Assessment of Nocturnal and Crepuscular Arthropods and Their Influence on Bat Species on the Eastern Highland Rim

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Carmen Rhiannon Black
May 2024

DEDICATION

To
Marlo Black
and
Staci Higdon
and
Ludivine Brunissen

My sister, my mother, and my partner have always supported me through every moment. Singularly the three most important people in my life, who have always been by my side. Thank you for always putting up with my fun insect facts (all the time) and encouraging me to fulfill my dreams. You all make me a better version of myself. I will be forever grateful to have you all in my life.

To my partner, friends, extended family, pets, and generous mentors: Without the support of my community, I would not be half the person I am today. With your care and guidance, you all have been integral in shaping the friend, scientist, partner, and person I am today. You have all brought me so much joy and knowledge in my life. Each of you inspires me. Thank you for everything.

ACKNOWLEDGEMENTS

A tremendous number of mentors and collaborators have made this research possible and my current graduate career possible. First and foremost, Dr. Jerome Grant cannot be thanked enough for believing in me as a student to take me into his lab. His guidance, mentorship, and teaching have greatly impacted me as a researcher, teacher, and person. I will be forever grateful for the tremendous amount of time and knowledge Dr. Grant has shared with me.

I would like to next acknowledge the contribution of John Lamb to this project. The amount of time and work he has dedicated to Arnold Air Force Base and their ongoing projects is the only reason this research is able to be conducted. He has been essential in coordinating with this research and provided the bat acoustic data presented in this thesis. Thank you so much for your countless hours of work and the kindness you have shown to me. Thank you to Amy Turner and other personnel from Arnold Air Force that make sharing this research possible.

This research would also not have been possible without the expertise and kindness of Dr. Joy O’Keefe and Dr. Elizabeth Beilke. Their mist-netting work and mentorship has been essential to this project. Thank you for patience and generosity in teaching an entomologist how to handle bats and understanding many facets of bat biology. I am so grateful for both of your continued support and guidance.

Thank you to all my mentors at University of Tennessee who have offered me lab space to sort specimens, allowed me to work in their labs, go to meetings, participate in research, or continuously supported one of the many projects I had decided to work on.

A special thanks to Dr. Ernest Bernard, Dr. Rebecca Trout-Fryxell, Dr. Richard Gerhold, Dr. Gary Phillips, Ash Cable, and Dr. Jennifer Weisent.

I also owe special thank-you’s to Matthew Longmire, Julian Cosner, Makhali Voss, Kassie Hollabaugh, and Matthew Brackin. Thank you for your help in collecting samples, reading my writing, giving feedback on my presentations, and sometimes letting some of many samples take up some of your lab space. You have all aided in the success of this project and been tremendously helpful and gracious to me.

ABSTRACT

Since the introduction of White Nose Syndrome (WNS) in the U.S. in 2007, there have been mass mortalities of bats around North America. WNS is caused by the parasitic and psychrophilic fungus *Pseudogymnoascus destructans*, which depletes lipid resources of bats. Researchers linked survival of infection of WNS to lipid concentrations of bats. This nutrition associated link has made it critical to understand prey availability as survival of WNS is dependent on food intake. Traditional insect research often overlooks nocturnal arthropods and for multiple seasonal periods. The increase of invasive species and global insect decline has also raised the need for arthropod diversity studies. Our objective was to determine the drivers of insects and how they influence bats. Insects were collected with either (1) ultraviolet traps or (2) CDC light traps. In collaboration with others, bats were monitored by mist netting and acoustic recordings in conjunction to insect sampling (From June 2022- August 2023 at Arnold Air Force Base, TN). Insect abundance was correlated with insect order biomass, insect family richness, and insect family diversity; indicating that higher number of insects were associated with higher prey biomass available to bats and family diversity in the available prey. Family diversity was correlated to insect abundance, but the composition of families was unique to each habitat and seasom. Abundance of six insect orders (Ephmeroptera, Plecoptera, Trichoptera, Coleoptera, Hemiptera, and Lepidoptera) were highly correlated with temporal variables. Habitat and habitat interactions with season influenced Coleoptera numbers. Mist-net models were poor and indicate that other variables may be more important in predicting presence or absence of a bat species. Evening bat calls were positively associated with the edge habitat and hymenopteran abundance. Eastern red bat calls had a positive relationship with edge habitat and negative ones with forested streams and forested wetlands. Gray bat calls were positively associated with total insect abundance and specifically Lepidoptera. These results suggest studies should consider specific insect orders and insect variables to understand bat foraging. These findings provide an ecological base for future studies at Arnold AFB and link arthropod diversity, abundance, and relative biomass to species of bats.

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Literature Review

Introduction

The importance of arthropods in ecosystems as pollinators, scavengers, decomposers, prey, carnivores, and herbivores has been well documented (Noriega et al., 2018; Schowalter, 2022). These arthropod species are essential food sources for bats (Kurta, 2001). The relationship between predator (chiropterans) and prey (arthropods) is integral to bat survival of due to their basic metabolic needs. However, this need is heightened by a recently discovered, fat-depleting parasitic fungus, *Pseudogymnoascus destructans* (Blehert & Gargas) Minnis & D. L. Lindner*,* (= *Geomyces destructans* Blehert & Gargas 2009). *Pseudogymnoascus destructans* is a fungal pathogen that causes White Nose Syndrome (WNS), an epitonic and fatal disease affecting bats in North America (Gargas et al., 2009; Cheng et al., 2019).

Several components of bat conservation have been identified by Fenton (1997) and Pierson (1998). The first and second components of bat conservation include protection of foraging habitat and protection of their prey base. The relationship between bats and arthropods has been traditionally studied by bat biologists using hand-sorting methods through guano to identify insect body parts and, in recent years, with PCR (Polymerase Chain Reaction) (Agosta et al., 2003; Deeley et al., 2023; Feldhamer et al., 2009; Hayes et al., 2019; Hughes et al., 202). However, little to no research in this area has been conducted by entomologists to expand knowledge on the relationship between chiropterans and their primary food source.

Research that includes monitoring arthropod populations during crepuscular periods, through the night, and throughout the year has been limited, specifically to locations in the southeastern United States (such as Arnold Air Force Base) (Arnold AFB). Arnold AFB is located on the eastern Highland Rim near Tullahoma, TN and spans around 162 sq km. Season-long, crepuscular, and night-time sampling of arthropods is crucial to better understand prey availability for bats. Bats with higher lipid content and high nutritional diet are more likely to survive hibernation, especially those that may be affected by WNS (Cheng et al., 2019). All these factors illustrate the importance of expanding the knowledge on the frequency of available prey and the associated nutritional content in each prey item when assessing bat health, including WNS.

One possible driver for fat production levels in bats is the abundance and diversity of prey. The increased number and diversity of prey results in reduced energy costs associated with feeding, and it allows more feeding that increases the chances of higher fat and mineral composition (Cheng et al., 2019). Studies focused on crepuscular and nocturnal insect availability may also allow for insight with other approaches. One example includes studies such as ‘Fat Bat’, a study using ultraviolet lights to attract prey to bats and, as a result, decrease mortality related to WNS (Electric Power Research Institute, 2021). Studies with emphasis on bat and prey interactions have the potential to inform strategies for mitigating environmental or biological factors by supplementing bat diets (O'Reilly, 2019).

Bats in the Southeastern United States and at Arnold Air Force Base

Nine species of bats, all of which are insectivores, have been documented from Arnold AFB on the eastern Highland Rim near Tullahoma, TN (Lamb, 2022). These nine species are big brown bat (*Eptesicus fuscus* (Beauvois, 1796)), eastern red bat

(*Lasiurus borealis* (Muller, 1776)), evening bat (*Nycticeius humeralis* (Rafinesque, 1818)), gray bat (*Myotis grisescens* (Howell, 1909)), hoary bat (*Lasiurus cinereus* (Allen, 1864)), Indiana bat (*Myotis sodalis* (*Miller & Allen*, 1928)), little brown bat (*Myotis lucifugus* (Le Conte, 1831)), northern long-eared bats (*Myotis septentrionalis* (Trouessart, 1897)) and tricolored bat (*Perimyotis* subflavus (F. Cuvier, 1832)). Six of the nine species of bats at Arnold AFB are affected by WNS including big brown bats, gray bats, Indiana bats, little brown bats, northern long-eared bats, and tricolored bats. Gray bats, Indiana bats, and northern long-eared bats are endangered. Tricolored bats and little brown bats are both currently under review for federal listing (Kitchell, 2022; U.S. Fish and Wildlife Services, 2022b). The scientific names, common names, species code, if species is affected by WNS, and current listing by the United States federal government of all nine bat species found at Arnold AFB are listed in Table 1.1.

Bats at and near Arnold AFB have been well studied over the last 20 years. Adjacent to Arnold AFB is a known colony of little brown bats and gray bats roost seasonally under Beth Paige Bridge (within 16 km of the base limits); gray bats roost in the Elk River Dam on the base (Wells, 2022). The U. S. Fish and Wildlife Services Gray Bat Recovery Plan also lists the gray bat colony that occurs on Arnold AFB at Woods Reservoir Dam as a priority two maternity colony, located 2.7 km away from Beth Page Bridge (Brady, 1982). Since 1988, Arnold AFB personnel have annually monitored colony counts of gray bats (both adult and juvenile) at the Woods Reservoir Dam and collected guano samples from the roost (Lamb, Unpublished). In conjunction with surveys at dams, personnel at Arnold AFB facilitate annual mist netting for bats in the summer months at five sites on the base and conduct acoustic monitoring throughout the year. In 2022 and 2023, researchers at the University of Illinois conducted a bat diet study through mist netting at Arnold AFB and the use of metagenomic analyses for collected guano samples to assess prey consumed. In 2021, personnel from Arnold AFB and Virginia Tech conducted a cooperative statewide cave survey to assess bats in caves on and surrounding Arnold AFB. Four of the eight caves surveyed contained bats that exhibited positive field signs for WNS, with tricolored bats the dominant species found in the caves surveyed (Lamb, 2020).

As mentioned previously, WNS is caused by the fungal pathogen, *P. destructans*. This fungus is an Ascomycete in the order Onygenales. It was first discovered in 2007 during a cave survey in Albany County, New York (Meteyer et al., 2009). This pathogen is one of the most devastating infectious pathogens to cause disease outbreaks in wild mammals to emerge in the last century (Hoyt et al., 2021). This multi-host and psychrophilic fungus (Minnis & Lindner, 2013) has infected bats in 38 states and led to substantial population declines in multiple species, particularly little brown, tricolored, and northern long-eared bats (U.S. Fish and Wildlife Services, 2022a, 2022b). The fungus is believed to have originated in Eurasia and may have historically reduced cave-hibernating bat populations in Europe (Hoyt et al., 2020). The fungus has now been confirmed in 12 species of bats in North America (U.S. Fish and Wildlife Services, 2022b).

Research indicates that bats infected with WNS may display unusual behaviors during cold winter months, including flying outside during the day and congregating near the entrances of caves and other hibernation areas. This behavior change induced by WNS infection is debatable, because of evidence that bats are active in winter months: e.g., Candan, pre-WNS, bats were active at temperatures of 0°C and only ceased at unexpected cold temperatures of -8°C (Lausen & Barclay, 2006). Another study conducted in Missouri captured big brown bats, evening bats, and eastern red bats pre-WNS in winter months and concluded the bats were feeding at the time of capture (Dunbar et al., 2007). Another study conducted in Indiana captured little brown bats, northern long-eared bats, and tricolored bats in winter months before WNS; however, data on feeding were lacking in that study (Whitaker & Rissler, 1992).

Previous studies suggest that winter activity of bats may be possible in the southeastern U.S. pre-WNS, but research on these behaviors pre-WNS is unclear. It is probable that bats in Tennessee have always shown activity in the winter independent of *P. destructans* (Carr et al., 2014). However, some researchers have suggested that winter activity of bats in Tennessee in the presence of *P. destructans* has increased and had more daytime appearances compared to uninfected individuals (Bernard & McCracken, 2017). Given the ambient temperatures and availability of prey during winter, the southeastern U.S. was once thought to be a possible regional refuge for bat populations (Bernard et al., 2017; Loeb & Winters, 2013). However, current studies have shown drastic population declines of small-bodied bats in the southeastern United States contradicting the concept of a possible WNS refuge (O'Keefe et al., 2019).

Much bat-related research has been conducted on Arnold AFB over the last 20 years. Specifically, biologist John Lamb has been actively mist netting and recording acoustic data throughout the year on Arnold AFB. Previous research also involved telemetry studies that showed that four species of bats (Indiana, northern long-eared, little brown, and tricolored) will travel from the Cumberland Plateau at night to forage on the base (Samoray, 2015). Bat-capture during mist-netting season of 2015 resulted in collection of 96 evening bats, 94 red bats, 59 gray bats, 29 little brown bats, 28 big brown bats and four tricolored bats. Of the four tricolored bats captured, three were fitted with radio transmitters for tracking their diurnal roosts and followed during foraging. No data were collected on northern long-eared bats or Indiana bats. Of the 29 little brown bats captured, 15 were fitted with radio transmitters, and tracked to their diurnal roosts and followed during foraging (Samoray, 2015). Observations on roosting and foraging of the three tricolored bats on Arnold AFB found nine tree roosts (roost switching did not occur daily). All of these bats were believed to be roosting in dead leaf clumps in trees. Tricolored bats were found to travel relatively quickly from roost trees to open water (e.g. Wood's Reservoir and Arnold Engineering Development Center [ADEC] Retention Pond) and were most often found feeding in or near the edge of these large bodies of water, often returning to the same site multiple times (Samoray, 2015). Little brown bats foraged over open water and rarely returned to the same foraging site (Samoray, 2015). A summary of the locations of the foraging and roosting sites of the little brown bat is shown in Figure 1.1.

The four most collected and recorded bat species at Arnold AFB for the past five years were big brown bats, eastern red bats, evening bats, and gray bats (Lamb, Unpublished). A review of basic life histories, ecologies, and known diets of each of these four bat species is provided below.

I. Ecology and Diet of Big Brown Bats

Big brown bats roost in a wide variety of structures, such as caves, cracks, tunnels, buildings, tree cavities, and other manufactured structures, and are found throughout North America (Whitaker & Gummer, 1992). Big brown bats are found widely throughout United States in a variety of habitats and primarily mate in fall and winter with pups being born in May and June, often giving birth to twins in the eastern United States (Harvey et al., 1999). Some habitat features are important to big brown bats when foraging, though they are considered habitat generalists. In the White Mountains of New Hampshire, the activity of big brown bats was highest near standing water and roads (Krusic et al., 1996). In Arizona, big brown bats displayed higher foraging activity in riparian zones compared to other habitats (Bell, 1980).

Microscopic analysis (where guano is dissected under microscope and insect parts are used for prey identification) was used to assess prey of big brown bats in guano from Pennsylvania to Maryland (Agosta et al., 2003). Big brown bats most common prey items were Coleoptera, especially Scarabaeidae. Hemiptera, primarily the green stink bug, *Chinavia hilaris* (Say) (= *Acrosternum hilare* (Say, 1832)), and Tettigoniidae, not previously reported, were prey of two populations of big browns (6%, 17%). Significant spatial and temporal variation existed in the diet composition among populations and the major prey types in this study (Agosta et al., 2003). In another study, comparable results showed that big brown bats consumed large numbers of coleopterans (Feldhamer et al., 2009). However, big brown bats have historically been classified as foraging generalists throughout many habitats with a wide range of prey types; and they have been considered flexible both temporally and spatially about prey use (Brigham & Saunders, 1990; Brigham, 1991; Whitaker, 1995; Hamilton & Barclay, 1998). In more recent years, DNA meta-barcoding studies in the mid-Atlantic region have shown that big brown bats had Coleoptera present in almost all guano samples, followed by Hemiptera, Blattodea, Hymenoptera, Lepidoptera, Trichoptera, and Megaloptera (Deeley et al., 2023); these samples were passed through multiple primer sets for a ‘stricter’ diet analysis. These researchers compared their meta-barcoding results to insect collections at each site and determined that multiple primer sets were needed to get accurate meta-barcoding results; however, many insects have not been barcoded for these types of analyses. These results serve aa a caution to future researchers to be careful with primer selection for insect meta-barcoding or to use multiple primer sets to achieve accurate results.

II. Ecology and Diet of Eastern Red Bats

The eastern red bat is a widely distributed, foliage-roosting species found throughout the eastern United States, southern parts of Canada, and northern Mexico (Shump & Shump, 1982; Boyles et al., 2003). Eastern red bats are known to be present in the southeastern United States all year, remaining in trees in relatively warmer winter days and roosting in leaf litter as temperature declines in the winter months (Cryan & Veilleux, 2007). This ecological trait is especially important to be aware of in landscapes that receive prescribed burning treatments, because eastern red bats have been documented flying from prescribed burns (Lyane, 2009). Leaf litter roosts offer cryptic cover and allows for passive warming (Barnes & Carey, 2004; Moorman et al., 1999). Torpor duration has been inversely correlated to ambient temperature and suggests that eastern red bats continue to forage throughout winter months (or go to water sources or new roosting sites) (Dunbar & Tomasi, 2006). Eastern red bats are reproductive (pregnancy to lactation) from May to July with juveniles usually appearing in late June in the southeastern United States (LaVal & LaVal, 1979).

Many studies have suggested that gaps or openings in highly forested or cluttered landscapes may be important for eastern red bat foraging and commuting (Amelon et al., 2014; Walters et al., 2007). A recent PCR study on diets of eastern red bats in Canada suggested that most prey species were in the orders Lepidoptera (16 families represented), but Coleoptera, Diptera, Ephemeroptera, and Hymenoptera were also identified. Members of the orders Hemiptera, Neuroptera, and Trichoptera were likely present based on sequence similarities to members of these orders although identifications were not possible given the incompleteness of the reference database (Clare et al., 2009). In southern Illinois, Feldhamer et al. (2009) determined eastern red bats fed primarily on lepidopterans followed by hemipterans. Hand methods of sampling guano revealed that eastern red bats primarily feed on coleopterans in the spring through summer and shift to moths (Lepidoptera) as the main prey item in the winter. Study site locality also influenced the composition of lepidopterans and coleopterans collected in the guano (Hayes et al., 2019). Another study described eastern red bats in the southeastern United States as potentially beneficial in integrated pest management of many economically important pest species (Hughes et al., 2021). In this study, analysis of fecal samples suggested that their diets included known hemipteran and lepidopteran pests. Eastern red bats had the highest likelihood of eating pest species compared to other bat species sampled.

III. Ecology and Diet of Evening Bats

In the southeastern U.S., mature forests have been documented as important summer roosting sites for evening bats, and they are well documented to roost in trees and sometimes in man-made structures (Hein et al., 2009; Timpone et al., 2006). Females typically give birth in May to July in tree maternal roosts (Harvey et al., 1999).

Overall, knowledge of evening bat diets is limited, and little is known about their fall and winter roosting and foraging. One study in the northeastern United States that examined guano pellets by hand sampling showed that most prey items were in the orders Coleoptera (40.7%), including Scarabaeidae, Carabidae, Hydrophilidae, and Chrysomelidae, Hymenoptera (no families identified) (23.3%) and Hemiptera (17.2%), including Cicadellidae, Corixidae, and Lygaeidae.

Lepidoptera (6.4%) and Diptera (0.6%) were minor constituents in the guano samples; the remaining 11.8% was unidentified or dispersed among many orders (Geluso et al., 2008).

In Texas, DNA meta-barcoding has been used to examine the diets of evening bats and suggests that evening bats had low diversity in foraging samples often only yielding, averaging two insect species per sample (Weinkauf et al., 2018); however, results like these are biased based on which arthropods have been sequenced and are part of the barcoding projects. In other words, they can state what was eaten but not everything that was eaten or available. In this study evening bats most frequently consumed Coleoptera, including Carabidae and Mycetophagidae, followed by Lepidoptera, including Erebidae and Gelechiidae, and then Diptera, including Chironomidae and Culicidae (Weinkauf et al., 2018). In southern Illinois, a similar study (Feldhamer et al., 2009) using microscopic analysis of evening bats concluded that their diet consisted of Coleoptera (52.8%) and Hemiptera (24.0%). Lepidoptera were not found in these samples (Feldhamer et al., 2009). Another study on microscopic guano analysis described evening bats in the southeastern United States as potentially beneficial in integrated pest management, as diets contained many economically important pest species including known hemipteran and lepidopteran pests (Hughes et al., 2021).

IV. Ecology and Diet of Gray Bats

The gray bat is federally listed as endangered due to historical declines and habitat loss (Tuttle, 1979). Despite recovery efforts, gray bats continue to face decline currently in the United States (Campbell, 2019; Powers et al., 2016). Gray bats are considered year-round cave obligates but have been documented using trees as diurnal roosts in Tennessee and North Carolina (Samoray et al., 2020). Typically, gray bats can be found in caves for both summer roosts and winter hibernacula. Mating occurs as bats return to winter caves in September and October. By November, most gray bats are hibernating (Kentucky Department of Fish & Wildlife, 2024). Recent telemetry with gray bats suggested that these individuals used two major areas during spring and fall migration: three high-likelihood pathways through central Tennessee and one primary migration route between northern Arkansas and central Missouri (Holliday et al., 2023). Following emergence from hibernation in late March to mid-May, pups are born in late May to early June (Kentucky Department of Fish & Wildlife, 2024; U.S. Fish and Wildlife Service, n.d.). Gray bats have been found to primarily use tributaries of large waterways as foraging areas and flyway routes from land-locked roosting sites to open-water habitats (Best & Hudson, 1996). At Arnold AFB, gray bats roost in Woods Dam and have been documented foraging in various habitats from forested streams to open woodlands. Both capture and acoustic monitoring have expanded the definition of foraging habitat for this species from strictly large waterways to forested wetlands and forested corridors (J. Lamb, 2020).

Microscopic analysis of guano showed that gray bats consume 11 families from nine orders of insects, with Coleoptera, Trichoptera, Diptera, and Lepidoptera accounting for the highest percentage volumes. Three coleopteran families (Carabidae, Chrysomelidae, Scarabaeidae) were common in the diet of gray bats. Ephemeropterans were not observed in fecal samples, despite their availability at foraging sites (Lacki et al., 1995); this study indicates the importance of meta-barcoding techniques in bat diet research. A more recent diet study using PCR analysis and insect light trapping showed trichopterans, coleopterans, and lepidopterans as important food sources in the diet and in light-trap samples (Brack & Laval, 2006). However, overall poor correlation was found between corresponding diet and light-trap samples. In contrast, plecopterans, ephemeropterans, and dipterans were occasionally common in light-trap and dietary samples, although again poor correlation was found between corresponding diet and light-trap samples (Brack & LaVal, 2006).

Insects as Food Sources and Predictor Variables for Bat Species

Though many studies in the past have evaluated bat diets, most of them used manual fecal analysis, which was largely biased and untrustworthy, but allows for identification of hard bodied insects that could potentially missed in meta-barcoding. Recent DNA sequencing techniques have been developed that allow for more accurate identification of arthropods. Traditional fecal analysis has rates of error based on insect order, often reporting more harder-bodied insects than soft-bodied insects. For example, up to 27.3% of lepidopterans fed to bats were not detected in traditional microscopic fecal analysis (Kunz & Whitaker, 1983). Neuropterans and small Dipterans were not recorded at all in fecal analysis (Kunz & Whitaker, 1983). Most research on bat diets has been conducted using this method, which has significant limitations to interpreting the full spectrum of bat diets. Another potential bias encountered in traditional fecal analysis, from either stomach contents or feces of insectivorous bats, is that some individuals may cull parts of their prey (such as wings) before ingestion (Kunz & Whitaker, 1983). This culling is important because bat diets have previously been shown to include a large diversity of insects and other arthropods, and hand methods of analyzing guano may lead to biases based on what is culled by foraging bats (Clare et al. 2011; Feldhamer et al., 2009).

Newer forms of fecal and stomach content analysis involve molecular sequencing to identify contents. This tool began to emerge in the early 2010s for research related to bat diets (Jedlicka et al., 2013). Though this method is much less biased than traditional fecal analysis, it still has limitations. First, it is not possible to tell whether identified diet components were directly consumed by bats or whether they were initially consumed by predatory insects and only secondarily detected in bat guano (Ingala et al., 2021). Guano collection and stomach contents represent, at most, a sampling of individual dietary components over a one- or two-night period (Ingala et al., 2021). Furthermore, primer bias is a concern that applies to any use of “universal” barcodes, as some consumed taxa may not be detected depending on which primers are used, and some taxa may be counted as a false positive (Deeley et al., 2023; Piñol et al., 2015). DNA sequencing is also limited by the lack of taxonomic and barcoding research on most insects.

One study suggests that, in some parts of the world, meta-barcoding analysis showed that barcoded specimens cover a proportion of less than 3.73% of the insect population identified by entomologist (Shashank et al., 2022). The proportion of barcoded species varies drastically based on region and current research, but it is widely considered incomplete in most areas. Comparison of captive feeding experiments with results from qPCR assay demonstrated that this method of identifying prey does not provide a direct measure of the number or biomass of prey consumed (McCracken et al., 2012). However, DNA sequencing techniques do provide important information about the number of bats and the species of bats that consumed a certain prey item, and it provides seasonal information and dates on which certain prey items were consumed (Jedlicka et al., 2013).

Some recent studies have used insect abundance and orders of insects as variables to predict bat foraging or presence. One study conducted in Australia aimed to understand how landscape variables and insect biomass predicted acoustic activity of bats (Threlfall et al., 2012). This study found that insect biomass was at least an order of magnitude greater within suburban landscapes in bushland and backyard elements located on the most fertile shale-influenced geologies compared to nutrient poor sandstone landscapes. Similarly, the feeding activity of bats was greatest in bushland, areas with riparian elements within suburbs, and areas with the most fertile shale. Regression tree analysis indicated that three variables explained the major proportion of the variation in insect biomass and bat foraging activity. These three variables were ambient temperature (positive), housing density (negative), and the percentage of fertile shale geologies (positive) in the landscape; however, variation in insect biomass did not directly explain bat foraging activity (Threlfall et al., 2012). In Sweden, bat acoustic activity is strongly correlated with weather conditions (de Jong et al., 2021). This study did suggest a low correlation between bat acoustic activity and relative insect abundance (de Jong et al., 2021).

Research in managed pine forested landscapes in the southeastern Coastal Plain of the United States suggests a different approach to understanding the interactions between insects and bat occurrences (Bender et al., 2021). These researchers used occupancy modeling in PRESENCE to analyze interactions of bat acoustics relative to insect size, insect order, insect abundance (relative to insect order), insect richness (relative to insect order), and site. They determined a positive correlation between the presence of big brown bats and 'medium' sized insects, total insects, lepidopterans, and coleopterans. The presence of tricolored bats had a positive relationship with lepidopterans. The presence of evening bats had a positive relationship with hymenopterans, insect richness, and specific sites. The presence of eastern red bats had a positive relationship with dipterans and insect richness (relative to insect order) (Bender et al., 2021). The results of this study suggest that future researchers should focus on specific insect orders as indicators of the presence of certain species of bats rather than using total insect abundance as a proxy for bat presence or abundance. Using specific orders of insects is a logical approach when modeling bat and insect interactions. The life history, biology, and ecology of each species of bat are distinct and likely to be unique in their own foraging behaviors.

Insects in the Southeastern United States and at Arnold Air Force Base

While the bat community at Arnold AFB is well studied and continues to be monitored, little is known about the arthropod community upon which it relies for food. A previous arthropod study, conducted in 1997, provided valuable information on established species in various habitats on Arnold AFB (Lambdin & Grant, 1999). However, in that study, oak forests (the most prevalent habitat on Arnold AFB) were just one of many habitats that were monitored. Importantly, the previous study did not include information on potential prey of any bat species.

The previous insect diversity study at Arnold AFB was focused on the unique Sinking Pond habitat (Lambdin & Grant, 1999 & Vlach et al., 2010). More than 13,000 insects were collected belonging to 19 different orders (coleopterans the most abundant) and 193 families, with a Shannon diversity index score of 4.98 (high diversity) and evenness recorded at 0.73 (moderately high). These sampling efforts resulted in the discovery of several insect species that were new state records, including the trichopteran *Wormaldia shawnee* (Ross) (Lambdin & Grant, 1999 & Vlach et al., 2010). In addition, four endangered or threatened species and 13 introduced species were identified and recorded at Sinking Pond (Vlach et al., 2010).

The previous insect diversity study also specifically listed hemipteran families found at all sampling sites (Lambdin & Grant, 1999 & Lambdin et al., 2003). It is important to note that at the time of the previous study, the Order Hemiptera was not combined with the Order Homoptera, so traditional homopterans were not included with Hemiptera in that study. Hemipterans were considered particularly important for plant damage given their piercing-sucking mouthparts and life history traits (Schaefer & Panizzi, 2000; Schuh & Slater, 1995). In the insect diversity study, species were sampled using nine collection methods (beat sheet, canopy fogging, direct hand-picking or aerial netting, leaf litter, light trap, Malaise trap, Manitoba trap, pitfall trap, and sweep-net). In total, 1,360 hemipteran specimens were collected, representing 97 species in 22 families. Four families (Pentatomidae, Lygaeidae, Reduviidae, and Coreidae) constituted 50% of all species collected, other major hemipteran families included families Miridae and Tingidae (Lambdin et al., 2003).

Research on insect diversity and abundance is urgently needed, especially as climate change, environmental change, increased globalization, and transportation (allowing movement of insects and pests) continue to pose threats to ecosystem health. Concerns about global insect loss continue to rise (Wagner et al., 2021; Donkersley et al., 2022). Global warming and climate change may affect insects negatively, such as changing insect-host plant interactions, affecting the synchrony between insect pests and their predators and parasitoids, shifting insect biodiversity, driving some species to extinction, and reducing effectiveness of plant protection management strategies. It may be expected that changes in climate will cause both temporal and spatial mismatches between plants and herbivores, plants and pollinators, and hosts and parasitoids (Mamay & Simsek, 2017). A survey of about 1,100 insect species revealed that climate change due to global warming may drive 15–37% of those species to extinction by 2050

(Thomas et al., 2004). In the United States, global insect loss hypothesis is disputed, but more studies are needed to confirm these trends (Crossley et al., 2020). With increased pressures from abiotic and biotic stressors, insect monitoring and diversity studies are needed, especially when monitoring relationships between predators and prey.

Habitat and management strategies are known to influence insect populations, and Arnold AFB has many identified habitats and management practices in it ~162 km area. Personnel at Arnold AFB continue to manage the Barrens habitat that ranges from grassland/shrubland to savanna/woodland. Management of these lands includes activities such as seasonal prescribed burning, logging, mowing, and recreational use for hunting. Studies have examined the prevalence of bat foraging activity along habitat gradients and vegetation ‘clutter’, including edge habitat; this work showed that differences in wing morphology of bats relates to different habitat use strategies (Loeb & Waldrop, 2008). Bat species with short, rounded wings, e.g., tricolored bat, are more maneuverable and tend to forage in cluttered environments, while bat species with long pointed wings, e.g., big brown bat, are more suited to open areas and edges (Caldwell et al., 2019). In a synthesis of available literature, Loeb (2020) found that a reduction in clutter as a result of thinning and fire had neutral to positive effects for clutter, edge, and open space-adapted species. However, Perry (2011) suggested that differential prey abundance/diversity may be a contributing factor to bat use. Research to compare arthropod diversity along the gradients of habitats and their ‘clutter’ is needed to provide information on availability of prey for bats in different seral stages and the applicability of fire management to benefit foraging bat species.

A one-year cooperative arthropod diversity study with the University of Tennessee and Arnold AFB was initiated in 2022 to monitor arthropod populations at 30 sites in multiple habitat types (edge burn sites, fields, upland forest, forested streams, pine stands, forested wetlands, etc.). Nine sampling techniques (pitfall traps, SLAM traps, beat-sheets, direct observations, sweep netting, Tullgren funnels, light traps, tick-dragging, and carbon dioxide traps) were used to collect arthropod specimens. The overall goal of that survey was to provide an up-to-date record of arthropod diversity and abundance at Arnold AFB throughout the year. This overall diversity project provides a unique opportunity for a research project to examine the predator-prey relationship.

The goal of the current project, an extension of the overall diversity project, is to examine the relationship between nocturnal and crepuscular insect diversity, prey availability, and abundance in relationship to bat activity in multiple habitats and examine specifically the role of fire on prey of foraging bat species. For the purposes of this study prescribed burning will be the most important management strategy considering its known impacts on foliage-roosting bats and insect populations (Cryan & Veilleux 2007). It is also important to include average biomass and weight categories of insect families so they can be compared to the frequency of insect families, seasonality of the insects and PCR analysis of fecal samples collected from bats on the base. Considering the nearly 25 years that has passed since the original insect study (Lambdin & Grant, 1999) and the increased anthropogenic and environmental factors, an insect diversity study is needed to assess prey availability, diversity, and biomass for bats on Arnold AFB.

Research Objectives

Little is known about the insect abundance and diversity relationship to the primary bat species in Tennessee specifically at Arnold AFB, an area protected by the United States Department of Defense on the Eastern Highland Rim. A current assessment of insect abundance and diversity throughout the year in this area is integral to gaining additional understanding of bat foraging behavior and predator/prey relationships. This information is crucial with proliferation of WNS, changing climate and increased movement of invasive species considering WNS.

The primary focus of this study was to determine insect diversity and abundance at night on Arnold AFB and the relationship of insects to bat species on Arnold AFB with mist netting and acoustic monitoring. This research will inform foraging behaviors of common bat species in middle Tennessee and better understand the role of insects in bat foraging and bat presence.

Specifically, the research objectives of this study were to:

1) Determine how habitat, season, and management decisions affect night prey (insect) abundance and diversity at Arnold AFB.
2) Determine how bat presence, monitored through mist nets, is related to insect abundance and diversity found at Arnold AFB.
3) Determine how bat presence, monitored through acoustic activity, is related to insect abundance and diversity found at Arnold AFB.

Literature Cited

Agosta, S. J., Morton, D., & Kuhn, K. M. (2003). Feeding ecology of the bat *Eptesicus fuscus*: ‘preferred’ prey abundance as one factor influencing prey selection and diet breadth. *Journal of Zoology*, *260*(2), 169-177.

Amelon, S. K., Thompson III, F. R., & Millspaugh, J. J. (2014). Resource utilization by foraging eastern red bats (*Lasiurus borealis*) in the Ozark Region of Missouri. *The Journal of wildlife management*, *78*(3), 483-493.

Barnes, B. M., & Carey, H. V. (Eds.). (2004). *Life in the Cold: Evolution, Mechanisms, Adaptations, and Application, Twelfth International Hibernation Symposium, 25 July to 1 August 2004*. Institute of Arctic Biology, University of Alaska Fairbanks.

Bell, G. P. (1980). Habitat use and response to patches of prey by desert insectivorous bats. *Canadian Journal of Zoology*, *58*(10), 1876-1883.

Bender, M. J., Perea, S., Castleberry, S. B., Miller, D. A., & Wigley, T. B. (2021). Influence of insect abundance and vegetation structure on site-occupancy of bats in managed pine forests. *Forest Ecology and Management*, *482*, 118839.

Bernard R. F., & McCracken G.F. (2017). Winter behavior of bats and the progression of white-nose syndrome in the southeastern United States. *Ecology and Evolution.*, *7*(5), 1487-1496. doi:10.1002/ece3.2772.

Bernard, R. F., Willcox, E. V., Parise, K. L., Foster, J. T., & McCracken, G. F. (2017). White-nose syndrome fungus, Pseudogymnoascus destructans, on bats captured emerging from caves during winter in the southeastern United States. *BMC Zoology*, *2*, 1-11.

Best, T. L., & Hudson, M. K. (1996). Movements of gray bats (*Myotis grisescens*) between roost sites and foraging areas. *Journal of the Alabama Academy of Science*, *67*(1), 6-14.

Boyles, J. G., Timpone, J. C., & Robbins, L. W. (2003). Late-winter observations of red bats, *Lasiurus borealis*, and evening bats, *Nycticeius humeralis*. *Missouri. Bat Research News*, *44*, 59-61.

Brack Jr, V., & LaVal, R. K. (2006). Diet of the gray myotis (*Myotis grisescens*): variability and consistency, opportunism, and selectivity. *Journal of Mammalogy*, *87*(1), 7-18.

Brady, J. (1982). *Gray Bat Recovery Plan*. U.S. Fish and Wildlife Service, Washington, D.C.Brigham, R. M. (1991). Flexibility in foraging and roosting behaviour by the big brown bat (*Eptesicus fuscus*). *Canadian Journal of Zoology*, *69*(1), 117-121.

Brigham, M. R., & Saunders, M. B. (1990). The diet of big brown bats (*Eptesicus fuscus*) in relation to insect availability in southern Alberta, Canada. *Northwest Science*, *64*(1). 7-10.

Caldwell, K. L., Carter, T. C., & Doll, J. C. (2019). A comparison of bat activity in a managed central hardwood forest. *The American Midland Naturalist*, *181*(2), 225-244.

Campbell, J. (2019). Tennessee winter bat population and white-nose syndrome monitoring report for 2018–2019. *Tennessee Wildlife Resources Agency Wildlife Technical Report*, 16-4.

Carr, J.A., Bernard, R.F., & Stiver, W.H., (2014). Unusual bat behavior during winter in Great Smoky Mountains National Park. *Southeastern naturalist*, *13*(2). doi:10.1656/058.013.0211

Cheng, T. L., Gerson, A., Moore, M. S., Reichard, J. D., DeSimone, J., Willis, C. K., ... & Kilpatrick, A. M. (2019). Higher fat stores contribute to persistence of little brown bat populations with white-nose syndrome. *Journal of Animal Ecology, 88*(4), 591-600.

Clare, E. L., B.R. Barber, B.W. Sweeny, P.D. Hebert, & M.B. Fenton. (2011). Eating local: influences of habitat on the diet of little brown bats *(Myotis lucifugus). Molecular Ecology,* doi: 10.1111/j.1365-294X.2011.05040.x.

Clare, E. L., Fraser, E. E., Braid, H. E., Fenton, M. B., & Hebert, P. D. (2009). Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. *Molecular Ecology*, *18*(11), 2532-2542.

Crossley, M. S., Meier, A. R., Baldwin, E. M., Berry, L. L., Crenshaw, L. C., Hartman, G. L., ... & Moran, M. D. (2020). No net insect abundance and diversity declines across US Long Term Ecological Research sites. *Nature Ecology & Evolution*, *4*(10), 1368-1376.

Cryan, P. M., & Veilleux, J. P. (2007). Migration and use of autumn, winter, and spring roosts by tree bats. *Bats in Forests. Johns Hopkins University Press, Baltimore, MD*, 153-175.

de Jong, J., Millon, L., Håstad, O., & Victorsson, J. (2021). Activity pattern and correlation between bat and insect abundance at wind turbines in south Sweden. *Animals*, *11*(11), 3269.

Deeley, S., Kang, L., Michalak, P., Hallerman, E., & Ford, W. M. (2023). DNA Metabarcoding-based Evaluation of the Diet of Big Brown Bats (*Eptesicus fuscus*) in the Mid-Atlantic Region. *Northeastern Naturalist*, *29*(4), 454-473.

Donkersley, P., Ashton, L., Lamarre, G. P., & Segar, S. (2022). Global insect decline is the result of wilful political failure: A battle plan for entomology. *Ecology and Evolution*, *12*(10), e9417.

Dunbar, M. B., & Tomasi, T. E. (2006). Arousal patterns, metabolic rate, and an energy budget of eastern red bats *(Lasiurus borealis*) in winter. *Journal of Mammalogy*, *87*(6), 1096-1102.

Dunbar, M. B., Whitaker, J. O., & Robbins, L. W. (2007). Winter feeding by bats in Missouri. *Acta Chiropterologica*, *9*(1), 305-310.

Electric Power Research Institute. (2021). (tech.). *Fat Bats: Research and Management Practices for Enhancing Foraging Habitat to Help Support Bats with White-Nose Syndromes*. Retrieved from https://www.epri.com/research/products/000000003002021572.

Feldhamer, G. A., Carter, T. C., & Whitaker, J. O. (2009). Prey consumed by eight species of insectivorous bats from southern Illinois. *The American Midland Naturalist*, *162*(1), 43-51.

Fenton, M. B. (1997). Science and the conservation of bats. *Journal of Mammalogy*, *78*(1), 1-14.

Gargas, A., Trest, M. T., Christensen, M., Volk, T. J., & Blehert, D. S. (2009). *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. *Mycotaxon, 108(1), 147-154.*

Geluso, K., Damm, J. P., & Valdez, E. W. (2008). Late-seasonal activity and diet of the evening bat (*Nycticeius humeralis*) in Nebraska. *Western North American Naturalist*, *68*(1), 21-24.

Hamilton, I. M., & Barclay, R. M. (1998). Diets of juvenile, yearling, and adult big brown bats (*Eptesicus fuscus*) in southeastern Alberta. *Journal of Mammalogy*, *79*(3), 764-771.

Harvey, M. J., Altenbach, J. S., & Best, T. L. (1999). *Bats of the United States*. Arkansas Game & Fish Commission. doi: 10.1656/058.013.0211

Hayes, C. R., Gumbert, M., Whitaker, J. O., Elliott, C. L., & Dodd, L. E. (2019). Spatial and temporal variation in the diet of the eastern red bat in Kentucky. *Journal of the Kentucky Academy of Science*, *79*(1), 12-17.

Hein, C. D., Miller, K. V., & Castleberry, S. B. (2009). Evening bat summer roost-site selection on a managed pine landscape. *The Journal of Wildlife Management*, *73*(4), 511-517.

Holliday, C., Wisby, J. P., Roby, P. L., Samoray, S. T., & Vannatta, J. M. (2023). Modeling migration and movement of gray bats. *The Journal of Wildlife Management*, *87*(3), e22364.

Hoyt, J. R., Langwig, K. E., Sun, K., Parise, K. L., Li, A., Wang, Y., ... & Kilpatrick, A. M. (2020). Environmental reservoir dynamics predict global infection patterns and population impacts for the fungal disease white-nose syndrome. *Proceedings of the National Academy of Sciences*, *117*(13), 7255-7262.

Hoyt, J. R., Kilpatrick, A. M., & Langwig, K. E. (2021). Ecology and impacts of white-nose syndrome on bats. *Nature Reviews Microbiology*, *19*(3), 196-210.

Hughes, M. J., de Torrez, E. C. B., & Ober, H. K. (2021). Big bats binge bad bugs: Variation in crop pest consumption by common bat species. *Agriculture, Ecosystems & Environment*, *314*, 10

Ingala, M. R., Simmons, N. B., Wultsch, C., Krampis, K., Provost, K. L., & Perkins, S. L. (2021). Molecular diet analysis of neotropical bats based on fecal DNA metabarcoding. *Ecology and Evolution*, *11*(12), 7474-7491.

Jedlicka, J. A., Sharma, A. M., & Almeida, R. P. (2013). Molecular tools reveal diets of insectivorous birds from predator fecal matter. *Conservation Genetics Resources*, *5*(3), 879-885.

Kentucky Department of Fish & Wildlife. (2024). Gray Bat. *Kentucky.gov* Retrieved February 12, 2024, from *https://fw.ky.gov/Wildlife/Pages/Gray-Bat.aspx.*

Kitchell, M. (2022). *Proposal to list the tricolored bat as Endangered: U.S. Fish & Wildlife Service*. FWS.gov. Retrieved January 9, 2023, from https://www.fws.gov/press-release/2022-09/proposal-list-tricolored-bat-endangered.

Krusic, R. A., Yamasaki, M., Neefus, C. D., & Pekins, P. J. (1996). Bat habitat use in white mountain national forest. *The Journal of Wildlife Management*, 625-631.

Kunz, T. H., & Whitaker Jr, J. O. (1983). An evaluation of fecal analysis for determining food habits of insectivorous bats. *Canadian Journal of Zoology = Revue Canadienne de Zoologie.*, *61*(6), 1317–1321. https://doi.org/10.1139/z83-177

Kurta, A. (2001). Bats on the surface: the need for shelter, food, and water. *Bat conservation and mining*, 197-204.

Lacki, M. J., Burford, L. S., & Whitaker Jr, J. O. (1995). Food habits of gray bats in Kentucky. *Journal of Mammalogy*, *76*(4), 1256-1259.

Lamb, J. (2022). *Trees, caves around Arnold AFB home to variety of bat species*. Arnold Air Force Base. Retrieved May 11, 2022, from https://www.arnold.af.mil/News/Article-Display/Article/3011722/trees-caves-around-arnold-afb-home-to-variety-of-bat-species/.

Lamb, J., (2020) Unpublished. "*Myotis grisescens*, *Myotis septentrionalis*, and *Myotis sodalis* Annual Report. Section 10 Permit TE88778B-2."

Lambdin, P.L., & Grant, J. F. (1999). Rare, Threatened, and Endangered (RTE) Terrestrial Invertebrate Survey. A Technical Report prepared for Arnold Air Force Base.

Lambdin, P. L., Grant, J. F., Wiggins, G. J., & Saxton, A. (2003). Diversity of the true bugs (Hemiptera: Heteroptera) on Arnold Air Force Base, Tullahoma, Tennessee. *Journal of the Tennessee Academy of Science*, *78*(3), 76-85.

Lausen, C. L., & Barclay, R. M. R. (2006). Winter bat activity in the Canadian prairies. *Canadian Journal of Zoology*, *84*(8), 1079-1086.

LaVal, R. K., & LaVal, M. L. (1979). Notes on reproduction, behavior, and abundance of the red bat, *Lasiurus borealis*. *Journal of Mammalogy*, *60*(1), 209-212.

Layne, J. T. (2009). *Eastern red bat (Lasiurus borealis) response to fire stimulus during torpor* (Doctoral dissertation, Missouri State University).

Loeb, S. C., & Waldrop, T. A.. (2008). Bat activity in relation to fire and fire surrogate treatments in southern pine stands. *Forest Ecology and Management,* 255: 3185-3192.

Loeb, S. C., & Winters, E. A. (2013). Indiana bat summer maternity distribution: effects of current and future climates. *Ecology and Evolution*, *3*(1), 103-114.

Loeb, S.C. (2020). Qualitative synthesis of temperate bat responses to silvicultural treatments-where do we go from here? *Journal of Mammalogy, 101(6):*1- 13.

Mamay, M., & Şimşek, E. (2017). The Expected Impact of Global Warming and Climate Change on Insect Diversity. *The 3rd International Symposium on EuroAsian Biodiversity*, pp. (173).

McCracken, G. F., Westbrook, J. K., Brown, V. A., Eldridge, M., Federico, P., & Kunz, T. H. (2012). Bats track and exploit changes in insect pest populations. *PLoS One 7(8)*: e43839. https://doi.org/10.1371/journal.pone.0043839

Meteyer, C. U., Buckles, E. L., Blehert, D. S., Hicks, A. C., Green, D. E., Shearn-Bochsler, V., ... & Behr, M. J. (2009). Histopathologic criteria to confirm white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation*, *21*(4), 411-414.

Minnis, A. M., & Lindner, D. L. (2013). Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biology*, *117*(9), 638-649.

Moorman, C. E. (1999). Bats roosting in deciduous leaf litter. *Bat Research News*, *40*, 74-75.

Noriega, J. A., Hortal, J., Azcárate, F. M., Berg, M. P., Bonada, N., Briones, M. J., ... & Santos, A. M. (2018). Research trends in ecosystem services provided by insects. *Basic and Applied Ecology*, *26*, 8-23.

O’Keefe, J. M., Pettit, J. L., Loeb, S. C., & Stiver, W. H. (2019). White-nose syndrome dramatically altered the summer bat assemblage in a temperate Southern Appalachian forest. *Mammalian Biology*, *98*(1), 146-153.

O'Reilly, K. (2019). Meet "team fat bat"-scientists try to save victims of white-nose syndrome. Sierra Club. Retrieved October 31, 2022, from https://www.sierraclub.org/sierra/slideshow/meet-team-fat-bat-scientists-try-save-victims-white-nose-syndrome

Pierson, E. D., & W E. Rainey. (1998). Distribution, Habitat Associations, Status, and Survey Methodologies for Three Molossid Bat Species (*Eumopsperotis, Nyctinomopsfemorosaccus, Nyctinomops macrotis)* and the vespertilionid *Euderma maculatum*. Final Report, Contract #FG2328WM, California Department of Fish and Game, Sacramento, USA.

Perry, R. W. (2011). A Review of Fire Effects on Bats and Bat Habitat in the Eastern Oak Region. In *Proceedings of the 4th fire in eastern oak forests conference* (pp. 170-191).

Piñol, J., Mir, G., Gomez-Polo, P., & Agustí, N. (2015). Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. *Molecular Ecology Resources*, 15, 819–830. https://doi.org/10.1111/1755-0998.12355

Powers, K. E., Reynolds, R. J., Orndorff, W., Hyzy, B. A., Hobson, C. S., & Ford, W. M. (2016). Monitoring the status of gray bats (*Myotis grisescens*) in Virginia, 2009–2014, and potential impacts of white-nose syndrome. *Southeastern Naturalist*, *15*(1), 127-137.

Samoray, S. (2015). *Roosting Ecology of Indiana, Northern Long-eared, Little Brown, and Tri-colored Bats on Arnold Air Force Base: A Technical Report for Arnold Air Force Base, Tennessee*. Copperhead Consulting Inc.

Samoray, S. T., Patterson, S. N., Weber, J., & O'keefe, J. (2020). Gray bat (*Myotis grisescens*) use of trees as day roosts in North Carolina and Tennessee. *Southeastern Naturalist*, *19*(3), N49.

Schowalter, T. D. (2022). *Insect Ecology: An Ecosystem Approach*, 5th Ed., Academic Press.

Shashank, P. R., Naveena, N. L., Rajgopal, N. N., Elliott, T. A., Sreedevi, K., Sunil, S., & Meshram, N. M. (2022). DNA barcoding of insects from India: Current status and future perspectives. *Molecular Biology Reports*, *49*(11), 10617-10626.

Schaefer, C. W., & Panizzi, A. R. (Eds.). (2000). *Heteroptera of Economic Importance*. CRC press.

Schuh, R. T., & Slater, J. A. (1995). *True Bugs of the World (Hemiptera: Heteroptera): Classification and Natural History*. Cornell University Press.

Shump, K. A., & Shump, A. U. (1982). *Lasiurus borealis*. *Mammalian Species*, *183*, 1-6.

Threlfall, C. G., Law, B., & Banks, P. B. (2012). Influence of landscape structure and human modifications on insect biomass and bat foraging activity in an urban landscape. *PloS One*, *7*(6), e38800.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., ... & Williams, S. E. (2004). Extinction risk from climate change. *Nature*, *427*(6970), 145-148.

Timpone, J. C., Boyles, J. G., & Robbins, L. W. (2006). Potential for niche overlap in roosting sites between *Nycticeius humeralis* (evening bats) and *Eptesicus fuscus* (big brown bats). *Northeastern Naturalist*, *13*(4), 597-602.

Tuttle, M. D. (1979). Status, causes of decline, and management of endangered gray bats. *The Journal of Wildlife Management*, *43* 1-17.

U.S. Fish and Wildlife Service. (2022a). *Endangered and Threatened Wildlife and Plants; Endangered Species Status for Northern Long-Eared Bat*. Federal Register :: Request Access. Retrieved January 9, 2023, from https://www.federalregister.gov/documents/2022/11/30/2022-25998/endangered-and-threatened-wildlife-and-plants-endangered-species-status-for-northern-long-eared-bat.

U.S. Fish and Wildlife Service. (2022b). *U.S. Fish & Wildlife Service White-Nose Syndrome*. the devasting disease of hibernating bats in North America. Retrieved December 7, 2022, from https://s3.us-west-2.amazonaws.com/prod-is-cms-assets/wns/prod/e2e0f150-a606-11ec-81da-ab16efc2070f-White-nose%20fact%20sheet%202022_edit.pdf.

U.S. Fish and Wildlife Service. (n.d.). *Gray bat (Myotis grisescens)*. ECOS. Retrieved January 9, 2023, from https://ecos.fws.gov/ecp/species/6329.

Vlach, J., Lambdin, P., Dilling, C., Grant, J., Paulsen, D., & Wiggins, G. (2010). Diversity of the insect fauna within the unique Sinking Pond habitat. *Journal of the Tennessee Academy of Science*, *85*(3-4), 62-86.

Wagner, D. L., Grames, E. M., Forister, M. L., Berenbaum, M. R., & Stopak, D. (2021). Insect decline in the Anthropocene: Death by a thousand cuts. *Proceedings of the National Academy of Sciences*, *118*(2), e2023989118.

Walters, B. L., Ritzi, C. M., Sparks, D. W., & Whitaker, J. O. (2007). Foraging behavior of eastern red bats (*Lasiurus borealis*) at an urban-rural interface. *The American Midland Naturalist*, *157*(2), 365-373.

Weinkauf, C. J., Comer, C. E., Conway, W. C., & Farrell, C. (2018). Dietary composition of four common chiropteran species in a bottomland hardwood forest. *Acta Chiropterologica*, *20*(1), 195-205.

Wells, M. C. (2022). Bat Diet Characterization at Arnold Air Force Base, Tennessee. Govtribe.com. Retrieved October 31, 2022, from https://govtribe.com/file/government-file/a04-rsoi-arnold-afb-bat-diet-study-21jan2022-dot-pdf.

Whitaker Jr, J. O. (1995). Food of the big brown bat *Eptesicus fuscus* from maternity colonies in Indiana and Illinois. *American Midland Naturalist*, *134*, 346-360.

Whitaker Jr, J. O., & Gummer, S. L. (1992). Hibernation of the big brown bat, *Eptesicus fuscus*, in buildings. *Journal of Mammalogy*, *73*(2), 312-316.

Whitaker Jr, J.O., & Rissler, L.J. (1992). Winter activity of bats at a mine entrance in Vermillion County, Indiana. *American Midland Naturalist, 127*, 52–59.

Appendix

Table 1.1: Bat species documented at Arnold Air Force Base, Tennessee.

Species and Genus	Common Name	Species Code*	Current Status**	WNS Been Found in Species?
Myotis grisescens	Gray Bat	MYGR	Endangered	Yes
Perimyotis subflavus	Tricolored Bat	PESU	Under Review	Yes
Myotis lucifugus	Little Brown Bat	MYLU	Under Review	Yes
Eptesicus fuscus	Big Brown Bat	EPFU	Least Concern	Yes
Lasiurus borealis	Eastern Red Bat	LABO	Least Concern	No
Lasiurus cinereus	Hoary Bat	LACI	Least Concern	No
Myotis sodalis	Indiana Bat	MYSO	Endangered	Yes
Myotis septentrionalis	Northern Long-Eared Bat	MYSE	Endangered	Yes
Nycticeius humeralis	Evening Bat	NYHU	Least Concern	No

*'Species Code' is provided because it will appear in reference to bat species in figures in later chapters where listing the full scientific or common name is not appropriate for space.

** 'Current Status' refers to the current federal listing of each species by International Union for Conservation of Nature including extinct, critically endangered, endangered, vulnerable, near threatened, least concern, and under review (listing is currently pending, and population changes has warranted a potential new listing).

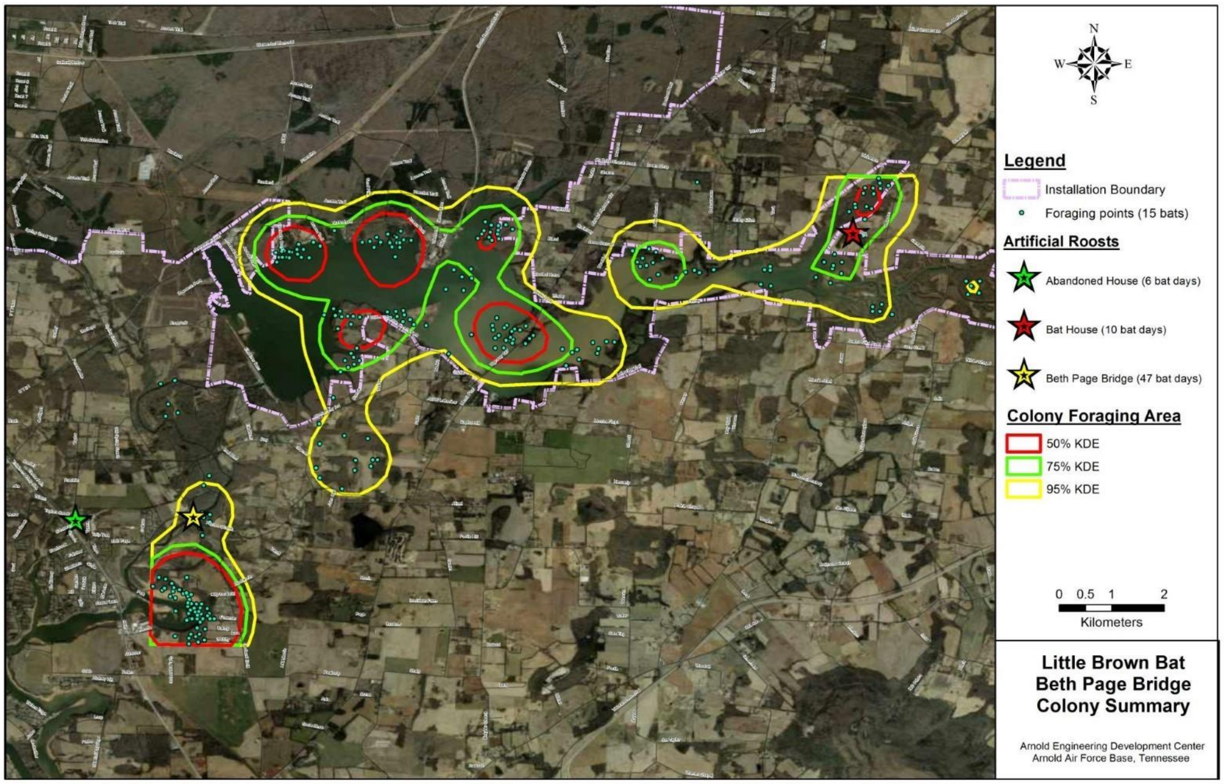

Figure 1.1. Roosting and foraging sites of the little brown bat near Arnold Air Force Base, TN, 2014 (from Samoray, 2015).

CHAPTER II

Seasonal Abundance and Diversity of Crepuscular and Nocturnal Insects at Arnold Air Force Base

Introduction

Insect populations, abundance, and diversity are essential variables in ecosystem balance as drivers of nutrient cycling, seed dispersal, biological control of pests, and pollination services, as well as often important indicators of ecosystem health as they help to modulate food webs (Macadam, 2015; Noriega et al., 2018; Schowalter, 2022; Yang & Gratton, 2014). Insects also serve as major food sources for many organisms, including fish, birds, bats, and even large mammals, such as bears and cattle (Beeman & Pelton, 1980; Fadila et al., 2020; Gunther et al., 2014; Shields & Bildstein, 1979; Tran et al., 2015). This relationship between predator and prey is becoming critical for insectivorous bats who rely on insects for fat storages to survive during torpor periods; this need is heightened by the effects of white nose syndrome (WNS) (Cheng et al., 2019; Warnecke et al., 2012).

WNS is a disease caused by a fungal pathogen, *Pseudogymnoascus destructans*, that infects bats and depletes fat storage causing millions of bats to starve to death since its introduction into the U.S. (Minnis et al., 2013; U.S. Fish and Wildlife Services, 2022). Insects are the sole source of food for many species of bats, but the nutrients and calories gained from each prey item is just as diverse as the organisms themselves varying by species, size, and nutrition (Chowdhury et al., 2017; Hawkey et al., 2021).

To understand the availability of prey as dietary resources for bats, arthropod diversity surveys have centered traditionally around diurnal active arthropods, with most sampling conducted in summer months (Didham et al., 2010; Gadagkar et al., 1990; Lambdin & Grant, 1999). Conversely, arthropod diversity studies focused on arthropod species active in nocturnal and crepuscular periods are largely lacking but nevertheless critically vital to better understand the factors affecting this relationship (Morningstar et al., 2019). These gaps in literature create a strong need for arthropod surveys conducted year-round focused on nocturnal and crepuscular populations as arthropod populations are known to differ through temporal and diurnal periods (Evans & Murdoch; 1968; Guevara & Avilés, 2013).

Therefore, the objective of this study was to determine how arthropod diversity, abundance, and biomass change based on habitat, season, and prescribed burning. This study was focused on nocturnal and crepuscular arthropods at Arnold Air Force Base (AFB) in middle Tennessee. I hypothesized that the composition of arthropods will differ by season and habitat type at Arnold AFB.

Materials and Methods

Insect Sampling

To assess the diversity and abundance of arthropods available for bats as food sources at Arnold AFB, seven sampling sites were predetermined by Arnold AFB biologist John Lamb. The seven sampling sites included four types of habitats, including one mixed deciduous and conifer forest, one mixed forest and wetland, four mixed forest and stream/creek, and one edge (mixed forest transition to grassland) in 2022 and 2023 (Figure 2.1 and Table 2.1). The edge site received prescribed burning treatments in March 2022 and 2023 and is hereafter referred to as edge (burn).

For each sampling event, one ultraviolet (UV) light trap (Figure 2.2) and CDC light trap (John W Hock company) (CO_2) trap (Figure 2.3) powered by 12-volt batteries were deployed at dusk at each site for six hours starting at dusk (time depended on time of year). UV light traps emitted wavelengths from 100-400 nm. The bulb in the UV light trap was located directly above the collection chamber and attached to a metal bucket with a funnel top. Two Hot Shot No Pest insect kill strips were placed inside the bottom of the metal bucket (separated from the rest of bucket by metal mesh) (Figure 2.2).

CDC light traps were made from a battery-operated fan with a plastic lid (30.5 cm diameter) attached above the fan, a plastic chamber attached by fabric underneath the fan (to funnel arthropods into chamber), and a plastic 3.8-liter cooler with holes drilled in it (Figure 2.3). Plastic Igloo coolers were filled with 0.23 kg of dry ice for each sampling event and tied next to fans. Dry ice in ambient temperatures sublimates into carbon dioxide (mimicking animals' exhalations) and is used to attract hematophagous insects. After six hours, samples were collected and the specimens were removed, placed in 3.8-liter Ziplock bags placed in a large cooler, and taken to the laboratory. Samples were stored in a refrigerator until they could be sorted and identified to family at minimum (using keys from Marshall 2006).

After each sample was collected, they were sorted to order (using keys from Marshall, 2006) and weighed (in grams) to determine the dry weight (in grams) (i.e., dry biomass) of each insect order. Once the biomass of each order was calculated, specimens in each order were then sorted to family, if not to a lower taxonomic group, and each specimen was weighed and assigned a classification based on weight. I then created weight classifications of each group, and these consisted of: Mega (>1.0 g), Macro (1.0-0.1 g), Standard (0.1-0.011 g), and Micro (< 0.01 g). This method allowed for biomass to be weighed and collected on an order level and on an individual specimen level. All specimens collected from the 13 samples were identified to at least family level. After specimens were identified to family, they were stored in 95% ethanol in vials (10 ml). Lepidopteran specimens were stored dry in Petri-dishes (100x15 mm), and Culicidae were sorted, stored in Petri-dishes (100x15 mm) (to preserve scales for future further identification) and placed in the refrigerator. Representative samples were spread and pinned as needed for voucher specimens.

Sampling began in July 2022 and was conducted every month until August 2023. All seven sites were visited at random. Seasonal periods included summer, fall, winter, and spring, and these periods were determined by equinox and solstice events. Three fall sampling events occurred four, three, and two days before the fall equinox, but substantial temperature declines and foliage decline had already occurred (Table 2.2).

Data Analysis

From the samples collected from 27 UV light traps and 27 corresponding CDC light traps from July 2022 to August 2023. Of the 27 sites, samples from 2 were not included in analysis (Table 2.2). Of the two samples not included in analysis, one sample was excluded from data analysis due to inclement weather and one sample did not contain any specimens because it was collected 10 days following a prescribed burn treatment. For all analyses, one sample corresponds to one UV light trap and CDC light trap operated at the same time and locality.

For all data analysis, figures, models, and statistics were constructed using R version 4.2.3. Arthropod sampling curves were computed using R package VEGAN and the rarefy function to calculate richness. Insect biomass and insect abundance were averaged across habitat and season to determine the fluctuation in insect orders throughout the year over all habitats. One way Analysis of Variance (ANOVA) tests followed by Tukey Honest Significant Differences (HSD) pairwise comparisons were used to determine the relationship between total insect abundance and habitat and season. It is important to note that for ANOVA tests fall and winter were separated for analysis but combined for visual comparison due to low sample sizes (Figure 2.12).

An analysis of variance was performed using GLMs to determine predictors for major insect order abundance. Insect orders chosen to be modeled were abundant or had high biomasses throughout the sampling period. A full model was developed to predict insect order abundance as a function of month (January- December), Season (Summer, Fall, Winter, and Spring), habitat (Forested, Forested Streams, Forested Wetlands, Edge (Burn)), and the interaction term between habitat and season. Therefore, the full model was insect order abundance~ Month + Season + Habitat + Habitat:Season. Models were compared to the null model, insect order abundance ~1. The best fit models were selected by lowest Akaike Information Criterion corrected (AICc) value, and if two models had the lowest AICc value then the most conservative model was selected and had to be significantly different from the null model, by having a delta AICc of 2 or greater (Anderson & Burnham, 2004). Variables selected as significant predictors had 95% confidence intervals that did not cross zero and a p-value of less than 0.05.

To examine insect abundance temporally for the four dominant habitats at Arnold AFB, insect order abundance was taken by month of sampling date and graphed. To understand the implications of insect orders as a food source, order biomasses were averaged by month to provide more information about the relevant biomass available for foraging bats. For each insect order overviewed (selected for abundance or importance in bat diets) the 10 most abundant families for all samples were identified. Shannon's diversity index and richness were calculated for each sample with R package VEGAN. To examine the effect of the sampling effort in relationship to total families, insect family curves were constructed using the VEGAN package in R and graphed together using ggplot. Finally, for each seasonal period and for each habitat type, tables were constructed to display the most abundant insect orders and families in each habitat type within each seasonal period on Arnold AFB. These tables excluded mixed forested sites in the winter due to a severe weather event and an edge (burn) site in the spring due to the lack of specimens after the prescribed burn treatment.

Results and Discussion

The relationship between insect family richness and total abundance of insects for all 25 samples is shown in Figure 2.4. For most samples, an increase in insects collected positively correlated (with a spearmen correlation < 0.6) to an increase in the number of families found in the sample; these individual relationships are expressed by each exponential curve (Figure 2.4). The smallest sample size (n=11 individuals) was found in a sample collected in December 2022 with the most abundant sample size (n=2,938 individuals) collected in July 2022 (Figure 2.4).

To understand the abundance of insects collected by order for each sample, individual samples were compiled to provide an overall view of each sampling event in relationship to order abundance (Figure 2.5). This comparison illustration shows the orders with the highest abundance throughout the year from each individual sampling event. Each sample is a combination of the collections from the UV light trap and corresponding carbon-dioxide trap at each site. Samples are ordered chronologically from July 2022 to August 2023.

Trends in average seasonal abundance by month for major insect orders on Arnold AFB are shown in Figure 2.6, 2.7. Figures 2.8, 2.9, and 2.10 show the distribution of major insect order abundance in 4-month periods. From January to March, Diptera was the most abundant insect order in all samples followed by Lepidoptera (Figure 2.6 & 2.8). From April to mid-May, Trichoptera was the most abundant order on the base, followed by Diptera and Plecoptera (Figure 2.6, 2.8, and 2.9). From late May through mid-September, Coleoptera was the most abundant order followed by Hemiptera (including Homoptera and Hemiptera), Lepidoptera, and Diptera (Figure 2.6, 2.9, and 2,10). Beginning in late September, Lepidoptera was the most abundant order, then Diptera and Coleoptera (Figure 2.6 & 2.10). In October Diptera was the most abundant order closely followed by Lepidoptera (Figure 2.6 & 2.10). Diptera remained the most abundant order from late fall to early spring (October-March) (Figure 2.6, 2.8, and 2.10). Based on these results, order abundance is closely linked to the month of sampling and seasonal periods. These data illustrate the possible shifts in diets of bats foraging on Arnold AFB based on insect order abundance (Figure 2.6, 2.8, 2.9, and 2.10). Sampling suggests increased numbers of ephemeral aquatic orders in spring and early summer (Trichoptera and Plecoptera), and high numbers of Coleoptera, Hemiptera, and Lepidoptera in mid-summer months. In late summer and early fall, before typical hibernation periods for bats, we see Lepidoptera and Diptera become most prominent and therefore likely to be reflected in bat diets. Dipterans are likely to be a constant food source throughout the year for foraging bats given their relative abundance in colder weather and relatively high abundance. Dipteran populations in each summer sample were over 100 individuals and dipterans represented 30-73% of total insects collected in each fall, winter, and spring sample.

Seasonal trends in insect order biomass allow for another metric besides abundance and provide a more holistic view of the availability of insects as a food source for bats at Arnold AFB (Figure 2.11). Starting in January, similar to the abundance of insect orders (Figure 2.6), Diptera and Lepidoptera had the highest biomass until April (Figure 2.11). In relationship to biomass, Trichoptera and Plecoptera had much lower biomass despite their abundance in April and May, while Coleoptera, Diptera, and Hemiptera had higher biomasses (Figure 2.11). After May, Coleoptera, Hemiptera, Diptera, and Lepidoptera had the highest biomass similar to their abundance, but high biomass is extended through November with the biomass of Coleoptera higher than both Diptera and Lepidoptera (Figure 2.11). In November through March, Diptera and Lepidoptera did not have the highest biomass despite having the highest abundances (Figure 2.6 and 2.11). It is also important to note that in mid-summer, other orders such as Orthoptera, Megaloptera, and Blattodea, had relatively high biomass per individual (Figure 2.11) although they were not as abundant as other orders. It is important to note that Blattodea, Orthoptera, and Megaloptera have not been considered important in bat diets (Best et al.,1997; Moosman et al., 2012). Biomass provides an alternative to understand the foraging strategy of bats not solely based around prey abundance or richness.

The average number of insects collected by habitat and season is shown in Figure 2.12. One way ANOVA test and Tukey HSD determined that habitat did not significantly influence total insect abundance ($p= 0.1$), but season did have a significant relationship with total insect abundance ($p<0.0001$). Summer had significantly higher populations of insects compared to Spring ($p=0.001$), Fall ($p=0.004$), and Winter ($p=0.0003$). No other seasons were significantly different from one another. Though habitat type and other seasonal periods were not significantly correlated to insect abundance, numerical differences in insect abundance by habitat were displayed across seasonal periods. In summer, insect abundance generally was highest in forested stream sites and lowest in edge (burn) sites. For fall and winter samples, forested wetlands had the lowest average number of insects and forested streams, and forested sites had the highest. Habitats were relatively comparable for spring season excluding edge (burn) site which had received a prescribed burning treatment prior to sampling. These data suggest that forested wetlands at Arnold AFB suffer major population declines from late summer into the fall and winter months, and that other habitats, such as forested sites, have slightly less population declines. By contrast, insect abundance was most evenly distributed during spring collections across all habitat types surveyed.

The results for predicting major insect order abundance are discussed below under each major insect orders section and variables selected for each order as predictor variables can be seen in Table 2.4.

Coleoptera

Coleoptera were abundant at Arnold AFB especially in mid-summer (Figure 2.7) and their average biomass was also high in spring through early fall (Figure 2.11). The most collected families, in order of abundance, were Staphylinidae, Heteroceridae, Carabidae, Hydrophilidae, Scarabaeidae, Anthicidae, Dytiscidae, Elateridae, Scirtidae, Noteridae, and Curculionidae.

The best-fit model for predicting Coleoptera abundance was Coleoptera Abundance~ Month+ Habitat+ Habitat:Season. Variables that were deemed significant ($p < 0.05$) included months of January ($p = 0.04$) and March ($p = 0.003$), forested wetland habitat ($p = 0.008$), and the interaction term between forested sites and spring ($p = 0.008$) (Table 2.4).

Coleoptera abundance was lower during the months March and January (Figure 2.7 &Table 2.4). Coleoptera abundance was highest in forested wetlands (Figure 2.13) (Table 2.4). Coleoptera abundance was also greater with forested habitats during spring (Table 2.4). Coleoptera abundance on average was highest in forested wetlands followed respectively by edge (burn) sites, forested streams, and forested sites (Figure 2.13).

Lepidoptera

Lepidoptera had relatively high abundance and biomass throughout the year. The most collected families of Lepidopterans, in order of abundance, were Torticidae, Crambidae, Tineidae, Gelechiidae, Noctuidae, Gracillariidae, Pyralidae, Geometeridae, Erebidae, and Megalopygidae.

The data was best described by the model Lepidoptera abundance~ Month+ Habitat + Season:Habitat. The best-fit model selected significant predictor variables that included the months of May (p=0.002), July (p =0.04), and September (p =0.03), which were the months with the greatest abundance of Lepidopterans (Table 2.4). May had the most positive association with Lepidoptera abundance followed by July and then September (Table 2.4). Data suggest that month of sampling may have a greater influence on Lepidoptera abundance than habitat or the interaction between habitat and season.

Average abundance distributions of lepidopterans change temporally through all habitats on Arnold AFB (Figure 2.7). Lepidopteran abundance was significantly higher in April and remained high through September. As abundance increased, so did average Lepdioptera biomass (Figure 2.11 & Figure 2.7). Average abundance of Lepidoptera varied by habitat type, with forested wetlands having the highest mean abundance, and forested streams having the lowest mean abundance (Figure 2.14). However, though this factor was included in our model, individual habitat types were not deemed as significant factors for predicting Lepidoptera abundance because parameter significance of a $p < 0.05$ and uncertainty in model predictions with 95% confidence intervals.

Diptera

Compared to other insect orders, Diptera populations were high throughout the year, especially in colder weather. The most collected families of Diptera, in order of abundance, were Culicidae, Chironomidae, Cecidomyiidae, Sciaridae, Chaoboridae, Ceratopogonidae, Tipulidae, Psychodidae, Rhagionidae, and Chloropidae.

Our null model, Diptera~1 ($p = 0.005$), was the only plausible which means that neither month, season, nor habitat, were not important predictors of Diptera abundance (Table 2.4). Populations of dipterans remained relatively stable compared to other insect orders temporally and over habitat types (Figure 2.7 & 2.14). This trend highlights the plasticity of this order and suggests that Diptera abundance may remain at consistent levels throughout the year in habitats on Arnold AFB. There was a tendency for higher Diptera abundance in forested streams (Figure 2.15), but the average abundance of dipterans was consistent for all four habitat types.

Hemiptera

Densities and biomass of hemipterans were particularly high in the summer months (Figure 2.11 & Figure 2.7). The most collected hemipteran families, in order of abundance, were Cicadellidae, Cercopidae, Corixidae, Cynidae, Rhyparochromidae, Pentatomidae, Meridae, Lygaeidae, Acanthosomatidae, and Flatidae.

These data were best described by the best-fit model Hemiptera~ Season. In relation to season, summer was the only variable that had a significant (p =0.024) positive correlation with Hemiptera populations (Table 2.4).

Densities of hemipterans increased from May to August (summer) (Figure 2.7). Edge (burn) habitats had the highest average densities of Hemiptera compared to other habitats followed by forested wetlands, forested, and forested streams, respectively (Figure 2.16). However, habitat was not considered a significant indicator of Hemiptera abundance.

Trichoptera

Densities of Trichoptera at times peaked in numbers due to mass emergences. The most collected Trichoptera families, in order of abundance, were Leptoceridae, Philopotamidae, Hydropsychidae, Hydroptilidae, Polycentropodidae, Psychomyiidae, Molannidae, Limnephilidae, Helicopsychidae, and Calamoceratidae.

These data were best described by the model Trichoptera~ Month + Habitat, and month (May) was the only significant (p >0.0001) factor (Table 2.4). The month of May had a positive relationship with Trichoptera abundance (Table 2.4).

The density of individual Trichopterans sharply increased in April and peaked in May, but sharply declined in June (Figure 2.7). This sharp increase in numbers of Trichoptera in May was likely due to a mass emergence event. Although habitat type was not considered significant as a predictor for Trichoptera abundance due to a p<0.05 and lack of confidence in predictor variables. Trichoptera abundance was, on average, much lower than other major insect orders, such as Lepidoptera, Coleoptera, Diptera, and Hemiptera (Figure 2.7). Densities of Trichoptera were consistent across all habitat types compared to other insect orders (Figure 17).

Plecoptera

Plecoptera, like Trichoptera, had relatively low densities and biomass compared to major orders, such as Diptera and Hemiptera, but had large numbers of individuals in certain temporal periods. The most collected Plecoptera families, by abundance, were Perlodidae, Leuctridae, and Nemouridae. More than 90% of Plecopterans collected belonged to the family Perlodidae.

The model selected was Plecoptera~ Month + Habitat. Of the categorical variables in month and habitat, the month of May was the only significant variable with a ($p > 0.0001$) (Table 2.4). The month of May had a positive relationship with Plecoptera abundance (Table 2.4).

Plecoptera densities were almost non-existent throughout most of the year, peaking in May and sharply declining in June. This trend was similar to the trends associated with trichopteran families. Again, this peak in density is likely due to a large emergence event of summer plecopterans. Densities of Plecoptera were, on average, much lower than that of other insect orders, such as Lepidoptera, Coleoptera, Diptera, Hemiptera, and even Trichoptera; plecopterans were found only in April, May, and June samples) (Figure 20 & Figure 21). These data suggest that at Arnold AFB most of the Plecoptera populations are summer families (Figure 2.7). Densities of Plecoptera were highest on average in forested habitats, but overall, average Plecoptera abundance was low across all four habitat types (Figure 2.18). Plecoptera abundance and biomass were closely correlated (Figure 2.7 & Figure 2.11).

Ephemeroptera

Ephemeroptera, like the other ephemeral aquatic insect orders, had relatively lower abundance and biomass compared to major orders such as Diptera and Hemiptera but had large number of individuals at certain times (Figure 2.11, 2.19, & 2.7). Ephemeroptera abundance and biomass were closely correlated (Figure 2.7 & Figure 2.11). Like Trichoptera and Plecoptera, a specific peak was associated with Ephemeroptera abundance. The most collected families of Ephemeroptera, in order of abundance, were Caenidae, Heptagoniidae, Leptophlebiidae, Ephemerellidae, Ephemeridae, Siphlonuridae, Baetidae, and Leptohyphidae.

These data were best described by the model Ephemeroptera~ Month. The month of May had a significant (p=0.04) positive relationship with Ephemeroptera abundance (Table 2.4). May was the only variable with a significant relationship to Ephemeroptera Abundance.

Densities of Ephemeroptera peaked in May through August and were almost non-existent the rest of the year (Figure 2.7). Though habitat was not considered an important predictor, Ephemeroptera abundance tended to vary by habitat type (Figure 2.19). For example, forested streams had the highest numbers of Ephemeroptera collected in a single sample, but, on average, ephemeropterans were found most often in forested sites. It is important to note that none of the trends between habitat and Ephemeroptera abundance was significant or included in the prediction model.

Other Arthropod Orders

Nine orders (Blattodea, Megaloptera, Hymenoptera, Neuroptera, Orthoptera, Psocodea, Dermaptera, Araneae, Acari) and one basal hexapod class (Collembola) were collected, but these orders did not have high abundances in the samples or during any temporal period. Blattodea, Megaloptera, and Orthoptera yielded relatively high biomasses compared to their respective abundances, likely due to the large sizes for individual specimens. As numbers were low for these orders, individual models were not developed for these orders; however, all ten taxa are included in models that predict overall insect abundance.

Diversity and Richness

Family diversity (Shannon Diversity Index) and family richness (number of different families per sample) at each sampling site and season are listed in Table 2.3. The Spearman correlation between family diversity and abundance was 0.6073 with a p=0.0047 The Spearman correlation between family diversity and family richness was 0.7030 with p=0.0032. Spearman correlation tests indicated that arthropod family diversity and total arthropod abundance were significantly correlated, and that insect family diversity and insect family richness were also significantly correlated. This trend is important when building models and understanding the relationship of variables to each other, and in analyzing how insect abundance affects insect family diversity and richness at Arnold AFB.

Season and Habitat Indices

For each seasonal period and for each habitat type, tables were developed to determine the most abundant orders and families in each habitat type within each seasonal period on Arnold AFB. Fourteen tables were calculated to determine the 20 most prevalent arthropod families and their relative dry biomass weight classes in each seasonal period and habitat; these are presented in Tables 2.5 - 2.18. If a habitat and seasonal period did not have 20 arthropod families found in it the highest number of families found was recorded in the index. When interpreting indices, families are listed in descending order of abundance. Dry biomass weight categories are recorded in percentages of individuals that were recorded from that habitat and season that fell into the weight categories: Mega (>1 g), Macro (1-0.1 g), Standard (0.1-0.011 g), and Micro (<0.01 g).

The majority of insects throughout all habitats and seasonal periods were considered Micro and with a biomass less than < 0.01 g (Tables 2.5 – 2.18). Summer collections overall had an increased number of insects with higher biomass compared to the other seasonal periods (Tables 2.5 – 2.18). These tables account for family abundance, diversity, and average weight throughout the year in four different habitats. These tables indicate that even though insect abundance is highly correlated to family richness and diversity, composition of families for each habitat and seasonal period is distinct (Tables 2.5 – 2.18).

In forested wetland habitats at Arnold AFB summer samples were dominated by Coleoptera and secondarily by Hemiptera; the four families with the highest abundance were Staphylinidae, Heteroceridae, Carabidae, and Ciccadellidae (Table 2.5). However, fall samples at forested wetland sites showed that this season was primarily dominated by Lepidoptera with Torticidae, Chaoboridae, Gracillariidae, and Tineidae as the four most collected families (Table 2.6). In winter months, forested wetlands yielded far fewer insects, with only four families present in samples, with most individuals belonging to Diptera, especially Chaoboridae and Mycetophilidae (Table 2.7). In spring, insect populations increased in forested wetlands and were dominated by Diptera and Lepidoptera; the four most collected families were Chironomidae, Pyralidae, Sciaridae, and Gelechiidae (Table 2.8).

Edge (burn) summer insect samples were very abundant with high numbers of Hemiptera, Coleoptera, Hymenoptera, and Lepidoptera; the four most collected families were Cicadellidae, Staphylinidae, Carabidae, and Formicidae (Table 2.9). In fall, edge (burn) site abundance and diversity dropped, with only four families present at the sampling site. The four most collected families found in edge (burn) sites in the fall included Noctuidae, Chaoboridae, Chironomidae, and Tettigoniidae (Table 2.10). Interestingly, in winter edge (burn) site had an increase in insect abundance and diversity. The four most abundant families found at the edge (burn) site in winter included Chironomidae, Noctuidae, Philopotamidae and Siphlonuridae (Table 2.11). In spring no specimens were found at the edge (burn) site due to a prescribed burning event that occurred prior to the sampling date.

Mixed forested sites in the summer were occupied readily by Diptera, Lepidoptera, Coleoptera, and Trichoptera; the four most collected families were

Cicadellidae, Culicidae, Chironomidae, and Cecidomyiidae (Table 2.12). In fall, forested sites retained high abundance and diversity relative to other habitat types, with Lepidoptera, Diptera, and Hemiptera comprising the majority of the specimen collected (Table 2.13). The four most collected families at forested sites in the fall were Tineidae, Tortricidae, Culicidae and Cicadellidae (Table 2.13). Winter samples for forested sites were not analyzed due to unforeseen weather events. Forested sites in the spring had relatively high abundance and diversity of orders and families (Table 2.14). The four most collected families included Chironomidae, Tineidae, Culicidae, and Cicadellidae. Mixed forested sites compared to other habitat types had relative high abundance of insects through each season's samples (excluding winter) and similar order compositions all year long compared to the other habitat types.

In summer, forested stream samples were highly diverse with relatively even number of insects in Hemiptera, Diptera, Lepidoptera, Trichoptera, and Coleoptera (Table 2.15). The four most collected families at forested stream sites in the summer were Cicadellidae, Culicidae, Chironomidae, and Cecidomyiidae (Table 2.15). In fall, forested stream habitats had similar composition of orders, but with fewer hemipterans (Table 2.16). The four most collected families at forested stream sites in the fall were Carabidae, Cecidomyiidae, Tortricidae, and Chironomidae (Table 2.16). Forested streams in winter had relatively high populations of Diptera and Lepidoptera, and the four most collected families were Chironomidae, Noctuidae, Sciaridae, and Geometridae (Table 2.17). In spring, specimens collected in forested streams shifted to predominantly ephemeral aquatic orders, such as Plecoptera, Trichoptera and Ephemeroptera, and the four most collected families were Perlodidae, Leptoceridae, Chironomidae and Caenidae (Table 2.18).

Discussion

This chapter describes the abundance, diversity, biomass, and richness of insect orders and families based on temporal and habitat influences. The goal of this research was to examine crepuscular and nocturnal insect populations over all seasonal periods (Didham et al., 2010; Didham et al., 2020). Nocturnal diversity studies are needed to understand trophic levels and relationships between insects and bats (Davy et al., 2022; Frick et al., 2020; Moiseienko & Vlaschenko, 2021). It is important to note that UV light traps and carbon dioxide traps were selected for sampling due to the high number of specimens collected, but present unique biases by using stimulants to attract insects (Hardwood et al., 2015; McDermott & Mullens, 2018). These biases are likely to result in high numbers of Culicidae and other Nematocerans from the CDC light traps and high numbers of Lepidoptera and Coleopteran families such as heteroceridae that are highly attracted to light.

Temporal predictor variables such as month and season were the most important to predicting insect populations. Temporal variables were likely the best indicator because of specific temperatures and plant resources available to insects being found at different temporal periods (Khaliq et al., 2014). High numbers of aquatic ephemeral orders in early summer late spring are common (Corbet, 1964), but to be the highest collected orders for these time periods was unexpected. This may indicate high water quality health to support such high populations of these aquatic ephemeral orders (Ab

Hamid & Rawi, 2017; Pandiarajan et al., 2019). Typically, highly abundant and diverse orders, such as Coleoptera, Lepidoptera, Diptera, and Hemiptera, are expected to be the most abundant because of how specious these orders are and the wide variety of ecological niches these orders fill (Mayhew, 2007). High populations of Dipterans throughout all temporal periods and habitats specifically of Nematocerans was unexpected and indicate these populations may be good food source for bats with disturbed torpor periods due to White Nose Syndrome (Cheng et al., 2019; Reeder et al., 2012).

Habitat was secondarily important to temporal factors. This was surprising given that many insect taxa are specialists in certain habitats or plants, such as trichopterans needing running water (Mackay & Wiggins, 1979). Overall, forested wetlands were important for coleopterans and forested sites in the spring were important for coleopteran populations to begin rising again in the spring. Though many insect orders average abundances did vary by habitat it was not an important predictor variable for most major insect orders. This may be due to the emphasis of trapping Pterygota (flying insects) as flying insects are much more mobile and may be often found moving from preferred habitat to preferred habitat. This work updates previous insect surveys done at Arnold AFB with limited nocturnal and crepuscular sampling to a more holistic view of insect fauna present (Lambdin et al., 2003; Lambdin & Grant, 1999).

These results suggest that based on the month or season the prey available to bats may change drastically and taxa availability would likely be reflected in their diets. Such as, high numbers of aquatic ephemeral orders in spring and early summer consumed, high numbers of Coleoptera and Hemiptera consumed mid-summer, and higher amounts of Lepidoptera and Diptera consumed in fall and winter. This finding may also indicate that bats foraging in forested wetlands and forest sites in the spring may consume higher proportions of coleopterans. This study also provides a detailed account of nocturnal and crepuscular arthropods in multiple habitats throughout a year period, which is work that is lacking in the southeastern united states at Arnold AFB (Lambdin et al., 2003; Lambdin & Grant, 1999). This study also emphasis the importance of understanding predator-prey dynamics by first understanding prey metrics and their primary influences. Many food chain relationships are studied top down, but often are lacking information about the primary food source and how they are influenced by the environment; understanding predator-prey relationships bottom-up may be equally as important (Burgos et al., 2023; Jiang & Morin, 2005). Work like this study aims to understand how prey dynamics are influenced and how prey in turn influences predators.

Given the large sample sizes of insects collected at UV and CDC light traps setting these attractants close to know foraging and roosting areas may be beneficial short-term solution to help bat populations (Electric Power Research Institute, 2021). CDC light traps may specifically be useful in colder weather to attract Nematocerans that comprise the majority of flying insects active in these periods. Supporting forested wetland habitats and monitoring these habitats around the base will be important to maintaining high coleopteran populations and other insect taxa (Balwan & Kour, 2021). Given the drastic decrease in insects in the edge (burn) site after the prescribed burn, it should be considered and further researched if changing seasonality of burns or increasing time between burns could be beneficial to supporting insect populations, but more research is needed on the base to make informed management decisions. Continuing to support large diversity of habitats and flora will in turn support high abundances and diversity of arthropods (Brown, 2003; Knuff et al., 2020; Sobeck et al., 2009).

Despite limitations due to sampling methodology and relatively low sample size (n=27 sampling surveys); data suggest that overall, at Arnold AFB, insect abundance is strongly tied to insect family richness and insect family diversity. Arthropod order abundance was determined to be primarily influenced by month and secondarily by habitat. Order abundance had a strong correlation with order biomass except for orders such as Blattodea, Megaloptera, and Orthoptera, which had relatively high biomass per individual specimen. Abundance was highly correlated to family diversity and family richness. However, in the 27 samples collected, individual family composition was distinct for each habitat and season. These results suggest that temporal factors and habitat influence the distribution of insect families across Arnold AFB and could potentially impact higher tropic levels based on foraging time and locality.

Literature Cited

Ab Hamid, S., & Rawi, C. S. M. (2017). Application of aquatic insects (Ephemeroptera, Plecoptera and Trichoptera) in water quality assessment of Malaysian headwater. *Tropical Life Sciences Research*, *28*(2), 143.

Anderson, D., & Burnham, K. (2004). Model selection and multi-mode inference. *Second. NY: Springer-Verlag*, *63*(2020), 10.

Balwan, W. K., & Kour, S. (2021). Wetland-an ecological boon for the environment. *East African Scholars Journal of Agriculture and Life Sciences*, *4*(3), 38-48.

Beeman, L. E., & Pelton, M. R. (1980). Seasonal foods and feeding ecology of black bears in the Smoky Mountains. *Bears: Their Biology and Management*, *4*, 141-147. https://doi.org/10.2307/3872858

Bender, M. J., Perea, S., Castleberry, S. B., Miller, D. A., & Wigley, T. B. (2021). Influence of insect abundance and vegetation structure on site-occupancy of bats in managed pine forests. *Forest Ecology and Management*, *482*, 118839.

Best, T. L., Milam, B. A., Haas, T. D., Cvilikas, W. S., & Saidak, L. R. (1997). Variation in diet of the gray bat (*Myotis grisescens*). *Journal of Mammalogy*, *78*(2), 569-583.

Brown, B. L. (2003). Spatial heterogeneity reduces temporal variability in stream insect communities. *Ecology Letters*, *6*(4), 316-325.

Burgos, T., Salesa, J., Fedriani, J. M., Escribano-Ávila, G., Jiménez, J., Krofel, M., ... & Virgós, E. (2023). Top-down and bottom-up effects modulate species co-existence in a context of top predator restoration. *Scientific Reports*, *13*(1), 4170.

Chowdhury, G. R., Datta, U., Zaman, S., & Mitra, A. (2017). Ecosystem services of insects. *Heart*, *17*, 23.

Cheng, T. L., Gerson, A., Moore, M. S., Reichard, J. D., DeSimone, J., Willis, C. K., ... & Kilpatrick, A. M. (2019). Higher fat stores contribute to persistence of little brown bat populations with white-nose syndrome. *Journal of Animal Ecology*, *88*(4), 591-600.

Corbet, P. S. (1964). Temporal patterns of emergence in aquatic insects. *The Canadian Entomologist*, *96*(1-2), 264-279.

Davy, C. M., von Zuben, V., Kukka, P. M., Gerber, B. D., Slough, B. G., & Jung, T. S. (2022). Rapidly declining body size in an insectivorous bat is associated with increased precipitation and decreased survival. *Ecological Applications*, *32*(7), e2639.

Didham, R. K., Basset, Y., & Leather, S. R. (2010). Research needs in insect conservation and diversity. *Insect Conservation and Diversity, 3*, 1-4.

Didham, R. K., Basset, Y., Collins, C. M., Leather, S. R., Littlewood, N. A., Menz, M. H., ... & Hassall, C. (2020). Interpreting insect declines: seven challenges and a way forward. *Insect Conservation and Diversity*, *13*(2), 103-114.

Electric Power Research Institute. (2021). (tech.). *Fat Bats: Research and Management Practices for Enhancing Foraging Habitat to Help Support Bats with White-Nose Syndromes*. Retrieved from https://www.epri.com/research/products/000000003002021572.

Evans, F. C., & Murdoch, W. W. (1968). Taxonomic composition, trophic structure and seasonal occurrence in a grassland insect community. *The Journal of Animal Ecology*, *37*, 259-273.

Fadila, M. I., Mardiastuti, A., & Mulyani, Y. A. (2020). Insects as food source for cattle egret (*Bubulcus ibis*) in Jatibarang landfill, Semarang. *IOP Conference Series: Earth and Environmental Science*, *528*(1):012016*,* doi:10.1088/1755-1315/528/1/012016.

Frick, W. F., Kingston, T., & Flanders, J. (2020). A review of the major threats and challenges to global bat conservation. *Annals of the New York Academy of Sciences*, *1469*(1), 5-25.

Gadagkar, R., Chandrashekara, K., & Nair, P. (1990). Insect species diversity in tropics: Sampling methods and a case study. *Journal of the Bombay Natural History Society*, *87*(3), 337-353.

Guevara, J., & Avilés, L. (2013). Community-wide body size differences between nocturnal and diurnal insects. *Ecology*, *94*(2), 537-543.

Gunther, K. A., Shoemaker, R. R., Frey, K. L., Haroldson, M. A., Cain, S. L., Van Manen, F. T., & Fortin, J. K. (2014). Dietary breadth of grizzly bears in the Greater Yellowstone Ecosystem. *Ursus*, *25*(1), 60-72.

Harwood, J. F., Arimoto, H., Nunn, P., Richardson, A. G., & Obenauer, P. J. (2015). Assessing Carbon Dioxide and Synthetic Lure-Baited Traps for Dengue and Chikungunya Vector Surveillance. *Journal of the American Mosquito Control Association*, *31*(3), 242-247.

Hawkey, K. J., Lopez-Viso, C., Brameld, J. M., Parr, T., & Salter, A. M. (2021). Insects: A potential source of protein and other nutrients for feed and food. *Annual Review of Animal Biosciences*, *9*, 333-354.

Jiang, L., & Morin, P. J. (2005). Predator diet breadth influences the relative importance of bottom-up and top-down control of prey biomass and diversity. *The American Naturalist*, *165*(3), 350-363.

Khaliq, A. M., Javed, M., Sohail, M., & Sagheer, M. (2014). Environmental effects on insects and their population dynamics. *Journal of Entomology and Zoology studies*, *2*(2), 1-7.

Knuff, A. K., Staab, M., Frey, J., Dormann, C. F., Asbeck, T., & Klein, A. M. (2020). Insect abundance in managed forests benefits from multi-layered vegetation. *Basic and Applied Ecology*, *48*, 124-135.

Lambdin, P. L., Grant, J. F., Wiggins, G. J., & Saxton, A. (2003). Diversity of the true bugs (Hemiptera: Heteroptera) on Arnold Air Force Base, Tullahoma, Tennessee. *Journal of the Tennessee Academy of Science*, *78*(3), 76-85.

Lambdin, P.L. and J.F. Grant. (1999). Rare, Threatened, and Endangered (RTE) Terrestrial Invertebrate Survey. A Technical Report prepared for Arnold AFB.

Macadam, C. R., & Stockan, J. A. (2015). More than just fish food: Ecosystem services provided by freshwater insects. *Ecological Entomology*, *40*, 113-123.

Mackay, R. J., & Wiggins, G. B. (1979). Ecological diversity in Trichoptera. *Annual Review of Entomology*, *24*(1), 185-208.

Marshall, S. A. (2006). *Insects: their natural history and diversity: with a photographic guide to insects of eastern North America* (p. 718). Buffalo, NY: Firefly Books.

Mayhew, P. J. (2007). Why are there so many insect species? Perspectives from fossils and phylogenies. *Biological Reviews*, *82*(3), 425-454.

McDermott, Emily G., and Bradley A. Mullens. (2018). The dark side of light traps. *Journal of Medical Entomology,* 55.2, 251-261.

Minnis, A. M., & Lindner, D. L. (2013). Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. Fungal Biology, 117(9), 638-649.

Moosman Jr, P. R., 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.

Morningstar, D. E., Robinson, C. V., Shokralla, S., & Hajibabaei, M. (2019). Interspecific competition in bats and diet shifts in response to white-nose syndrome. *Ecosphere*, *10*(11), e02916.

Moiseienko, M., & Vlaschenko, A. (2021). Quantitative evaluation of individual food intake by insectivorous vespertilionid bats (Chiroptera, Vespertilionidae). *Biology Open*, *10*(6), bio058511.

Noriega, J. A., Hortal, J., Azcárate, F. M., Berg, M. P., Bonada, N., Briones, M. J., ... & Santos, A. M. (2018). Research trends in ecosystem services provided by insects. *Basic and Applied Ecology*, *26*, 8-23.

Pandiarajan, S., Thambiratnam, S., & Sivaruban, I. R. B. (2019). Bio-monitoring and detection of water quality using Ephemeroptera, Plecoptera and Trichoptera (EPT) complex in Karanthamalai Stream of Eastern Ghats. *Indian Journal of Ecology*, *46*(4), 818-822.

Reeder, D. M., Frank, C. L., Turner, G. G., Meteyer, C. U., Kurta, A., Britzke, E. R., ... & Blehert, D. S. (2012). Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PloS One*, *7*(6), e38920.

Schowalter, T. D. (2022). *Insect Ecology: An Ecosystem Approach*, 5th Ed., Academic Press.

Shields, W. M., & Bildstein, K. L. (1979). Bird versus bats: Behavioral interactions at a localized food source. *Ecology*, *60*(3), 468-474.

Sobek, S., GOßNER, M. M., Scherber, C., STEFFAN-DEWENTER, I. N. G. O. L. F., & Tscharntke, T. (2009). Tree diversity drives abundance and spatiotemporal β-diversity of true bugs (Heteroptera). *Ecological Entomology*, *34*(6), 772-782.

Tran, G., Heuzé, V., & Makkar, H. P. (2015). Insects in fish diets. *Animal Frontiers*, *5*(2), 37-44.

U.S. Fish and Wildlife Service. (2022, March). U.S. Fish & Wildlife Service White-Nose Syndrome. the devasting disease of hibernating bats in North America. Retrieved December 7, 2022, from https://s3.us-west-2.amazonaws.com/prod-is-cms-assets/wns/prod/e2e0f150-a606-11ec-81da-ab16efc2070f-White-nose%20fact%20sheet%202022_edit.pdf.

Warnecke, L., Turner, J. M., Bollinger, T. K., Lorch, J. M., Misra, V., Cryan, P. M., ... & Willis, C. K. (2012). Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proceedings of the National Academy of Sciences*, *109*(18), 6999-7003.

Yang, L. H., & Gratton, C. (2014). Insects as drivers of ecosystem processes. *Current Opinion in Insect Science*, *2*, 26-32.

Appendix

Table 2.1. Description of seven sampling sites and sampling periods used to assess arthropod diversity at Arnold Air Force Base, 2022 and 2023.

Site	Habitat	Prescribed Burning	Seasons Collected
BBI021	**Mixed Forested Stream** (Brumalow Creek)	No	Summer, Fall, Winter, and Spring
BBI125	**Mixed Forested Wetland** (Sinking Pond)	No	Summer, Fall, Winter, and Spring
BBI051	**Mixed Forested Stream** (Bradley Creek)	No	Summer, Fall, Winter, and Spring
BBI052	**Mixed Forested Stream** (Rowland Creek)	No	Summer, Fall, Winter, and Spring
BBI087	**Mixed Forested Stream** (Upper Rowland Creek)	No	Summer, Fall, Winter, and Spring
BBI097	**Edge** (Mixed Forest to Grassland)	Yes	Summer, Fall, Winter, and Spring
BBI105	**Mixed Forest** (Road Rut Close)	No	Summer, Fall, Winter, and Spring

Table 2.2. List of sampling locations and dates, Arnold Air Force Base, 2022 and 2023.

Sample Number	Site*	Date and Season	Additional Comments
1	BBI097 (E)	26 July2022- Summer	
2	BBI125 (MFW)	27 July 2022- Summer	
3	BBI021 (MFS)	16 August 2022- Summer	
4	BBI125 (MFW)	17 August 2022- Summer	
5	BBI021 (MFS)	18 August 2022- Summer	
6	BBI051 (MFS)	16 September 2022- Fall	4 days before Fall equinox
7	BBI105 (MF)	17 September 2022- Fall	3 days before Fall equinox
8	BBI125 (MFW)	18 September 2022- Fall	2 days before Fall equinox
9	BBI087 (MFS)	28 October 2022- Fall	
10	BBI097 (E)	29 October 2022- Fall	
11	BBI087(MFS)	21 November 2022- Fall	
12	BBI105 (MF)	23 December 2022- Winter	Excluded (Trap Malfunction)
13	BBI125 (MFW)	18 January 2023- Winter	
14	BBI097 (E)	28 February 2023- Winter	
15	BBI021 (MFS)	29 February 2023- Winter	
16	BBI087 (MFS)	29 March 2023- Spring	
17	BBI125 (MFW)	30 March 2023- Spring	
18	BBI105 (MF)	28 April 2023- Spring	
19	BBI097 (E)	29 April 2023- Spring	0 specimen- After Burn
20	BBI052(MFS)	25 May 2023- Spring	
21	BBI125 (MFW)	26 June 2023- Summer	
22	BBI125 (MFW)	27June 2023- Summer	
23	BBI105 (MF)	28 June 2023- Summer	
24	BBI097 (E)	24 July 2023- Summer	
25	BBI052 (MFS)	25 July 2023- Summer	
26	BBI051 (MFS)	21 August 2023- Summer	
27	BBI051 (MFS)	22 August 2023- Summer	

*Sites are E = Edge, MFS = Mixed Forest Stream, MFW = Mixed Forest Wetland, and MF = Mixed Forest.

Table 2.3. Insect diversity and family diversity at each sampling site and season, Arnold Air Force Base, 2022 and 2023.

Sample Number	Site* and Season	Shannon Diversity Index	Arthropod Family Richness
1	BBI097 (E)- Summer	3.20	80
2	BBI125 (MFW)- Summer	3.18	77
3	BBI021 (MFS)- Summer	2.84	79
4	BBI125 (MFW)- Summer	2.12	59
-5	BBI021 (MFS)- Summer	3.13	69
6	BBI051 (MFS)- Fall	2.95	47
7	BBI105 (MF)- Fall	3.25	54
8	BBI105 (MF)- Fall	3.16	47
9	BBI087 (MFS)- Fall	3.38	47
10	BBI097 (E)- Fall	0.54	3
11	BBI087(MFS)- Fall	0.56	6
12	BBI105 (MF)- Winter	Excluded	Excluded
13	BBI125 (MFW)- Winter	1.27	5
14	BBI097 (E)- Winter	1.61	11
15	BBI021 (MFS)- Winter	1.70	15
16	BBI087 (MFS)- Spring	2.22	23
17	BBI125 (MFW)- Spring	1.58	14
18	BBI105 (MF)- Spring	2.56	62
19	BBI097 (E)- Spring	0 (After Burn)	0 (After Burn)
20	BBI052(MFS)- Spring	3.41	81
21	BBI125 (MFW)- Summer	3.25	76
22	BBI125 (MFW)- Summer	3.35	78
23	BBI105 (MF)- Summer	3.16	67
24	BBI097 (E)- Summer	3.01	73
25	BBI052 (MFS)- Summer	2.91	70
26	BBI051 (MFS)- Summer	2.24	77
27	BBI051 (MFS)- Summer	2.24	77

*Sites are E = Edge, MFS = Mixed Forest Stream, MFW = Mixed Forest Wetland, and MF = Mixed Forest.

Table 2.4. Temporal and site-specific variables that were significantly associated with insect orders Coleoptera, Lepidoptera, Diptera, Hemiptera, Trichoptera, Ephemeroptera, and Plecoptera from Arnold Air Force Base. Regular GLMs were built for all insect orders. 2022- 2023.

Order	Variables	Estimate	Standard Error	Lower 95	Upper 95	Model Results
Coleoptera	~Janurary	-887	218.8	-1,324.6	-449.4	F= 8.73; T=0.041
Coleoptera	~March	-887	218.8	-1,324.6	-449.4	F=8.73; T=4.05
Coleoptera	~Forested Wetland Habitat	927.5	264.51	398.5	1,456.5	F=8.73; T=3.56
Coleoptera	~Forseted Habitat: Spring	927.5	264.51	398.5	1,456.5	F=8.73 ; T=3.51
Lepidoptera	~May	414	91.37	231.3	596.7	F=9.31 ; T=4.53
Lepidoptera	~July	220	91.37	37.26	402.74	F=9.31 ; T=2.41
Lepidoptera	September	177	69.07	38.86	315.14	F=9.31; T=2.56
Diptera	~1	158.62	51.68	55.08	261.98	T=3.07
Hemiptera	~Summer	297.0	122.9	51.2	542.8	F=3.86; T=2.42
Trichoptera	~May	541.12	92.21	356.7	725.5	F=6.14; T=5.87;
Ephemeroptera	~May	263.00	66.18	136.6	395.4	F=2.97; T=3.97
Plecoptera	~May	393.65	0.95	391.75	395.55	F=3.35+ e04; T=413.97;

Table 2.5. The 20 most collected insect families by size class for forested wetlands in Summers 2022 and 2023 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1 g)*	% Macro (1-0.1 g)**	% Std (0.1- 0.011 g)***	% Micro (<0.01 g)****
Coleoptera	Staphylinidae	0	0	0	100
Coleoptera	Heteroceridae	0	0	0	100
Coleoptera	Carabidae	0	1.1	4.6	94.3
Hemiptera	Cicadellidae	0	0	0	100
Diptera	Chironomidae	0	0	0	100
Lepidoptera	Tortricidae	0	0	0	100
Hemiptera	Corixidae	0	0	0	100
Coleoptera	Hydrophilidae	2.9	9.2	0	87.9
Trichoptera	Leptoceridae	0	0	0	100
Coleoptera	Anthicidae	0	0	0	100
Coleoptera	Dytiscidae	1.1	7.2	31.1	60.6
Lepidoptera	Gelechiidae	0	0	0	100
Coleoptera	Scarabaeidae	1.8	55.8	16.9	25.5
Coleoptera	Noteridae	0	0	0	100
Lepidoptera	Crambidae	0	0	66.7	33.3
Hymenoptera	Figitidae	0	0	0	100
Coleoptera	Elateridae	2.1	21.1	8.4	68.4
Lepidoptera	Gracillariidae	0	0	0	100
Coleoptera	Scirtidae	0	0	0	100
Hemiptera	Rhyparochromidae	0	0	0	100

*% Mega (>1 g) indicates the percentage of individuals in that family found in summer at forested wetlands with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in summer at forested wetlands with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family found in summer at forested wetlands with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in summer in forested wetlands with a dry biomass less than <0.011 g.

Table 2.6. The 20 most collected insect families for forested wetlands in Fall 2022 at Arnold Airforce Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1 g)*	% Macro (1-0.1 g)**	% Std (0.1- 0.011 g)***	% Micro (<0.011 g)****
Lepidoptera	Torticidae	0	0	0	100
Diptera	Chaoboridae	0	0	0	100
Lepidoptera	Gracillaridae	0	0	0	100
Lepidoptera	Tineidae	0	0	0	100
Diptera	Cecidomyiidae	0	0	0	100
Coleoptera	Elateridae	0	0	23.5	76.5
Lepidoptera	Geometridae	0	0	23.5	76.5
Hemiptera	Cicadellidae	0	0	0	100
Diptera	Chironomidae	0	0	0	100
Lepidoptera	Gelechiidae	0	0	0	100
Lepidoptera	Erebidae	0	0	0	100
Hymenoptera	Formicidae	0	0	0	100
Coleoptera	Curculionidae	0	0	0	100
Lepidoptera	Pyralidae	0	0	0	100
Lepidoptera	Noctuidae	0	37.5	62.5	0
Coleoptera	Carabidae	0	0	100	0
Psocodea	Psocidae	0	0	0	100
Coleoptera	Cleridae	0	0	0	100
Hemiptera	Cercopidae	0	0	100	0
Diptera	Culicidae	0	0	0	100

*% Mega (>1 g) indicates the percentage of individuals in that family found in fall at forested wetlands with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in fall at forested wetlands with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family found in fall at forested wetlands with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in fall in forested wetlands with a dry biomass less than <0.011 g.

Table 2.7. All collected families for forested wetlands in Winter 2023 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1 g)*	% Macro (1-0.1 g)**	% Std (0.1- 0.011 g)***	% Micro (<0.011 g)****
Diptera	Chaoboridae	0	0	0	100
Diptera	Mycetophilidae	0	0	0	100
Coleoptera	Scarabaeidae	0	0	100	0
Hymenoptera	Formicidae	0	0	0	100

*% Mega (>1 g) indicates the percentage of individuals in that family found in winter at forested wetlands with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in winter at forested wetlands with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family found in winter at forested wetlands with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in winter in forested wetlands with a dry biomass less than <0.011 g.

Table 2.8. All collected insect families for forested wetlands in Spring 2023 at Arnold Airforce Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1 g)*	% Macro (1-0.1 g)**	% Std (0.1- 0.011 g)***	% Micro (<0.01 g)****
Diptera	Chironomidae	0	0	0	100
Lepidoptera	Pyralidae	0	0	0	100
Diptera	Sciaridae	0	0	0	100
Lepidoptera	Gelechiidae	0	0	0	100
Diptera	Mycetophilidae	0	0	0	100
Coleoptera	Carabidae	0	0	0	100
Diptera	Dolichopodidae	0	0	0	100
Diptera	Empididae	0	0	0	100
Trichoptera	Erebidae	0	0	0	100
Trichoptera	Philopotamidae	0	0	0	100
Diptera	Phoridae	0	0	0	100
Diptera	Canacidae	0	0	0	100
Blattodea	Ectobiidae	0	100	0	0

*% Mega (>1 g) indicates the percentage of individuals in that family found in spring at forested wetlands with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in spring at forested wetlands with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family found in spring at forested wetlands with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in spring in forested wetlands with a dry biomass less than <0.011 g.

Table 2.9. The 20 most collected insect families for edge (burn) in Summers 2022 and 2023 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1 g)*	% Macro (1-0.1 g)**	% Std (0.1- 0.011 g)***	% Micro (<0.01 g)****
Hemiptera	Cicadellidae	0	0	0	100
Coleoptera	Staphylinidae	0	0	0.5	95.5
Coleoptera	Carabidae	0.3	2.8	7.6	89.3
Hymenoptera	Formicidae	0	0	0	100
Lepidoptera	Torticidae	0	0	0	100
Hemiptera	Cercopidae	0	0	0	100
Coleoptera	Heteroceridae	0	0	0	100
Lepidoptera	Tineidae	0	0	0	100
Trichoptera	Philopotamidae	0	0	0	100
Lepidoptera	Geometridae	0	3.2	80.4	16.4
Lepidoptera	Gracillariidae	0	0	0	100
Diptera	Chironomidae	0	0	0	100
Lepidoptera	Crambidae	0	0	82.9	17.1
Lepidoptera	Gelechiidae	0	0	0	100
Lepidoptera	Noctuidae	0	84.8	15.2	0
Hemiptera	Pentatomidae	0	100	0	0
Coleoptera	Chrysomelidae	0	0	0	100
Coleoptera	Anthicidae	0	0	0	100
Coleoptera	Hydrophilidae	0	0	36	64
Coleoptera	Scirtidae	0	0	0	100

*% Mega (>1 g) indicates the percentage of individuals in that family found in summer at edge site with prescribed burn with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in summer at edge site with prescribed burn with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family in summer at edge site with prescribed burn with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in summer at edge site with prescribed burn with a dry biomass less than <0.011 g

Table 2.10. All collected insect families for edge (burn) in Fall 2022 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1 g)*	% Macro (1-0.1 g)**	% Std (0.1- 0.011 g)***	% Micro (<0.01 g)****
Lepidoptera	**Noctuidae**	**0**	**100**	**0**	**0**
Diptera	**Chaoboridae**	**0**	**0**	**0**	**100**
Diptera	**Chironomidae**	**0**	**0**	**0**	**100**
Orthoptera	**Tettigonidae**	**100**	**0**	**0**	**0**

*% Mega (>1 g) indicates the percentage of individuals in that family found in fall at edge site with prescribed burn with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in fall at edge site with prescribed burn with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family in fall at edge site with prescribed burn with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in fall at edge site with prescribed burn with a dry biomass less than <0.011 g.

Table 2.11. All collected insect families for edge (burn) in Winter 2023 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1g)*	% Macro (1-0.1g)**	% Std (0.1- 0.011g)***	% Micro (<0.01g)****
Diptera	Chironomidae	0	0	0	100
Lepidoptera	Noctuidae	0	44.4	56.6	0
Trichoptera	Philopotamidae	0	0	0	100
Ephemeroptera	Siphlonuridae	0	0	0	100
Coleoptera	Staphylinidae	0	0	0	100
Hymenoptera	Figitidae	0	0	0	100
Trichoptera	Leptoceridae	0	0	0	100
Araneae	Salticidae	0	0	0	100
Hymenoptera	Xyelidae	0	0	0	100

*% Mega (>1 g) indicates the percentage of individuals in that family found in winter at edge site with prescribed burn with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in winter at edge site with prescribed burn with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family in winter at edge site with prescribed burn with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in winter at edge site with prescribed burn with a dry biomass less than <0.011 g.

Table 2.12. The 20 most collected insect families for mixed forests in Summers 2022 and 2023 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1 g)*	% Macro (1-0.1 g)**	% Std (0.1- 0.011 g)***	% Micro (<0.01 g)****
Hemiptera	Cicadellidae	0	0	0	100
Diptera	Culicidae	0	0	0	100
Diptera	Chironomidae	0	0	0	100
Diptera	Cecidomyiidae	0	0	0	100
Lepidoptera	Crambidae	0	0	54	46
Coleoptera	Heteroceridae	0	0	0	100
Coleoptera	Carabidae	0	1	4	95
Trichoptera	Philopotamidae	0	0	0	100
Trichoptera	Leptoceridae	0	0	0	100
Lepidoptera	Geometridae	0	15.8	52.6	31.6
Lepidoptera	Tineidae	0	0	0	100
Lepidoptera	Torticidae	0	0	0	100
Lepidoptera	Noctuidae	0	54	24.3	21.7
Collembola	Entomobryidae	0	0	0	100
Trichoptera	Polycentropodidae	0	0	0	100
Diptera	Sciaridae	0	0	0	100
Lepidoptera	Erebidae	0	100	0	0
Coleoptera	Staphylinidae	0	0	0	100
Diptera	Ceratopogonidae	0	0	0	100
Lepidoptera	Pryalidae	0	0	100	0

*% Mega (>1 g) indicates the percentage of individuals in that family found in summer at forested sites with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in summer at forested sites with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family in summer at forested sites with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in summer at forested sites with a dry biomass less than <0.011 g.

Table 2.13. The 20 most collected insect families for mixed forests in Fall 2022 at Arnold Air Force Base.

Order	Family	% Mega (>1g)*	% Macro (1-0.1g)**	% Std (0.1- 0.011g)***	% Micro (<0.01g)****
Lepidoptera	Tineidae	0	0	0	100
Lepidoptera	Torticidae	0	0	0	100
Diptera	Culicidae	0	0	0	100
Hemiptera	Cicadellidae	0	0	0	100
Diptera	Cecidomyiidae	0	0	0	100
Lepidoptera	Geometridae	0	0	23.7	76.4
Coleoptera	Elateridae	0	0	40.6	59.4
Diptera	Chironomidae	0	0	0	100
Lepidoptera	Noctuidae	0	40	60	0
Diptera	Chaoboridae	0	0	0	100
Lepidoptera	Gracillariidae	0	0	0	100
Lepidoptera	Gelechiidae	0	0	0	100
Lepidoptera	Erebidae	0	0	0	100
Hemiptera	Cercopidae	0	0	100	0
Diptera	Cecidomyiidae	0	0	0	100
Hymenoptera	Formicidae	0	0	0	100
Coleoptera	Curculionidae	0	0	0	100
Lepidoptera	Pyralidae	0	0	0	100
Diptera	Ceratopogonidae	0	0	0	100
Coleoptera	Carabidae	0	0	100	0

*% Mega (>1 g) indicates the percentage of individuals in that family found in fall at forested sites with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in fall at forested sites with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family in fall at forested sites with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in fall at forested sites with a dry biomass less than <0.011 g.

Table 2.14. The 20 most collected insect families for mixed forests in Spring 2023 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1g)*	% Macro (1-0.1g)**	% Std (0.1- 0.011g)***	% Micro (<0.01g)****
Diptera	Chironomidae	0	0	0	100
Lepidoptera	Tineidae	0	0	0	100
Diptera	Culicidae	0	0	0	100
Hemiptera	Cicadellidae	0	0	0	100
Hymenoptera	Ichneumonidae	0	0	60	40
Lepidoptera	Geometridae	0	50	0	50
Diptera	Cecidomyiidae	0	0	0	100
Plecoptera	Nemouridae	0	0	0	100
Trichoptera	Hydroptilidae	0	0	0	100
Diptera	Tipulidae	0	0	0	100
Figitidae	Figitidae	0	0	0	100
Elateridae	Elateridae	0	0	0	100
Hymenoptera	Formicidae	0	0	0	100
Diptera	Lonchaeidae	0	0	0	100
Lepidoptera	Gelechiidae	0	0	0	100
Diptrea	Phoridae	0	0	0	100
Lepidoptera	Noctuidae	0	100	0	0
Lepidoptera	Pyralidae	0	0	100	0
Trichoptera	Philopotamidae	0	0	0	100
Diptera	Sciaridae	0	0	0	100

*% Mega (>1 g) indicates the percentage of individuals in that family found in spring at forested sites with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in spring at forested sites with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family in spring at forested sites with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in spring at forested sites with a dry biomass less than <0.011 g.

Table 2.15. The 20 most collected insect families for forested streams in Summers 2022 and 2023 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1g)*	% Macro (1-0.1g)**	% Std (0.1- 0.011g)***	% Micro (<0.01g)****
Hemiptera	Cicadellidae	0	0	0	100
Diptera	Culicidae	0	0	0	100
Diptera	Chironomidae	0	0	0	100
Diptera	Cecidomyiidae	0	0	0	100
Lepidoptera	Crambidae	0	0	14	86
Coleoptera	Heteroceridae	0	0	0	100
Coleoptera	Carabidae	0	15.6	20.2	62.2
Trichoptera	Philopotamidae	0	0	0	100
Trichoptera	Leptoceridae	0	0	0	100
Lepidoptera	Geometridae	0	8	30	62
Lepidoptera	Tineidae	0	0	0	100
Lepidoptera	Torticidae	0	0	0	100
Lepidoptera	Noctuidae	0	37.5	54.2	8.3
Collembola	Entomobryidae	0	0	0	100
Trichoptera	Polycentropodidae	0	0	0	100
Coleoptera	Staphylinidae	0	0	0	100
Diptera	Ceratopogonidae	0	0	0	100
Lepidoptera	Pyralidae	0	0	54.5	45.5

*% Mega (>1 g) indicates the percentage of individuals in that family found in summer at forested stream sites with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in summer at forested stream sites with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family in summer at forested stream sites with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in summer at forested stream sites with a dry biomass less than <0.011 g.

Table 2.16. The 20 most collected insect families for forested streams in Fall 2022 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1g)*	% Macro (1-0.1g)**	% Std (0.1- 0.011g)***	% Micro (<0.01g)****
Coleoptera	Carabidae	0	0	35	65
Diptera	Cecidomyiidae	0	0	0	100
Lepidoptera	Torticidae	0	0	0	100
Diptera	Chironomidae	0	0	0	100
Lepidoptera	Crambidae	0	0	88.6	11.4
Diptera	Culicidae	0	0	0	100
Lepidoptera	Gelechiidae	0	0	0	100
Hemiptera	Cicadellidae	0	0	0	100
Trichoptera	Philopotamidae	0	0	0	100
Coleoptera	Heteroceridae	0	0	0	100
Lepidoptera	Tineidae	0	0	0	100
Lepidoptera	Geometridae	0	0	25	75
Coleoptera	Anobiidae	0	0	0	100
Lepidoptera	Gracillariidae	0	0	0	100
Diptera	Tipulidae	0	0	0	100
Lepidoptera	Noctuidae	0	50	37.5	12.5
Diptera	Psychodidae	0	0	0	100
Diptera	Sciaridae	0	0	0	100
Lepidoptera	Erebidae	0	20	40	40

*% Mega (>1 g) indicates the percentage of individuals in that family found in fall at forested stream sites with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in fall at forested stream sites with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family found in fall at forested stream sites with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in fall at forested stream sites with a dry biomass less than <0.011 g.

Table 2.17. All collected insect families for forested streams in the Winter 2023 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1g)*	% Macro (1-0.1g)**	% Std (0.1- 0.011g)***	% Micro (<0.01g)****
Diptera	Chironomidae	0	0	25.9	74.1
Lepidoptera	Noctuidae	0	0	0	100
Diptera	Sciaridae	0	0	0	100
Lepidoptera	Geometridae	0	0	88.6	11.4
Diptera	Cecidomyiidae	0	0	0	100
Lepidoptera	Erebidae	0	0	0	100
Diptera	Tipulidae	0	0	0	100
Hemiptera	Cercopidae	0	0	100	0
Coleoptera	Curculionidae	0	0	0	100
Hymenoptera	Formicidae	0	0	0	100
Coleoptera	Anobiidae	0	0	0	100
Lepidoptera	Gracillariidae	0	0	0	100
Diptera	Tipulidae	0	0	0	100
Diptera	Chaoboridae	0	0	0	100
Lepidoptera	Noctuidae	0	50	37.5	12.5
Diptera	Psychodidae	0	0	0	100
Diptera	Sciaridae	0	0	0	100

*% Mega (>1 g) indicates the percentage of individuals in that family found in winter at forested stream sites with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in winter at forested stream sites with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family found in winter at forested stream sites with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in winter at forested stream sites with a dry biomass less than <0.011 g.

Table 2.18. The 20 most collected insect families for forested streams in Spring 2023 at Arnold Air Force Base. Ordered from most abundant to least abundant family.

Order	Family	% Mega (>1g)*	% Macro (1-0.1g)**	% Std (0.1- 0.011g)***	% Micro (<0.01g)****
Plecoptera	Perlodidae	0	0	0	100
Trichoptera	Leptoceridae	0	0	0	100
Diptera	Chironomidae	0	0	0	100
Ehemeroptera	Caenidae	0	0	0	100
Trichoptera	Philopotamidae	0	0	0	100
Hemiptera	Cicadellidae	0	0	0	100
Trichoptera	Hydropsychidae	0	0	0	100
Diptera	Sciaridae	0	0	0	100
Ephemeroptera	Ephemerellidae	0	0	0	100
Trichoptera	Heptageniidae	0	0	0	100
Diptera	Cecidomyiidae	0	0	0	100
Lepidoptera	Geometridae	0	0	57.9	42.1
Coleoptera	Scarabaeidae	35.3	64.7	0	0
Diptera	Culicidae	0	0	0	100
Coleoptera	Elateridae	0	50	50	0
Lepidoptera	Noctuidae	0	0	0	100
Diptera	Ceratopogonidae	0	0	0	100
Lepidoptera	Crambidae	0	0	33.5	66.5
Trichoptera	Hydroptilidae	0	0	0	100
Coleoptera	Tenebrionidae	0	0	0	100

*% Mega (>1 g) indicates the percentage of individuals in that family found in winter at forested stream sites with a dry biomass greater than 1 g.

**% Macro (1-0.1 g) indicates the percentage of individuals in that family found in winter at forested stream sites with a dry biomass between than 1 g- 0.1 g.

***% Std (0.1-0.011 g) indicates the percentage of individuals in that family found in winter at forested stream sites with a dry biomass between 0.1g -0.011 g.

****% Micro (<0.01g) indicates the percentage of individuals in that family found in winter at forested stream sites with a dry biomass less than <0.011 g.

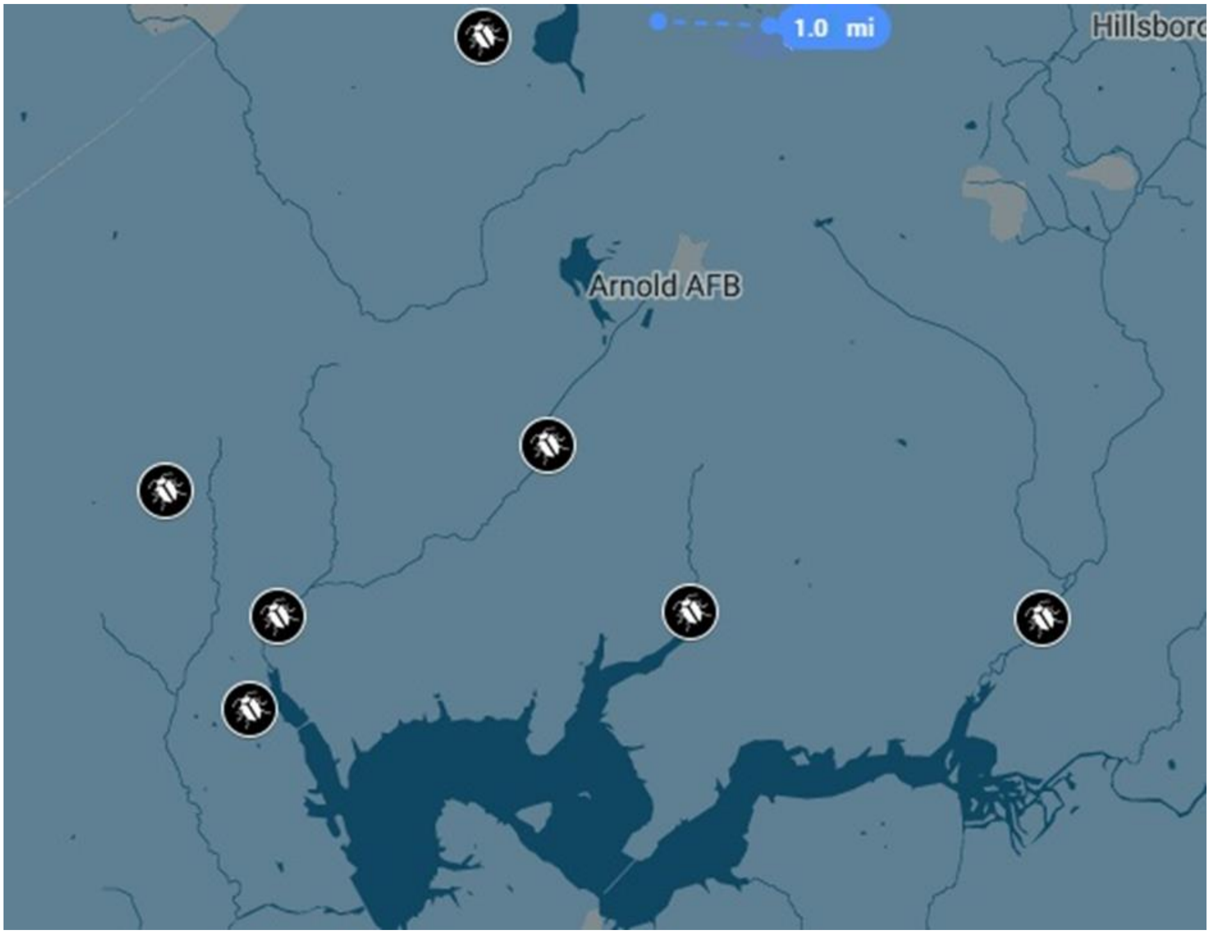

Figure 2.1. Locations of seven sites used to assess arthropod diversity at Arnold Air Force Base, 2022 and 2023.

Figure 2.2. Ultraviolet (UV) light trap used for insect sampling at Arnold Air Force Base, 2022 and 2023.

Figure 2.3. CDC light traps (CO_2) used for insect sampling at Arnold Air Force Base, 2022 and 2023.

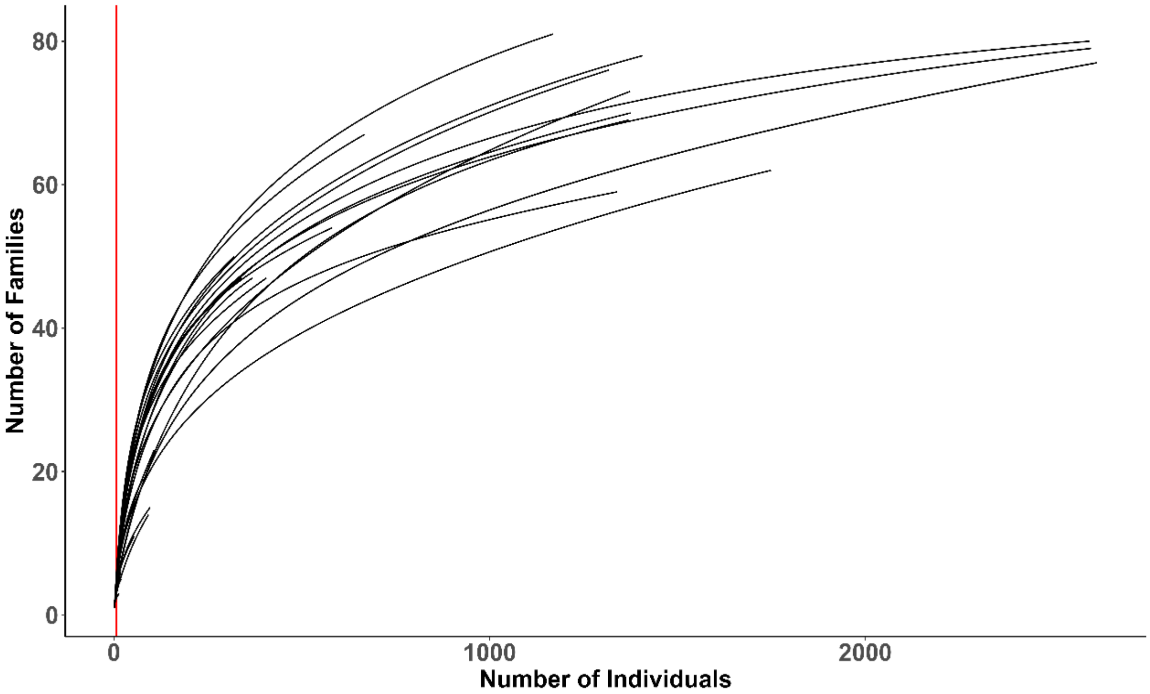

Figure 2.4. Insect family curves for all samples collected, Arnold Air Force Base, 2022 and 2023. The more individuals collected, the more families were represented in the sample. The red line indicates the smallest sample size (n = 11 individuals), and each black line represents an individual sampling event.

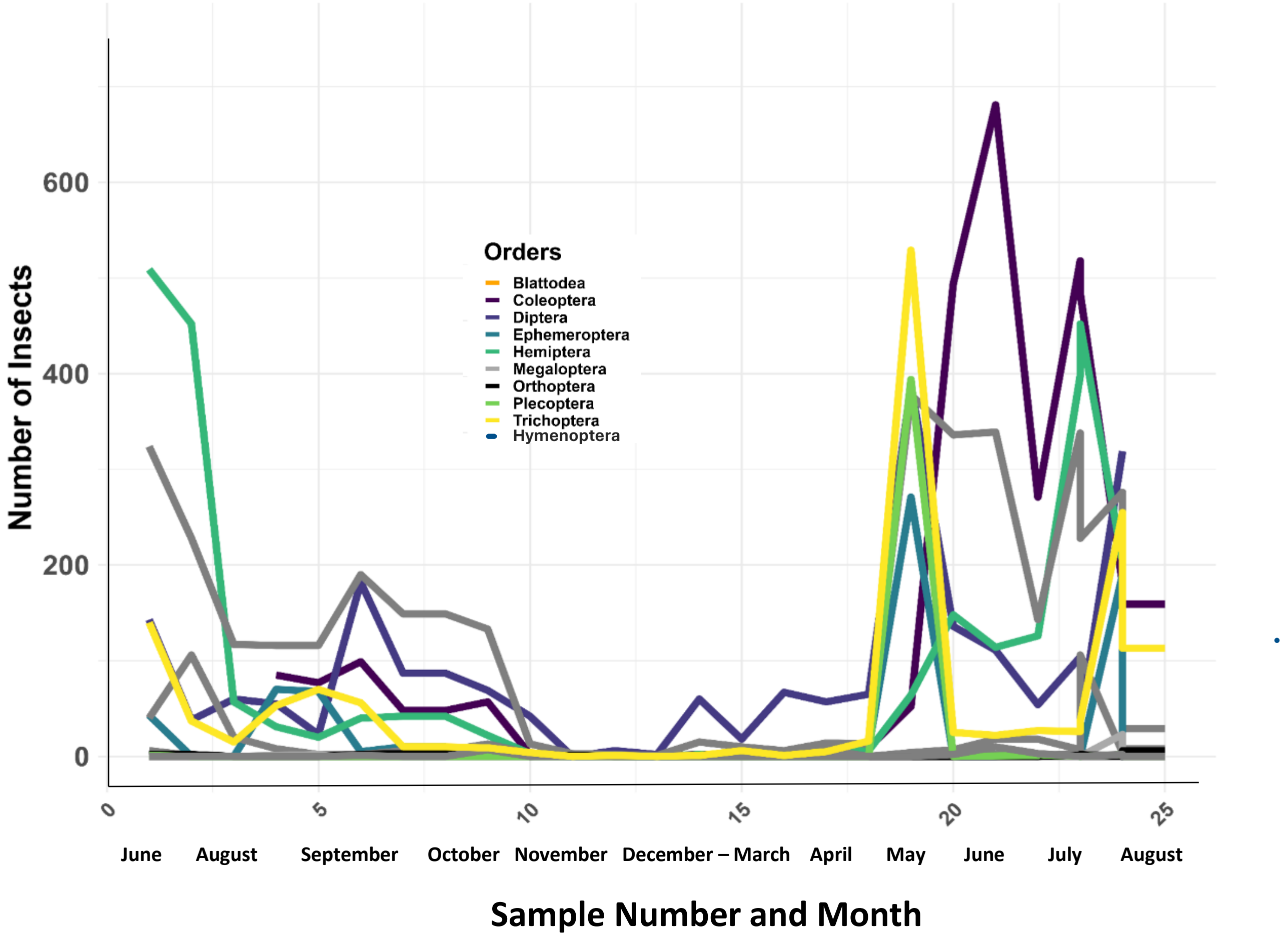

Figure 2.5. Comparison of order abundance by each sampling event, Arnold Air Force Base, 2022 and 2023. (*Ordered chronologically from June 2022- August 2023.)

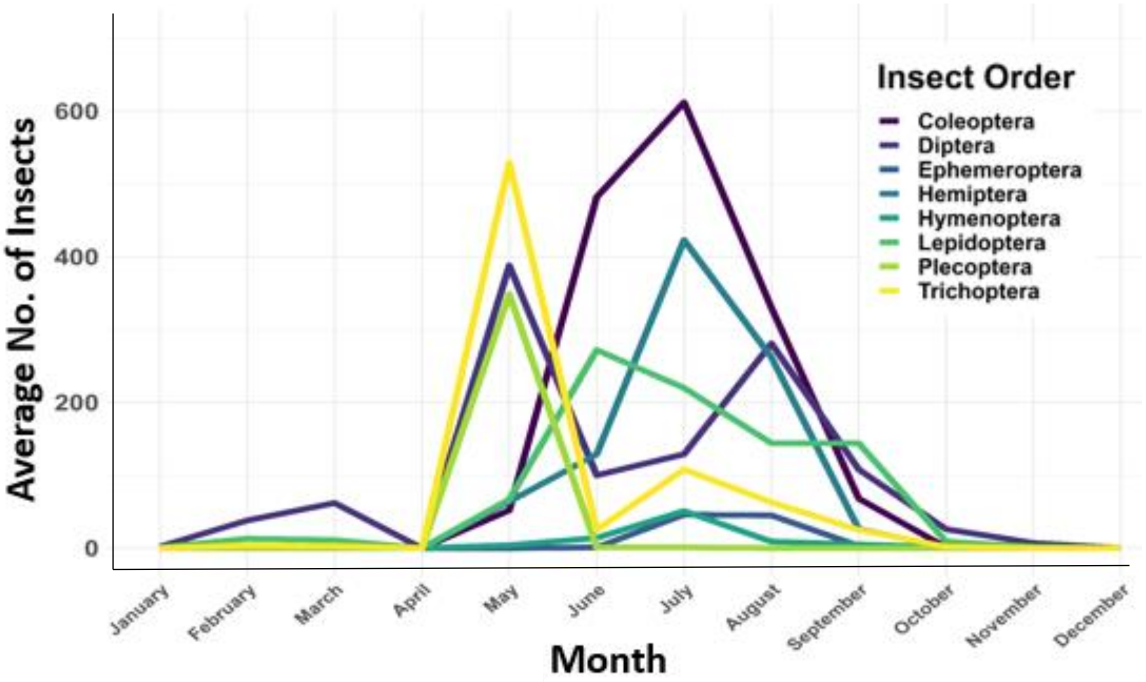

Figure 2.6. Relative seasonal abundance of major insect orders, Arnold Air Force Base, 2022 and 2023. The months of January, November, December, and May contained one sampling event, all other months had a minimum of two sampling events.

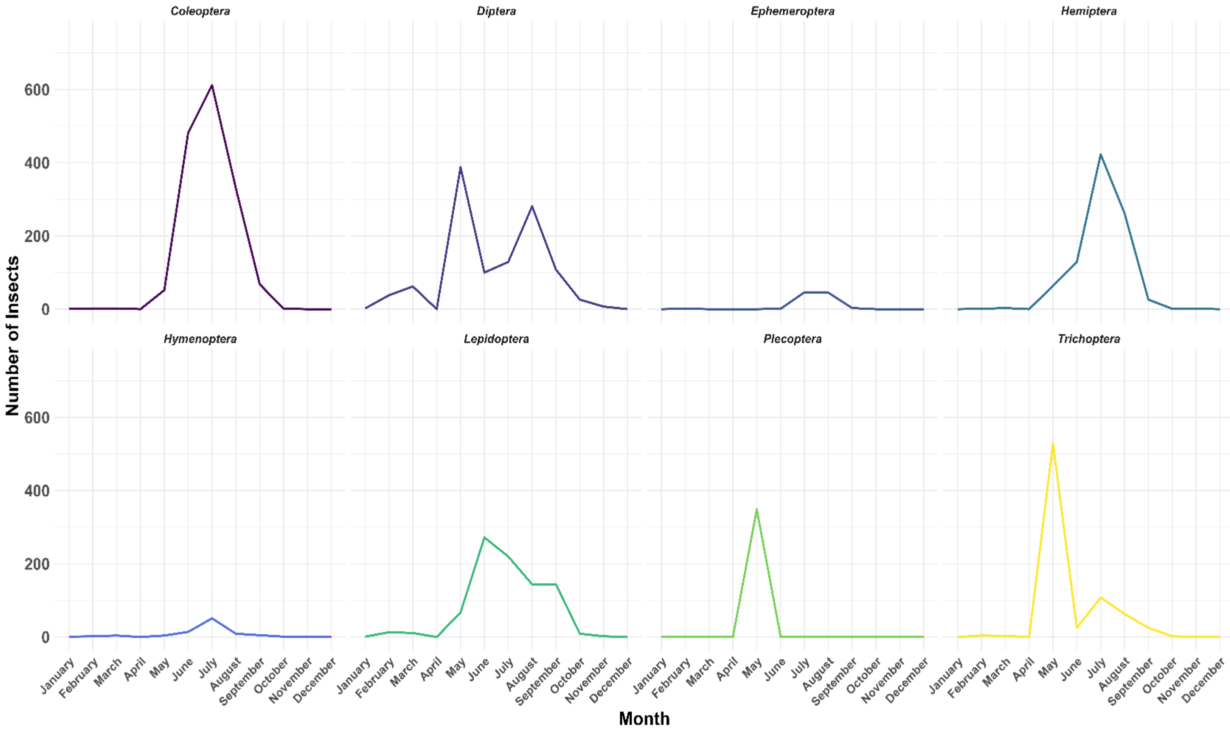

Figure 2.7. Relative seasonal abundance of major insect orders by individual insect order, Arnold Air Force Base, 2022 and 2023. The months of January, November, December, and May contained one sampling event, all other months had a minimum of two sampling events.

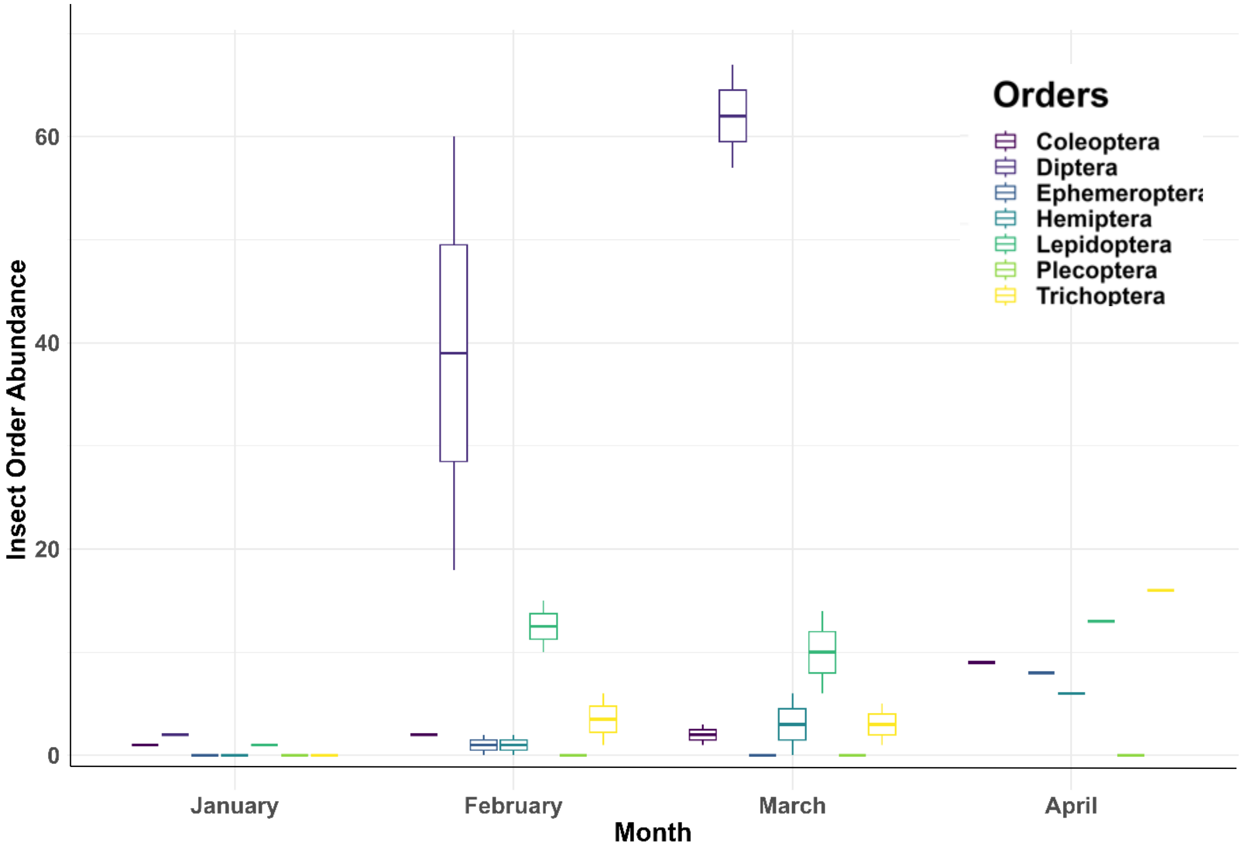

Figure 2.8. Average seasonal abundance of major insect orders for the months of January through April with standard deviation, Arnold Air Force Base, 2022 and 2023. The month of January contained one sampling event, all other months had a minimum of two sampling events.

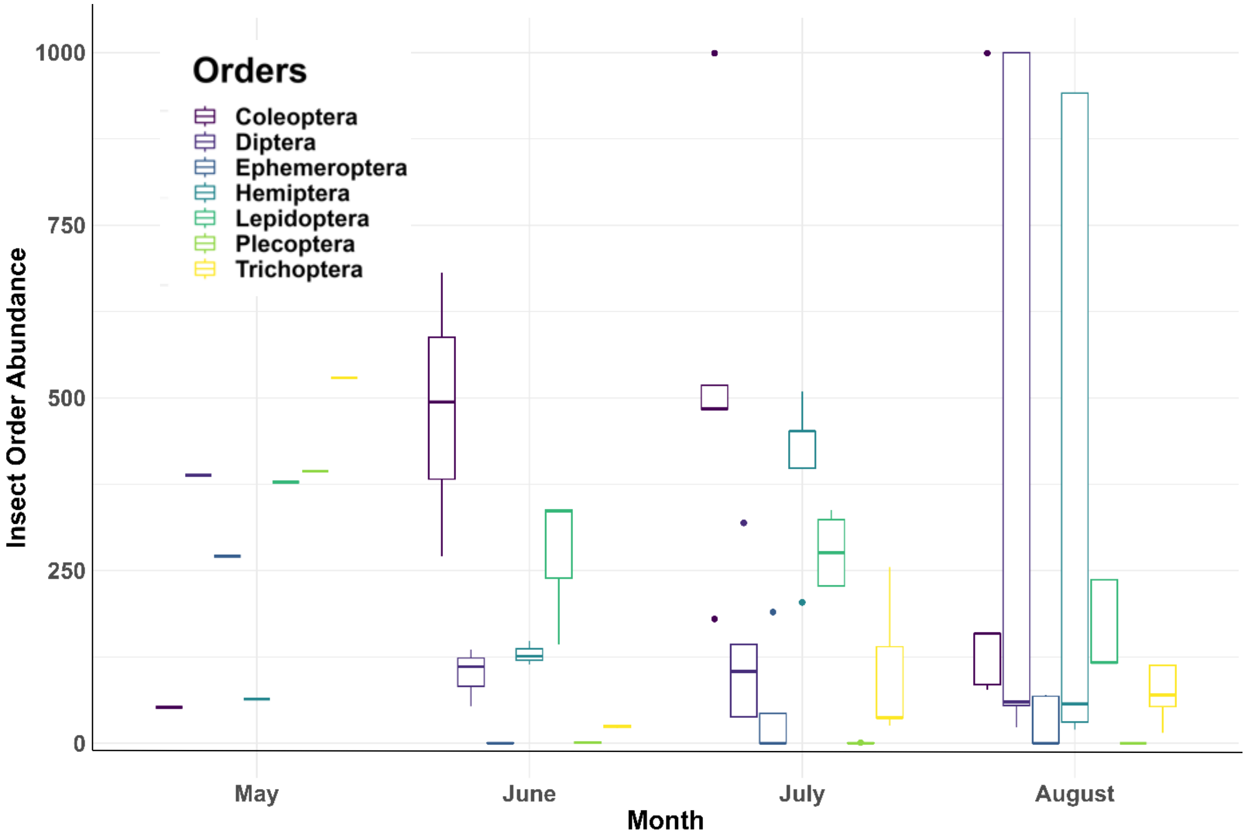

Figure 2.9. Average seasonal abundance of major insect orders for the months of May through August with standard deviation, Arnold Air Force Base, 2022 and 2023. The month of May contained one sampling event, all other months had a minimum of two sampling events.

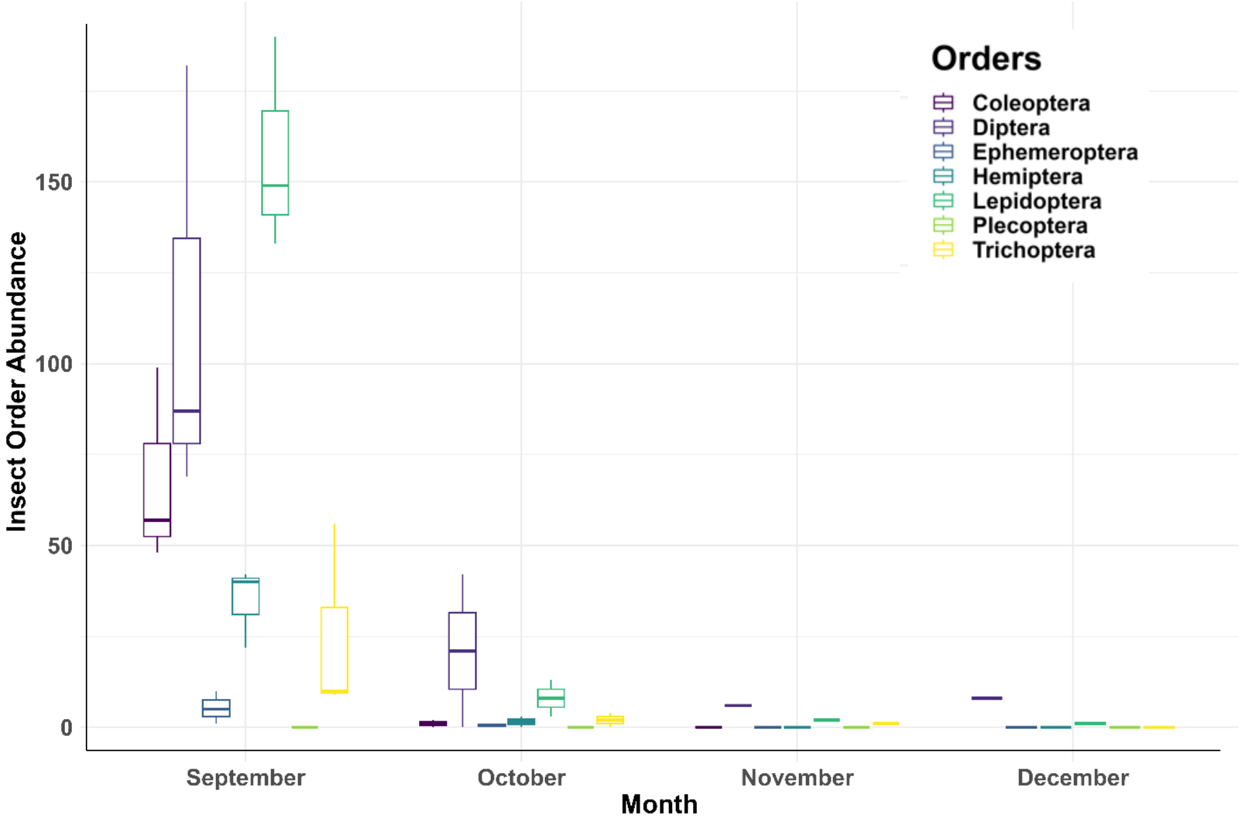

Figure 2.10. Average seasonal abundance of major insect orders for the months of September through December with standard deviation, Arnold Air Force Base, 2022 and 2023. The months of November and December contained one sampling event, all other months had a minimum of two sampling events.

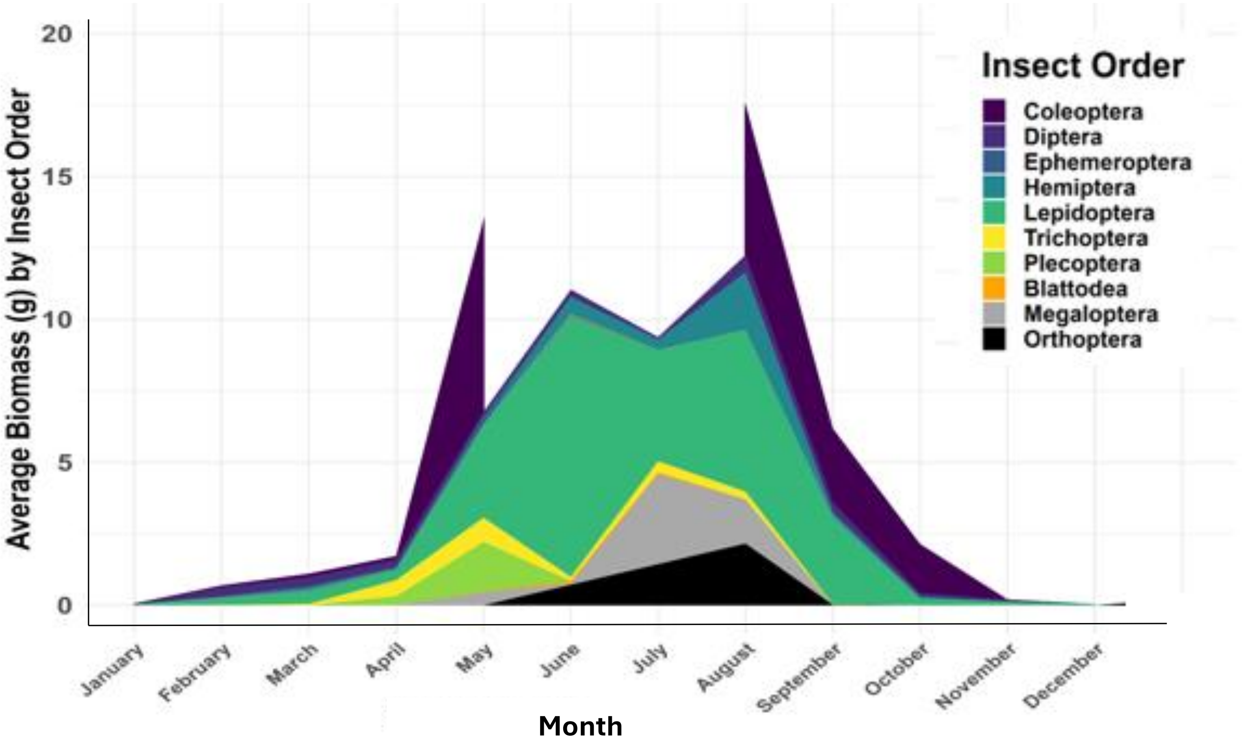

Figure 2.11. Average biomass of insect order by month, Arnold Air Force Base, 2022 and 2023. The months of January, November, December, and May contained one sampling event, all other months had a minimum of two sampling events.

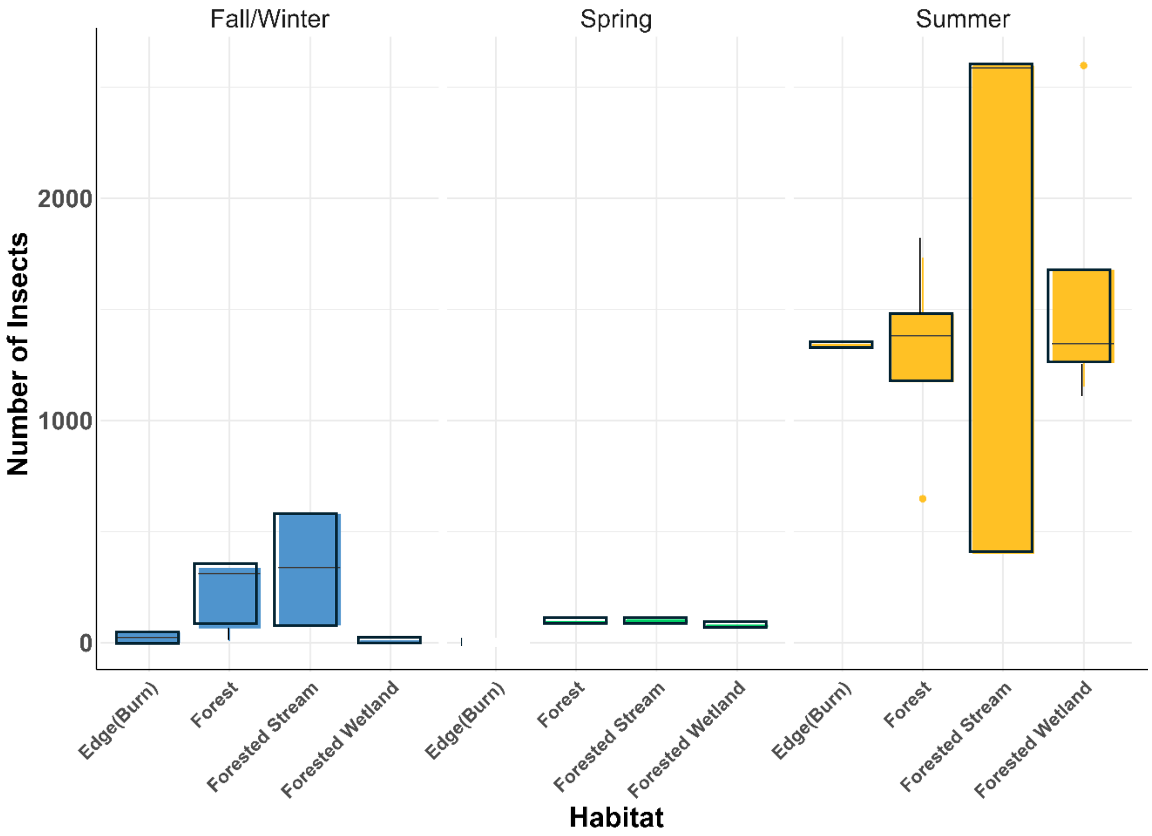

Figure 2.12. Relative insect abundance by habitat and season, Arnold Air Force Base, 2022 and 2023. Sample sizes for habitat included forested streams (n=4 sites), forested sites (n=1 site), forested wetlands (n= 1), and edge (burn) sites (n= 1 site)**.**

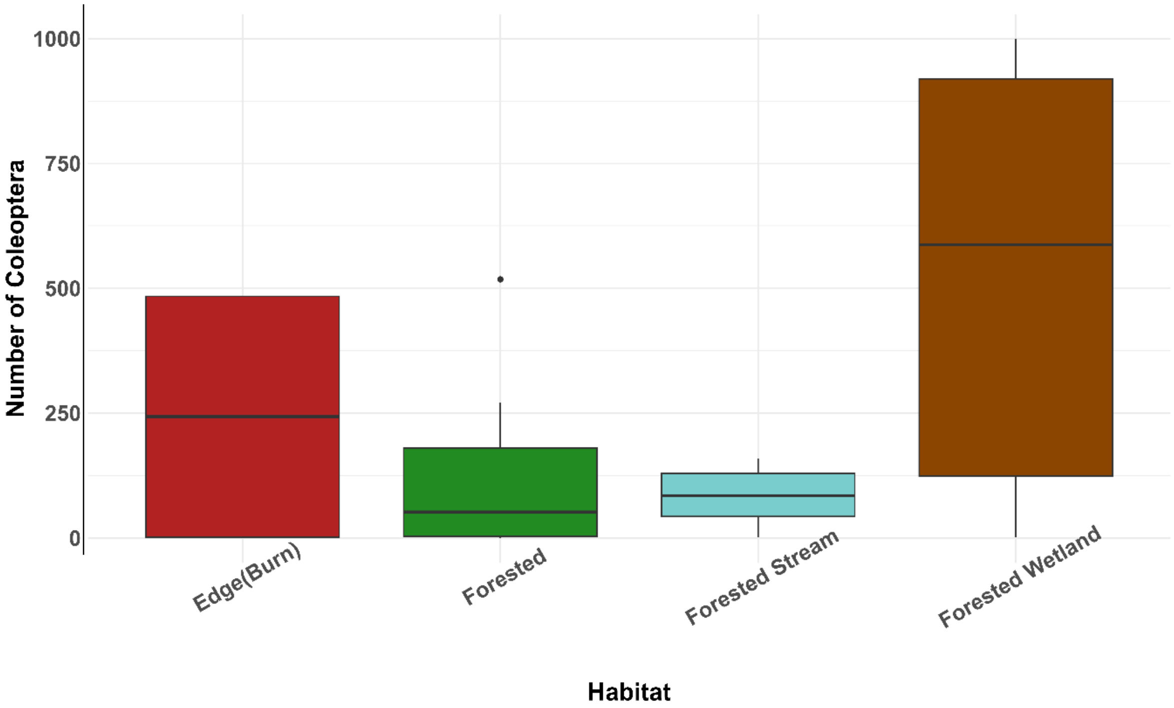

Figure 2.13. Abundance of Coleoptera by habitat across all sampling dates, Arnold Air Force Base, 2022 and 2023.

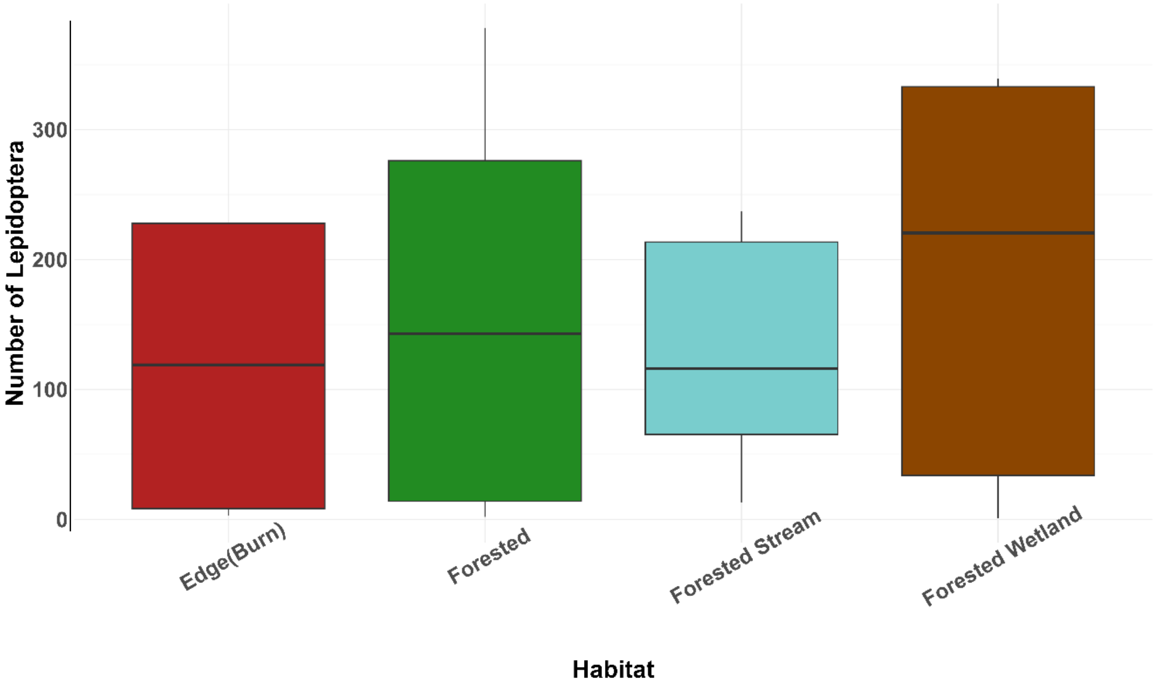

Figure 2.14. Abundance of Lepidoptera collected by habitat type across all sampling dates, Arnold Air Force Base, 2022 and 2023.

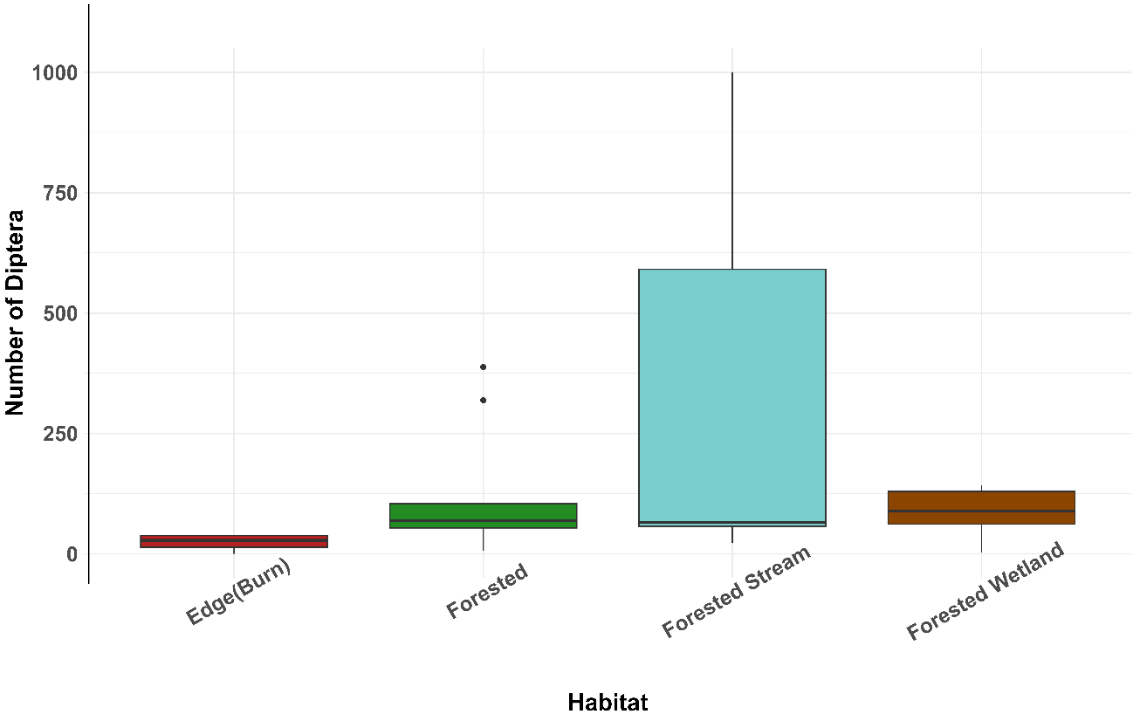

Figure 2.15. Abundance of Diptera by habitat type across all sampling dates, Arnold Air Force Base, 2022 and 2023.

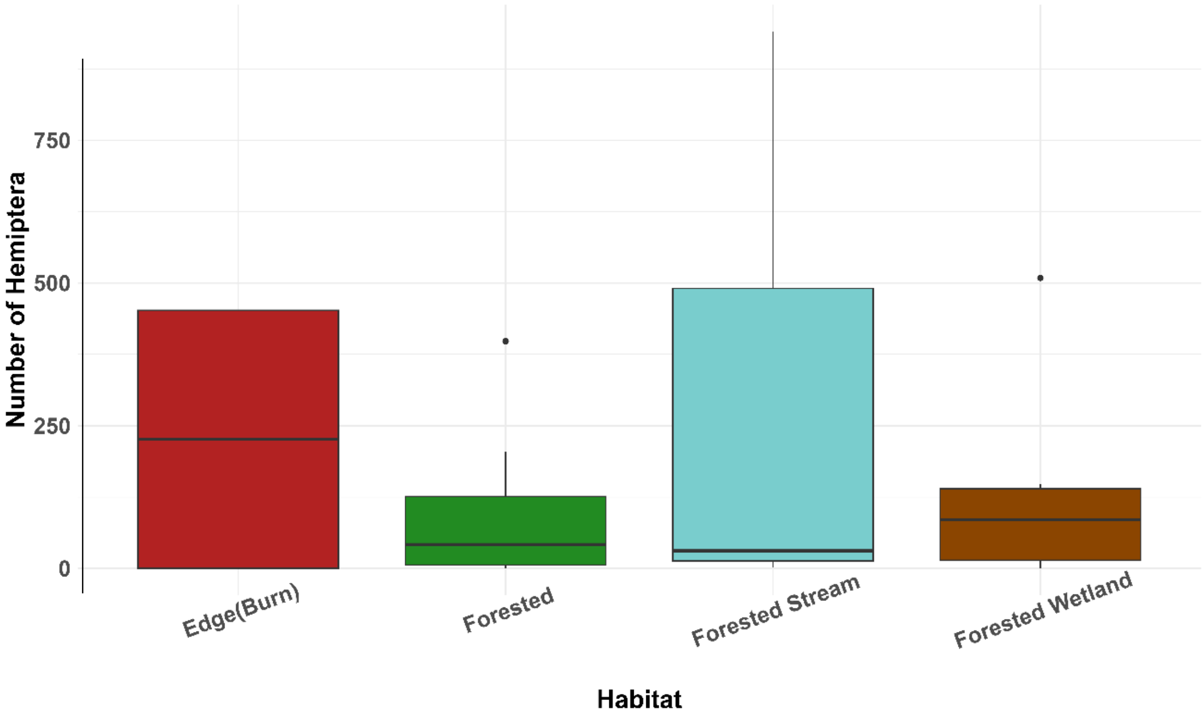

Figure 2.16. Abundance of Hemiptera by habitat across all sampling dates, Arnold Air Force Base, 2022 and 2023.

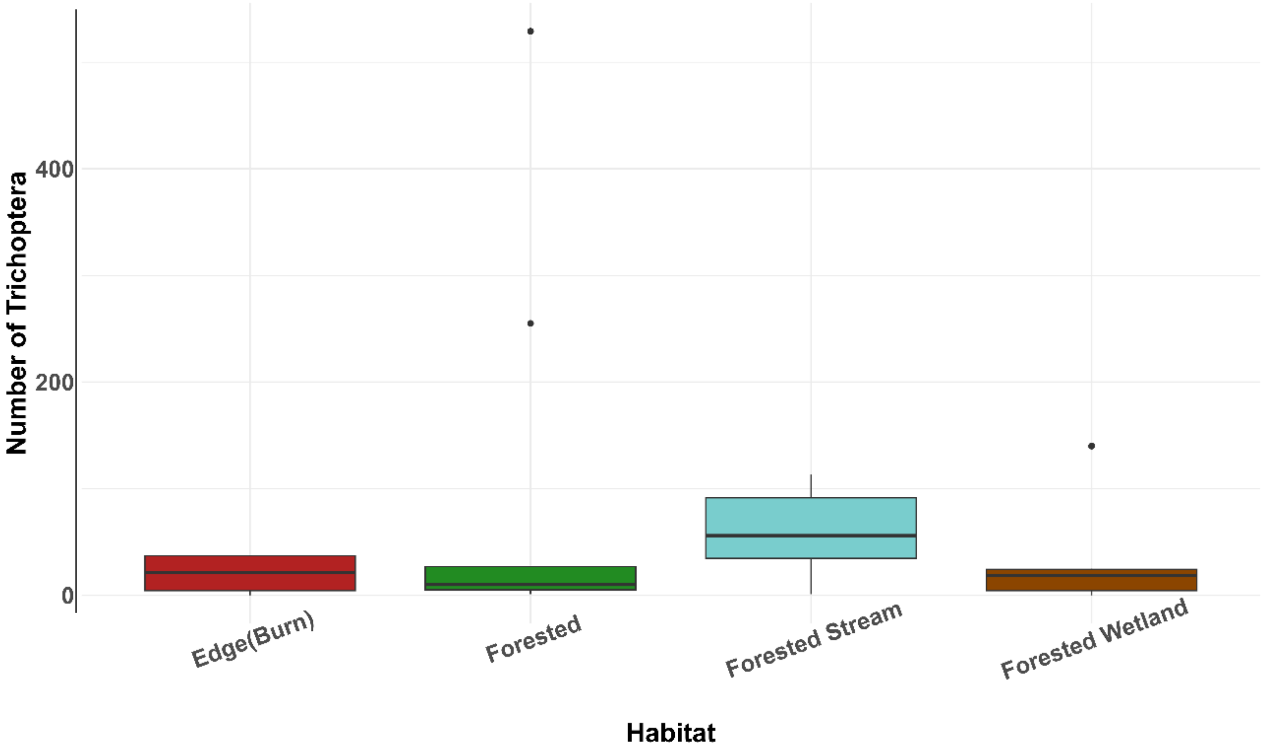

Figure 2.17. Abundance of Trichoptera by habitat across all sampling dates, Arnold Air Force Base, 2022 and 2023.

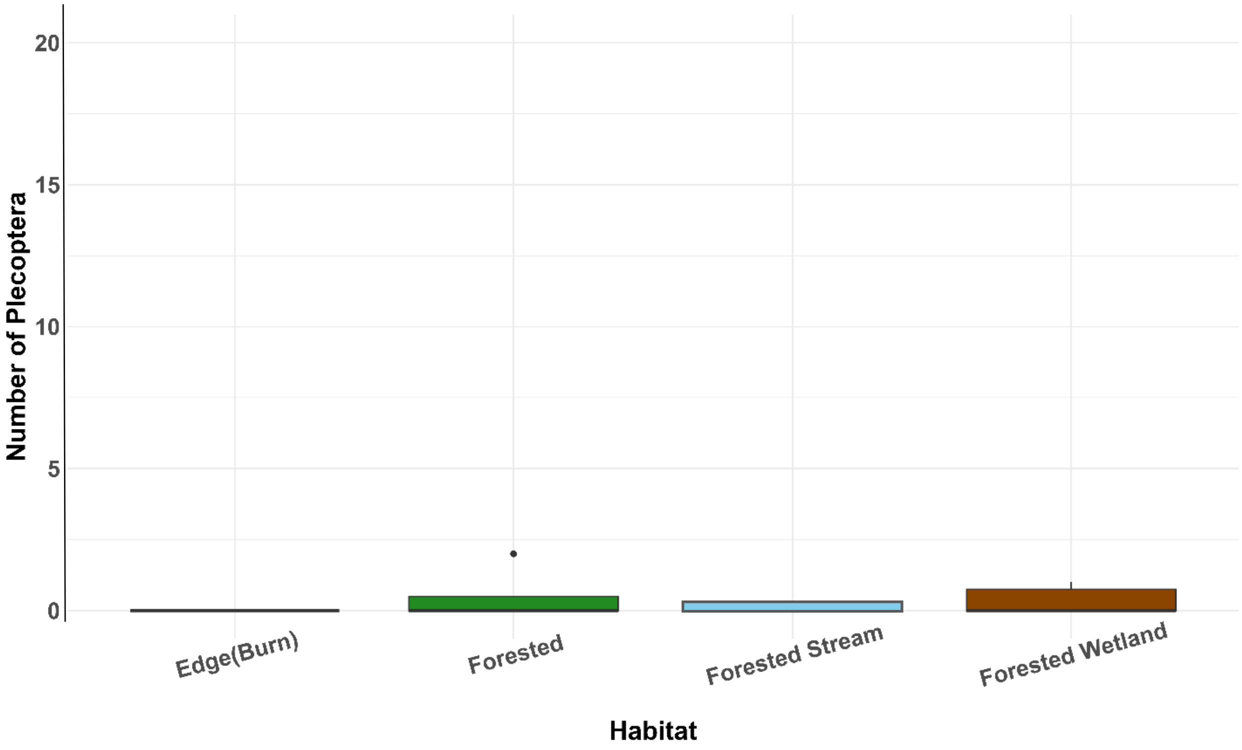

Figure 2.18. Abundance of Plecoptera by habitat type across all sampling dates.

Arnold Air Force Base, 2022 and 2023.

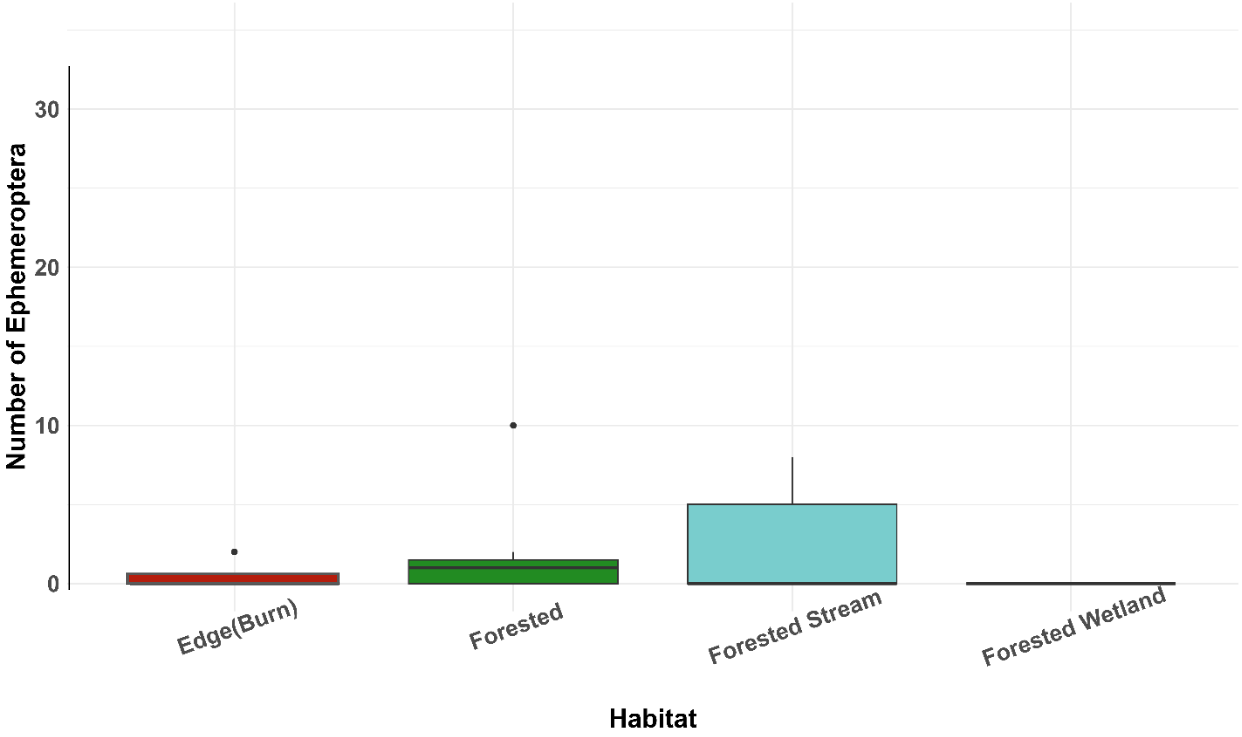

Figure 2.19. Abundance of Ephemeroptera by habitat type across all sampling dates, Arnold Air Force Base, 2022 and 2023.

CHAPTER III

Relationships of Insects and Bats at Arnold Air Force Base:

Mist-Net Sampling

Introduction

Bats are critical to ecosystem balance for their mitigation of agricultural and forest pests and limiting the propagation of vectors that transmit disease-causing pathogens (Maslo et al., 2022; Russo et al., 2018). Bats are also important in ecosystem regulation and provide other resources, such as energy via guano to cave ecosystems (Augusto et al., 2024; Ghanem & Voigt, 2012). However, in the United States, the combination of habitat loss and White Nose Syndrome (WNS) has led to the collapse of many bat populations and restructuring of species communities in the U.S. (Hoyt et al., 2021).

Pseudogymnoascus destructans, the causal agent of WNS, presumably spread from Europe and has caused significant mortality in bat populations in North America (Wilson et al., 2017). Since the introduction of *P. destructans* into North America, the fungus has become well adapted to infecting epidermal tissue of hibernating bats (Lorch et al., 2011; Warnecke et al., 2012). The fungus grows on hibernating bats in the winter, depleting fat storage and disturbing natural torpor periods (Mayberry et al., 2018). Infected bats arouse more often than normal in torpor periods (Reeder et al., 2012). Emaciated bats infected with the fungus often die of starvation in winter months (Reeder & Moore., 2013). The only known traits to reduce mortality of bats when infected with WNS are higher weight and lipid content, which makes it more likely bats will survive infection (Cheng et al., 2019; Pannkuk et al., 2021). Water loss may also contribute to WNS-induced mortality of bats, but has not been confirmed (Willis et al., 2011).

Researchers continue to evaluate ways to slow the spread of mortality associated with WNS and preserve bat populations in North America. However, little research has been conducted by entomologists to assess bat diets. As insect consumption and, in turn, fat deposition is critical for the survival of individual bats infected with WNS, it is important that research efforts assess arthropod abundance, diversity and biomass (Cheng et al., 2019). Understanding the role that arthropods play in predicting bat presence would enable conservationists to assess and integrate management strategies to reduce bat mortality. Little research has been done using arthropod orders as predictors of bat populations (Bender et al., 2021). As arthropod communities experience changes due to globalization, transportation, climate change, and habitat modification, continued assessment of arthropod and bat relationships is essential (Donkersley et al., 2022; Mamay & Simsek, 2017; Thomas et al., 2004).

This study was designed to assess the relative influence of insect order, abundance, diversity, and size on foraging bats at Arnold Air Force Base (AFB) to determine the relationship between bats and their arthropod prey.. Based on previous studies, it is hypothesized that total arthropod abundance is unlikely to predict the presence of bat populations, but the abundance of individual insect orders and the relative size of insects are more likely to predict the presence of bat species. This hypothesis will be addressed by answering the following question: How do insect order, insect abundance, insect diversity, insect size, and habitat influence a bat species to be present at a mist-net site at Arnold AFB?

Materials and Methods

Bat Sampling

All mist-net sampling was led by Dr. Elizabeth Beilke and Dr. Joy O'Keefe of the University of Illinois in a cooperative study with Arnold AFB. Mist netting occurred at six sites at Arnold AFB (Table 3.1). Dates and sites for each mist-netting event (n=13) are listed in Table 3.2. In 2022, sampling began in August and ended in September; in 2023, sampling began in May and ended in August. Mist nets were all made of nylon mesh screen, ranging in size from 8–12 m. Mist nets were hung between two poles and varied from single high nets (about 2 m tall), double high nets (around 4 meters tall), and triple high nets (7.3 m tall) based on the surrounding environment of the site. The number of nets per site ranged from three to five, depending on site parameters. A mist-net collection area is shown in Figure 3.1.

Nets were opened at dusk for six hours with all nets checked for bats every ten minutes. Bats were extracted from nets and placed in see-through nylon mesh bags to hold bats on the paracord line (strung between two structures to clip mesh bat bags on). After 30 minutes bats were identified to species, weighed, sexed, aged (juvenile or adult), given a wing score (0 – 3, 0 is a perfect wing or only one or two pin holes; Reichard and Kunz, 2009). If the bat deposited guano into their bag, a fecal sample was collected and stored in a plastic tube with desiccant beads. After bats were evaluated, they were released and all items that were in contact with the bats were removed or cleaned with ethanol. To slow the spread of WNS all field materials were cleaned with ethanol, discarded, or placed in boiling water for five minutes to prevent the spread of the fungus, as required by WNS protocols (White-Nose Syndrome Response Team, 2024). All personnel were required to wear disposable gloves, as well as handling gloves (thicker, often leather gloves) under the disposable gloves if working directly with bats, n-95 masks while around bats or bat guano, coveralls, and waders (as needed for stream or wetland sites).

Insect Sampling

Insect sampling was conducted concurrently each evening while mist netting occurred for the same six-hour duration. For each insect sampling event, one ultraviolet light (UV trap) (Figure 2.2) and one CDC light trap (John W Hock company) (CO_2 trap) powered by 12-volt batteries (Figure 2.3) were deployed at dusk at a site for six hours at the same time as mist nets were open. UV light traps and CDC light traps were installed at least 100 m (about 328.08 ft) away from mist nets to not interfere with bat trapping. UV light traps emitted wavelengths from 100-400 nm. The bulb in the UV light trap was located directly above the collection chamber and attached to a metal bucket with a funnel top. Two Hot Shot No Pest insect kill strips were placed inside the bottom of the metal bucket (separated from the rest of bucket by metal mesh) (Figure 2.2).

CDC light traps were made from a battery-operated fan with a plastic lid (30.5 cm diameter) attached above the fan, a plastic chamber attached by fabric underneath the fan (to funnel arthropods into chamber), and a plastic 3.8-liter cooler with holes drilled in it (Figure 2.3). Plastic Igloo coolers were filled with 0.23 kg of dry ice for each sampling event and tied next to fans. Dry ice in ambient temperatures sublimates into carbon dioxide (mimicking animals' exhalations) and is used to attract hematophagous insects.

After six hours, samples were collected and the specimens were removed, placed in 3.8-liter Ziplock bags placed in a large cooler, and taken to the laboratory. Samples were stored in a refrigerator until they could be sorted and identified to family at minimum (using keys from Marshall, 2006).

After each sample was collected, they were sorted to order (using keys from Marshall, 2006) and weighed (in grams) to determine the dry weight (in grams) (i.e., dry biomass) of each insect order. Once the biomass of each order was calculated, specimens in each order were then sorted to family, or a lower taxonomic group when feasible, and each specimen was weighed and assigned a classification based on weight. I then created weight classifications of each group, and these consisted of: Mega (>1.0 g), Macro (1.0-0.1 g), Standard (0.1-0.011 g), and Micro (< 0.01 g). This method allowed for biomass determinations by order and specimen. Samples were stored in 95% ethanol in vials (10 ml). Lepidopteran specimens were stored dry in Petri-dishes (100x15 mm), and Culicidae were sorted, stored in Petri-dishes (100x15 mm) (to preserve scales for future further identification) and placed in the refrigerator. Representative samples were spread and pinned as needed for voucher specimens.

Data Analysis

For all data analysis, figures, models, and statistics were constructed using R version 4.2.3. For each mist-net sample, bat species were recorded for abundance, richness, and presence (0 or 1). For each insect sample (UV light trap and carbon dioxide trap combined) in correlation to the mist-net sampling event, insect abundance, insect family diversity, insect family richness, and the number of insects in each weight class (Mega (> 1.0 g), Macro (1.0-0.1 g), Standard (0.1-0.011 g), and Micro (< 0.01 g)) were calculated. Shannon's family diversity index and family richness were calculated for each sample using R package VEGAN. The number of insects belonging to each insect order and family was also calculated for each sampling event. Spearman's correlation test was performed on total insect abundance and insect diversity and richness. Trends between total insect abundance and total bat abundance were graphed.

Binomial generalized linear models (GLMs) were used to analyze the relationship between bat presence or absence at mist nets. Fixed effects for the binomial GLMs included insect abundance, insect order abundance, insect size class abundance, and habitat type to determine what factors, if any, influenced individual bat species presence and bat species richness (Table 4.4). Insect family diversity and insect family richness were not included in models due to their high correlation values with insect abundance (Spearman correlation > 0.6). Best fitting models were determined by the lowest Akaike Information Criterion corrected (AICc) value, and if two models had the lowest AICc value then the most conservative model was selected. AICc is used to correct low sample sizes in models (Brewer et al., 2016). Models were deemed to be significantly different from one another with a delta AICc of 2 (Anderson & Burnham, 2004). A null model was used for comparison on all constructed models. Null models were used to provide a basic guide for understanding predictor variables and were set so bat presence was not predicted by any variable. For a fixed effect to be considered significant, 95% confidence intervals should not cross 0 and $p \leq 0.05$. If bat species richness or bat species presence was significantly correlated to insect order abundance the five highest family abundances in that order were also tested using binomial GLM.

Results and Discussion

During 13 simultaneous mist netting and insect trapping events at Arnold AFB; 273 bats were caught, and more than 20,000 insects were collected. The four most captured bat species and their seasonality on the base during the are shown in Figure 3.2. Bats collected in 2022-2023, included 91 eastern red bats, 86 evening bats, 65 gray bats, 26 big brown bats, 2 tri-color bats, and 1 hoary bat. A summary of bat common names, scientific names, and species code can be found in Table 3.3. Arthropod orders represented in the collections made in conjunction with mist netting were Coleoptera, Blattodea, Collembola, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, Plecoptera, Psocoptera, Megaloptera, Neuroptera, and Trichoptera. Seasonality of insects collected in 2022 and 2023 is depicted in Figure 3.3. For more information regarding specific arthropod families, biomass of insects surveyed in summer months, diversity of families, and order abundance please refer to Chapter II.

The relationship between bat abundance and insect abundance in the four different habitat types, forested streams, forested sites, and edge (burn) sites all had positive relationships with bat abundance, while forested wetlands had a negative correlation between insect abundance and bat abundance (Figure 3.4). The forested site was the site with the most positive relationship between insect abundance and bat abundance (Figure 3.5).

Insect richness and insect diversity were first correlated separately with insect abundance. Insect richness and insect abundance were found to be highly correlated (Spearman's coefficient of 0.8154, p=0.0006), while insect diversity and insect abundance were poorly correlated (Spearman's coefficient of -0.0093, p=0.7). Thus, insect diversity and insect abundance were used in Binomial GLM models to limit repetition in the results. Insect abundance was chosen for its prevalence in prior literature over insect richness for importance with bat foraging.

Though some variables were selected as being better than indicators of presence or absence of bat species than the null models (~1) all these variables had very large confidence intervals passing zero and p>0.05 (Table 3.5 & 3.6). Figures 3.6, 3.7, 3.8, and 3.9 visualize the variables selected as better than the null models, these graphs show very large standard errors associated with these relationships. These metrics suggest that our models were overall poor and could potentially benefit from a different analysis. These findings suggest that model confidence and significance is likely linked to small sample sizes or distribution of the dataset, suggesting this study would benefit from continuation or repetition.

Discussion

Overall, binomial GLM models constructed to predict the presence of bat species and bat species richness were not well supported. Results indicate the lack of significance of these models may be due to over or under-dispersion related to the small sample size (n=13) or that other variables than habitat and insect metrics are important to predicting bat species at a mist net site, such as proximity to roosts, height of flight, presence of water or other ecological and landscape factors (Beilke et al., 2023; Threlfall et al., 2012). These results may also indicate there may be a large amount of chance in which species are captured at a mist net site at any given point in time (Larsen et al., 2007). These models would likely improve with a repeated study and a higher sample size, but also may benefit by the inclusion of other predictor variables.

Researchers commonly use UV light and carbon dioxide traps because they can collect large numbers of insects in relatively short time periods, but this trapping method does present unique biases (Truxa & Fiedler, 2012). Traps that use attractants can often attract more families or species that are naturally attracted to UV wavelengths (such as mud-loving beetles - Heteroceridae) or carbon dioxide (such as mosquitos - Culicidae) and lead to skewed interpretations of insect biodiversity (McDermott & Mullens, 2018). Mist-net sampling also has its own biases with bats that are caught often flying at a lower height or bats that forage over water as mist nets have height limitations and are often installed over bodies of water and in corridors (Larsen et al., 2007). Mist netting can be biased towards certain species, leading to some species of bats being under or over-sampled (Flaquer et al., 2007).

It is important to interpret the results of these sampling efforts with care. Though these models were overall poor, previous research suggests unique selection of prey items and habitats by foraging bat species (Burgar et al., 2014; Agosta et al., 2003). These parameters may be useful in addressing current questions about predator-prey relationships and used as templates for future work. While we didn’t find insects nor habitat to be very important as predictors, others have (Bender et al., 2021; Charbonnier et al., 2014; Thomas & Jacob, 2013). We suggest in the future combing data on insect abundance, biomass, and diversity with other larger scale factors to better understand the multivariate factors predicting bat distributions during the summer.

Literature Cited

Agosta, S. J., Morton, D., & Kuhn, K. M. (2003). Feeding ecology of the bat *Eptesicus fuscus:* ‘preferred’ prey abundance as one factor influencing prey selection and diet breadth. *Journal of Zoology*, *260*(2), 169-177.

Anderson, D., & Burnham, K. (2004). Model selection and multi-model inference. *Second. NY: Springer-Verlag*, *63*(2020), 10.

Augusto, A. M., Raposeira, H., Horta, P., Mata, V. A., Aizpurua, O., Alberdi, A., ... & Rebelo, H. (2024). Bat diversity boosts ecosystem services: Evidence from pine processionary moth predation. *Science of the Total Environment*, *912*, 169387.

Beilke, E. A., Haulton, G. S., & O'Keefe, J. M. (2023). Foliage-roosting eastern red bats select for features associated with management in a central hardwood forest. *Forest Ecology and Management*, *527*, 120604.

Bender, M. J., Perea, S., Castleberry, S. B., Miller, D. A., & Wigley, T. B. (2021). Influence of insect abundance and vegetation structure on site-occupancy of bats in managed pine forests. *Forest Ecology and Management*, *482*, 118839.

Brewer, M. J., Butler, A., & Cooksley, S. L. (2016). The relative performance of AIC, AICC and BIC in the presence of unobserved heterogeneity. *Methods in Ecology and Evolution*, *7*(6), 679-692.

Burgar, J. M., Murray, D. C., Craig, M. D., Haile, J., Houston, J., Stokes, V., & Bunce, M. (2014). Who's for dinner? High-throughput sequencing reveals bat dietary differentiation in a biodiversity hotspot where prey taxonomy is largely undescribed. *Molecular Ecology*, *23*(15), 3605-3617.

Charbonnier, Y., Barbaro, L., Theillout, A., & Jactel, H. (2014). Numerical and functional responses of forest bats to a major insect pest in pine plantations. *PLoS One*, *9*(10), e109488.

Cheng, T. L., Gerson, A., Moore, M. S., Reichard, J. D., DeSimone, J., Willis, C. K., ... & Kilpatrick, A. M. (2019). Higher fat stores contribute to persistence of little brown bat populations with white-nose syndrome. *Journal of Animal Ecology*, *88*(4), 591-600.

Donkersley, P., Ashton, L., Lamarre, G. P., & Segar, S. (2022). Global insect decline is the result of willful political failure: A battle plan for entomology. *Ecology and Evolution*, *12*(10), e9417.

Flaquer, C., Torre, I., & Arrizabalaga, A. (2007). Comparison of sampling methods for inventory of bat communities. *Journal of Mammalogy*, *88*(2), 526-533.

Ghanem, S. J., & Voigt, C. C. (2012). Increasing awareness of ecosystem services provided by bats. In *Advances in the Study of Behavior* (Vol. 44, pp. 279-302). Academic Press.

Hoyt, J. R., Kilpatrick, A. M., & Langwig, K. E. (2021). Ecology and impacts of white-nose syndrome on bats. *Nature Reviews Microbiology*, *19*(3), 196-210.

Larsen, R. J., Boegler, K. A., Genoways, H. H., Masefield, W. P., Kirsch, R. A., & Pedersen, S. C. (2007). Mist netting bias, species accumulation curves, and the rediscovery of two bats on Montserrat (Lesser Antilles). *Acta Chiropterologica*, *9*(2), 423-435.

Lorch, J. M., Meteyer, C. U., Behr, M. J., Boyles, J. G., Cryan, P. M., Hicks, A. C., ... & Blehert, D. S. (2011). Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature*, *480*(7377), 376-378.

Mamay, M., & Şimşek, E. (2017). The Expected Impact of Global Warming and Climate Change on Insect Diversity. *The 3rd International Symposium on EuroAsian Biodiversity*, pp. (173).

Marshall, S. A. (2006). *Insects: their natural history and diversity: with a photographic guide to insects of eastern North America* (p. 718). Buffalo, NY: Firefly Books.

Maslo, B., Mau, R. L., Kerwin, K., McDonough, R., McHale, E., & Foster, J. T. (2022). Bats provide a critical ecosystem service by consuming a large diversity of agricultural pest insects. *Agriculture, Ecosystems & Environment*, *324*, 107722.

Mayberry, H. W., McGuire, L. P., & Willis, C. K. (2018). Body temperatures of hibernating little brown bats reveal pronounced behavioural activity during deep torpor and suggest a fever response during white-nose syndrome. *Journal of Comparative Physiology B*, *188*, 333-343.

McDermott, E. G., & Mullens, B. A. (2018). The dark side of light traps. *Journal of Medical Entomology*, *55*(2), 251-261.

Pannkuk, E. L., Dorville, N. A. Y., Dzal, Y. A., Fletcher, Q. E., Norquay, K. J., Willis, C. K., ... & Laiakis, E. C. (2021). Hepatic lipid signatures of little brown bats (*Myotis lucifugus*) and big brown bats (*Eptesicus fuscus*) at early stages of white-nose syndrome. *Scientific Reports*, *11*(1), 11581.

Reeder, D. M., Frank, C. L., Turner, G. G., Meteyer, C. U., Kurta, A., Britzke, E. R., ... & Blehert, D. S. (2012). Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PloS One*, *7*(6), e38920.

Reeder, D. M., & Moore, M. S. (2013). White-nose syndrome: A deadly emerging infectious disease of hibernating bats. *Bat Evolution, Ecology, and Conservation*, 413-434.

Reichard, J. D., & Kunz, T. H. (2009). White-nose syndrome inflicts lasting injuries to the wings of little brown *Myotis* (*Myotis lucifugus*). *Acta Chiropterologica*, *11*(2), 457-464.

Russo, D., Bosso, L., & Ancillotto, L. (2018). Novel perspectives on bat insectivory highlight the value of this ecosystem service in farmland: Research frontiers and management implications. *Agriculture, Ecosystems & Environment*, *266*, 31-38.

Threlfall, C. G., Law, B., & Banks, P. B. (2012). Influence of landscape structure and human modifications on insect biomass and bat foraging activity in an urban landscape. *PloS One*, *7*(6), e38800.

Thomas, A. J., & Jacobs, D. S. (2013). Factors influencing the emergence times of sympatric insectivorous bat species. *Acta Chiropterologica*, *15*(1), 121-132.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., ... & Williams, S. E. (2004). Extinction risk from climate change. *Nature*, *427*(6970), 145-148.

Truxa, C., & Fiedler, K. (2012). Attraction to light-from how far do moths (Lepidoptera) return to weak artificial sources of light?. *European Journal of Entomology*, *109*(1). 77-84.

Warnecke, L., Turner, J. M., Bollinger, T. K., Lorch, J. M., Misra, V., Cryan, P. M., ... & Willis, C. K. (2012). Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proceedings of the National Academy of Sciences*, *109*(18), 6999-7003.

Willis, C. K., Menzies, A. K., Boyles, J. G., & Wojciechowski, M. S. (2011). Evaporative water loss is a plausible explanation for mortality of bats from white-nose syndrome. *Integrative and Comparative Biology*, *51*(3), 364-373.

Wilson, M. B., Held, B. W., Freiborg, A. H., Blanchette, R. A. & Salomon, C. E. (2017). Resource capture and competitive ability of non-pathogenic *Pseudogymnoascus* spp. and *P. destructans*, the cause of white-nose syndrome in bats. *PLoS One, 12*, e0178968.

White-Nose Syndrome Response Team. (2024). (rep.). *National White-Nose Syndrome Decontamination Protocol.* Retrieved from https://s3.us-west-2.amazonaws.com/prod-is-cms-assets/wns/prod/669f6310-dd5e-11ee-9008-8dbb177a1768-March2024-%20revised%20WNS%20Decon%20Protocol%20-%20Final.pdf.

Appendix

Table 3.1. Overview of mist-net sites and congruent insect sampling at Arnold Air Force Base, 2022 and 2023.

Site	Mist Net*	UV/CO2**	Prescribed Burning***	Habitat	Year(s) Collected
BBI021	Yes	Yes	No	**Mixed Forested Stream** (Brumalow Creek)	2022
BBI125	Yes	Yes	No	**Mixed Forested Wetland** (Sinking Pond)	2022, 2023
BBI051	Yes	Yes	No	**Mixed Forested Stream** (Bradley Creek)	2022, 2023
BBI052	Yes	Yes	No	**Mixed Forested Stream** (Rowland Creek)	2023
BBI097	Yes	Yes	Yes	**Edge** (Mixed Forest to Grassland)	2023
BBI105	Yes	Yes	No	**Mixed Forest** (Road Rut Close)	2022, 2023

*Mist-net column indicates if mist netting occurred at the corresponding site.

**UV/CO2 column indicates if insect sampling occurred in conjunction with mist netting.

***Prescribed burning column indicates which sites receive yearly prescribed burning treatments.

Table 3.2. Site and date for each mist-net sample collected at Arnold Air Force Base, 2022 and 2023.

Sample Number	Site*	Summer Date	Season
1	BBI021 (MFS)	16 August 2022	Summer
2	BBI021 (MFS)	18 August 2022	Summer
3	BBI051 (MFS)	16 September 2022	Early Fall Sample
4	BBI105 (MF)	17 September 2022	Early Fall Sample
5	BBI125 (MFW)	18 September 2022	Early Fall Sample
6	BBI052 (MFS)	25 May 2023	Spring Sample
7	BBI125 (MFW)	26 June 2023	Summer
8	BBI125 (MFW)	27June 2023	Summer
9	BBI105 (MF)	28 June 2023	Summer
10	BBI097 (E)	24 July 2023	Summer
11	BBI052 (MFS)	25 July 2023	Summer
12	BBI051 (MFS)	21 August 2023	Summer
13	BBI051 (MFS)	22 August 2023	Summer

*Sites are E = Edge, MFS = Mixed Forest Stream, MFW = Mixed Forest Wetland, and MF = Mixed Forest.

Table 3.3. Bat species documented at Arnold Air Force Base, Tennessee.

Species and Genus	Common Name	Species Code*	Current Status**	WNS Been Found in Species?
Myotis grisescens	Gray Bat	MYGR	Endangered	Yes
Perimyotis subflavus	Tricolored Bat	PESU	Under Review	Yes
Myotis lucifugus	Little Brown Bat	MYLU	Under Review	Yes
Eptesicus fuscus	Big Brown Bat	EPFU	Least Concern	Yes
Lasiurus borealis	Eastern Red Bat	LABO	Least Concern	No
Lasiurus cinereus	Hoary Bat	LACI	Least Concern	No
Myotis sodalis	Indiana Bat	MYSO	Endangered	Yes
Myotis septentrionalis	Northern Long-Eared Bat	MYSE	Endangered	Yes
Nycticeius humeralis	Evening Bat	NYHU	Least Concern	No

*Species Codes are abbreviations of the scientific names of bat species that use the first two letters of the genus and species to make the species code.

**Current Status refers to the current listing of each species by International Union for Conservation of Nature (IUCN) : extinct, critically endangered, endangered, vulnerable, near threatened, least concern, or under review (listing is currently pending, and population changes have warranted a potential new listing.

Table 3.4. Models tested to predict presence or absence of bat species at mist-net sites at Arnold Air Force Base, Tennessee.

Model Name	Parameters	Hypothesis
Habitat	Habitat included the 4 habitats surveyed with acoustic monitoring. This model included forested, forested wetlands, forested streams, and edge site with prescribed burn treatment.	Bat activity is influenced by the habitat.
Insect Abundance	This model included total arthropod abundance from each sampling event from the combined UV light trap and CDC light trap.	Bat activity is influenced by total prey availability.
Insect Diversity	This model included Shannon diversity from each sampling event from the combined UV light trap and CDC light trap.	Bat activity is influenced by the diversity of prey availability.
Insect Order Abundance	This model included arthropod orders with the highest abundances from each sampling event from the combined UV light trap and CDC light trap. These variables included Coleoptera, Lepidoptera, Diptera, Hemiptera, Trichoptera, Plecoptera, and Ephemeroptera.	Bat activity is influenced by specific prey groups' availability.

Table 3.4. Continued.

Insect Weight	This model has an abundance of insects in each weight class from each sampling event from the combined UV light trap and CDC light trap. These variables included Mega (>1.0 g), Macro (1.0-0.1 g), Standard (0.1-0.011 g), and Micro (< 0.01 g).	Bat activity is influenced by the biomass of prey available.

Table 3.5. Models predicting the presence of bat species using mist-net sampling, Arnold Air Force Base, 2022 and 2023.

Bat Species	Parameter*	△AICc**
Big Brown Bat	~ Coleoptera	0
	~ Trichoptera	0.02
	~ Plecoptera	0.77
Eastern Red Bat	~ 1	0
Gray Bat	~ Mega	0
Evening Bat	~ Insect Abundance	0
	~ Micro	0.11

*Parameter indicates which predictor variable was modeled to predict the presence of the corresponding bat species.

**Delta AICc was used to analyze the differences between AICc values for constructed models. Delta AICc subtracts the lowest AICc value from the AICc of the corresponding model. To significantly differentiate between two models a delta AICc value (often of 2) is employed. The AIC is the "Akaike information criterion" and it is an estimate of how well a model is describing the patterns in the data. It is mainly used for comparing models trained on the same dataset, and delta AICc is adjusted AIC for small sample sizes.

Table 3.6. Parameter estimates, standard errors, and 95% confidence intervals for plausible models that predicted the presence of bat species presence using mist-net sampling, Arnold Air Force Base, 2022 and 2023.

Bat Species	Parameter*	Estimate**	SE***	Lower 95****	Upper 95****
Big Brown Bat	~ Coleoptera	0.0046	0.0027	-0.0008	0.01
	~ Trichoptera	-0.0387	0.0332	-0.1051	0.0277
	~ Plecoptera	-17.168	3972.3	-3989.5	3955.1
Eastern Red Bat	~1				
Gray Bat	~ Mega	0.042	0.112	-0.182	0.644
Evening Bat	~ Insect Abundance	1.621	290.61	-579.59	582.23
	~ Micro	0.855	234.3	-467.75	469.45

*Parameter indicates which predictor variable was modeled to predict the presence of the corresponding bat species.

**Estimate corresponds to the covariance between the predictor variable and the predicted variable; these values show the correlation between the bat species and the predictor variable (positive or negative and to what extent). For fixed variables such as habitat, the estimate is based on whether it is occurring. For random variables this increases as if the predictor variable were one unit higher.

***SE describes the standard error associated with the model predictions based on the dataset used to construct the model.

***** Lower and Upper 95 show the 1st and 3rd quarter confidence intervals for the model. This value can be used to allude to the distribution of the provided dataset and confidence level of overall model.

Figure 3.1. Example of mist-net site with nets closed, Arnold Air Force Base. 2022 (flagging indicates location of closed net).

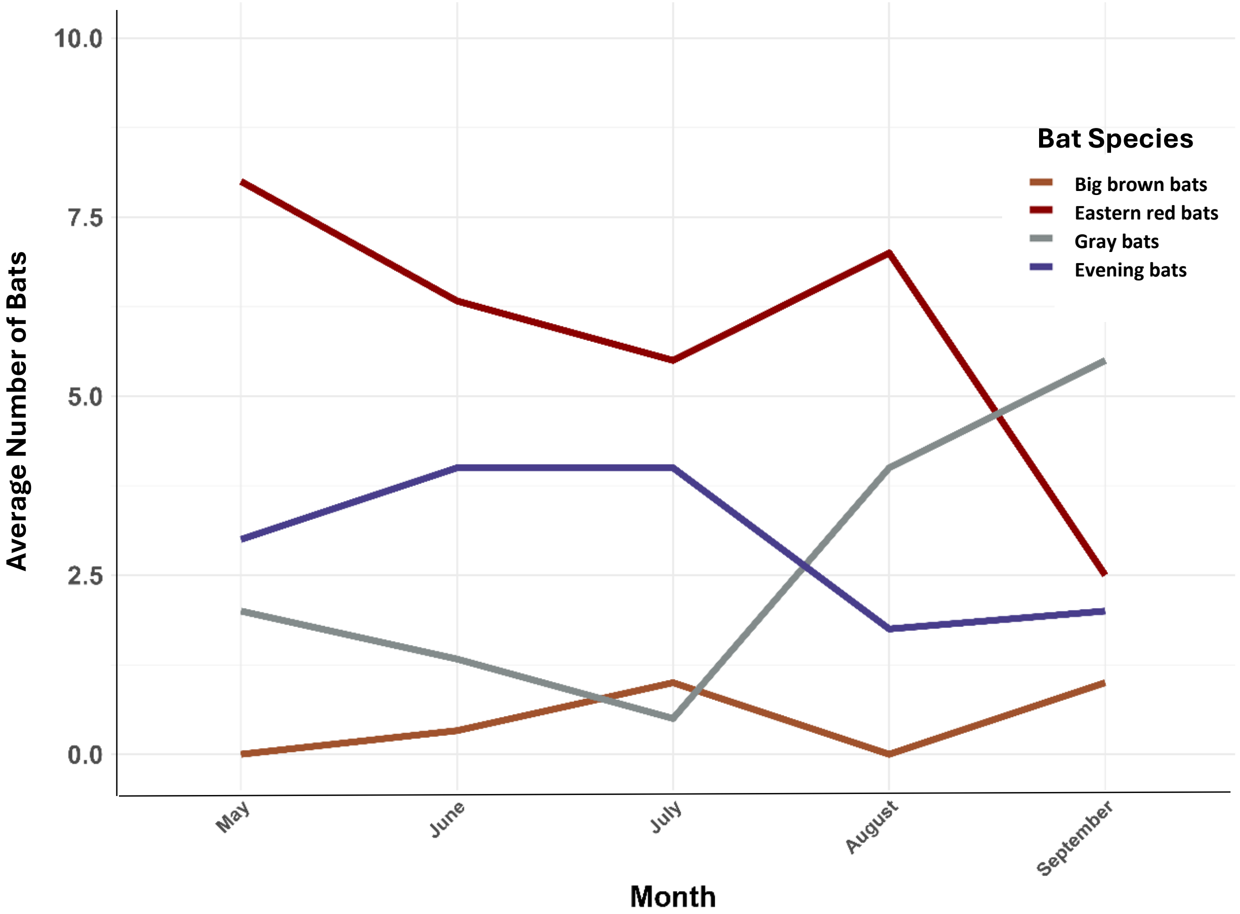

Figure 3.2. Average number of bats captured each month by species collected from mist-net sampling (n= 13) at Arnold Air Force Base, 2022 and 2023. Tricolor and Hoary bats were captured but were not graphed due to small sample size.

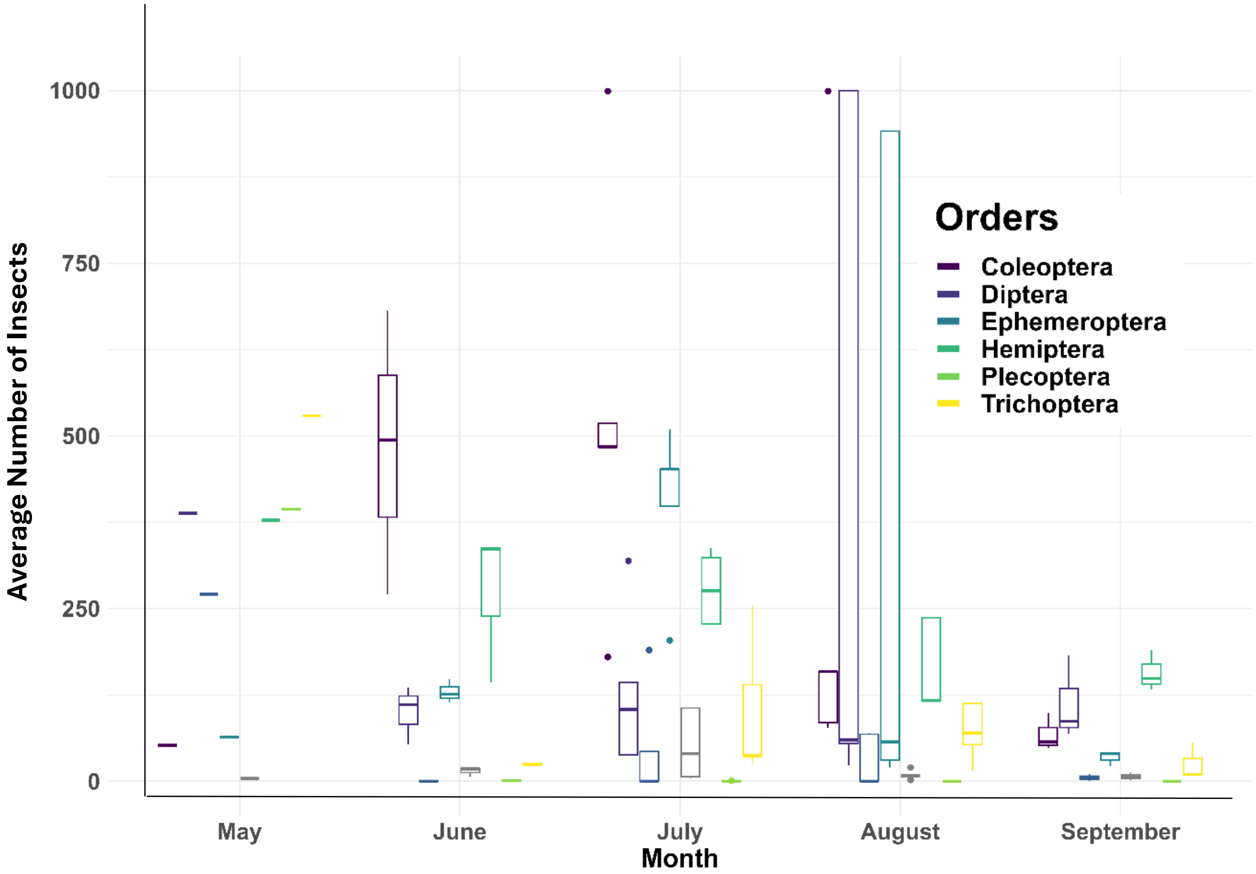

Figure 3.3. Abundance of insects in the major insect orders collected in carbon dioxide traps plus UV traps, Arnold Air Force Base, 2022 and 2023. May 2023 only had one sampling event associated with it**.**

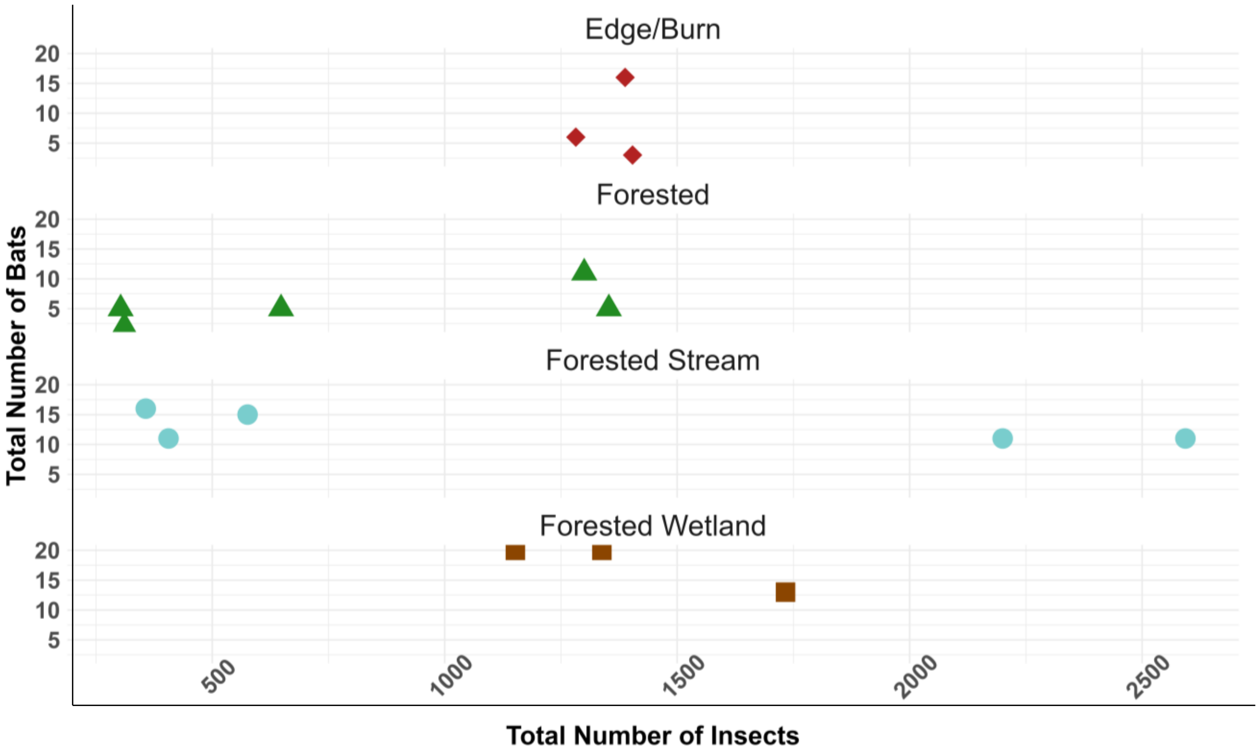

Figure 3.4. Relationship between insect abundance and bat abundance for each of the four habitat types sampled by mist nets, Arnold Air Force Base, 2022 and 2023.

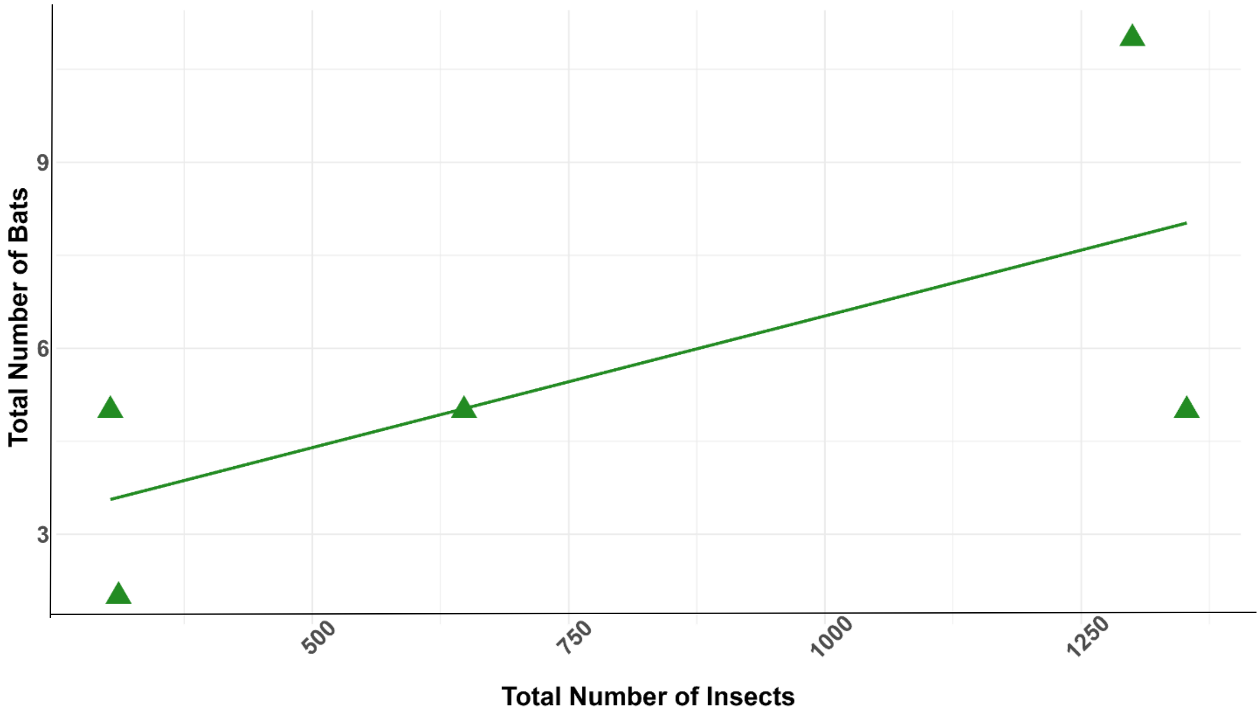

Figure 3.5. Relationship between insect abundance and bat abundance for forested habitats, Arnold Air Force Base, 2022 and 2023. The trend line is loess smoothed best fit.

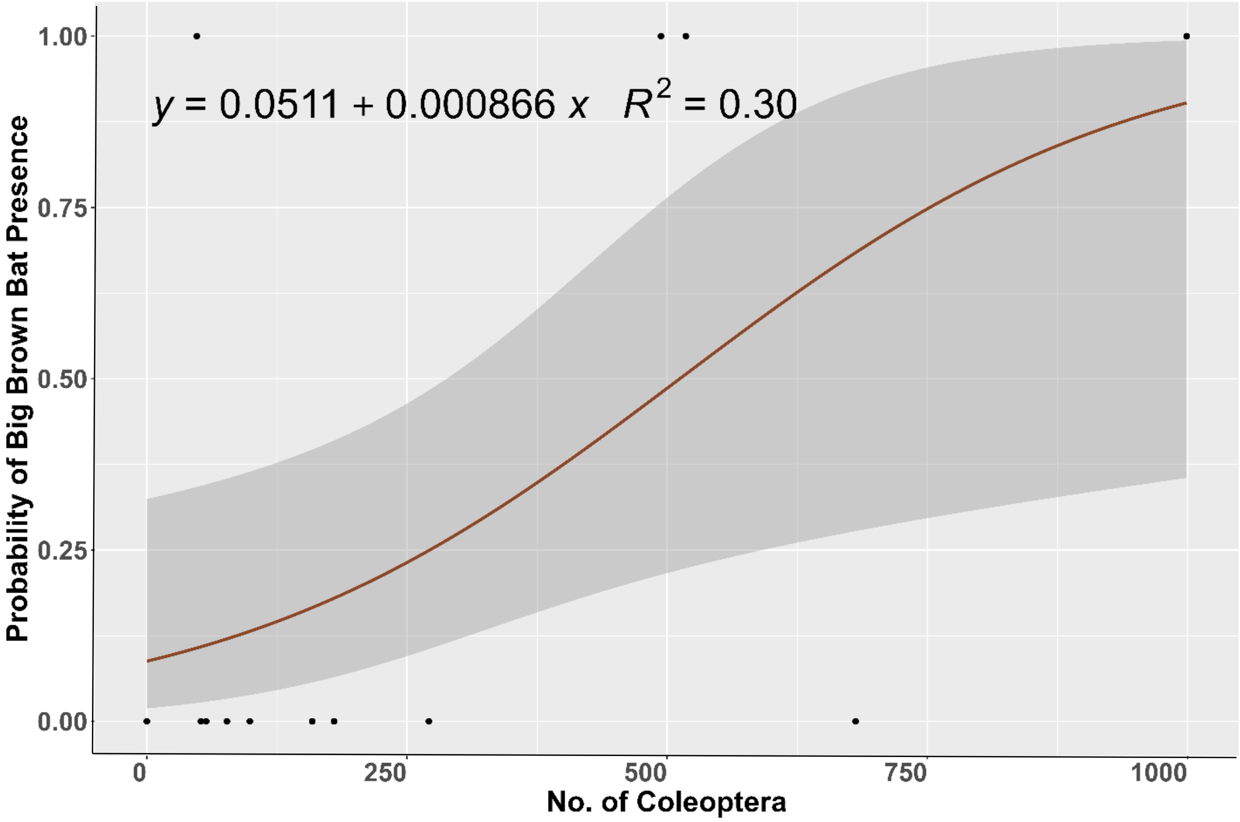

Figure 3.6. The influence of Coleoptera abundance on big brown bat presence at mist-netting sites, Arnold Air Force Base, 2022 and 2023.

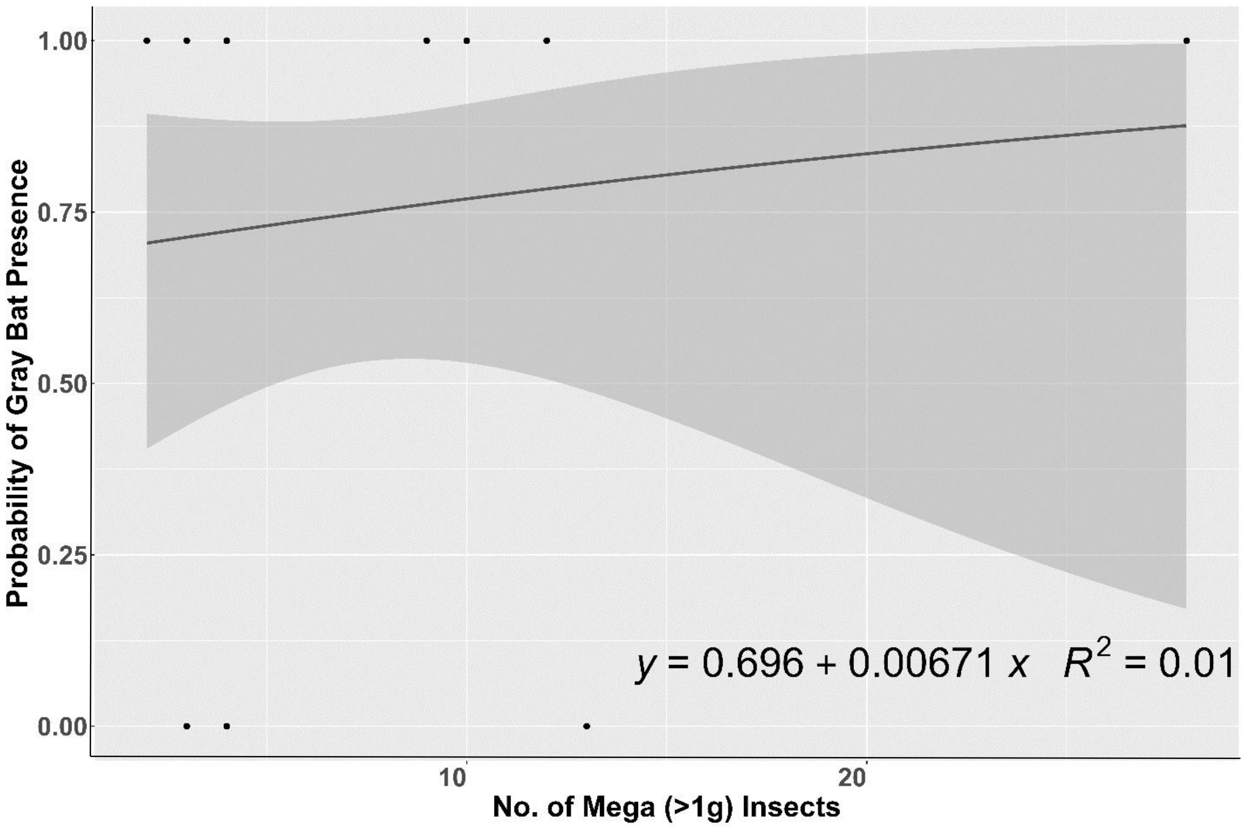

Figure 3.7. The influence of the abundance of mega (>1g) insects on gray bat presence at mist-netting sites, Arnold Air Force Base, 2022 and 2023.

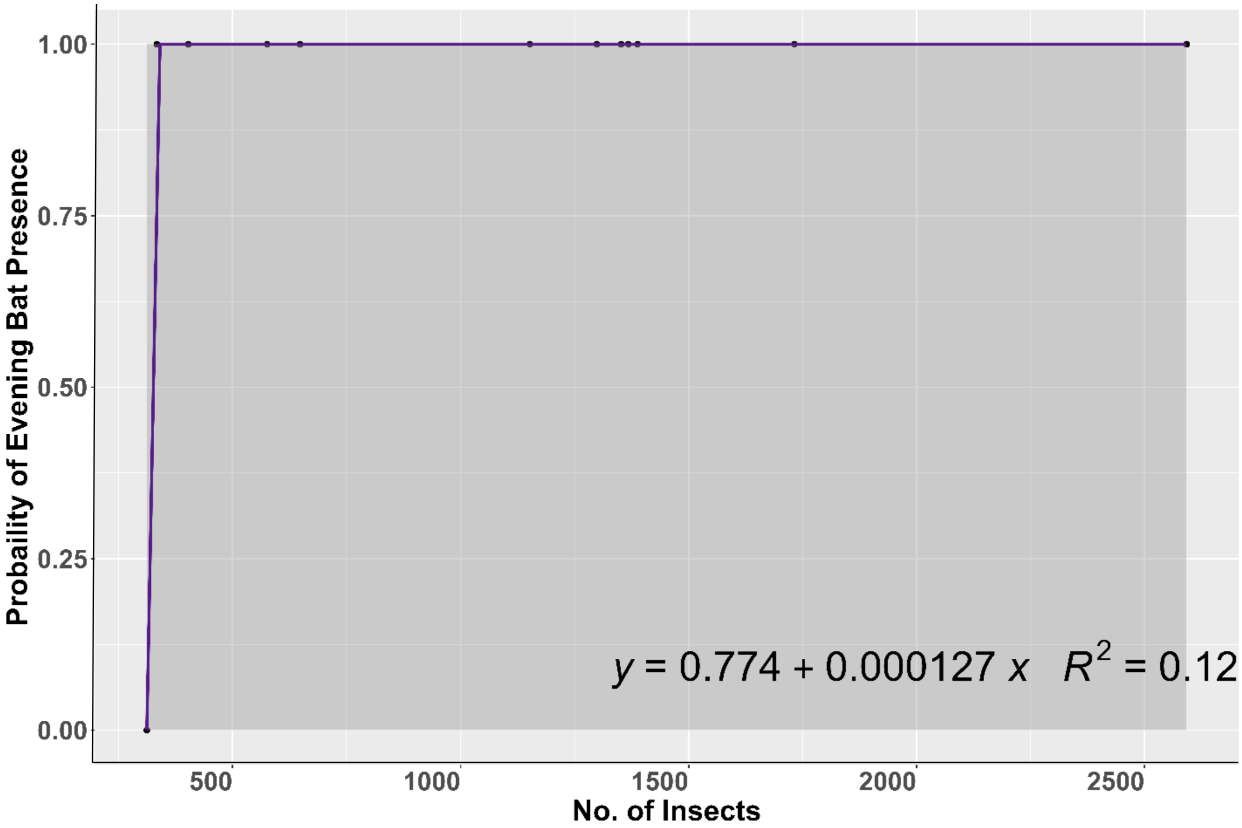

Figure 3.8. The influence of insect abundance on evening bat presence at mist-netting sites, Arnold Air Force Base, 2022 and 2023.

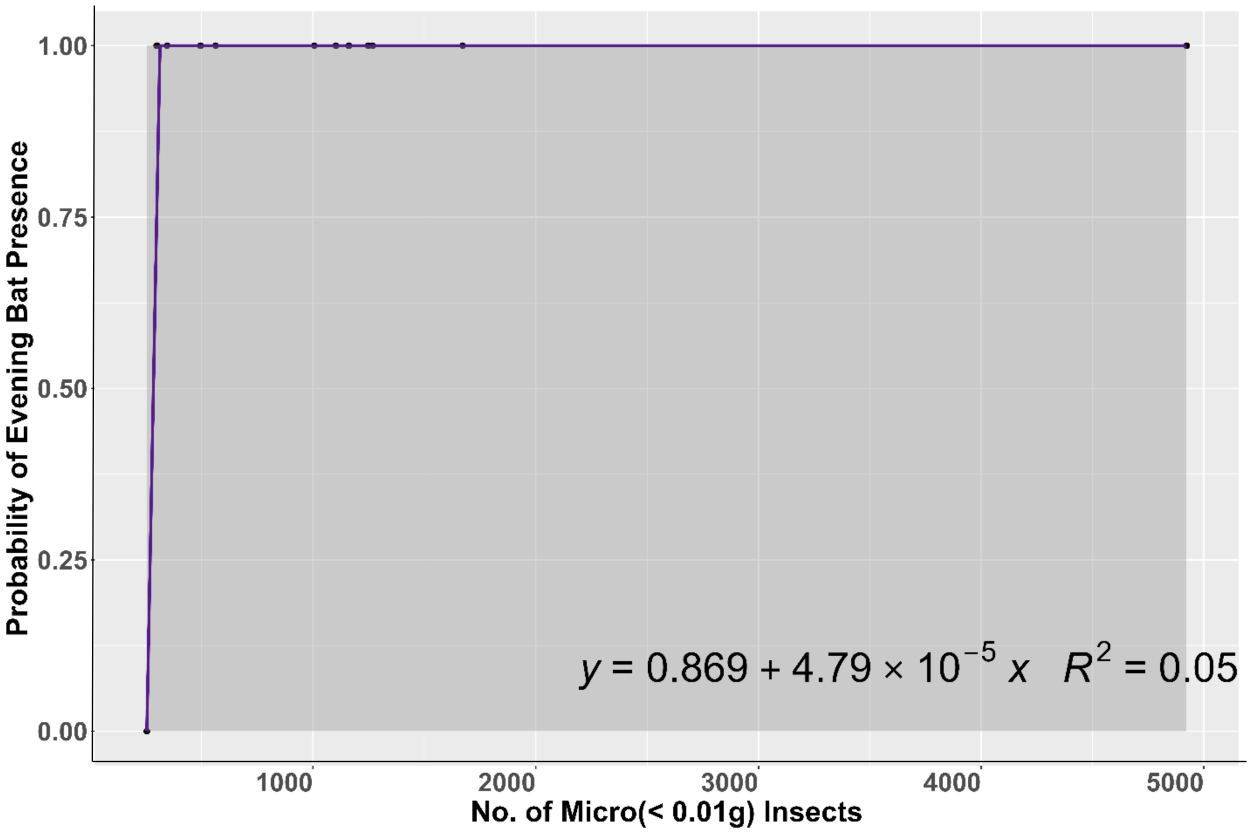

Figure 3.9. The influence of abundance of micro (<0.01g) insects on evening bat presence at mist-netting sites, Arnold Air Force Base, 2022 and 2023.

CHAPTER IV

Relationships of Insects and Bats at Arnold Air Force Base:

Acoustic Sampling

Introduction

Bats are essential to ecosystem balance and are important in pest management for both agriculture and forest systems (Riccucci & Lanza, 2014). Although it is challenging to estimate foraging quantity for bats, studies suggest that insectivorous bats consume more than 25% of their body weight in prey items each night (Coutts et al., 1973). Bats have diverse feeding and foraging behaviors that are determined by body size and ecology of individual bat species. Some species of bats are considered generalists, feeding on a wide variety of taxonomic groups, and some are considered more specialized in their foraging behaviors, but much is still unknown about the foraging behaviors of individual species (Kasso & Balakrishnan, 2013). Bats opportunistically consume prey of varying sizes (depending on the bat's size), as small as a gall midge to large moths, beetles, and katydids (Anthony & Kunz, 1977).

The relationship between prey and predators (arthropods and bats) is not only essential to the systems in which they live by contributing to pest management but also important for the survival of bats themselves. Since the introduction of White Nose Syndrome (WNS), caused by the fungus *Pseudogymnoascus destructans,* to the United States in 2008, bat populations have suffered major decline, causing many species to become threatened or endangered and permanently shifting ecosystems (Hoyt et al., 2021). *Pseudogymnoascus destructans* has made investments in parasitic traits making it both reliant and well adapted to infecting epidermal tissue of hibernating bats (Lorch et al., 2011; Warnecke et al., 2012). The fungus grows on hibernating bats in the winter, depleting stored fat and disturbing natural torpor periods (Mayberry et al., 2018).

Infected bats arouse more often than normal in periods of torpor (Reeder et al., 2012). Emaciated bats infected with the fungus often die of starvation in winter months (Reeder & Moore, 2013). Research has shown that one of the main factors in surviving infection of *P. destructans* is increasing the fat content of bats before hibernation (Cheng et al., 2019; Johnson et al., 2014; Pannkuk et al., 2021). Water loss may also be a link to mortality of bats from WNS, but it is not confirmed (Willis et al., 2011). As fat stores are imperative to bat survival, it is critical to understand the relationship between bats and their prey.

Acoustic monitoring may be the best option to monitor populations of bats that have suffered decline in populations, as it may be much harder to capture them in traditional mist netting (Barratt et al., 1997; Hammesfahr, 2020). Though acoustic monitoring has many benefits it also has limitations based on microphone placement and number of calls does not equally equate to abundance (Freeze et al., 2021; O’Keefe et al., 2014). Bat calls also vary based on their environment and can be difficult to identify to species (Fenton, 2000; Hayes, 2009). To increase confidence of identification calls can be grouped into phonic groups (Beilke et al., 2021; Norberg & Rayner, 1987).

Little research has been conducted on arthropod orders as predictors for bat populations (Bender et al., 2021). As arthropod communities experience changes due to globalization, transportation, climate change, and habitat modification, continued assessment of arthropod and bat relationships is essential (Donkersley et al., 2022; Mamay & Simsek, 2017; Thomas et al., 2004). The present study was designed to assess the relative influence of insect order, abundance, diversity, and size on foraging bats at Arnold Air Force Base (AFB). It is hypothesized based on previous studies that total arthropod abundance is unlikely to predict the abundance of bat populations, but the abundance of individual insect orders and relative size of insects are more likely to predict the presence of bat species. This hypothesis will be addressed by answering the following questions:

1. How does insect order, insect abundance, insect diversity, and insect size influence the abundance of bat group call files (low, mid, and high frequency by *Myotis* spp.) recorded at Arnold AFB?

2. How does insect order, insect abundance, insect diversity, and insect size influence the abundance of call flies for bat species recorded at Arnold AFB?

Materials and Methods

Bat Sampling

Acoustic monitoring is one of three methods used to survey bats on Arnold AFB, in addition to mist netting (see Chapter 3) and roost surveys (see Chapter 1) in efforts to provide a holistic depiction of the bat populations on the base (Flaquer et al., 2007). Acoustic monitoring at Arnold AFB was conducted using the Anabat system by biologist John Lamb (Figure 4.1). To evaluate year-round activity and relative abundance, bat call files were recorded using an Anabat, and calls were identified using Bat Call Identification software (BCID version 2.8b). Species identifications were restricted to those bats that have been physically captured on the base. All activity is quantified as the number of files identified to each species confirmed with at least a maximum likelihood estimator (MLE) of 0.05 or less and a three-pulse minimum (Lamb, 2020).

Acoustic monitoring stations were installed at five sites (i.e., acoustic sites) by Lamb (Figure 4.2). One site is in the restricted access security area of the base and was not used in this study (indicated by a red circle on the map - BBI073).

Sites were chosen to represent the varied habitats used by bats at Arnold AFB including forested streams (BBI021 and BBI087), forested wetland (BBI060), and upland woodland managed by fire (BBI073 and BBI097) (Table 4.1). The two woodland sites differ in that BBI073 is in a pond at the edge of the woodland and BBI097 is along the edge of woodland transitioning to grassland/shrubland. Acoustic data were recorded at these five sites from dusk till dawn for a 7-night period. Anabat stations consisted of an Anabat SD2 bat detector and a Waterproof Hi Mic Head connected by a 3-m cable (Titley Scientific). Anabats were powered by a 12-volt battery that was charged by a 10-watt solar panel. Components were connected through a 0-12 VDC power regulator. The solar panel and Hi Mic Head were attached to the frame of a tripod (Lamb, 2020). All remaining components were housed in a water-resistant box that was attached to the same tripod (Figure 4.1). A small amount of desiccant was put in each detector box to eliminate interference with humidity or water. Each site was monitored weekly to check batteries and download acoustic data. Anabats, cables, and Mic Heads were returned to Titley Scientific for testing and certification (Lamb, 2020).

Insect Sampling

Insect sampling began in June 2022 and ended in August 2023. All sites were visited in the middle of the 7-night observational period. In other words, insect samples were taken from the fourth night of acoustic sampling with three days of acoustic recording before and after insect samples collection. Sites were visited at random with all sites being visited at minimum once per season. This resulted in 20 distinct corresponding bat acoustic and insect sampling events. A summary of acoustic monitoring durations and corresponding insect collections is provided in Table 4.2.

For each sampling event, one ultraviolet (UV) light trap (Figure 2.2) and one CDC light trap (CO_2 trap) (Figure 2.3) powered by 12-volt batteries were deployed at dusk at a site for six hours at the 3-day mark (middle of the acoustic sampling period). The 4-day mark was used to get representative samples of insect diversity, richness, and abundance for the duration of the acoustic monitoring period. UV light traps emitted wavelengths from 100-400 nm. The bulb in the UV light trap was located directly above the collection chamber and attached to a metal bucket with a funnel top. Two Hot Shot No Pest insect kill strips were placed inside the bottom of the metal bucket (separated from the rest of bucket by metal mesh) (Figure 2.2).

CDC light traps were made from a battery-operated fan with a plastic lid (30.5 cm diameter) attached above the fan, a plastic chamber attached by fabric underneath the fan (to funnel arthropods into chamber), and a plastic 3.8-liter cooler with holes drilled in it (Figure 2.3). Plastic coolers were filled with 0.23 kg of dry ice for each sampling event and tied next to fans. Dry ice in ambient temperatures releases carbon dioxide slowly (mimicking animals' exhalations) and is used to attract predominantly blood feeding insects. After six hours, the samples were collected and the specimens were removed, placed in 3.8-liter Ziplock bags placed in a large cooler and taken to the laboratory. Samples were stored in a refrigerator until they could be sorted and identified.

After each sample was collected, they were sorted to order and weighed (in grams) to determine the dry weight (in grams) (i.e., dry biomass) of each insect order. Once the biomass of each order was calculated, specimens in each order were then sorted to family, if not to a lower taxonomic group, and each specimen was weighed and assigned a classification based on weight. Weight classifications were: Mega (>1.0 g), Macro (1.0-0.1 g), Standard (0.1-0.011 g), and Micro (< 0.01 g). This methodology allowed for biomass to be weighed and collected on an order level and on an individual specimen level. All specimens collected from the 13 samples were identified to at least family level. After specimens were identified to family, they were stored in 95% ethanol in vials (10 ml). Lepidopteran specimens were stored dry in Petri-dishes (100x15 mm), and Culicidae were sorted, stored in Falcon tubes (100 ml), and placed in the refrigerator. Representative samples were spread and pinned as needed for voucher specimens.

Data Analysis

For all data analysis, figures, models, and statistics were constructed using R version 4.2.3. For each acoustic sample, individual call files were counted and added for each bat species found on the base, unknown bats, and total calls recorded. Bat call files were then grouped into three phonic groups for analysis with high (*Myotis*), mid, and low frequency calls; a summary of groups is provided in Table 4.3 (Beilke et al., 2021; Norberg & Rayner, 1987). For an overview of bats present on the base, their scientific name, status, and WNS history refer to Table 1.1.

For each insect sample (UV light trap and carbon dioxide trap combined) correlation analysis was used for acoustic sampling events, insect abundance, insect family diversity, insect family richness and the number of insects in each weight class (Mega (> 1.0 g), Macro (1.0-0.1 g), Standard (0.1-0.011 g), and Micro (< 0.01 g)) were calculated. Shannon family diversity index and family richness were calculated for each sample using R package VEGAN. The number of insects belonging to each insect order and family was also calculated for each sampling event. Spearman's correlation test was performed on total insect abundance and on insect diversity and richness.

First trends between total insect abundance and total acoustic calls were graphed. An analysis of variance was performed using negative binomial GLMs to examine the number of call files of each 4 most abundant species of bats and each phonic group caught in the mist nets using the R package MASS. Fixed effects for the negative binomial GLMs included insect abundance, insect order abundance, insect size class abundance, habitat type, and season to determine what factors, if any, influenced the presence of individual bat species or bat species richness. Insect family diversity and insect family richness were not included in models due to their high correlation values with insect abundance (> 0.6). Insect abundance and insect order abundance were log transformed to make the data set more parametric and better suited for analysis. Best-fit models were determined by the lowest Akaike Information Criterion corrected (AICc) value, and if two models had the lowest AICc value then the most conservative model was selected. For a fixed effect to be considered significant if 95% confidence intervals did not cross zero and $p \leq 0.05$.

All models were constructed as single variable models and listed in order of their lowest AICc scores. All models presented were significantly better than the null model constructed for each species and overall richness using a delta AICc value of 2 to determine significance. Null models were used to provide a guide for understanding predictor variables and are set so bat presence was not predicted by any variable.

Results and Discussion

The 20 corresponding samples of acoustic and insect collections yielded 50,000+ bat call files and 20,000+ insects. An overview of acoustic call files by month is provided in Figure 4.3. Average call files of individual bat species throughout the year (determined by John Lamb) are shown in Figure 4.4. These calls included gray bats, big brown bats, eastern red bats, evening bats, tri-color bats, Indiana bats, little brown bats, northern-long eared bats, and hoary bats. Gray bats, Indiana bats, northern-long eared bats, and little brown bats comprised High phonic group (Table 4.3). Eastern red bats, evening bats, and tricolor bats comprised Mid phonic group (Table 4.3). Big brown bats and hoary bats made up the Low phonic group (Table 4.3). Insects collected in conjunction with acoustic sampling periods spanned the insect orders Coleoptera, Lepidoptera, Diptera, Hemiptera, Hymenoptera, Plecoptera, Ephemeroptera, Trichoptera, Megaloptera, Blattodea, Orthoptera, Psocoptera, Collembola, and Neuroptera. An overview of insect abundance from 2022 to 2023 is shown in Figure 4.6.

Insect richness and insect diversity were first correlated with insect abundance using Spearman's correlation test. Insect richness and insect abundance were found to be highly correlated, with a Spearman's coefficient of 0.9727, p=0.0005. Thus, insect abundance was used in models in place of insect richness. Insect diversity and insect abundance had a Spearman's coefficient of 0.7345, p=0.0006. Thus, only total insect abundance was used as a metric in model construction in place of both insect diversity and insect richness.

In forested wetlands, forested streams, and edge(burn) acoustic call files were positively related to insect abundance (Figure 4.6). These positive trends between acoustic call file abundance and insect abundance were highest in forested stream habitats (Figure 4.7) and forested wetlands (Figure 4.8). Theses visualizations indicate that relative bat activity increases as prey availability increases in forested wetlands, forested streams, and edge(burn) sites.

Secondarily, models were constructed for bat phonic groups (high (*Myotis*), mid, and low) (Table 4.5), the results for selected models are provided in Table 4.6. All models were tested against the null model (assuming no effect), and a summary of models tested, and model variables can be found in Table 4.4. For the high (*Myotis)* group (all *Myotis* spp.) insect abundance was selected to be the best predictor of high (*Myotis*) call files. High (*Myotis*) call files had a significant positive relationship with total insect abundance (p=0.005). The relationship between *Myotis* spp. call files and insect abundance is displayed in Figure 4.9. Suggesting as insect abundance increases at a foraging site in turn so does the number *Myotis* spp. call files. The lower and upper confidence intervals for insect abundance model for the high (*Myotis*) phonic group did not pass zero, but $p < 0.05$, making this relationship significant.

For the mid phonic group (eastern red bats, evening bats, and tricolored bats), the habitat model was selected, suggesting that for low and mid phonic groups habitat is the primary limiting factor to predict call activity. Mid phonic group call files had a significant positive correlation with the edge (burn) site and mid phonic group call files had a significant negative relationship with forested wetlands. Mid phonic group call files had a negative relationship with forested streams but was not considered significant. The relationship between Mid phonic group acoustic call files and habitat type is shown in Figure 4.10. The lower and upper confidence intervals for habitat models for the low phonic group did not pass zero, making this relationship significant. Hymenoptera abundance was also selected as an important predictor variable for Mid phonic group calls (p=0.04). Hymenoptera abundance and Mid phonic group call files had a positive relationship, and as hymenopteran populations increased so did Mid phonic group calls (Figure 4.11). The lower and upper 95% confidence intervals for habitat models for the low phonic group did not pass zero and had p=0.04 indicates this model as a good predictor for the number of Mid phonic group calls.

For the low phonic group (big brown bats and hoary bats), the only model selected to predict low phonic call abundance was Habitat; low phonic group call files had a significant positive relationship with the edge (burn) site and low phonic group call files had a significant negative relationship with both forested wetlands and forested streams. The relationship between Low phonic group acoustic call files and habitat type is shown in Figure 4.12. The lower and upper confidence intervals for habitat models for the low phonic group did not pass zero, suggesting high model confidence.

Models were then constructed for the four most often detected and caught bat species on the base (eastern red bats, evening bats, gray bats, and big brown bats). These models were included to examine variables that may predict species call abundance but are overall less certain than the grouping of calls into phonic groups. Models constructed for the four bat species are listed in Table 4.4. Results for selected models are provided in Table 4.7 and 4.8. All models were compared against the null model (assuming no effect). For big brown bats, habitat model was selected to be beneficial to predicting the number of big brown bat call files. Big brown bat call files had a significant positive relationship to the edge (burn) site and a significant negative relationship with both forested streams and forested wetlands. The relationship between big brown bat acoustic call files and habitat type is shown in Figure 4.13. The lower and upper confidence intervals for habitat model for the big brown bats did not pass zero, suggesting high model confidence.

For eastern red bats, the habitat model was selected, suggesting that insect orders, abundance, and season may not be important variables to predict call abundance for this species. Eastern red bats had a positive significant relationship with the edge (burn) site and a significant negative relationship with wetlands. Eastern red bats had a negative relationship with forested stream sites, but this relationship was not significant. The relationship between eastern red bat acoustic call files and habitat type is shown in Figure 4.14. The habitat model for eastern red bat calls confidence intervals did not pass zero indicating high model confidence.

For evening bats, two models were selected as significant predictors of evening bat call files. Habitat was the first model selected, and evening bat call files had a significant positive relationship to the edge (burn) sites. Evening bat call files in turn had a significant negative relationship to forested streams and forested wetlands. The relationship between evening bat acoustic call files and habitat type are shown in Figure 4.15. The habitat model for evening bat calls confidence intervals did not pass zero, suggesting high model confidence. The second model selected for predicting evening bat call file abundance was Hymenoptera abundance. Evening bat call files had a significant positive relationship with Hymenoptera abundance ($p = 0.01$). Suggesting as hymenopteran populations increased at a foraging site in turn so did relative number of evening bat call files. The relationship between the number of evening bat acoustic call files and Hymenoptera abundance is shown in Figure 4.16. The confidence intervals for models predicting evening bat calls by hymenopteran abundance did not cross zero suggesting an even distribution of data, and significance with $p < 0.05$. These data suggest that evening bats calls were primarily influenced by habitat and secondary by insect order abundance of hymenopterans.

For gray bats, one model was selected as significantly better than the null model. The model selected was total insect abundance predicting number of gray bat call files. Gray bat call files had a significant positive relationship with insect abundance ($p=0.008$). The relationship between gray bat acoustic call files and total insect abundance is shown in Figure 4.17, indicating that as total insect populations increased at a foraging site so did relative number of gray bat call files. This model proposes that insect abundance is important to foraging gray bats and in predicting their calls. The model's confidence intervals did not cross zero, suggesting high model confidence.

Discussion

The use of acoustic bat call files as a metric for measuring bat activity must be carefully interpreted, as the numbers of acoustic call files are not always directly correlated to numbers of individuals (Hayes, 2009). Also, the placement of acoustic recording stations may have impacts on call files recorded. For example, areas with less clutter often receive more recordings based on the habitat surrounding the recording station (Freeze et al., 2021; O'Keefe et al., 2014). The limitations of current software to differentiate bat call files to species also is a concern, as considerable intra-species variation in echolocation can occur based on factors such as habitat type and foraging mode (Fenton, 2000; Hayes, 2009). However, as certain populations of bats become low and other ecological behaviors are altered, bats may become more difficult to capture in mist nets; therefore, acoustic recordings may be the best method for monitoring populations of some bat species (Barratt et al., 1997; Hammesfahr, 2020).

A combination of mist-net sampling, acoustic sampling, and roost surveys is recommended to sample bat populations to obtain the most accurate results for bats with differing ecologies and behaviors (Flaquer et al., 2007). These results suggest that the bats species (Big brown bats, eastern red bats, evening bats, and gray bats) and phonic groups (high (*Myotis*), Mid, and Low) were primarily influenced by habitat. For three of the bat species (Big brown bats, eastern red bats, and evening bats) and two phonic groups (mid and low) calls had a strong positive relationship with the edge (burn) site. These results suggest the importance of transition sites and openings needed for foraging and transit. Many species such as eastern red bats have been noted to use logging roads or transition zones for foraging or passage for flight (Amelon et al., 2014; Beilke et al., 2023; Grindal & Brigham, 1999; LESIŃSKI et al., 2011). These findings reiterate the importance of controlled burns, and their long-term effects on the landscape (Austin et al., 2018; Tormanen & Garrie, 2021) These results suggest further monitoring of insect populations and bat relationships in these transition zones that receive prescribed burning. At the start of April, no specimens were found in the sample taken after the prescribed burn event (Chapter II). As winter torpor periods typically end in spring, retaining insect abundance in this habitat may be important for these emerging bats (Cheng et al., 2019; Salvarina et al., 2018). Management for these strategies may include prescribed burning events in alternate times for prescribed burning events or for longer durations between prescribed burning events to allow insect populations to be higher in the spring period (Ferrenberg et al., 2006; Harper et al., 2000). These results indicate that insect abundance and taxa are secondarily important to evening bats. For *Myotis* spp. and gray bats insect abundance and taxa were the primary predictor variables isolated for predicting call abundance. These results highlight the need for insect metrics to be included in foraging studies. These results confirm insect abundance is important for *Myotis* spp. on Arnold AFB, and specifically lepidopteran abundance is important to gray bats. Insect abundance and insect taxa abundance (lepidopteran, hemipteran, and hymenopteran) were deemed important to a bat phonic group and bat species and are critical to maintaining bat populations especially in the face of WNS (Cheng et al., 2019). Management strategies for increasing insect populations close to known roosting sites would be advised. To increase insect populations temporally could be done using UV light traps, which has proven effective in the ‘fat bat’ studies (Electric Power Research Institute, 2021). More permanent measures would include supporting plant populations that host high insect diversity or high densities of important insect taxa. Examples include supporting deciduous tree growth over pine growth; especially oak populations are known to support high diversity and abundance of canopy insects (Crowley et al., 2023; Hammond & Miller, 1998; Southwood, 1961; Vaca-Sánchez et al., 2021). Supporting insect populations may also include planting increased grass and spring and summer ephemerals close to roosting and foraging areas on Arnold AFB (Curran et al., 2022; Hammond & Miller,1998).

These models can be used to compare past and future foraging studies and dietary studies. It is important to note the limitations of both the insect and bat methodologies. CDC light traps and UV light traps both use attractants to pull insects to the trap inherently biasing samples collected from them with often increased proportions of lepidopterans, coleopteran families such as Heteroceridae, and dipteran families such as Culicidae and other nematoceran families (Acuff, 1976; Marshall, 2006; McDermott & Mullens, 2018; Muirhead-Thompson, 2012). Mist-net and acoustic monitoring does not inherently equate to bat numbers and may be biased to where the mics are located or at what height the species fly at or habitat preference (de Torrez et al., 2017; Kaiser & O’Keefe, 2015; Mancini et al., 2022; Russo & Voigt, 2016)

These results suggest that strategies for multiple bat species in the southeastern United States may be more influenced by prey than historically perceived. Habitat is still an important predictor variable for predicting call file abundance, but incorporating arthropod data may increase the efficacy of these predictions. This research also highlights the need for entomologists to conduct long-term nocturnal insect research, trophic level (predator-prey relationships) research, and integrative and collaborative research.

Literature Cited

Acuff, V. R. (1976). Trap biases influencing mosquito collecting. *Mosq. News*, *36*(2), 173-176.

Anthony, E. L., & Kunz, T. H. (1977). Feeding strategies of the little brown bat, *Myotis lucifugus*, in southern New Hampshire. *Ecology*, *58*(4), 775-786.

Austin, L. V., Silvis, A., Ford, W. M., Muthersbaugh, M., & Powers, K. E. (2018). Bat activity following restoration prescribed burning in the central Appalachian upland and riparian habitats. *Natural Areas Journal*, *38*(2), 183-195.

Amelon, S. K., Thompson III, F. R., & Millspaugh, J. J. (2014). Resource utilization by foraging eastern red bats (*Lasiurus borealis*) in the Ozark Region of Missouri. *The Journal of Wildlife Management*, *78*(3), 483-493.

Barratt, E. M., Deaville, R., Burland, T. M., Bruford, M. W., Jones, G., Racey, P. A., & Wayne, R. K. (1997). DNA answers the call of pipistrelle bat species. *Nature*, *387*(6629), 138-139.

Beilke, E. A., Blakey, R. V., & O'Keefe, J. M. (2021). Bats partition activity in space and time in a large, heterogeneous landscape. *Ecology and Evolution*, *11*(11), 6513-6526.

Beilke, E. A., Haulton, G. S., & O'Keefe, J. M. (2023). Foliage-roosting eastern red bats select for features associated with management in a central hardwood forest. *Forest Ecology and Management*, *527*, 120604.

Bender, M. J., Perea, S., Castleberry, S. B., Miller, D. A., & Wigley, T. B. (2021). Influence of insect abundance and vegetation structure on site-occupancy of bats in managed pine forests. *Forest Ecology and Management*, *482*, 118839.

Cheng, T. L., Gerson, A., Moore, M. S., Reichard, J. D., DeSimone, J., Willis, C. K., ... & Kilpatrick, A. M. (2019). Higher fat stores contribute to persistence of little brown bat populations with white-nose syndrome. *Journal of Animal Ecology*, *88*(4), 591-600.

Coutts, R. A., Fenton, M. B., & Glen, E. (1973). Food intake by captive *Myotis lucifugus* and *Eptesicus fuscus* (Chiroptera: Vespertilionidae). *Journal of Mammalogy*, *54*(4), 985-990.

Crowley, L. M., Ivison, K., Enston, A., Garrett, D., Sadler, J. P., Pritchard, J., ... & Hayward, S. A. (2023). A comparison of sampling methods and temporal patterns of arthropod abundance and diversity in a mature, temperate, Oak woodland. *Acta Oecologica*, *118*, 103873.

Curran, M. F., Robinson, T. J., Guernsey, P., Sorenson, J., Crow, T. M., Smith, D. I., & Stahl, P. D. (2022). Insect abundance and diversity respond favorably to vegetation communities on interim reclamation sites in a semi-arid natural gas field. *Land*, *11*(4), 527.

Donkersley, P., Ashton, L., Lamarre, G. P., & Segar, S. (2022). Global insect decline is the result of wilful political failure: A battle plan for entomology. *Ecology and Evolution*, *12*(10), e9417.

Electric Power Research Institute. (2021). (tech.). *Fat Bats: Research and Management Practices for Enhancing Foraging Habitat to Help Support Bats with White-Nose Syndromes*. Retrieved from https://www.epri.com/research/products/000000003002021572.

Fenton, M. B. (2000). Choosing the correct bat detector. *Acta Chiropterologica*, *2*(02).

Ferrenberg, S. M., Schwilk, D. W., Knapp, E. E., Groth, E., & Keeley, J. E. (2006). Fire decreases arthropod abundance but increases diversity: early and late season prescribed fire effects in a Sierra Nevada mixed-conifer forest. *Fire Ecology*, *2*, 79-102.

Flaquer, C., Torre, I., & Arrizabalaga, A. (2007). Comparison of sampling methods for inventory of bat communities. *Journal of Mammalogy*, *88*(2), 526-533.

Freeze, S. R., Shirazi, M., Abaid, N., Ford, M., Silvis, A., & Hakkenberg, D. (2021). Effects of environmental clutter on synthesized chiropteran echolocation signals in an anechoic chamber. *Acoustics* (Vol. 3, No. 2, pp. 391-410). MDPI.

Grindal, S. D., & Brigham, R. M. (1999). Impacts of forest harvesting on habitat use by foraging insectivorous bats at different spatial scales. *Ecoscience*, *6*(1), 25-34.

Hammesfahr, A. (2020). Solutions for imperiled bat conservation: Integrating ecology, technology, and the public. *Electronic Theses & Dissertations*. 343. https://digitalcommons.pittstate.edu/etd/343

Hammond, P. C., & Miller, J. C. (1998). Comparison of the biodiversity of Lepidoptera within three forested ecosystems. *Annals of the Entomological Society of America*, *91*(3), 323-328.

Harper, M. G., Dietrich, C. H., Larimore, R. L., & Tessene, P. A. (2000). Effects of prescribed fire on prairie arthropods: an enclosure study. *Natural Areas Journal*, *20*(4), 325-335.

Hayes, J.P. (2009). Survey and monitoring of bats. *Ecological and Behavioral Methods for the Study of Bats*. T.H. Kunz & S. Parsons, Eds.: 112–129. The Jones Hopkins University Press.

Hoyt, J. R., Kilpatrick, A. M., & Langwig, K. E. (2021). Ecology and impacts of white-nose syndrome on bats. *Nature Reviews Microbiology*, *19*(3), 196-210.

Johnson, J. S., Reeder, D. M., McMichael III, J. W., Meierhofer, M. B., Stern, D. W., Lumadue, S. S., ... & Field, K. A. (2014). Host, pathogen, and environmental characteristics predict white-nose syndrome mortality in captive little brown myotis (*Myotis lucifugus*). *PLoS One*, *9*(11), e112502.

Kaiser, Z. D., & O'Keefe, J. M. (2015). Factors affecting acoustic detection and site occupancy of Indiana bats near a known maternity colony. *Journal of Mammalogy*, *96*(2), 344-360.

Kasso, M., & Balakrishnan, M. (2013). Ecological and economic importance of bats (Order Chiroptera). *Isrn Biodiversity*, *2013*, 1-9.

Lamb, J. (2020). "*Myotis grisescens*, *Myotis septentrionalis*, and *Myotis sodalis* Annual Report. Unpublished. Section 10 Permit TE88778B-2."

LESIŃSKI, G., Olszewski, A., & Popczyk, B. (2011). Forest roads used by commuting and foraging bats in edge and interior zones. *Polish Journal of Ecology*, *59*(3), 611-616.

Lorch, J. M., Meteyer, C. U., Behr, M. J., Boyles, J. G., Cryan, P. M., Hicks, A. C., ... & Blehert, D. S. (2011). Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature*, *480*(7377), 376-378.

Mamay, M., & Şimşek, E. (2017). The Expected Impact of Global Warming and Climate Change on Insect Diversity. *The 3rd International Symposium on EuroAsian Biodiversity*, pp. (173).

Mancini, M. C. S., Hintze, F., de Souza Laurindo, R., de Macêdo Mello, R., & Gregorin, R. (2022). Tradition vs. innovation: comparing bioacoustics and mist-net results to bat sampling. *Bioacoustics*, *31*(5), 575-593.

Marshall, S. A. (2006). *Insects: their natural history and diversity: with a photographic guide to insects of eastern North America* (p. 718). Buffalo, NY: Firefly Books.

Mayberry, H. W., McGuire, L. P., & Willis, C. K. (2018). Body temperatures of hibernating little brown bats reveal pronounced behavioural activity during deep torpor and suggest a fever response during white-nose syndrome. *Journal of Comparative Physiology B*, *188*, 333-343.

McDermott, E. G., & Mullens, B. A. (2018). The dark side of light traps. *Journal of Medical Entomology*, *55*(2), 251-261.

Muirhead-Thompson, R. C. (2012). *Trap responses of flying insects: the influence of trap design on capture efficiency*. San Diego, CA: Academic Press.

Norberg, U. M., & Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): Wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *316*(1179), 335-427.

O'Keefe, J. M., Loeb, S. C., Hill Jr, H. S., & Lanham, J. D. (2014). Quantifying clutter: A comparison of four methods and their relationship to bat detection. *Forest Ecology and Management*, *322*, 1-9.

Pannkuk, E. L., Dorville, N. A. Y., Dzal, Y. A., Fletcher, Q. E., Norquay, K. J., Willis, C. K., ... & Laiakis, E. C. (2021). Hepatic lipid signatures of little brown bats (*Myotis* *lucifugus*) and big brown bats (*Eptesicus fuscus*) at early stages of white-nose syndrome. *Scientific Reports*, *11*(1), 11581.

Reeder, D. M., Frank, C. L., Turner, G. G., Meteyer, C. U., Kurta, A., Britzke, E. R., ... & Blehert, D. S. (2012). Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PloS One*, *7*(6), e38920.

Reeder, D. M., & Moore, M. S. (2013). White-nose syndrome: A deadly emerging infectious disease of hibernating bats. *Bat Evolution, Ecology, and Conservation*. https://doi.org/10.1007/978-1-4614-7397-8_20.

Riccucci, M., & Lanza, B. (2014). The importance of bats to agriculture and forestry. *Georgofili*, *11*(2), 664-703.

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.

Salvarina, I., Gravier, D., & Rothhaupt, K. O. (2018). Seasonal bat activity related to insect emergence at three temperate lakes. *Ecology and Evolution*, *8*(7), 3738-3750.

Southwood, T. R. E. (1961). The number of species of insect associated with various trees. *The Journal of Animal Ecology*, *30(1)*,1-8.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., ... & Williams, S. E. (2004). Extinction risk from climate change. *Nature*, *427*(6970), 145-148.

Tormanen, A. P., & Garrie, J. (2021). Responses of bat-prey to prescribed burns and mechanical thinning. *Forest Ecology and Management*, *491*, 119162.

Vaca-Sánchez, M. S., Maldonado-López, Y., González-Rodríguez, A., Oyama, K., Fernandes, G. W., Fagundes, M., ... & Cuevas-Reyes, P. (2021). Canopy arthropod diversity associated with *Quercus laurina*: importance of an oak species diversity gradient on abundance, species richness and guild composition. *Journal of Insect Conservation*, *25*, 859-874.

Warnecke, L., Turner, J. M., Bollinger, T. K., Lorch, J. M., Misra, V., Cryan, P. M., ... & Willis, C. K. (2012). Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proceedings of the National Academy of Sciences*, *109*(18), 6999-7003.

Willis, C. K., Menzies, A. K., Boyles, J. G., & Wojciechowski, M. S. (2011). Evaporative water loss is a plausible explanation for mortality of bats from white-nose syndrome. *Integrative and Comparative Biology*, *51*(3), 364-373.

Appendix

Table 4.1. Permanent acoustic monitoring sites operated by John Lamb at Arnold Air Force Base.

Site	UV/CO2 Collections	Prescribed Burning	Habitat	Year(s) Collected
BBI021	Yes	No	**Mixed Forested Stream** (Brumalow Creek)	2022, 2023
BBI125	Yes	No	**Mixed Forested Wetland** (Sinking Pond)	2022, 2023
BBI073	No	Yes	**Mixed Forested Pond** (Inside Base Security)	Not Sampled
BBI087	Yes	No	**Mixed Forested Stream** (Upper Rowland Creek)	2022,2023
BBI097	Yes	Yes	**Edge** (Mixed Forest to Grassland)	2022, 2023
BBI105	Yes	No	**Mixed Forest** (Road Rut)	2022, 2023

Table 4.2. Summary of acoustic monitoring durations and corresponding insect collections at Arnold Air Force Base, 2022-2023.

Sample Number	Site*	Insect Sample Date	Start Acoustic Monitoring	End Acoustic Monitoring
1	BBI087 (MFS)	22 June 2022	19 June 2022	25 June 2022
2	BBI097 (E)	27 July 2022	24 July 2022	30 July 2022
3	BBI125 (MFW)	27 July 2022	24 July 2022	30 July 2022
4	BBI021 (MFS)	17 August 2022	15 August 2022	20 August 2022
5	BBI125 (MFW)	17 August 2022	15 August 2022	20 August 2022
6	BBI087 (MFS)	28 October 2022	25 October 2022	31 November 2022
7	BBI097 (E)	28 October 2022	25 October 2022	31 November 2022
8	BBI087 (MFS)	22 November 2022	19 November 2022	25 December 2022
9	BBI125 (MFW)	18 January 2023	15 January 2023	21 January 2023
10	BBI021 (MFS)	28 February 2023	25 February 2023	2 March 2023
11	BBI097 (E)	28 February 2023	25 February 2023	2 March 2023
12	BBI125 (MFW)	29 February 2023	26 February 2023	3 March 2023
13	BBI087 (MFS)	29 March 2023	26 March 2023	2 April 2023
14	BBI097 (E)	21 April 2023	18 April 2023	25 April 2023
15	BBI125 (MFW)	26 June 2023	23 June 2023	29 June 2023
16	BBI021 (MFS)	27 June 2023	24 June 2023	1 July 2023
17	BBI125 (MFW)	27 June 2023	24 June 2023	1 July 2023
18	BBI021 (MFS)	28 June 2023	25 June 2023	2 July 2023
19	BBI125 (MFW)	28 June 2023	25 June 2023	2 July 2023

Table 4.2. Continued.

20	BBI097 (E)	26 July 2023	23 July 2023	29 July 2023

Table 4.3. Bat call phonic groups of species detected on Arnold Air Force Base, 2022-2023.

Phonic Group	**Common Name**	**Scientific Name (Species Code)**	**Caught in Mist nets in 2022-2023 Surveys?**
Myotis (High)	Indiana bat	*Myotis sodalis* (MYSO)	No, historically captured on base.
	Northern long-eared bat	*Myotis septentrionalis* (MYSE)	No, historically captured on base.
	Little brown bat	*Myotis lucifugus* (MYLU)	Yes, one captured and historically captured on base.
	Gray bat	*Myotis grisescens* (MYGR)	Yes, captured frequently.
Mid	Eastern red bat	*Lasiurus borealis* (LABO)	Yes, captured frequently.
	Tricolored bat	*Permytois subflavus* (PESU)	Yes, two captured and historically captured on base.
	Evening bat	*Nycticeius humeralis* (NYHU)	Yes, captured frequently.
Low	Big brown bat	*Eptesicus fuscus* (EPFU)	Yes, captured regularly.

Table 4.3. Continued.

	Hoary bat	*Lasiurus cinereus* (LACI)	Yes, one captured and historically captured on base.

Table 4.4. Models tested to predict the number of call files for bats at Arnold Air Force Base, 2022 to 2023, Acoustic Sampling.

Model Name	Parameters	Hypothesis
Habitat	Habitat included the 3 habitats surveyed with acoustic monitoring. This model included Forested Wetlands, Forested Streams, and Edge site with prescribed burn treatment.	Bat activity is influenced by the habitat.
Season	Season included the temporal periods in which acoustic monitoring occurred. This model included summer, fall, winter, and spring (determined by solstice and equinox)	Bat activity is influenced by the season.
Insect Abundance	This model included total arthropod abundance from each sampling event from the combined UV light trap and CDC light trap.	Bat activity is influenced by total prey availability.
Insect Order Abundance	This model included arthropod orders with the highest abundances from each sampling event from the combined UV light trap and CDC light trap. These variables included Coleoptera, Lepidoptera, Diptera, Hemiptera, Trichoptera, Plecoptera, and Ephemeroptera.	Bat activity is influenced by specific prey groups' availability.
Insect Weight	This model has an abundance of insects in each weight class from each sampling event from the combined UV light trap and CDC light trap. These variables included Mega (>1.0 g), Macro (1.0-0.1 g), Standard (0.1-0.011 g), and Micro (< 0.01 g).	Bat activity is influenced by the biomass of prey available.

Table 4.5. Models predicting abundance of bat phonic group call files at Arnold Air Force Base, 2022 to 2023, Acoustic Sampling.

Phonic Group*	Parameter**	△AICc***
Myotis (High)	~ log (Insect Abundance)	0
Mid	~ Edge (Burn)	0
	~ Forested Wetlands	0
	~ log (Hymenoptera)	6.64
Low	~ Edge (Burn)	0
	~ Forested Stream	0
	~ Forested Wetlands	0

*Phonic group is a bat acoustic call classification. Summary of phonic groups can be found in Table 4.3

**Parameter indicates which predictor variable was modeled to predict the presence of the corresponding bat species.

***Delta AICc was used to analyze the differences between AICc values for constructed models. Delta AICc subtracts the lowest AICc value from the AICc of the corresponding model. Delta AICc value is used to determine if models are significantly different from one another (often at delta AICc of 2). The AIC is the "Akaike information criterion" and it is an estimate of how well a model describes the patterns in the data. It is mainly used for comparing models trained on the same dataset, and delta AICc is adjusted AIC for small sample sizes.

Table 4.6. Parameter estimates, standard errors, and 95% confidence intervals for plausible models that predicted the number of bat phonic group call files, Arnold Air Force Base, 2022 and 2023.

Species	Parameter*	Estimate**	SE***	Lower 95****	Upper 95****
Myotis (High)	~log (Insect Abundance)	0.295	0.107	0.081	**0.509**
Mid	~ Edge (Burn)	4.749	0.605	3.535	**5.953**
	~ Forested Wetland	-2.947	0.751	-4.449	**-1.443**
	~ log (Hymenoptera)	0.448	0.218	0.012	**0.884**
	~ Edge (Burn)	1.18	0.313	0.558	**1.810**
Low	~ Forested Stream	-1.258	0.527	-2.312	**-0.203**
	~ Forested Wetland	-1.946	0.575	-3.096	**-0.796**

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*Parameter indicates which predictor variable was modeled to predict the presence of the corresponding bat species.

**Estimate corresponds to the covariance between the predictor variable and the predicted variable; these values show the correlation between the bat species and the predictor variable (positive or negative and to what extent). For fixed variables such as habitat, the estimate is based on whether it is occurring. For random variables this increases as if the predictor variable were one unit higher.

***SE describes the standard error associated with the model predictions based on the dataset used to construct the model.

***** Lower and Upper 95 show the 1st and 3rd quarter confidence intervals for the model. This value can be used to allude to the distribution of the provided dataset and confidence level of overall model.

Table 4.7. Models predicting abundance of bat species call files at Arnold Air Force Base, 2022 to 2023, acoustic sampling.

Bat Species	Parameter*	△ AICc**
Big Brown Bat	~ Edge (Burn)	0
	~ Forested Stream	0
	~ Forested Wetland	0
Eastern Red Bat	~ Edge (Burn)	0
	~ Forested Stream	0
	~ Forested Wetland	0
Evening Bat	~ Edge (Burn)	0
	~ Forested Wetland	0
	~ log (Hymenoptera)	16.85
Gray Bat	~ log (Insect Abundance)	0

*Parameter indicates which predictor variable was modeled to predict the presence of the corresponding bat species.

**Delta AICc was used to analyze the differences between AICc values for constructed models. Delta AICc subtracts the lowest AICc value from the AICc of the corresponding model. Delta AICc value is used to determine if models are significantly different from one another (often at delta AICc of 2). The AIC is the "Akaike information criterion" and it is an estimate of how well a model describes the patterns in the data. It is mainly used for comparing models trained on the same dataset, and delta AICc is adjusted AIC for small sample sizes.

Table 4.8. Parameter estimates, standard errors, and 95% confidence intervals for plausible models that predicted the number of bat species call files, Arnold Air Force Base, 2022 and 2023.

Species	Parameter*	Estimate**	SE***	Lower 95****	Upper 95****
Big Brown Bat	~ Edge (Burn)	1.053	0.294	0.4657	1.640
	~ Forested Stream	-1.390	0.546	-2.482	-0.298
	~ Forested Wetland	-2.315	0.669	-3.653	-0.977
Eastern Red Bat	~ Edge (Burn)	3.428	0.654	2.12	4.736
	~ Forested Wetland	-3.348	0.855	-5.058	-1.638
Evening Bat	~ Edge (Burn)	4.043	0.423	3.197	4.889
	~ Forested Stream	-2.616	0.582	-3.780	-1.452
	~ Forested Wetland	-3.847	0.591	-5.029	-2.665
	~ log (Hymenoptera)	0.343	0.162	0.019	0.667
Gray Bat	~ log (Insect Abundance)	0.297	0.113	0.071	0.523

*Parameter indicates which predictor variable was modeled to predict the presence of the corresponding bat species.

**Estimate corresponds to the covariance between the predictor variable and the predicted variable; these values show the correlation between the bat species and the predictor variable (positive or negative and to what extent). For fixed variables such as habitat, the estimate is based on whether it is occurring. For random variables this increases as if the predictor variable were one unit higher.

***SE describes the standard error associated with the model predictions based on the dataset used to construct the model.

*****Lower and Upper 95 show the 1st and 3rd quarter confidence intervals for the model. This value can be used to allude to the distribution of the provided dataset and confidence level of overall model.

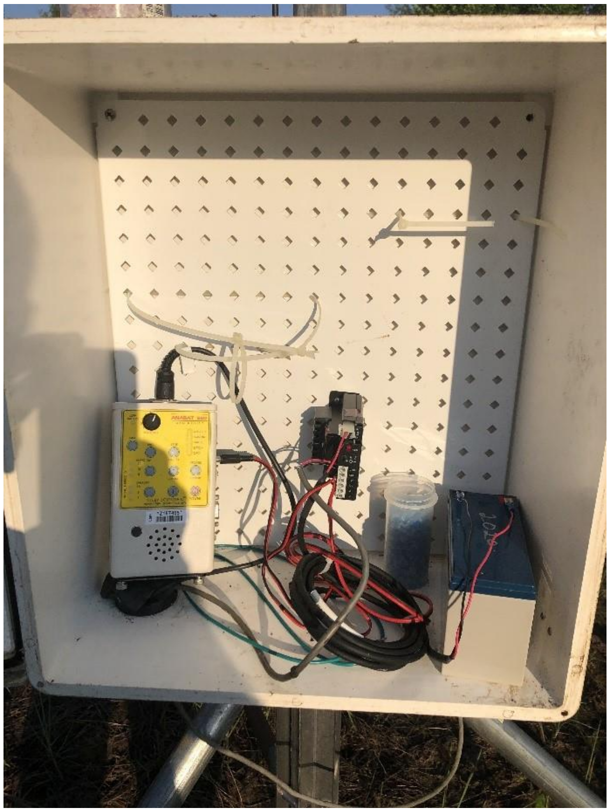

Figure 4.1. Anabat hardware, battery, and desiccant used to record bat calls files, Arnold Air Force Base, 2022-2023.

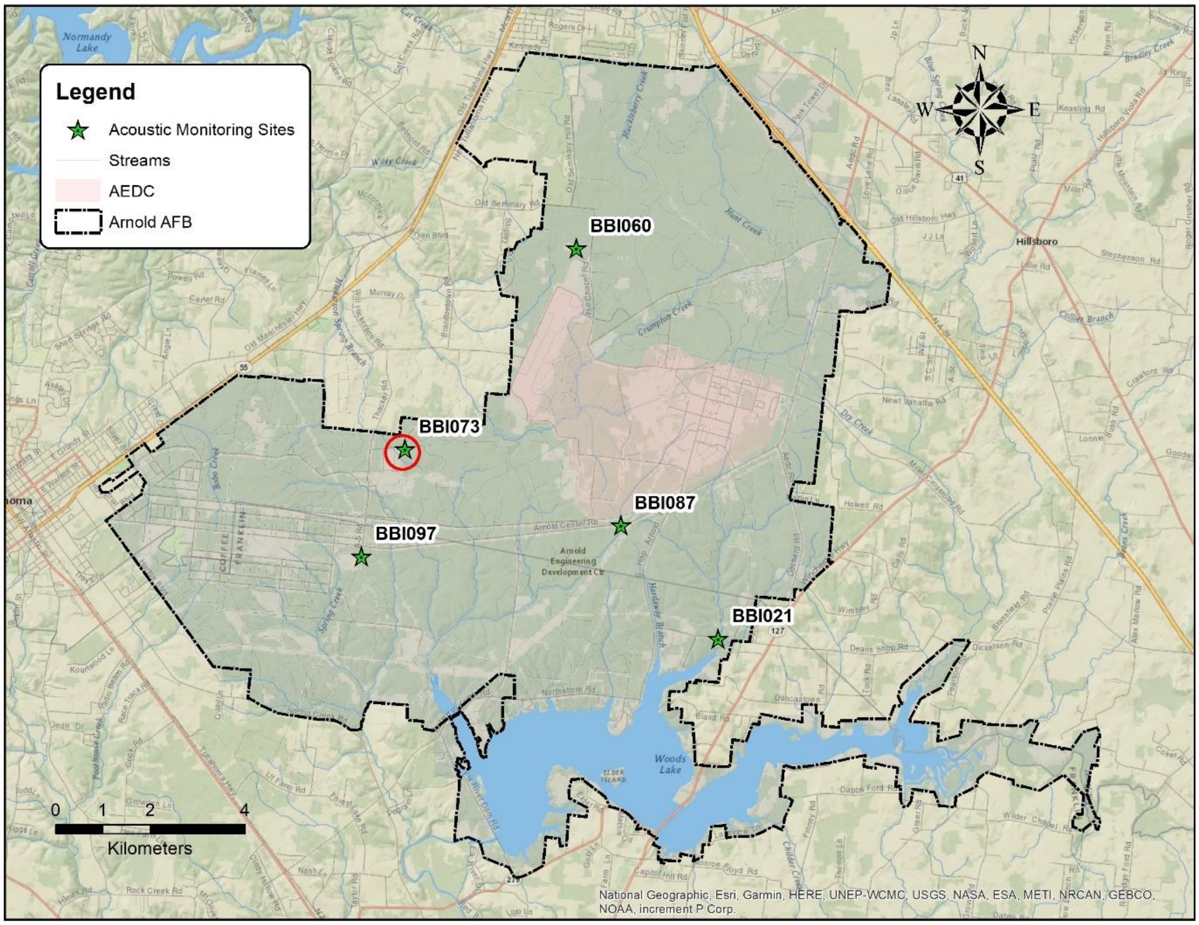

Figure 4.2. Locations of permanent acoustic monitoring sites, Arnold Air Force Base, 2022-2023. Provided by John Lamb. (*Red circle in base security area; Green stars without red circle outside of base security area.)

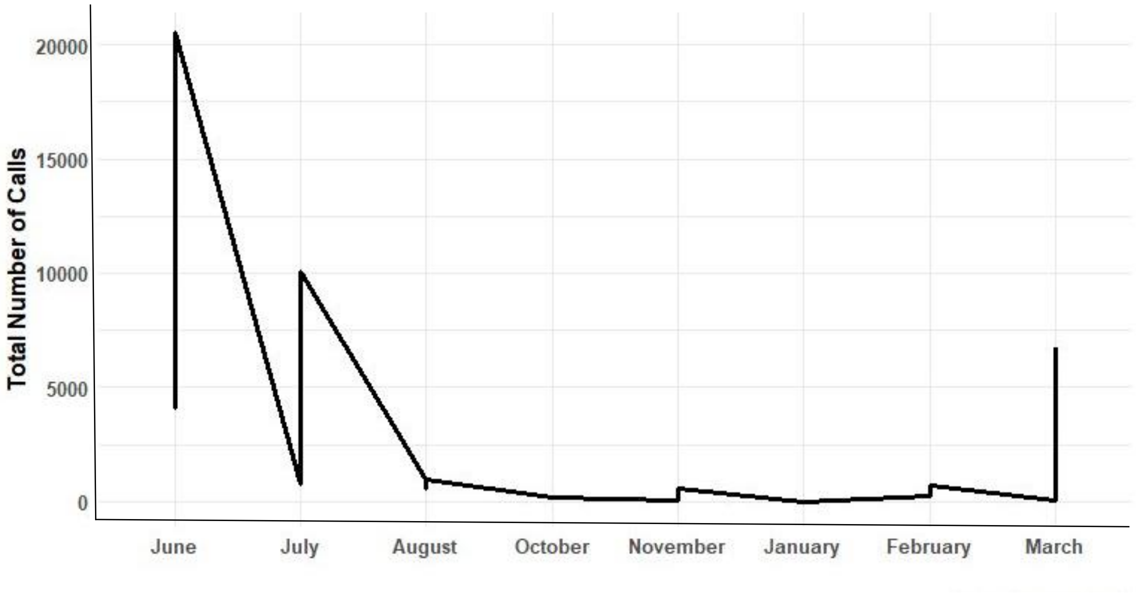

Figure 4.3. Total number of acoustic call files recorded annually, Arnold Air Force, 2022 and 2023. Provided by John Lamb.

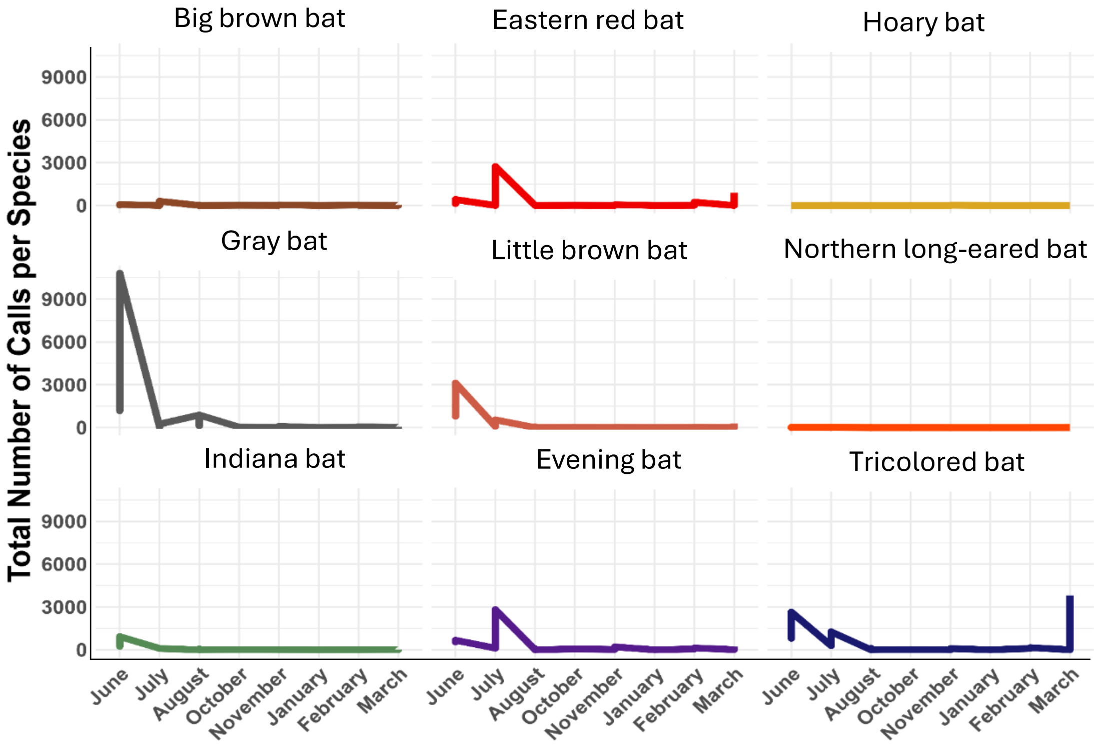

Figure 4.4. Total number of acoustic call files recorded by species annually, Arnold Air Force, 2022 and 2023. Provided by John Lamb.

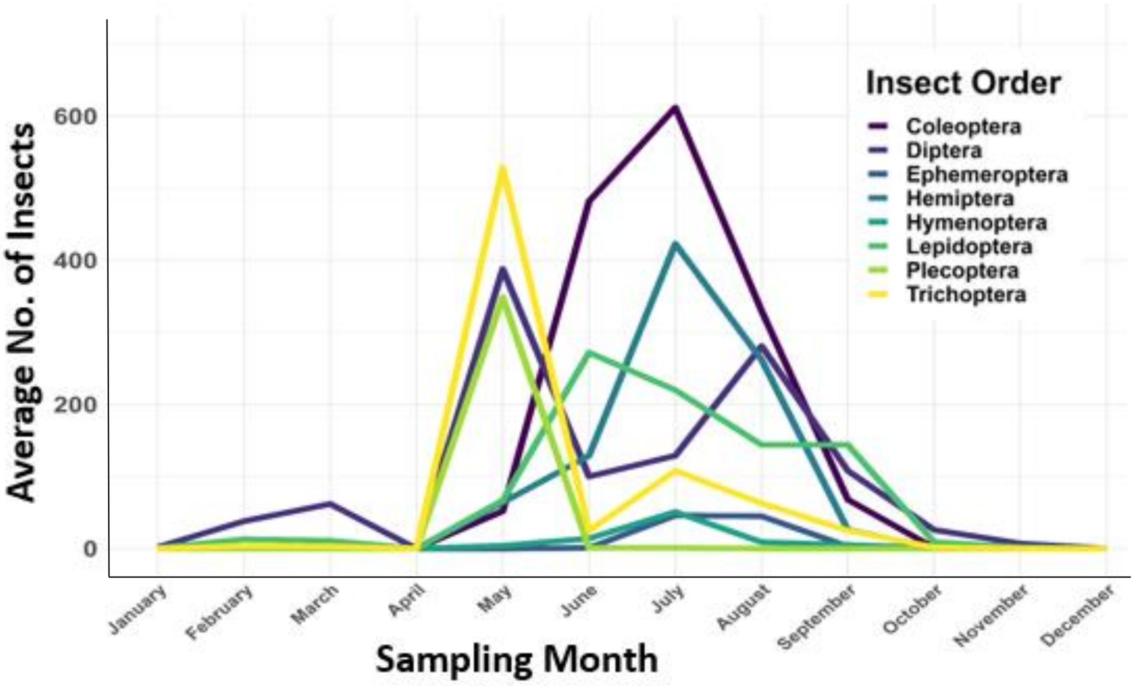

Figure 4.5. Average insect abundance captured monthly by order, Arnold Air Force Base, 2022 and 2023.

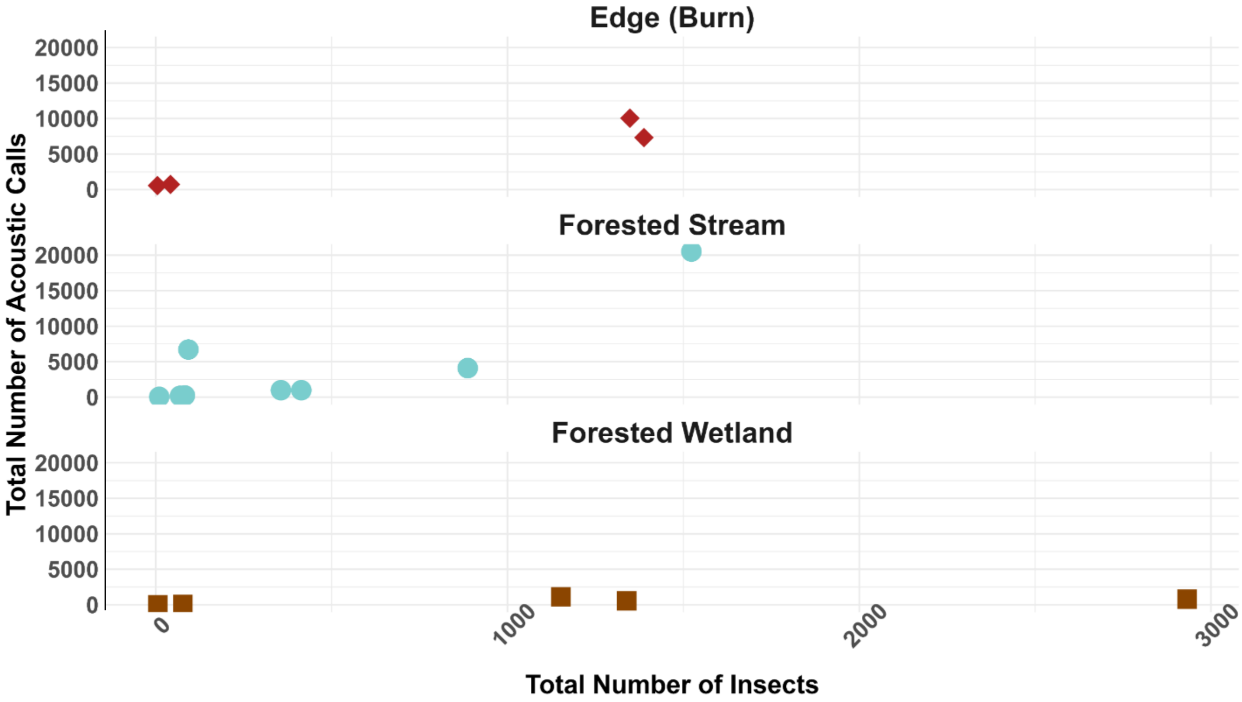

Figure 4.6. Relationship of insect abundance to acoustic bat call file abundance by habitat, Arnold Air Force Base, 2022 and 2023.

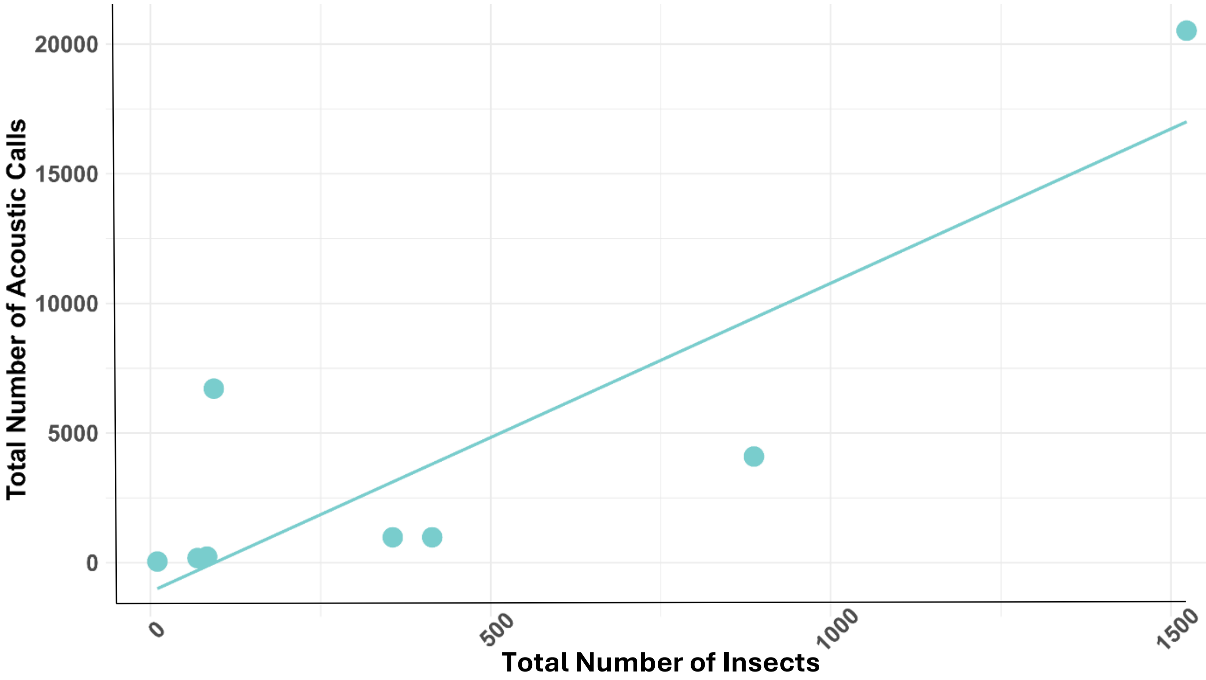

Figure 4.7. Relationship of insect abundance to acoustic call file abundance in forested stream habitats, Arnold Air Force Base, 2022 and 2023. Line is loess smoothed best fit.

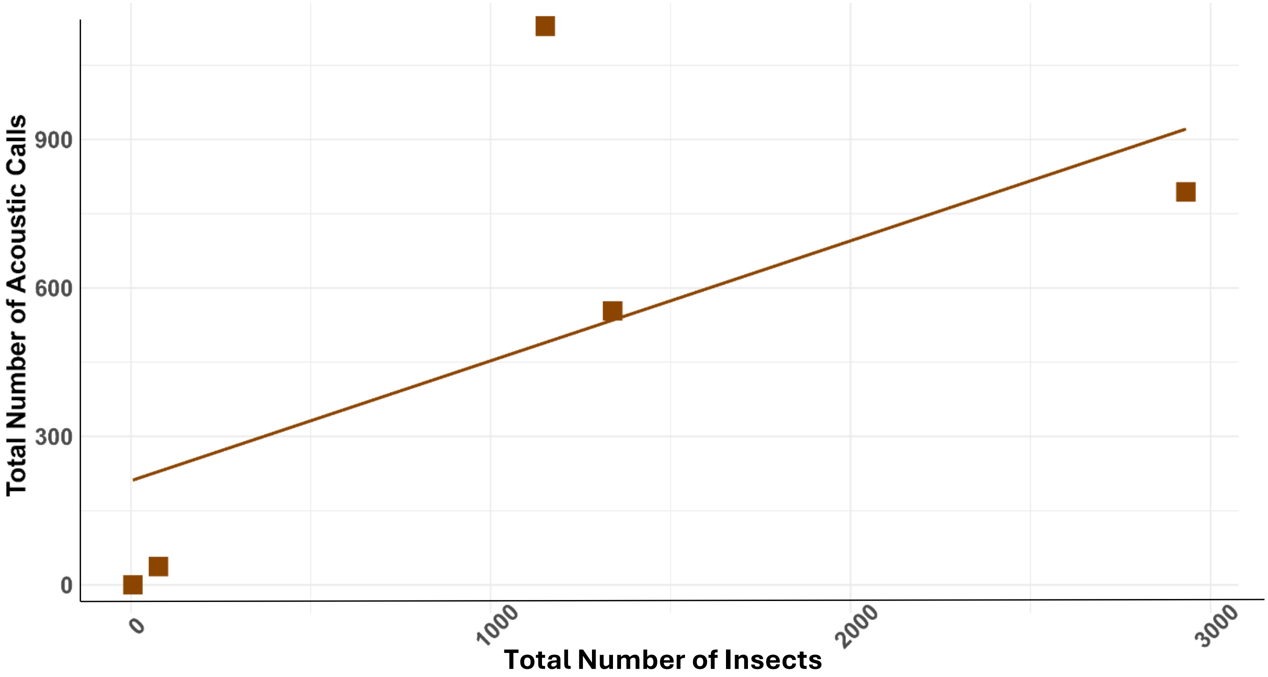

Figure 4.8. Relationship of insect abundance to acoustic call file abundance in forested wetland habitats, Arnold Air Force Base, 2022 and 2023. Line is loess smoothed best fit.

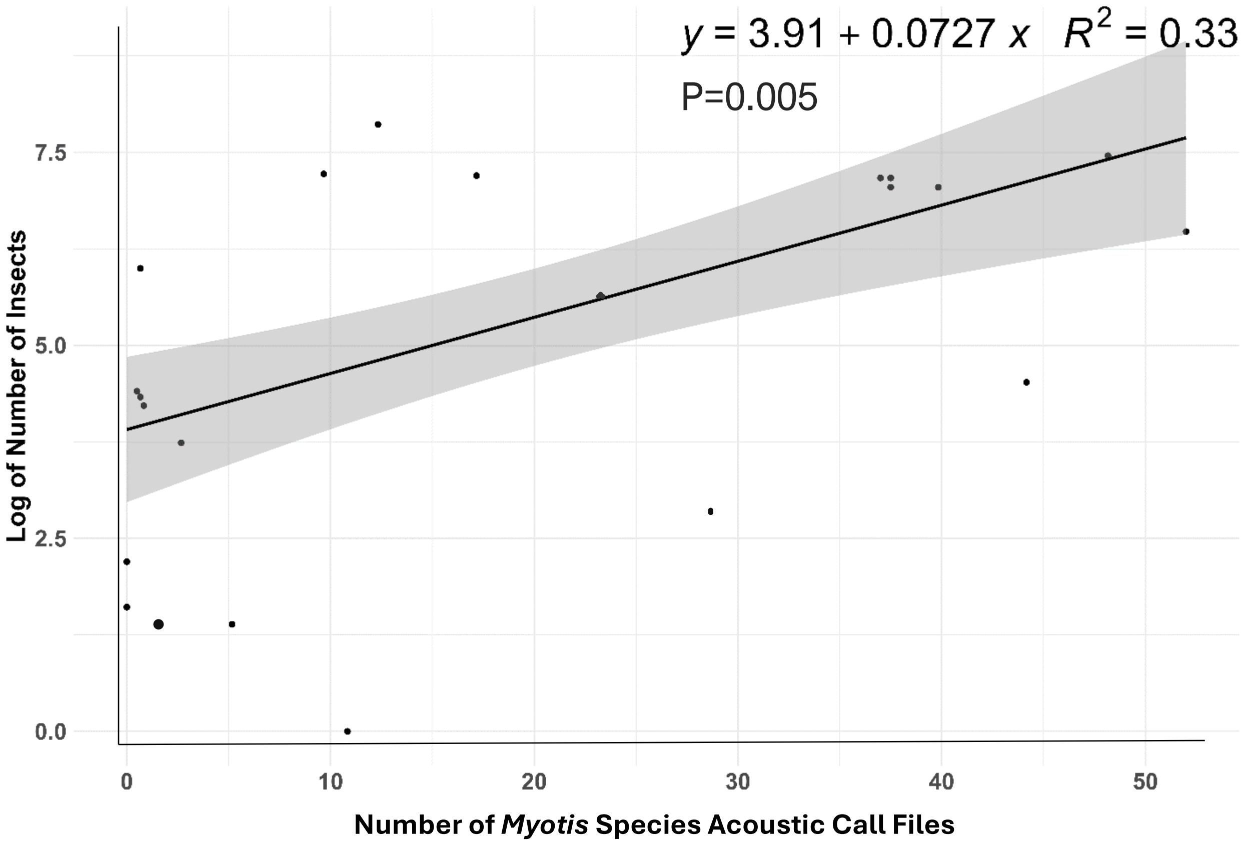

Figure 4.9. Relationship between *Myotis* (High) phonic group call files and insect Abundance, Arnold Air Force Base, 2022 and 2023.

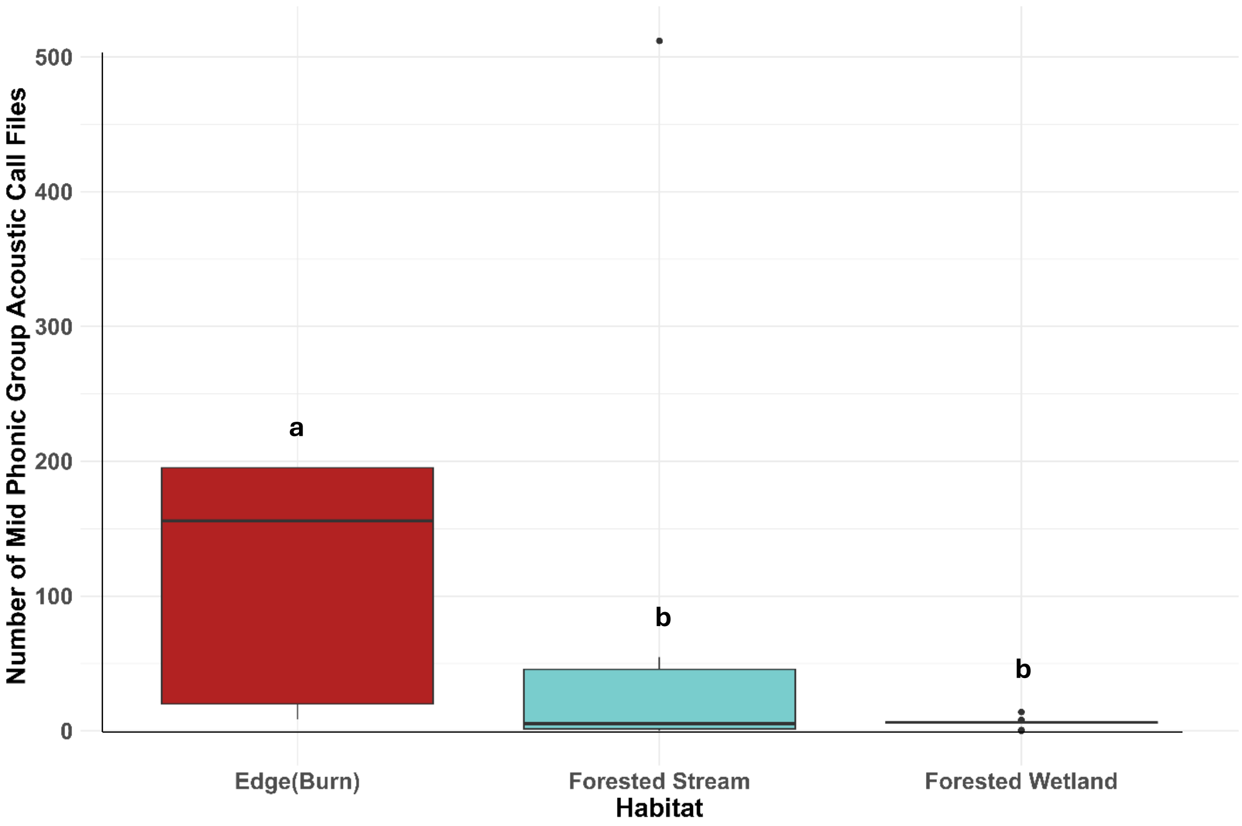

Figure 4.10. Relationship between the Mid phonic group call files and habitat, Arnold Air Force Base, 2022 and 2023.

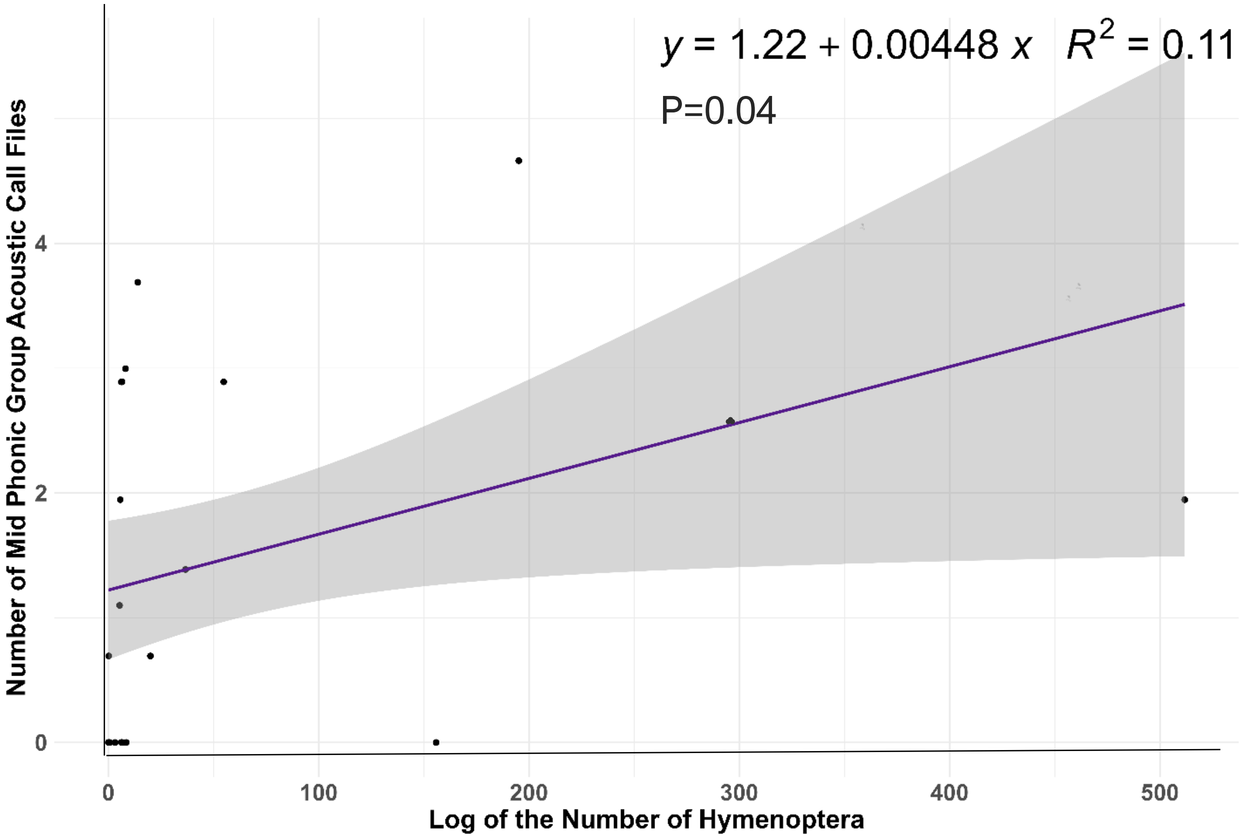

Figure 4.11. Relationship between the number of Mid phonic group call files and log of Hymenoptera abundance, Arnold Air Force Base, 2022 and 2023.

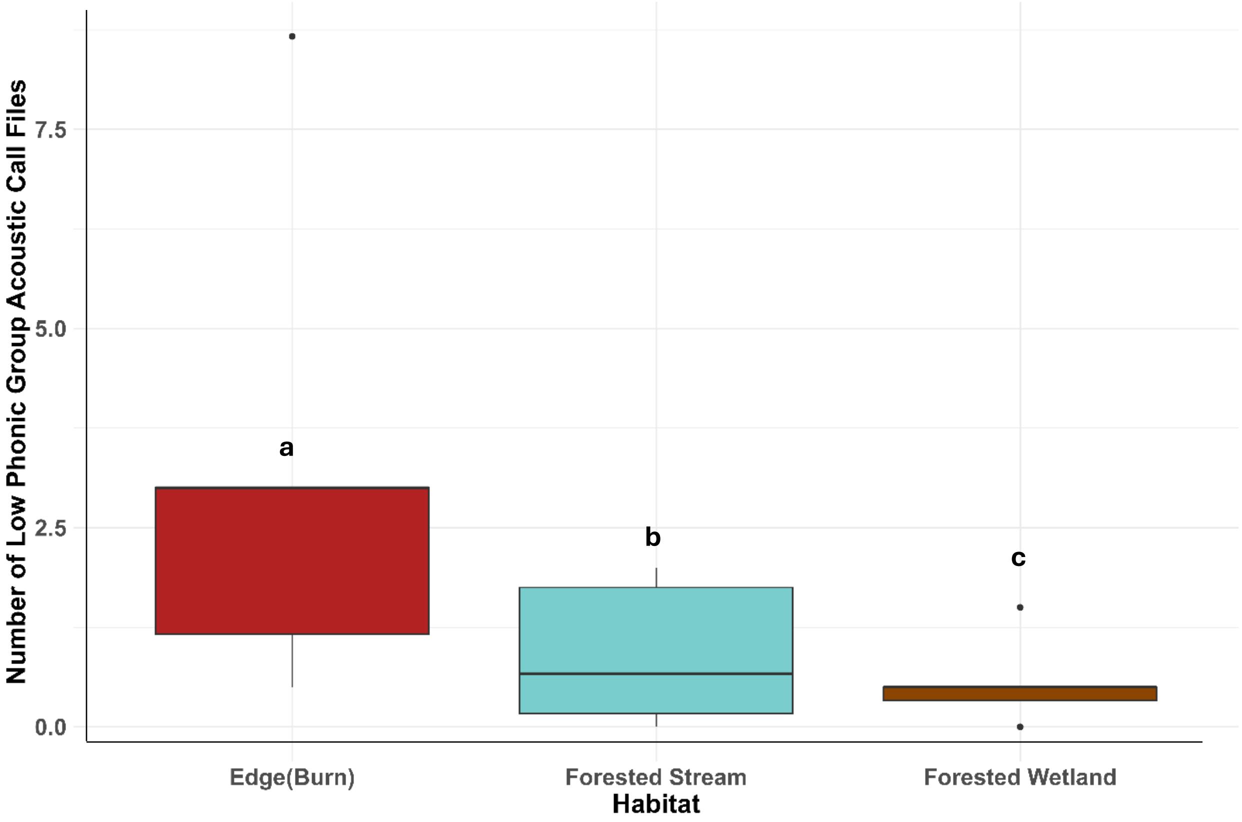

Figure 4.12. Relationship between the number of the Low phonic group call files and habitat, Arnold Air Force Base, 2022 and 2023.

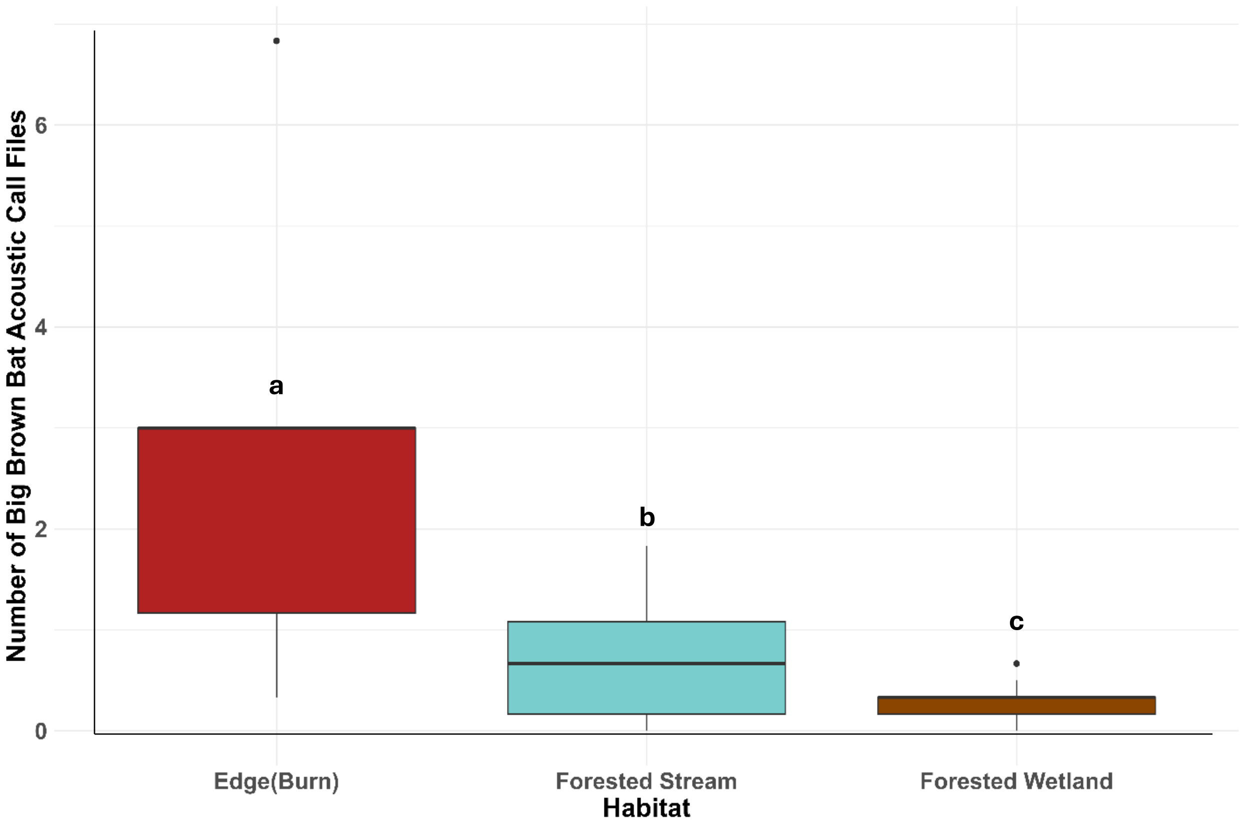

Figure 4.13. Relationship between big brown bat call files and habitat, Arnold Air Force Base, 2022 and 2023.

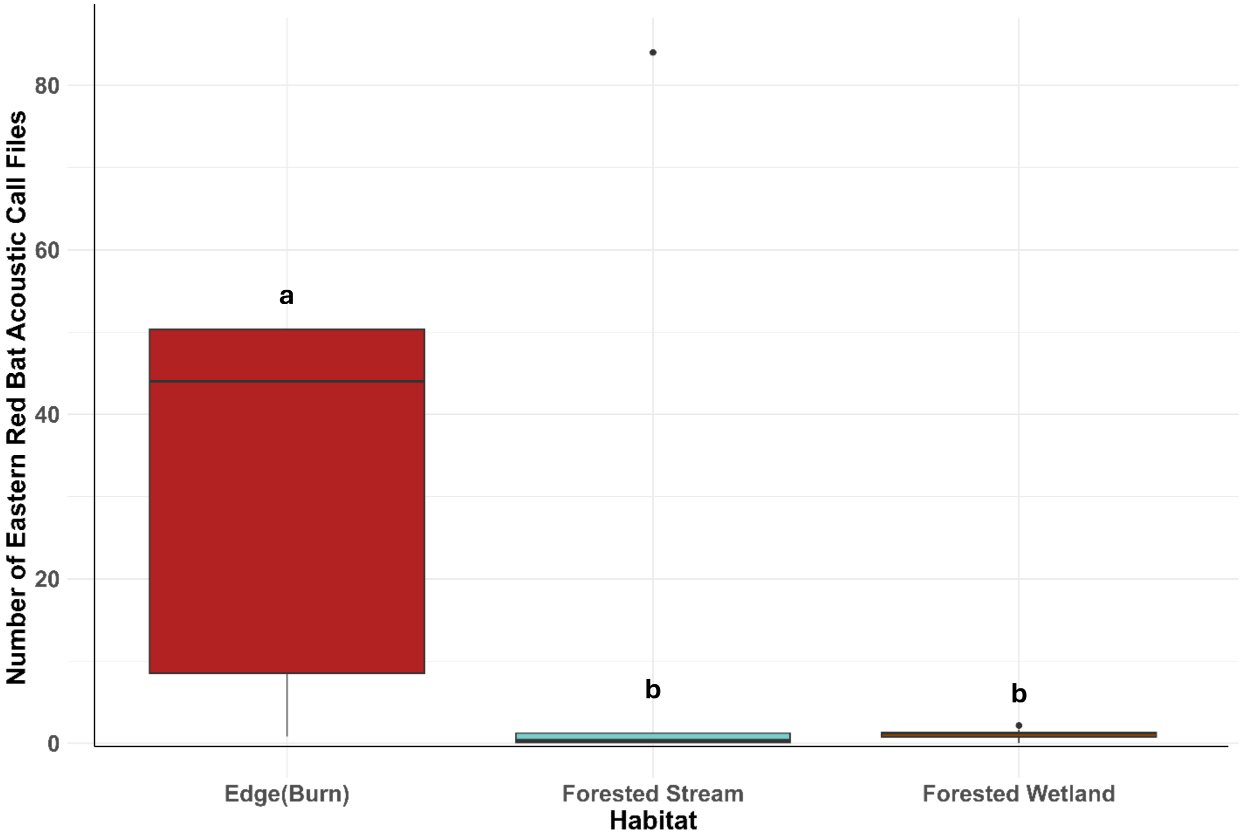

Figure 4.14. Relationship between eastern red bat call files and habitat, Arnold Air Force Base, 2022 and 2023.

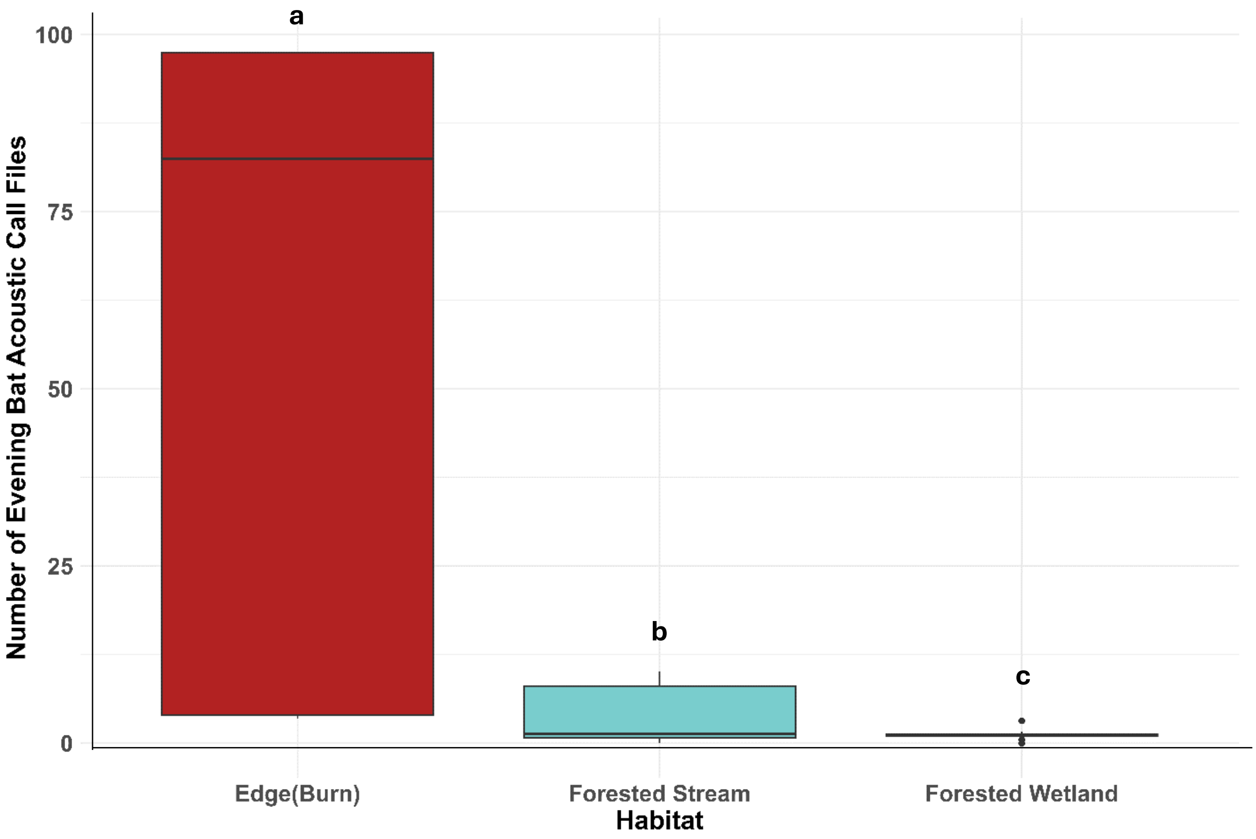

Figure 4.15. Relationship between the number of evening bat call files and habitat, Arnold Air Force Base, 2022 and 2023.

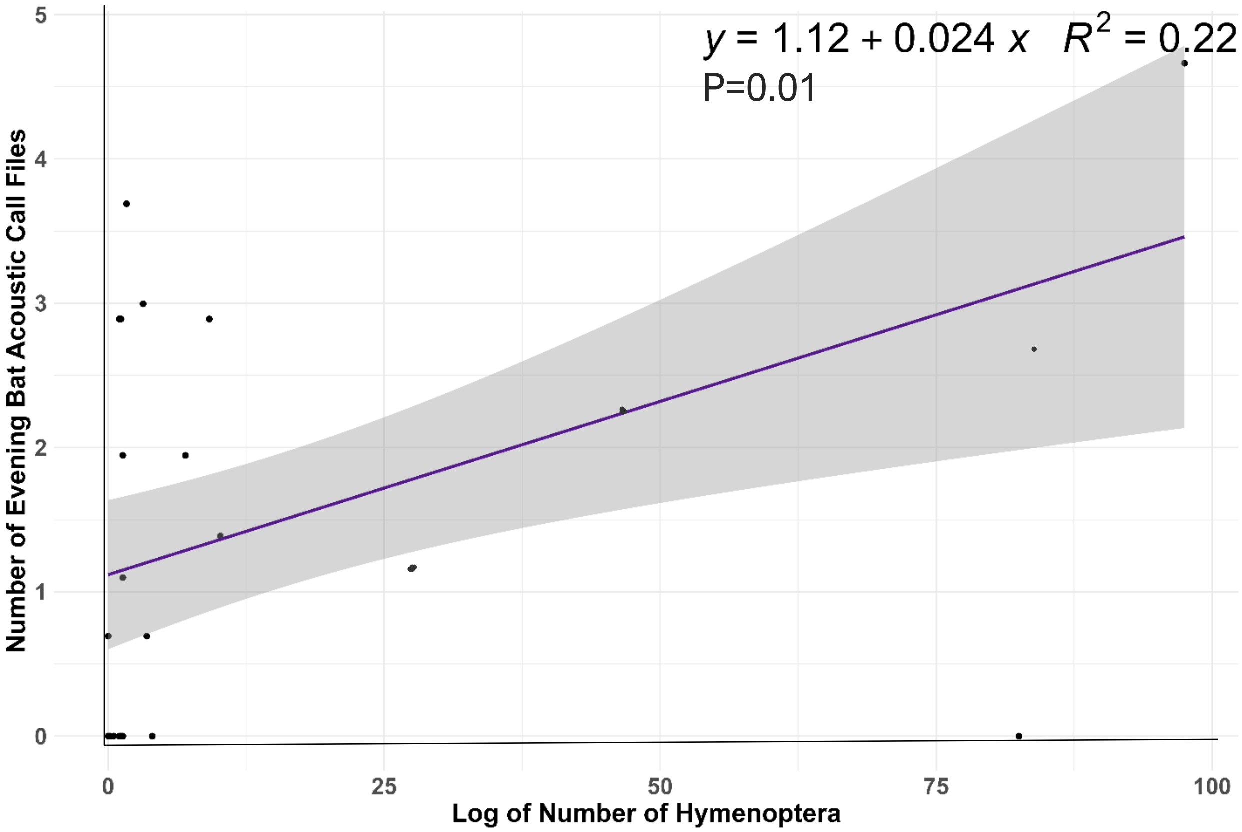

Figure 4.16. Relationship between the number of evening bat call files and Hymenoptera abundance, Arnold Air Force Base, 2022 and 2023.

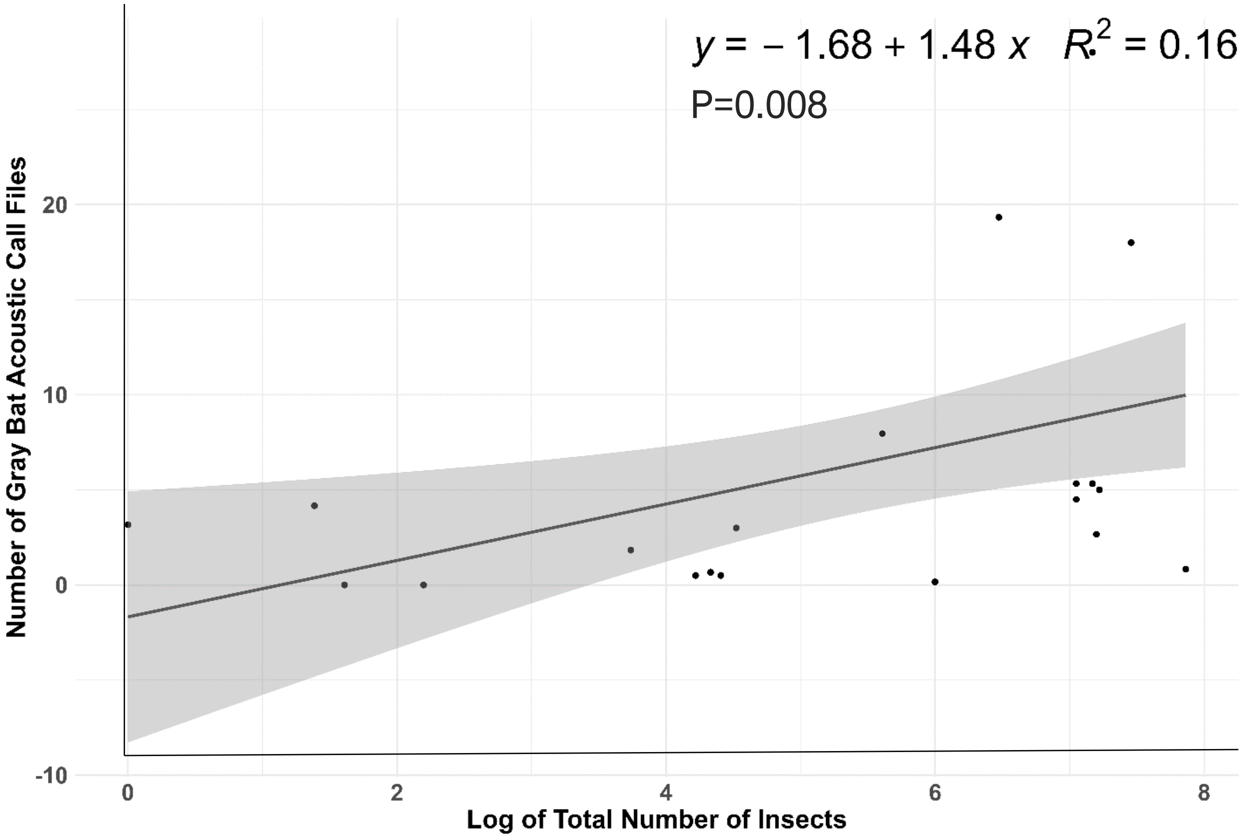

Figure 4.17. Relationship between the number of gray bat call files and insect abundance, Arnold Air Force Base, 2022 and 2023.

CHAPTER V

Conclusions

White Nose Syndrome (WNS) was first reported in the United States in Albany, NY in 2008 (Hoyt et al., 2021; Meteyer et al., 2009). WNS is caused by the fungal pathogen *Pseudogymnoascus* [*Geomyces*] *destrucans.* Since its introduction into the United States, this pathogen has caused mass mortality of bat species resulting in drastic declines in many species and causing shifts in ecosystem communities (Morningstar et al., 2019: Simonis et al., 2020). Once infected with the fungal pathogen, bats experience around 90% mortality (Frank et al., 2019; Reeder & Moore., 2013). Infected individuals that survive winter have higher lipid concentrations and higher body masses compared to individuals that succumb to WNS in the same species (Cheng et al., 2019).

Historically, the influence of insect order, family, diversity, and abundance on bat presence and abundance has been poorly studied (Bender et al., 2021). Crepuscular and nocturnal insect populations are less researched than their diurnal counterparts by entomologists (Evans & Murdoch; 1968; Guevara & Avilés, 2013). Current surveys of insects and other arthropods are especially critical as many insect taxa experience global loss, as well as shifts in ranges due to urbanization, climate change, environmental change, and globalization (Donkersley et al., 2022; Mamay & Simsek, 2017; Thomas et al., 2004). Because insects provide the primary food source for species of bats in North America, it is crucial to better understand the availability of different insect taxa across habitats and seasons to inform conservation efforts more fully for bats (Chowdhury et al., 2017; Hawkey et al., 2021). Given the importance of the relationship between bats and their insect prey there is a strong need for focused, integrated research.

Thus, the objectives of this study were to:

1) Determine crepuscular and nocturnal arthropods throughout various seasons and habitats at Arnold Air Force Base (AFB).
2) Explore the relationship of arthropods to bat presence caught in mist nets at Arnold AFB.
3) Explore the relationship of arthropods to the abundance of bat acoustic call files at Arnold AFB.

Crepuscular and nocturnal arthropods assessed were trapped through ultraviolet (UV) light and carbon dioxide (CO2) trapping at Arnold AFB over four habitat types (forested sites, forested streams, forested wetlands, and edge (transition shrubland/grassland from forest) with annual prescribed burning treatment) and four seasons (summer, fall, winter, and spring). Biomass, insect order abundance, insect family abundance, insect family diversity, insect family richness, and overall insect abundance were determined. This study showed that as arthropod abundance increased family richness increased (Chapter II). Results also showed that overall insect abundance was positively correlated with both insect family diversity and insect family richness with Spearman correlation values > 0.7 (Chapter II). Insect abundance and family diversity did vary across habitats and seasonal periods, but they were significantly correlated only with summer (positive correlation) (Chapter II). Insect populations were relatively high in summer and lowest in winter samples (Chapter II). Insect populations were significantly highest in summer in forested wetlands and lowest in summer in forested habitats. In contrast, though not significantly different, forested habitats had the highest insect abundance in the winter (Chapter II). Though not significantly different retention of insects in forested habitats in winter months could be important for emaciated bats.

Insect order biomass corresponded to insect order abundance except for orders that had relatively high biomass to low number of individuals, such as Blattodea, Orthoptera, and Meglaoptera (Chapter II). Despite the lack in overall influence of habitat on insect family diversity, habitat and season did have impacts on the families that were present and the abundances of those families (Chapter II, Tables: 2.5 - 2.18).

Overall, abundance of insect orders was influenced by a variety of factors, such as month, season, and habitat. Aquatic ephemeral orders (Ephemeroptera, Plecoptera, and Trichoptera) were significantly correlated with month; abundance of Ephemeroptera was positively correlated with July, and abundances of Plecoptera and Trichoptera were positively correlated with May (Chapter II). Abundance of Hemiptera (and Homoptera) was significantly and positively correlated with the summer season (Chapter II). Abundance of Diptera remained relatively stable all year and throughout all habitats compared to other orders, and in turn they had no significant predictor variables (Chapter II). Lepidoptera abundance was positively correlated with the months of May, July, and September (Chapter II). Abundance of Coleoptera was significantly correlated with many factors. For example, Coleoptera abundance had a negative significant relationship with March and January (Chapter II), while it had a positive significant relationship with forested wetlands (Chapter II). Coleoptera abundance was also positively correlated with forested habitats during spring (Chapter II).

To address the second objective of this study, mist-net surveys were used in conjunction with insect sampling events to evaluate the overall influence of insects on the presence of bats and richness of bat species found at Arnold AFB. Overall model confidence and significance were low but could be used to compare with other results from this study or with future studies (Chapter III). These results may suggest other variables, such as proximity to roost, are important for predicting presence or absence of bat species at mist-net sites. These results also may indicate that is large amount of chance in which species may be captured in mist nets on a particular evening. This lack of correlation is likely due to the small sample size of mist-netting events to insect sampling events, especially since species of bats are known to have some habitat preferences, such as gray bats foraging on waterways (Best & Hudson, 1996; Moore et al., 2017). The models developed for bat presence are best used in conjunction with previous/future studies and acoustic models in Chapter IV to make accurate assessments of the influence of insects on bat species at Arnold AFB.

To address the third objective of this study, acoustic surveys were used in conjunction with insect sampling events to evaluate the overall influence of insects on the abundance of calls for the four most prevalent bat species in three bat phonic groups (*Myotis* (High)*,* Mid, and Low) located on Arnold AFB. For the *Myotis* phonic group (all *Myotis* species) many predictor variables were found to be significant indicators for call file abundance. Insect abundance was positively and significantly correlated with *Myotis* call files (Chapter IV). In relationship to the Mid phonic group (eastern red bats, evening bats, and tricolor bats) had a positive significant relationship with edge (burn) sites and hymenopteran abundance (Chapter IV). Regarding the low phonic group (big brown bats and hoary bats) habitat was important in predicting number of call files. The low phonic group call files had a positive relationship with the edge (burn) site and negative relationship with forested stream and forested wetland sites (Chapter IV).

Big brown bat call files had a positive relationship with the edge (burn) site and negative relationship with forested stream and forested wetland sites (Chapter IV). Eastern red bats had a positive relationship with the edge (burn) site and negative relationship with forested stream and forested wetland sites (Chapter IV). On the other hand, gray bat call files were positively associated with insect abundance (Chapter IV). In relationship to evening bat call files, forested streams and forested wetlands were negatively correlated to call file abundance (Chapter IV). However, edge sites had a significantly correlated positive relationship with evening bat call files (Chapter IV). Hymenoptera abundance had a positive relationship to evening bat call files (Chapter IV).

Overall, models selected for acoustic call files were predominantly driven by habitat and secondarily by insect abundance and order abundance. However, insect order abundance did significantly increase the ability to predict species of bats and phonic groups of bats compared to the use of traditional indicators, such as habitat and season (Chapter IV). Season and biomass variables were not selected to be good predictors for any bat species or phonic group. These results indicate that future studies should consider adding specific insect orders and variables to better understand the abundance and presence of bat species.

These research findings increase knowledge on diversity, abundance, and relative biomass of arthropods on Arnold AFB and their influences on bat species present on the base. A better understanding of nocturnal arthropod availability and variation at Arnold AFB allows for the assessment of available prey items for foraging bats. Dry weight classes for insects were used to allude to relative nutritional content available for bats. The predictor variables for bat presence in reference to mist netting and the number of acoustic calls may provide greater insight to the foraging strategies of bats and provide insight into how bats consume their prey.

Management strategies to support insect populations include maintaining and monitoring forested wetland habitats around the base to encourage high coleopteran populations and other insect taxa (Balwan & Kour, 2021; Chapter II). Given the drastic decrease in insects in the edge (burn) site after the prescribed burn, it should be considered and further researched if rescheduling the seasonality of burns or increasing time between burns could be beneficial to support insect populations, but more research on these strategies is needed to make informed management decisions. Future research should improve knowledge on coordinating prescribed burning efforts to maximize insect abundance. Continuing to promote large diversity of habitats and flora will in turn encourage high abundance and diversity of arthropods (Brown, 2003; Knuff et al., 2020; Sobeck et al., 2009).

Possible ramifications of this research include promoting insect abundance for bat species, such as gray bats, and increasing insect abundance in edge sites for many bat species known to forage in edge habitats. Implementing management strategies close to known roosting sites to increase insect populations would be advised. Insect populations may be increased temporally by using UV light traps, which has proven effective in the 'fat bat' studies (Electric Power Research Institute, 2021). More permanent measures would include supporting plant populations that host high insect diversity or high densities of important insect taxa. Examples include supporting deciduous tree growth over pine growth; especially enhancing oak populations that are known to support high diversity and abundance of canopy insects (Crowley et al., 2023; Hammond & Miller, 1998; Southwood, 1961; Vaca-Sánchez et al., 2021). Supporting insect populations may also include planting more grasses, as well as spring and summer ephemerals, close to roosting and foraging areas on Arnold AFB (Curran et al., 2022; Hammond & Miller,1998). Continued monitoring and support of heterogeneity in habitats and known foraging areas could benefit insect populations in the long term at Arnold AFB.

It is important to note the limitations of both the insect and bat methodologies. CDC light traps and UV light traps both use attractants to pull insects to the trap inherently biasing samples collected from them with often increased proportions of lepidopterans, coleopteran families, such as Heteroceridae, and dipteran families, such as Culicidae and other nematoceran families (Acuff, 1976; Marshall, 2006; McDermott & Mullens, 2018; Muirhead-Thompson, 2012).

The overall goal of this study was to be complementary with ongoing studies at Arnold AFB, such as guano meta-barcoding (University of Illinois) and surveys to assess the use of prescribed burnings on arthropods (University of Tennessee). These studies together aim to provide more informed management decisions. By understanding insect composition and availability, their relationships to individual bat species can be further explored, enhancing the development of management practices centered around these relationships. This study highlights the importance of understanding the primary influences in insect populations and how in turn these potentially affect foraging bats. This type of research highlights the importance of understanding bottom-up predator-prey relationships as well as top-down. This study ultimately aims to be a tool combined with past and future research to aid in conservation management of insect populations and in turn the bat species they support on Arnold AFB.

Literature Cited

Acuff, V. R. (1976). Trap biases influencing mosquito collecting. *Mosquito News*, *36*(2), 173-176.

Balwan, W. K., & Kour, S. (2021). Wetland-an ecological boon for the environment. *East African Scholars Journal of Agriculture and Life Sciences*, *4*(3), 38-48.

Bender, M. J., Perea, S., Castleberry, S. B., Miller, D. A., & Wigley, T. B. (2021). Influence of insect abundance and vegetation structure on site-occupancy of bats in managed pine forests. *Forest Ecology and Management*, *482*, 118839.

Best, T. L., & Hudson, M. K. (1996). Movements of gray bats (*Myotis grisescens*) between roost sites and foraging areas. *Journal of the Alabama Academy of Science*, *67*(1), 6-14.

Brown, B. L. (2003). Spatial heterogeneity reduces temporal variability in stream insect communities. *Ecology Letters*, *6*(4), 316-325.

Cheng, T. L., Gerson, A., Moore, M. S., Reichard, J. D., DeSimone, J., Willis, C. K., ... & Kilpatrick, A. M. (2019). Higher fat stores contribute to persistence of little brown bat populations with white-nose syndrome. *Journal of Animal Ecology*, *88*(4), 591-600.

Chowdhury, G. R., Datta, U., Zaman, S., & Mitra, A. (2017). Ecosystem services of insects. *Heart*, *17*, 23.

Curran, M. F., Robinson, T. J., Guernsey, P., Sorenson, J., Crow, T. M., Smith, D. I., & Stahl, P. D. (2022). Insect abundance and diversity respond favorably to vegetation communities on interim reclamation sites in a semi-arid natural gas field. *Land*, *11*(4), 527.

Donkersley, P., Ashton, L., Lamarre, G. P., & Segar, S. (2022). Global insect decline is the result of wilful political failure: A battle plan for entomology. *Ecology and Evolution*, *12*(10), e9417.

Electric Power Research Institute. (2021). (tech.). *Fat Bats: Research and Management Practices for Enhancing Foraging Habitat to Help Support Bats with White-Nose Syndromes*. Retrieved from https://www.epri.com/research/products/000000003002021572.

Evans, F. C., & Murdoch, W. W. (1968). Taxonomic composition, trophic structure and seasonal occurrence in a grassland insect community. *The Journal of Animal Ecology*, *37(1),* 259-273.

Frank, C. L., Davis, A. D., & Herzog, C. (2019). The evolution of a bat population with white-nose syndrome (WNS) reveals a shift from an epizootic to an enzootic phase. *Frontiers in Zoology*, *16*, 1-9.

Guevara, J., & Avilés, L. (2013). Community-wide body size differences between nocturnal and diurnal insects. *Ecology*, *94*(2), 537-543.

Hammond, P. C., & Miller, J. C. (1998). Comparison of the biodiversity of Lepidoptera within three forested ecosystems. *Annals of the Entomological Society of America*, *91*(3), 323-328.

Hawkey, K. J., Lopez-Viso, C., Brameld, J. M., Parr, T., & Salter, A. M. (2021). Insects: a potential source of protein and other nutrients for feed and food. *Annual Review of Animal Biosciences*, *9*, 333-354.

Hoyt, J. R., Kilpatrick, A. M., & Langwig, K. E. (2021). Ecology and impacts of white-nose syndrome on bats. *Nature Reviews Microbiology*, *19*(3), 196-210.

Knuff, A. K., Staab, M., Frey, J., Dormann, C. F., Asbeck, T., & Klein, A. M. (2020). Insect abundance in managed forests benefits from multi-layered vegetation. *Basic and Applied Ecology*, *48*, 124-135.

Mamay, M., & Şimşek, E. (2017). The Expected Impact of Global Warming and Climate Change on Insect Diversity. *The 3rd International Symposium on EuroAsian Biodiversity*, pp. (173).

Marshall, S. A. (2006). *Insects: their natural history and diversity: with a photographic guide to insects of eastern North America* (p. 718). Buffalo, NY: Firefly Books.

McDermott, E. G., & Mullens, B. A. (2018). The dark side of light traps. *Journal of Medical Entomology*, *55*(2), 251-261.

Meteyer, C. U., Buckles, E. L., Blehert, D. S., Hicks, A. C., Green, D. E., Shearn-Bochsler, V., ... & Behr, M. J. (2009). Histopathologic criteria to confirm white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation*, *21*(4), 411-414.

Moore, P. R., Risch, T. S., Morris, D. K., & Rolland, V. (2017). Habitat use of female gray bats assessed using aerial telemetry. *The Journal of Wildlife Management*, *81*(7), 1242-1253.

Morningstar, D. E., Robinson, C. V., Shokralla, S., & Hajibabaei, M. (2019). Interspecific competition in bats and diet shifts in response to white-nose syndrome. *Ecosphere*, *10*(11), e02916.

Muirhead-Thompson, R. C. (2012). Trap responses of flying insects: the influence of trap design on capture efficiency. San Diego, CA: Academic Press.

Reeder, D. M., & Moore, M. S. (2013). White-nose syndrome: a deadly emerging infectious disease of hibernating bats. *Bat Evolution, Ecology, and Conservation*, https://doi.org/10.1007/978-1-4614-7397-8_20.

Simonis, M. C., Brown, B. K., & Bahn, V. (2020). Mobile bat acoustic routes indicate cavity-roosting species undergo compensatory changes in community composition following white-nose syndrome. *Acta Chiropterologica*, *22*(2), 315-326.

Sobek, S., GOßNER, M. M., Scherber, C., STEFFAN-DEWENTER, I. N. G. O. L. F., & Tscharntke, T. (2009). Tree diversity drives abundance and spatiotemporal β-diversity of true bugs (Heteroptera). *Ecological Entomology*, *34*(6), 772-782.

Southwood, T. R. E. (1961). The number of species of insect associated with various trees. *The Journal of Animal Ecology*, *30(1),*1-8.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., ... & Williams, S. E. (2004). Extinction risk from climate change. *Nature*, *427*(6970), 145-148.

Vaca-Sánchez, M. S., Maldonado-López, Y., González-Rodríguez, A., Oyama, K., Fernandes, G. W., Fagundes, M., ... & Cuevas-Reyes, P. (2021). Canopy arthropod diversity associated with *Quercus laurina*: importance of an oak species diversity gradient on abundance, species richness and guild composition. *Journal of Insect Conservation*, *25*, 859-874.

VITA

Carmen Black, along with her twin sister Marlo Black, was born on February 24, 1999, in Murfreesboro, Tennessee. Carmen grew up in middle Tennessee with her sisters (Marlo Black, Sidra Levenhagen, and Violet Black), her dad (Jeff Black), her mom (Staci Higdon), and her stepmom (Mindy Black). Growing up, Carmen was always fascinated by the outdoors. In both her personal and professional lives (working a variety of jobs) she has always centered her life around outdoor recreation and wildlife.

In 2017, Carmen graduated as valedictorian from Central Magnet School in Murfreesboro, Tennessee. After graduating Carmen attended college at the University of Tennessee, Chattanooga, where she obtained a Bachelor of Science in Environmental Science and a minor in Biology in May 2021. After college Carmen worked on a trail crew until her acceptance to the University of Tennessee in Dr. Jerome Grant’s lab in the Department of Entomology and Plant Pathology at the University of Tennessee in Knoxville, Tennessee. Carmen completed her Master of Science requirements in May of 2024, and accepted her enrollment for a Doctor in Philosophy in the Department of Ecology and Evolutionary Biology at University of Colorado, Boulder. Carmen is a member of the Entomological Society of America, the Tennessee Entomological Society, and the Tennessee Bat Working Group.