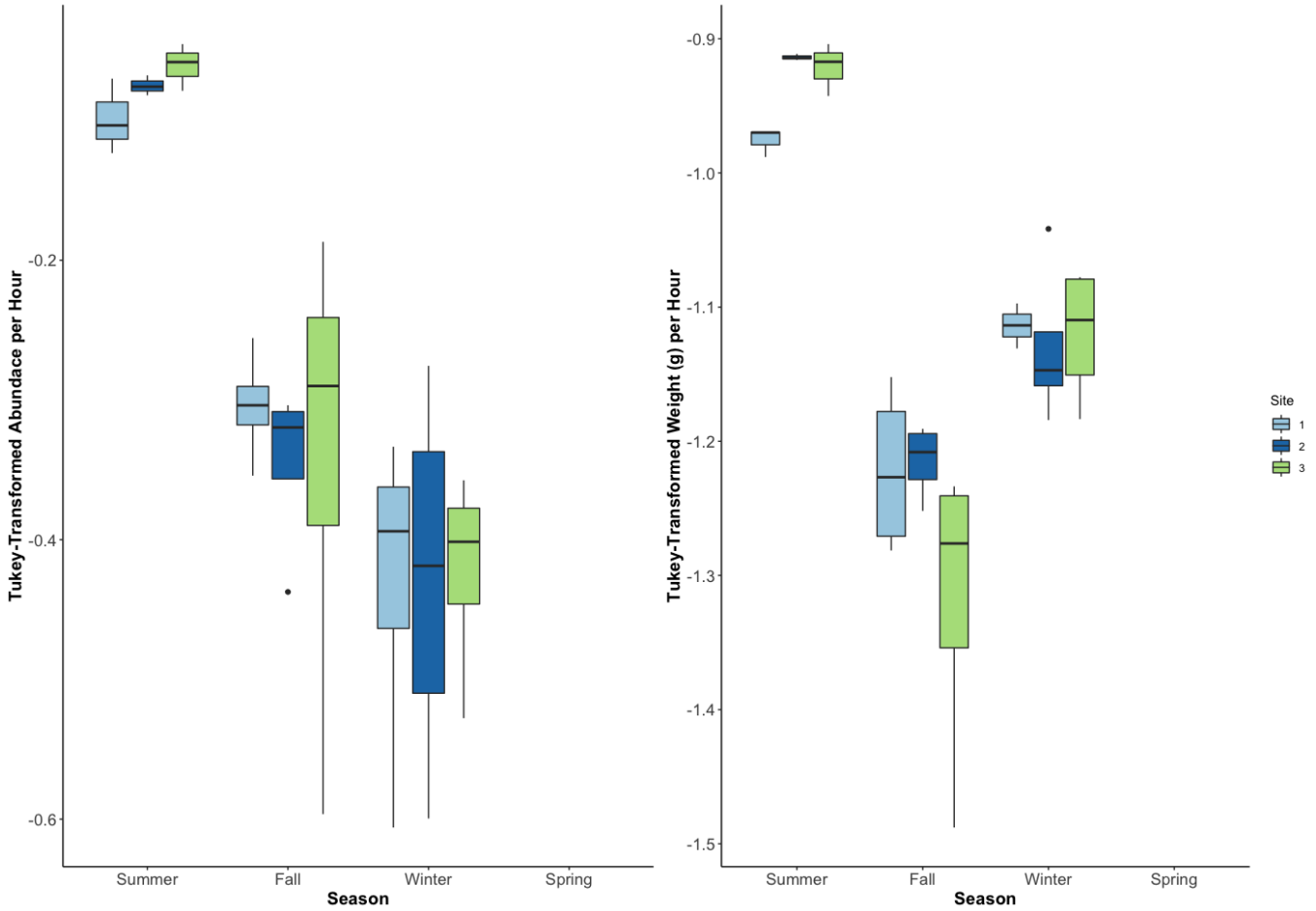

Figure 8. Box plots show mean arthropod abundance per site during each season (A) and mean arthropod weight per hour per site (B). Ggplot removes outliers for visual purposes. No significant differences were found between sites.

Stacked boxplots showed relative frequencies of arthropods throughout the night at each site (Figure 9). During the summer, Coleoptera abundance decreased throughout the night in all sites. Diptera relative abundance increased as the night progressed. During the summer, we detected an increase of Lepidoptera during the second collection time across all sites. During the fall, Coleoptera relative abundance was lower than previously detected. We saw a decrease in Lepidoptera abundance as the night progresses in all three sites. In sites one and three, we detected a large increase of Diptera as the night progresses; in site two, we detected a decrease of Diptera at the end of the night. The fall sample sites showed the greatest abundance of Hemiptera. Winter sample sites showed a similar pattern as seen in other seasons sampled. Coleoptera relative abundance decreased throughout the night across all three sites. Lepidoptera relative abundance was greatest during the second and third collection times. Finally, Diptera relative abundance increased as the night progressed (Figure 9).

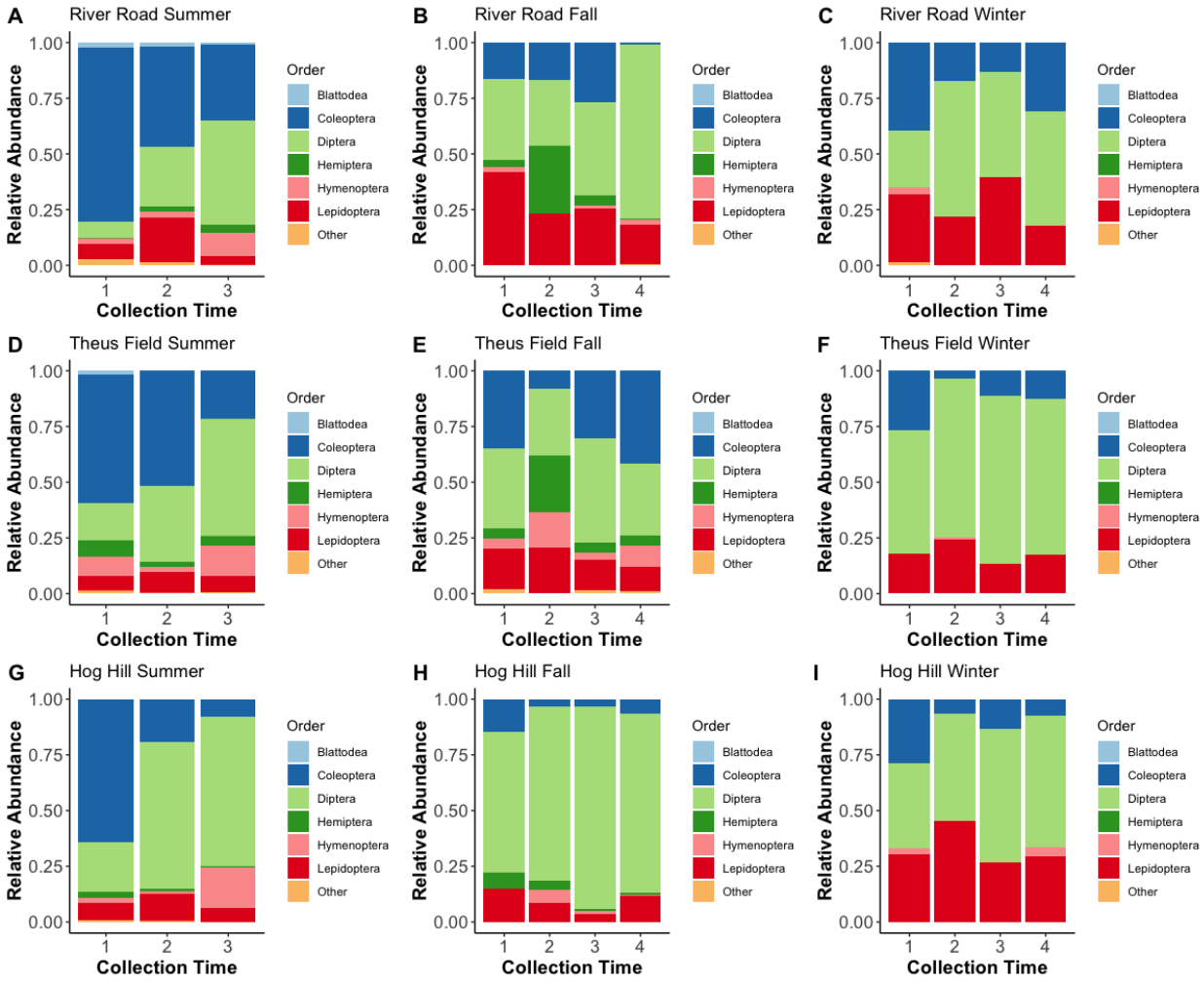

Figure 9. Relative abundance of arthropods collected throughout the night of different sites throughout the year. Only orders with a total abundance of over 100 are shown for clarity purposes. Since nights are longer during the fall and winter, there is an additional sample time. Panels A, B, and C belong to Site 1, panels D, E, and F belong to Site 2, and panels G, H, and I belong to Site 3.

Bat Activity

Seasonal Analysis

We predicted that bat activity would be highest during the same period when insect abundance was highest. This prediction was supported in an increase of bat activity during in the summer; however, activity was observed in ever season (Figure 10). ~20 kHz bats were rarely active during the summer, their activity increased in other seasons. During the fall, ~20kHz were the most active and showed two spikes of activity; one spike was ~2 hours after sunset and the other was ~10 hours after sunset. ~30 kHz bat species were active as the sun set, activity then plateaued as the night progressed. ~40 kHz species of bats showed the highest activity in our sample site during the summer, winter, and spring, however ~40 kHz group was among the lowest during the fall. Throughout the summer, winter, and spring we noted a spike of ~40 kHz activity as the sun set, and another spike ~8 hours after sunset. 40-50 kHz non-Myotis species were most active during the summer.

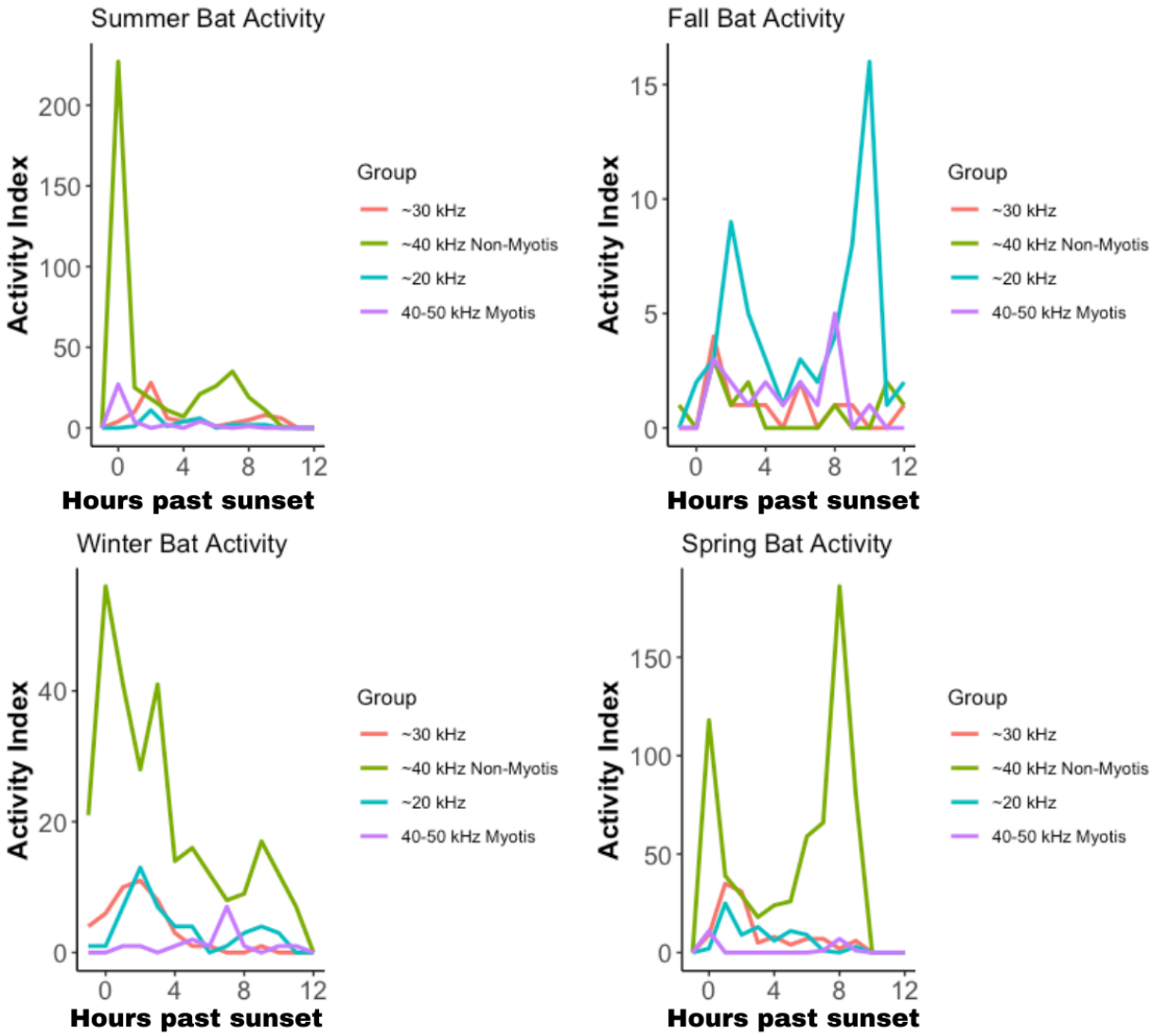

Figure 10. Activity maps of different bat groups. The activity index represents the total number of bat passes. These activity maps are made on characteristic call frequencies to account for misidentification from the software

Activity indexes differed across seasons. An ANOVA shows a significant difference of bat activity among different seasons ($F_{3,40} = 11.343$, p = 1.6e-05). Tukey's post hoc shows significance between summer and fall (Tukey's; p = 0.0002), spring and fall (Tukey's; p = 0.00004), and fall and winter (Tukey's; p = 0.031).

Site Analysis

Bat activity index maps showed the greatest activity early in the night throughout the summer (Figure 11). Throughout sites, the highest activity index occurs within ~4 hours of sunset. Site 3 showed the greatest spike of activity. ~40 kHz non-myotis shows the greatest activity index across sites 1, 2, and 3. ~30 kHz and 40-50 kHz myotis show the highest activity in site 4 (Figure 11).

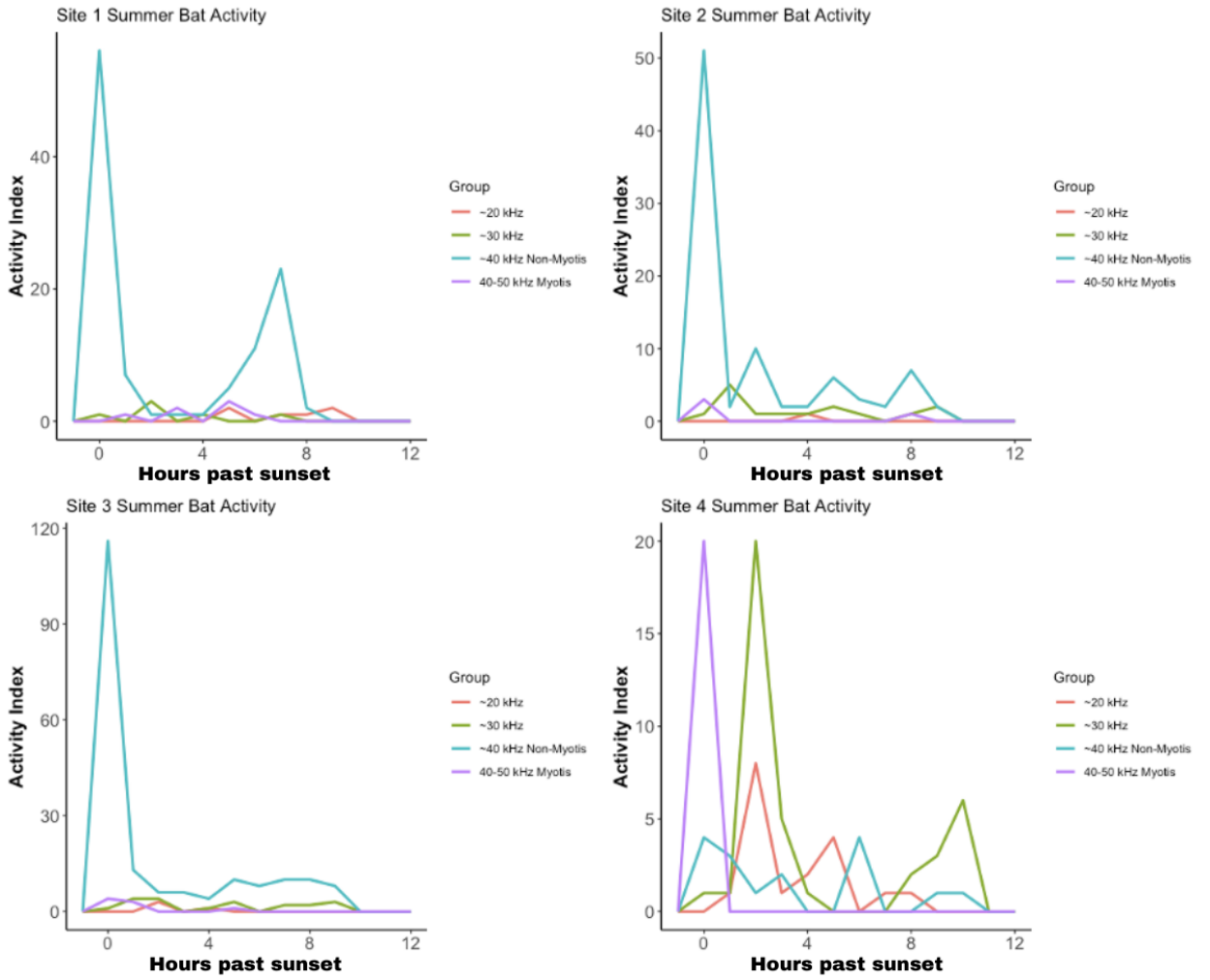

Figure 11. Activity maps of different bat groups specific to the site. These maps encompass the summer sampling efforts. The activity index represents the total number of bat passes. These activity maps are made on characteristic call frequencies to account for misidentification of software.

Bat activity index maps showed the lowest overall activity during the fall (Figure 12). There was activity throughout the entire night in sites 1, 3, and 4. Site 2 showed a spike in activity early in the night and a decrease in activity ~2 hours after sunset. The fall sample also showed the least amount of ~40 kHz non-myotis bat activity. ~20 kHz bat group was active through the different sites; however, was most active ~10 hours after sunset in site 3. ~30 kHz bat group was most active in site 2. The activity map in site 1 and site 4 was identical during the fall.

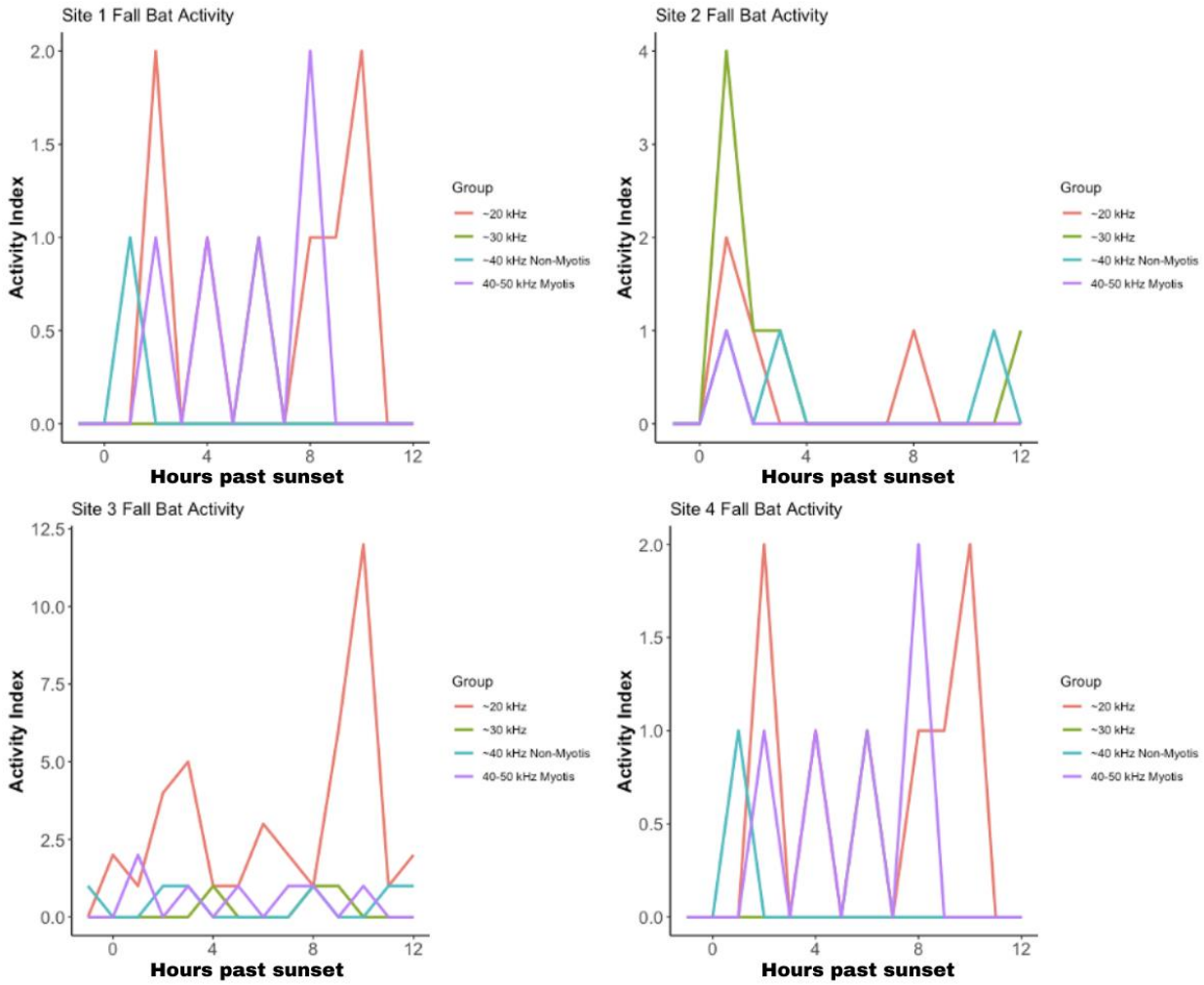

Figure 12. Activity maps of different bat groups specific to the site. These maps encompass the fall sampling efforts. The activity index represents the total number of bat passes. These activity maps are made on characteristic call frequencies to account for misidentification of software.

Winter activity maps showed the greatest activity early in the night throughout the different sites. The highest peaks of activity occurred within 1-2 hours after sunset. ~40 kHz non-myotis showed the highest activity peaks in all four sites; however, activity was greatest in site four. ~20 kHz and ~30 kHz bat groups had their highest spike in activity in site 2, ~2 hours after sunset. 40-50 kHz myotis had peak activity in site 3, ~7 hours after sunset (Figure 13).

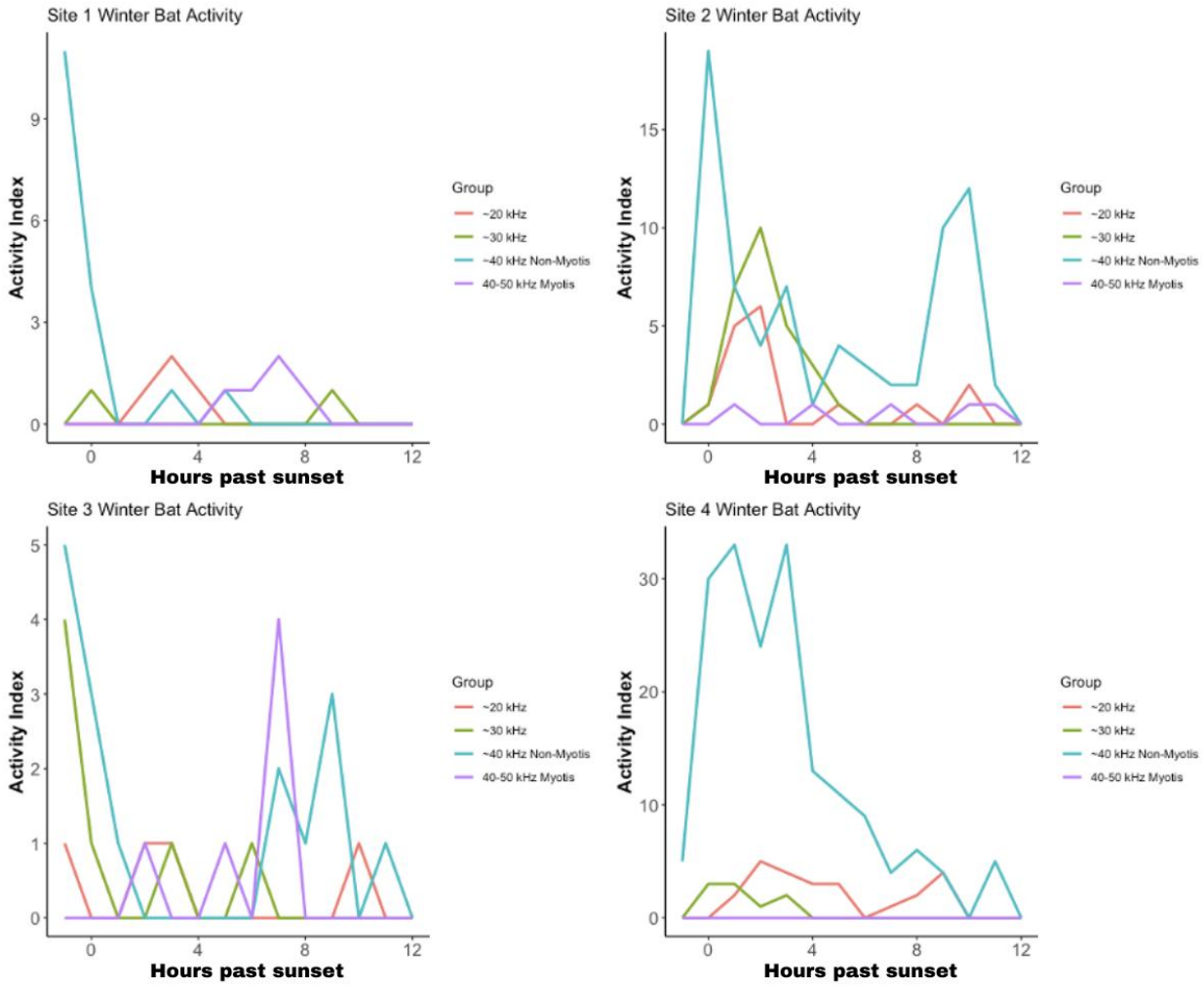

Figure 13. Activity maps of different bat groups specific to the site. These maps encompass the winter sampling efforts. The activity index represents the total number of bat passes. These activity maps are made on characteristic call frequencies to account for misidentification of software.

Spring bat activity index showed the highest peaks early in the night (~2 hours after sunset) and later in the night (~8 hours after sunset) across all four sites. Peaks primarily represent ~40 kHz non-myotis bat passes; however, all other groups were detected. Site 2 shows the highest peak activity, ~80 bat passes 8 hours after sunset (Figure 14).

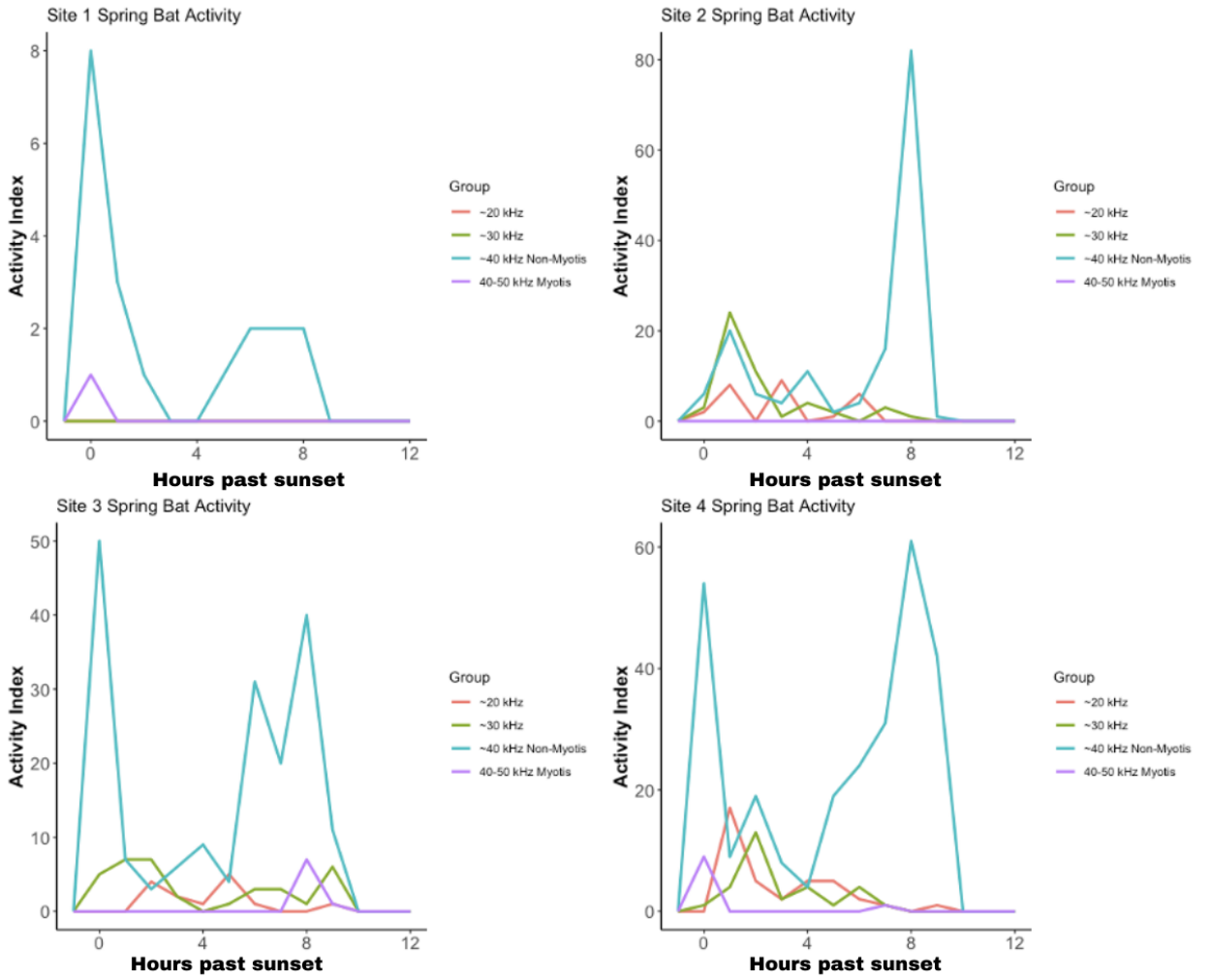

Figure 14. Activity maps of different bat groups specific to the site. These maps encompass the spring sampling efforts. The activity index represents the total number of bat passes. These activity maps are made on characteristic call frequencies to account for misidentification of software.

An ANOVA showed significant differences in bat activity indexes across sites per season ($F_{12,40} = 3.1$, $p = 0.0036$). Tukey's posthoc analysis showed a significant difference between site 1 and site 4 during the spring (Tukey's; $p = 0.01$).

Correlations

Regressions show a positive relationship between temperature and bat passes, temperature and arthropod abundance, temperature and arthropod weight, and temperature and arthropod richness (Figure 15). Spearman's correlation shows a significant correlation between temperature and bat passes ($R=0.44$, $p = 0.001$, Figure 15 (A)), temperature and arthropod abundance ($R = 0.67$, $p = 2.3e\text{-}05$, Figure 15 (B)), temperature and arthropod weight ($R = 0.5$, $p = 0.003$, Figure 15 (C)), and temperature and arthropod richness ($R = 0.58$, $p = 0.0004$, Figure 15 (D)).

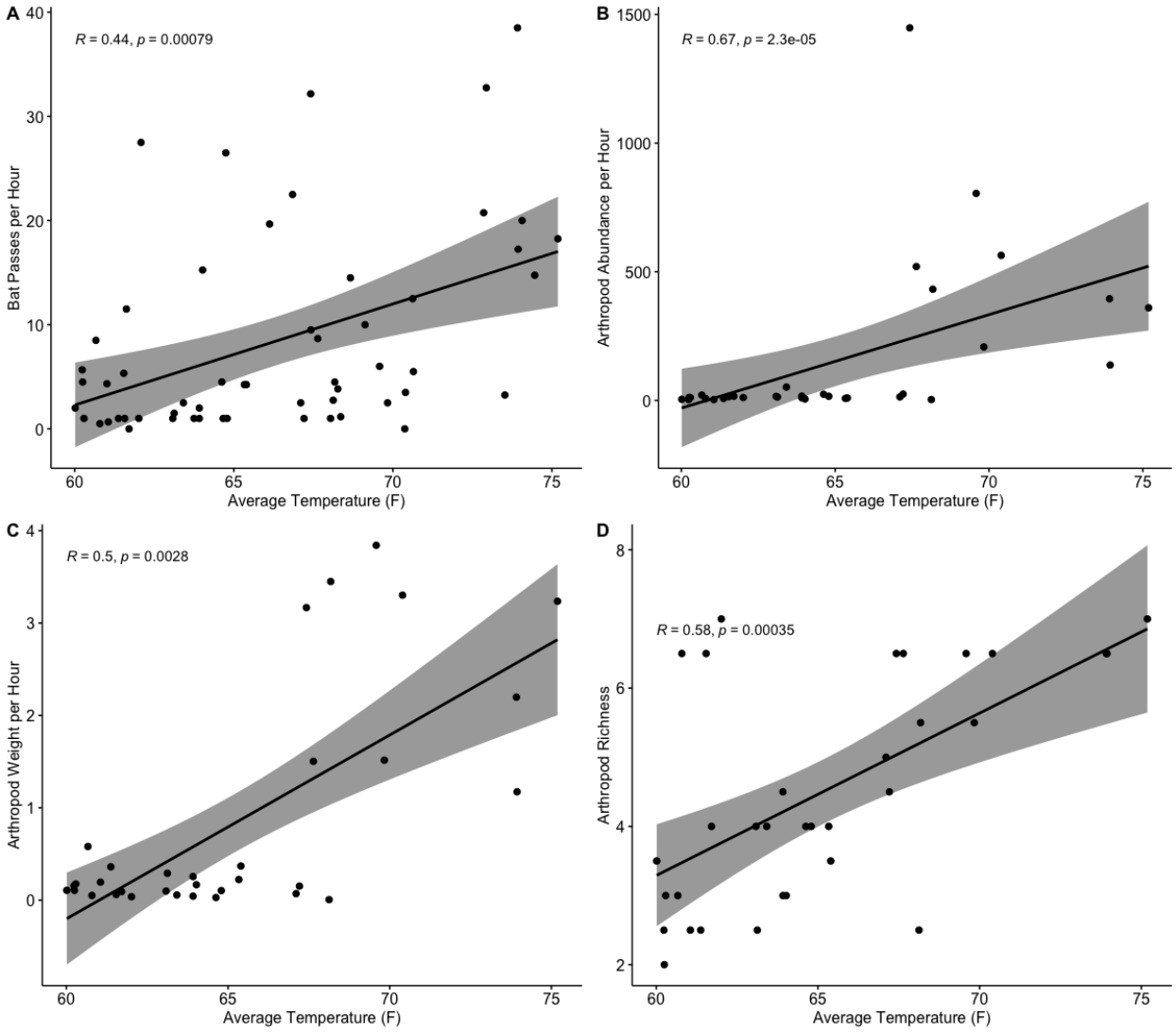

Figure 15. Regressions comparing Average temperature to bat passes per hour (A), temperature to arthropod abundance per hour (B), temperature to arthropod weight per hour (C), and temperature to arthropod richness (D). The slope indicates the relationship between factors, while R is a measure of the strength of predictors. Strong relationships between temperature and arthropod variables, only abundance per hour and richness have a p-value < 0.05.

Regressions show a weak negative relationship between humidity and bat passes per hour, a weak positive relationship between humidity and arthropod weight per hour, and a weak positive relationship between humidity and arthropod richness (Figure 16). The strongest correlation regarding humidity is associated with arthropod abundance per hour (R = 0.41, p = 0.02, Figure 16 (B)).

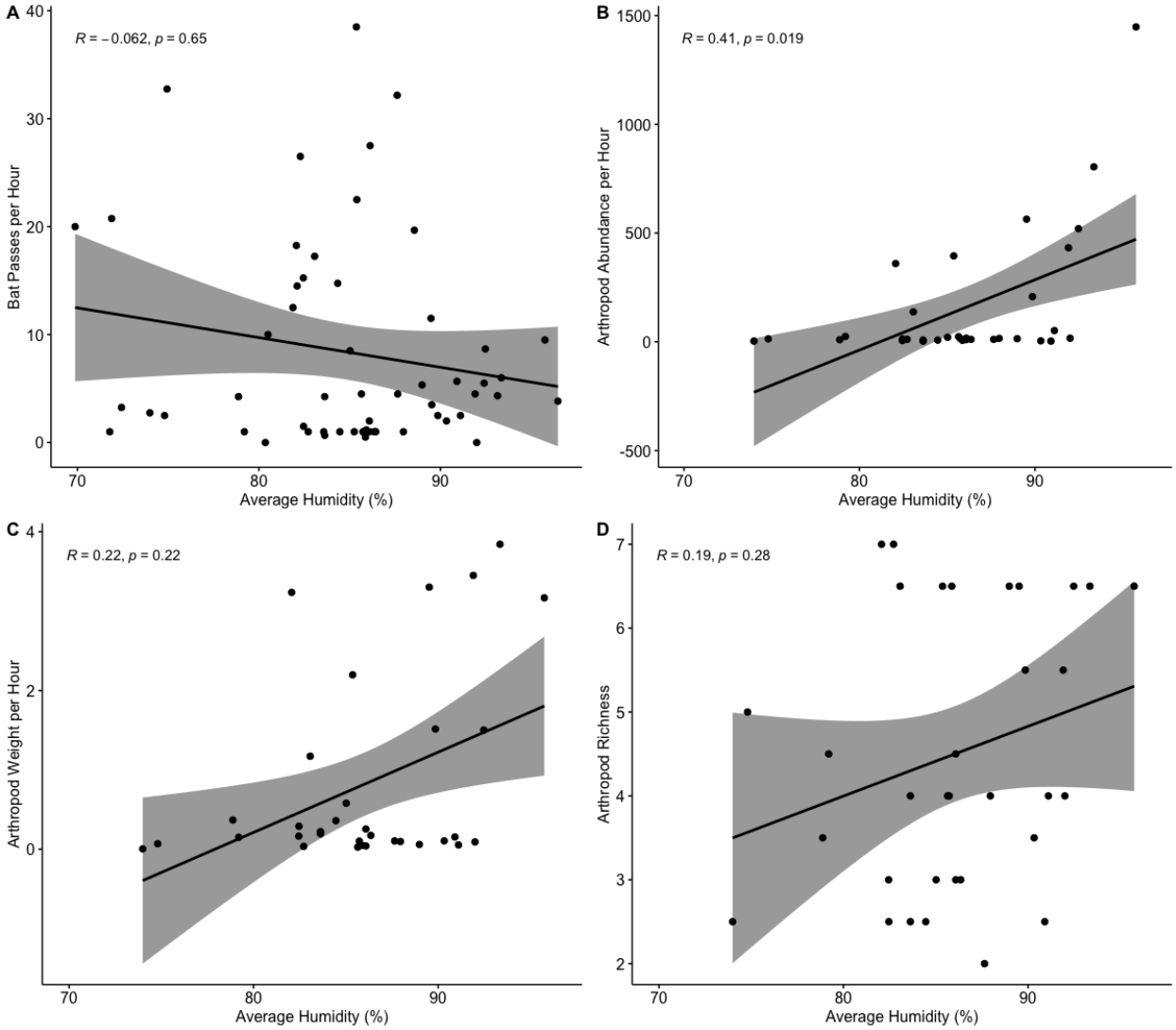

Figure 16. Regressions comparing average humidity to bat passes per hour (A), humidity to arthropod abundance per hour (B), humidity to arthropod weight per hour (C), and humidity to arthropod richness (D). The slope indicates the relationship between factors, while R is a measure of the strength of predictors.

Regressions show no relationship between canopy coverage and bat passes per hour (R = -0.12, p = 0.66, Figure 17(A)), no relationship between canopy coverage and arthropod abundance (R = 0.54, p = 0.13, Figure 17(B)), no relationship between canopy coverage and arthropod weight (R = 0.34, p = 0.37, Figure 17(C)), and no relationship canopy coverage and arthropod richness (R = 0.87, p = 0.33, Figure 17(D)). The p value was > 0.05 for all these correlations.

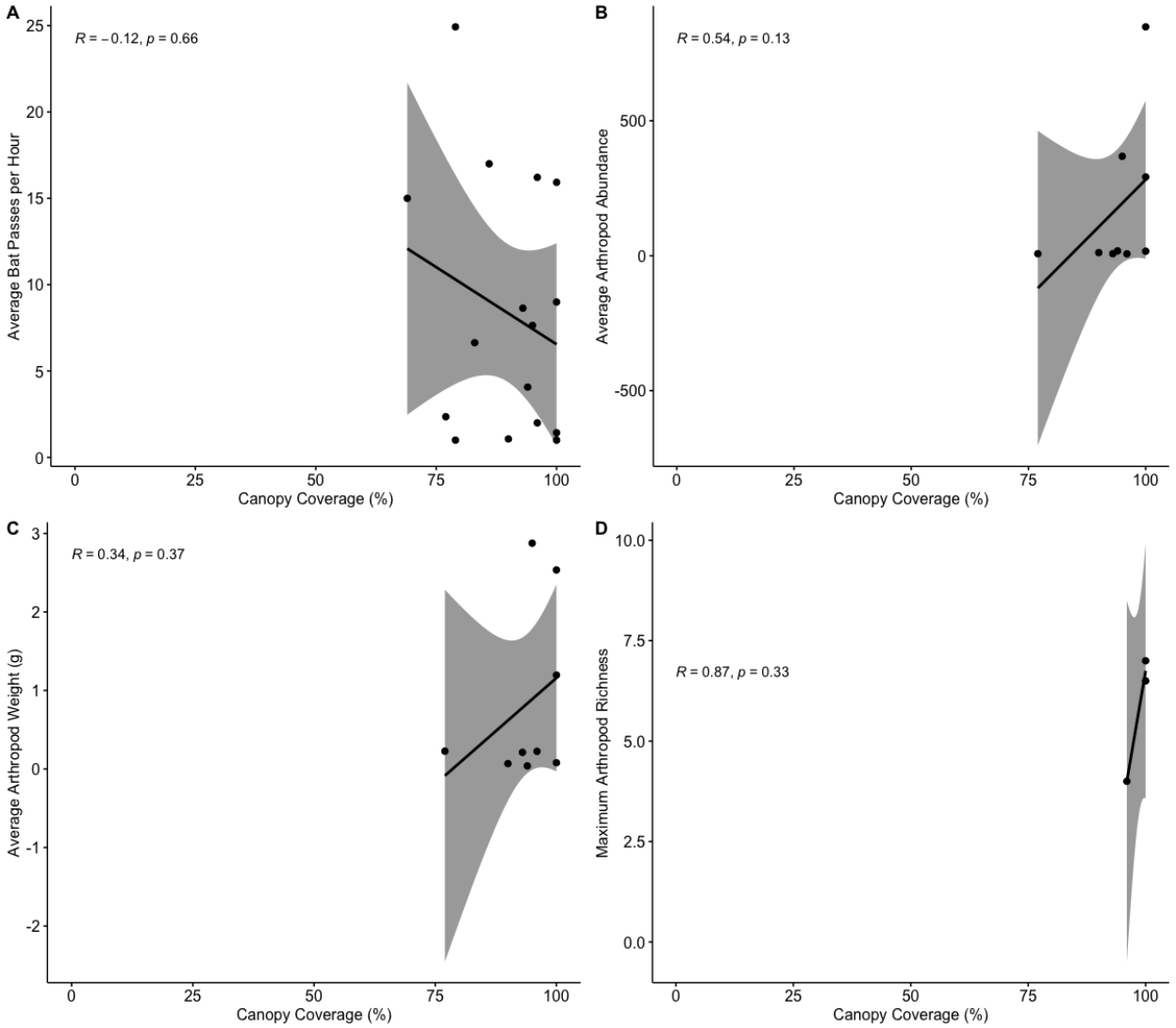

Figure 17. Regressions comparing average canopy coverage to bat passes per hour (A), average canopy coverage to arthropod abundance per hour (B), average canopy coverage to arthropod weight per hour (C), and average canopy coverage to arthropod richness (D). The slope indicates the relationship between factors, while R is a measure of the strength of predictors. High correlations (R) between canopy coverage and arthropod variables, however, no significance ($p > 0.05$).

Average understory height shows no significant relationship to bat activity, arthropod abundance, arthropod weight, or arthropod richness. Spearman's correlation coefficient states a weak relationship, making it a poor predictor variable. No significance was noted from regressions (Figure 18).

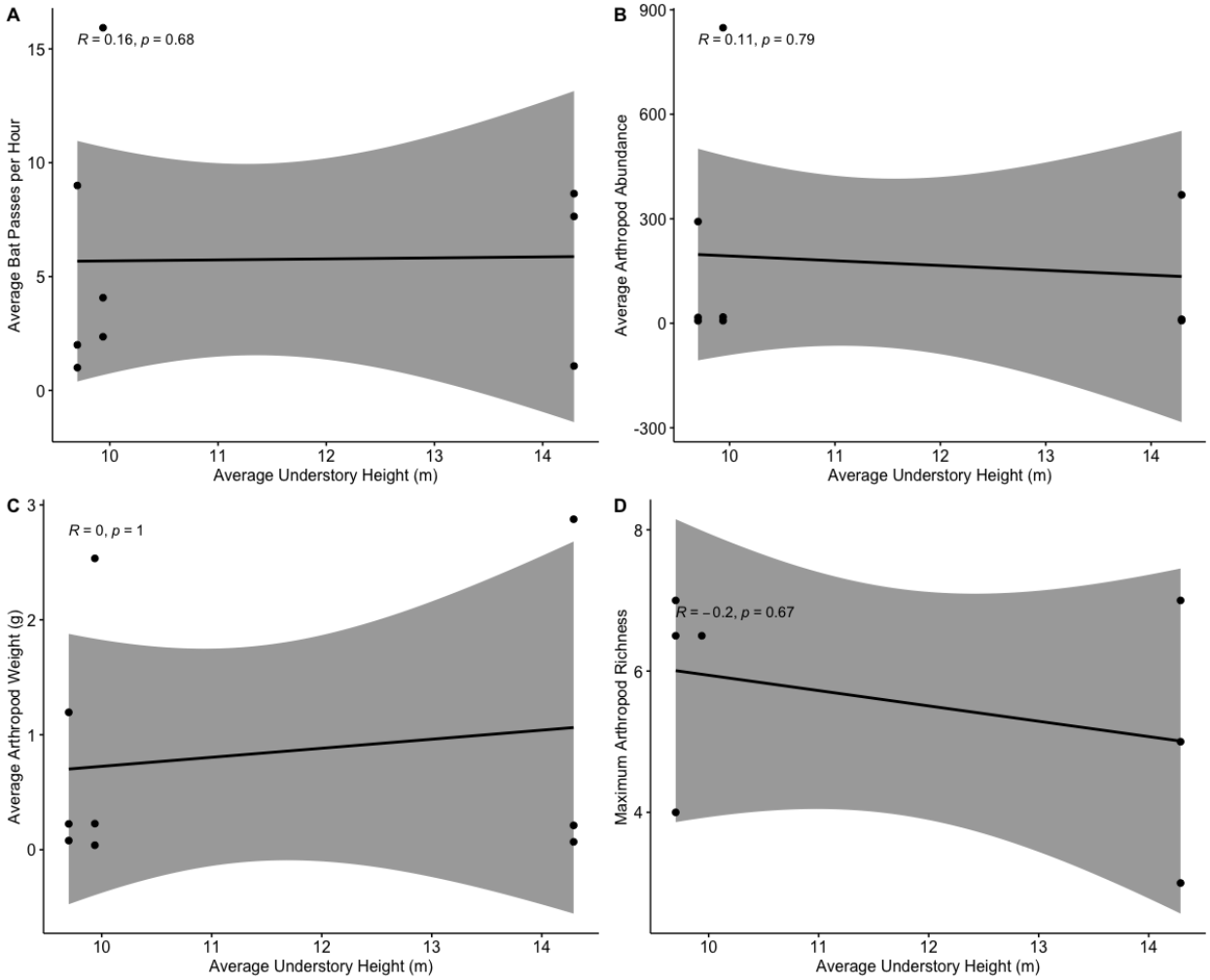

Figure 18. Regressions comparing average understory height to bat passes per hour (A), average understory height to arthropod abundance per hour (B), average understory height to arthropod weight per hour (C), and average understory height to arthropod richness (D). The slope indicates the relationship between factors, while R is a measure of the strength of predictors. No significance ($p > 0.05$).

Regressions between arthropod variables and bat activity show a strong significant relationship between arthropod abundance and bat passes ($R = 0.41$, $p = 0.02$(Figure 19(A)) and arthropod weight and bat passes ($R = 0.51$, $p = 0.002$(Figure 19(B)). Arthropod weight shows no significant relationship to bat passes ($R = 0.27$, $p = 0.12$(Figure 19(C)).

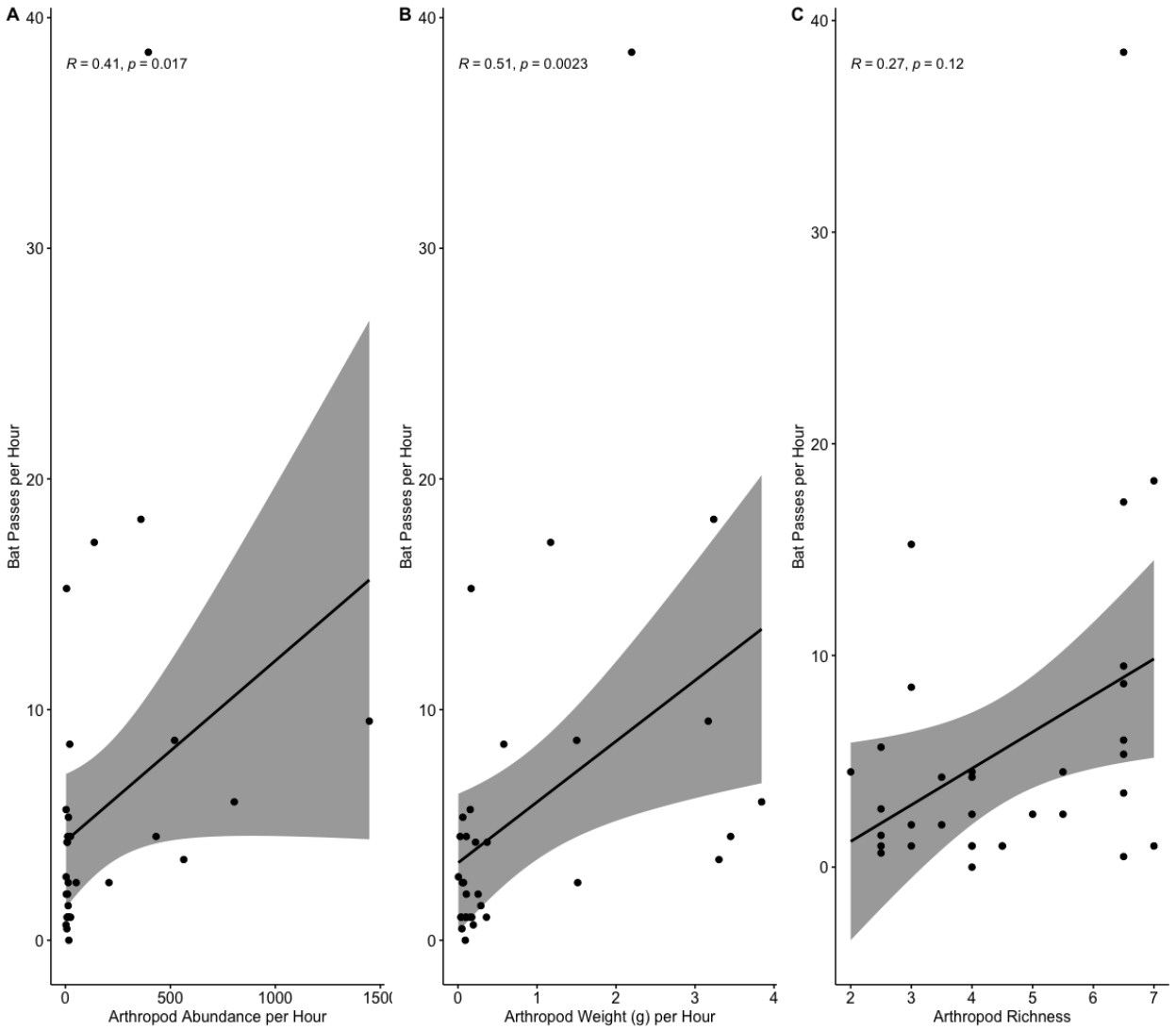

Figure 19. Regressions comparing arthropod abundance per hour to bat passes per hour (A), arthropod weight per hour to bat passes per hour (B), arthropod richness to bat passes per hour (C). The slope indicates the relationship between factors, while R is a measure of the strength of predictors.

Table 3. Shows the details pertaining to the different collection times. This table shows information per hour to standardize the values.

Season	Collection Time	Arthropod Weight (g)/Hour	Arthropod Abundance/Hour	Bat Passes/Hour
Summer	1	2.202083333	297.5416668	88.75
Summer	2	2.886666667	525.3333335	17.5
Summer	3	2.706666667	800.0555555	26.5
Fall	1	0.075416667	13.91666667	7.25
Fall	2	0.075833334	18.66666667	7.5
Fall	3	0.063333334	28.33333334	4.5
Fall	4	0.049166667	11	7.833333333
Winter	1	0.2525	7.666666668	50.25
Winter	2	0.398333334	12.91666667	39
Winter	3	0.19	11.91666667	18.5
Winter	4	0.151666667	3.694444445	12.5

Discussion

Classic ecological theory suggests organisms can reduce interspecies competition by partitioning resources. To better understand the underlying mechanisms behind bat diversity in the coastal plains of South Carolina, we attempted to observe and quantify resource use by bat groups with overlapping distribution. Across multiple metrics, we found data which supports that bats do indeed partition resources when foraging. First, we collected data that shows a positive correlation between bat activity and arthropod abundance. Second, we found evidence that bat diets change during different seasons. Finally, we found that bats tend to forage early in the night, which is when beetles are also most active.

Within a given habitat, we suspected bats would maximize foraging efforts in high-energy patches. We predicted bats would spend more time in patches when insect abundances were greatest. When comparing insect abundances per hour and insect weights per hour to bat passes per hour, we observed greatest bat activity early in the night (Table 3). A Spearman correlation indicated a strong positive relationship between arthropod abundance per hour and bat activity per hour (Figure 19 (A)) and arthropod weight per hour and bat activity per hour (Figure 19 (B)).

To determine if our second hypothesis was supported, we compared the relative frequency of specific insect orders detected in bat guano (from preliminary analysis) to the relative frequency of those same orders during our collections. Assuming order relative abundance does not dramatically change between our studied regions, we noticed a preference for specific food items. Our final hypothesis was that abiotic factors would lead to changes in bat feeding behaviors. As seasons changed, temperature, humidity significantly changed. We suspect these changes in abiotic factors affected arthropod abundance. When arthropods are abundant, we suspected bats would partition food groups as a method to reduce competition; however, when arthropods become more scarce, we suspected bats would consume anything available. The dietary analysis is needed to determine if changes in abundance affected bat feeding behaviors.

Bats were present throughout all four study sites during every season sampled. We hypothesized that bats would be most active when abundances were greatest. Our data did support this hypothesis, arthropod abundance per hour appears to have a positive relationship with bat passes per hour (Figure 19 (A)), most of the bat passes occurred early in the night even though abundance was not greatest at this time (Table 3). Instead, arthropod weight per hour appears to significantly affect bat passes (Figure 19 (B)). Similar to the findings of Skalak 2012, activity was greatest while the sun was setting. We also saw an increase in the relative abundance of beetles early in the night during every sample time. We suspect bats are most active early in the night while beetles are mostly present since they provide a higher nutritional value for the same cost of foraging. The content of the pray could be driving the feeding behaviors of the bats. We see a decrease in bat activity during the fall. This decrease in activity could have been due to the rain experienced during that sampling time, which is consistent with the findings of Audet 1990 and Erickson and West 2002.

Our second hypothesis was that bats would specialize prey while insect populations are most abundant. During the late summer sample from our preliminary study (Figure 3), which best overlapped with our summer sample in SC, we observed a relative frequency of 99% Coleoptera DNA in the late summer big brown bat fecal sample, however the relative frequency of Coleoptera during our insect collections was 23% (Table 5). We also observed a relative frequency of 87% Lepidoptera DNA in the late summer red bat fecal sample, however the

relative abundance of Lepidoptera from our relative sample was 8% (Table 5). Although these samples are from different places and times, we suspect the results from DNA samples will not differ dramatically. This works is a preliminary method to determine if bats are actively selecting insect orders. Future work will incorporate a Chesson's index, to determine selective feeding on specific food items.

Table 5. Table compared preliminary findings in Rome, GA to those in Bluffton, SC. This shows the relative abundance of specific insect order DNA present in Guano and the relative abundance of insect orders detected in environmental samples.

EPFU (Big Brown Bat)	Guano	Environmetal
Spring Coleoptera	0.77	Spring still being sorted
Summer Coleoptera	0.99	0.23

LABO (Red Bat)	Guano	Environmetal
Spring Diptera	0.85	Spring still being sorted
Summer Lepidoptera	0.87	0.08

We hypothesized bats would specify prey items when temperatures decreased. We suspected a decrease in temperature would result in a decrease in arthropod abundance and richness. Surrounded by fewer options, we predicted bats would consume any prey they happen to encounter instead of focusing on specific orders. We noticed a slight overlap in diets during the summer in our preliminary study (Figure 3). Big brown bat focused primarily on Coleoptera, while other species ate combinations of Diptera and Lepidoptera. However, Bray-Curtis distances do not show similarity within species or temporally. This same analysis must be done with samples from SC. The temperature has the highest correlation with the arthropod community (Figure 15). Further analysis, such as model building, must be done to determine the significance of predictor variables.

This study demonstrates the importance of a multifaceted approach. Behaviors as complex as feeding tendencies are influenced by many external factors. We attempted to incorporate various data types to understand better the coexistence of various bat species in a overlapping site. We expect to see patterns regarding arthropods available and those consumed by incorporating the DNA data and the spring collections. Future studies should incorporate more types of data to understand resource use better. Just looking at what an organism is consuming does not give a clear perspective on what is occurring in nature.

References

Altringham, J. D. (2011). Bats: From Evolution to Conservation. Oxford England ; New York: Oxford University Press.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of molecular biology*, *215*(3), 403-410.

Audet, D. (1990). Foraging behavior and habitat use by a gleaning bat, Myotis myotis (Chiroptera: Vespertilionidae). *Journal of Mammalogy*, *71*(3), 420-427.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37(8), 852-857. doi:10.1038/s41587-019-0209-9 [doi]

Britzke, E., Gillam, E., & Murray, K. (2013). Current state of understanding of ultrasonic detectors for the study of bat ecology. Acta Theriologica, 58(2), 109-117. doi:10.1007/s13364-013-0131-3

Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. Theoretical Population Biology, 9(2), 129-136. doi:10.1016/0040-5809(76)90040-X

Chesson, P. (2000). Mechanisms of maintenance of species diversity. Annual review of Ecology and Systematics, 31(1), 343-366.

Choate, P. M. Introduction to the Identification of Adult Insects and Related Arthropods-2010.

Emlen, J. M. (1966). The role of time and energy in food preference. The American Naturalist, 100(916), 611-617.

Erickson, J. L., & West, S. D. (2002). The influence of regional climate and nightly weather conditions on activity patterns of insectivorous bats. *Acta Chiropterologica*, *4*(1), 17-24.

Fath, B. D., & Jørgensen, S. E. (2008). Encyclopedia of ecology. Amsterdam: Elsevier.

Finke, D. L., & Snyder, W. E. (2008). Niche partitioning increases resource exploitation by diverse communities. Science, 321(5895), 1488-1490.

Flygare, S., Simmon, K., Miller, C., Qiao, Y., Kennedy, B., Di Sera, T., . . . Schlaberg, R. (2016). Taxonomer: An interactive metagenomics analysis portal for universal pathogen detection and host mRNA expression profiling. Genome Biology, 17(1), 111-1. doi:10.1186/s13059-016-0969-1 [doi]

Gaston, K. J., & Lawton, J. H. (1988). Patterns in the distribution and abundance of insect populations. Nature, 331, 709-712.

Ingersoll, T. E., Sewall, B. J., & Amelon, S. K. (2016). Effects of white-nose syndrome on regional population patterns of 3 hibernating bat species. Conservation Biology, 30(5), 1048-1059. doi:10.1111/cobi.12690

Jones, G., Jacobs, D., Kunz, T., Willig, M., & Racey, P. (2009). Carpe noctem: The importance of bats as bioindicators. Endangered Species Research, 8, 93-115. doi:10.3354/esr00182

Justin G. Boyles, Paul M. Cryan, Gary F. McCracken, & Thomas H. Kunz. (2011). Economic importance of bats in agriculture. Science, 332(6025), 41-42. doi:10.1126/science.1201366

Kasso, M., & Balakrishnan, M. (2013). Ecological and economic importance of bats (order chiroptera). ISRN Biodiversity, 2013, 1-9. doi:10.1155/2013/187415

Lang, A. B., Kalko, E. K., Römer, H., Bockholdt, C., & Dechmann, D. K. (2006). Activity levels of bats and katydids in relation to the lunar cycle. Oecologia, 146(4), 659-666.

Langwig, K. E., Hoyt, J. R., Parise, K. L., Frick, W. F., Foster, J. T., & Kilpatrick, A. M. (2017). Resistance in persisting bat populations after white-nose syndrome invasion.

Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 372(1712), 20160044. doi:10.1098/rstb.2016.0044

Loeb, S. C., Rodhouse, T. J., Ellison, L. E., Lausen, C. L., Reichard, J. D., Irvine, K. M., . . . Johnson, D. H. (2015). A plan for the north american bat monitoring program (NABat)

MacArthur, R. H., & Pianka, E. R. (1966). On optimal use of a patchy environment. The American Naturalist, 100(916), 603. Retrieved from https://search.proquest.com/docview/1308335653

McArthur, R. H. (1970). Species packing and competitive equilibrium for many species. Theor. Pop. Biol, 1, 1-11.

Moosman, P. R., Jr., Thomas, H. H., & Veilleux, J. P. (2012). Diet of the widespread insectivorous bats eptesicus fuscus and myotis lucifugus relative to climate and richness of bat communities. Journal of Mammalogy, 93(2), 491-496. doi:10.1644/11-MAMM-A-274.1

Ollivier FJ, Samuelson DA, Brooks DE, Lewis PA, Kallberg ME, Komáromy AM. Comparative morphology of the tapetum lucidum (among selected species). Vet Ophthalmol. 2004 Jan-Feb;7(1):11-22. doi: 10.1111/j.1463-5224.2004.00318.x. PMID: 14738502.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ratnasingham, S., & Hebert, P. D. (2007). BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). Molecular ecology notes, 7(3), 355-364.

Russo, D., & Voigt, C. C. (2016). The use of automated identification of bat echolocation calls in acoustic monitoring: A cautionary note for a sound analysis. Ecological Indicators, 66, 598-602. doi:10.1016/j.ecolind.2016.02.036

Rydell, J., Entwistle, A., & Racey, P. A. (1996). Timing of foraging flights of three species of bats in relation to insect activity and predation risk. Oikos, 243-252.

Schoener, T. W. (1971). Theory of feeding strategies. Annual Review of Ecology and Systematics, 2, 369-404.

Skalak, S. L., Sherwin, R. E., & Brigham, R. M. (2012). Sampling period, size and duration influence measures of bat species richness from acoustic surveys. Methods in Ecology and Evolution, 3(3), 490-502.

South Carolina Department of Natural Resources. (2019). South carolina bat conservation plan. Columbia, South Carolina:

Teeling, E. C., Springer, M. S., Madsen, O., Bates, P., O'brien, S. J., & Murphy, W. J. (2005). A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science*, *307*(5709), 580-584.

Zeale, M. R. K., Butlin, R. K., Barker, G. L. A., Lees, D. C., & Jones, G. (2011). Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. Molecular Ecology Resources, 11(2), 236-244. doi:10.1111/j.1755-0998.2010.02920.x