ECOLOGICAL CONSEQUENCES OF DISEASE-RELATED BAT DECLINES

by

AMY KRISTINE WRAY

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

(Wildlife Ecology)

at the

UNIVERSITY OF WISCONSIN-MADISON

2020

Date of final oral examination: 1 December 2020

This dissertation is approved by the following members of the final oral committee:

M. Zachariah Peery, Professor, Forest & Wildlife Ecology

Claudio Gratton, Professor, Entomology

Daniel Lindner, Research Plant Pathologist, USFS NRS Center for Forest Mycology Research

Jonathan Pauli, Associate Professor, Forest & Wildlife Ecology

Benjamin Zuckerberg, Associate Professor, Forest & Wildlife Ecology

"They turned then to the necessary animal life—burrowing creatures to open the soil and aerate it: kit fox, kangaroo mouse, desert hare, sand terrapin . . . and the predators to keep them in check: desert hawk, dwarf owl, eagle and desert owl; and insects to fill the niches these couldn't reach: scorpion, centipede, trapdoor spider, the biting wasp and the wormfly . . . and the desert bat to keep watch on these."

– Frank Herbert, *Dune,* Appendix I

Acknowledgements

First of all, I thank my advisor, Zach Peery, who has constantly encouraged me to "think outside the bat box". By spending the past five years under the (owl) wings of your lab I have grown so much as a scientist, as a writer, and as a person, and for that I will always be thankful. I thank Claudio Gratton for showing me the world of entomology, without which I would have never realized how much I am fascinated by Diptera in particular. I also thank Dan Lindner, who has been a wonderfully supportive mentor and opened my eyes to the fantastic field of the fungi. My committee members, Ben Zuckerberg and Jon Pauli, have been a constant source of feedback and advice, and offered many thoughtful suggestions that helped me improve this dissertation and well as enrich my perspectives on ecology, for which I am most appreciative.

This dissertation is the product of many collaborations, which brought together a truly unique team of awesome people. I would particularly like to thank Heather Kaarakka and Paul White from the Wisconsin Department of Natural Resources for their continual insights on bat ecology and for being the best people you could ever hope to spend time with in the field, at the Field Museum, in a mine full of sand, or just about anywhere on the planet. I also thank Mark Banik and Jon Palmer from the Center for Forest Mycology Research for ensuring the soundness of our methodology and for holding our work up to the highest standards. I am, of course, indebted to Michelle Jusino — who not only trained me in prepping next-gen libraries, but who also was a constant source of expertise, encouragement, and endless ideas for presentation titles with puns.

I could never have survived the unreasonably cruel winter or the many hours in the windowless basement without the remarkable community at UW-Madison. I thank the Peery lab and its alumni, especially Tiffany Bougie, Kristin Brunk, Nathan Byer, Gavin Jones, Brendan Hobart, Kevin Kelly, Nick Kryshak, Mario Garcés-Restrepo, Elena West, Evan Wilson, Connor Wood, and Ceeanna Zulla

for being outstanding friends and colleagues. Additionally, I thank Phil Manlick, Marie Martin, and Mauriel Rodriguez-Curras for always being willing to nerd out about stable isotopes and predator ecology. I am especially grateful to field & lab technician extraordinaire, Jade Kochanski, who helped me figure out the first field season, kept me sane during the second field season, and counted a truly monstrous quantity of arthropods. And for the Rusty Spikers volleyball team, I would like to record in this citable document that we definitely would have won the intramural championship if there had not been a pandemic.

Many other friends and colleagues ("the extended bat family") have been a huge source of both professional and emotional support, and were frequently willing to offer advice, read abstracts, watch practice presentations, or discuss ideas, often when requested at the last minute. This group includes Aja Sherman, Catherine Doyle-Capitman, Nicole Edmison, Grace Vaziri, Caylee Falvo, Kyle Shute, Lois Wray (a.k.a. my mom), and the many members of the North American Society for Bat Research and the Midwest Bat Working Group. Without your help, I would be plagued by typos and I cannot imagine going through this process without you all.

Finally, I want to thank the many state and county park officials, private landowners, and community scientists who contributed to this project by allowing sampling on their lands and/or by fastidiously collecting samples. Thank you for sharing the greatest gifts a scientist could ask for: so many bugs and so much bat guano. In particular, I would like to thank Mike and Jane Balch, Rhonda (and Ruby) Enge, Marci and Jim Hess, Gary Jansen, and Elaine Swanson, who were overwhelmingly kind and welcoming to me, especially when I was first starting out. In addition to appreciating your roles in the project, I also admire your commitment to your bat colonies and cherish your friendships!

Overall, I consider myself so fortunate to have had the opportunity to work with and learn from such an amazing group of people, and I hope I can make all of you proud as I move forward.

Dissertation Abstract

The spread of infectious disease in wildlife represents an emerging threat to biodiversity. Particularly among predators, the effects of emerging infectious diseases not only include population declines and potential extirpation, but also can result in top-down effects on prey communities. In North America, several hibernating bat species face serious population declines due to the emergence of white-nose syndrome, a devastating disease caused by the fungal pathogen *Pseudogymnoascus destructans*. While arthropodivorous bats are often lauded for providing ecosystem services in the form of agricultural pest suppression, other regulatory effects on the arthropod food web as a whole have seldom been assessed. In light of the impending westward spread of white-nose syndrome and corresponding predicted bat population declines, this dissertation seeks to characterize the role of bats as top predators in the nocturnal arthropod food web and to assess the broader ecological consequences of disease-related bat population declines. Specifically, this research focuses on two common bat species, the little brown bat (*Myotis lucifugus*) and the big brown bat (*Eptesicus fuscus*), to explore the response of bats to changing prey abundance (Chapter 1), changes in bat foraging patterns over the past century (Chapter 2), top-down consequences of bat declines on arthropod communities (Chapter 3), and the possibility of the functional replacement of one bat species by another (Chapter 4). Overall, this dissertation demonstrates that the function of bats in the nocturnal arthropod food web is complex, and that declines among little brown bats in particular can have top-down effects which are unlikely to be ameliorated by other persisting bat species. As such, these results emphasize the necessity of promoting the conservation of bats and other aerial arthropodivores, while highlighting their importance as predators that influence their respective food webs.

Table of Contents

CHAPTER 1: PREDATOR PREFERENCES SHAPE THE DIETS OF ARTHROPODIVOROUS BATS MORE THAN QUANTITATIVE LOCAL PREY ABUNDANCE

Amy K. Wray[1,2], M. Zachariah Peery[1], Michelle A. Jusino[3,4], Jade M. Kochanski[2], Mark T. Banik[3], Jon M. Palmer[3], Daniel L. Lindner[3], and Claudio Gratton[2]

[1]Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, Madison, WI, USA; [2]Department of Entomology, University of Wisconsin-Madison, Madison, WI, USA; [3]Center for Forest Mycology Research, Northern Research Station, USDA Forest Service, Madison, WI, USA; [4]Department of Plant Pathology, University of Florida, Gainesville, FL, USA

Citation:

Wray, A. K., Peery, M. Z, Jusino, M. A., Kochanski, J. M., Banik, M. T., Palmer, J. M., Lindner, D. L., & Gratton, C. Predator preferences shape the diets of arthropodivorous bats more than quantitative local prey abundance. *Molecular Ecology,* in revision.

Abstract

Although most predators are generalists, the majority of studies on the association between prey availability and prey consumption have focused on specialist predators. To investigate the role of highly generalist predators in a complex food web, we measured the relationships between prey consumption and prey availability in two common arthropodivorous bats. Specifically, we used high-throughput amplicon sequencing coupled with a known mock community to characterize seasonal changes in little brown and big brown bat diets. We then linked spatio-temporal variation in prey consumption with quantitative prey availability estimated from intensive prey community sampling. We found that although quantitative prey availability fluctuated substantially over space and time, the most commonly consumed prey items were consistently detected in bat diets independently of their respective abundance. Positive relationships between prey abundance and probability of consumption were found only among prey groups that were less frequently detected in bat diets. While the probability of prey consumption was largely unrelated to abundance, the community structure of prey detected in bat diets was influenced by the local or regional abundance of prey. Observed patterns suggest that while little brown and big brown bats maintain preferences for particular prey independently of quantitative prey availability, total dietary composition may reflect some degree of opportunistic foraging. Overall, our findings suggest that generalist predators can display strong prey preferences that persist despite quantitative changes in prey availability.

Introduction

Though capable of consuming many types of prey resources, generalist predators are often selective. Predator preference, that is, any deviation from a random sampling of available prey, is a particularly useful measure for describing which prey resources are sought out or avoided by a selective predator (Chesson 1978; Manly et al. 1972). Under the assumption that foraging is optimal, predator

preferences for certain prey are expected to maximize net energy gains, and consequently, consumption of non-preferred prey should be expected only when preferred prey are absent or low in abundance (Pulliam 1974; Schoener 1971). However, empirical studies often demonstrate patterns that deviate from optimal foraging, particularly for predators that consume mobile prey (Sih & Christensen 2001). Additionally, despite the importance of understanding how predator preferences influence their role in food webs, many generalist predators lack detailed descriptions of the prey that they consume, and even fewer have corresponding information on their responses to fluctuating resource availability (Thompson et al. 2012; Pringle 2020).

For generalist predators, prey preferences can influence functional responses (sensu Holling 1959a,b), although the relationship between prey consumption and prey availability may not necessarily resemble classic response types (Murdoch & Oaten 1975; Oksanen et al. 2001). For example, generalist predators foraging in multi-prey systems have been shown to consume prey irrespective of changing availability or even reduce the consumption of increasing prey as a result of preferences related to nutritional demands, energy requirements, or ease of prey capture (Baudrot et al. 2016; Chesson 1984; Dale et al. 1994). Therefore, the relationship between prey consumption and quantitative availability in generalist predators can be highly context dependent (Novak et al. 2017; Preston et al. 2018; Symondson et al. 2002). The occurrence of individual specialization, even among the most archetypal generalist predators, also suggests that prey preferences are not necessarily static over time or between conspecifics (Bolnick et al. 2002; Panzacchi et al. 2008; Sacks & Neale 2002; Woo et al. 2008). Recent studies have provided additional evidence that generalist predators may display consistent selectivity despite low preferred prey availability or abundant alternative prey (Krey et al. 2017; Ritger et al. 2020; Roubinet et al. 2018; Whitney et al. 2018). Overall, the growing body of empirical studies on generalist predators suggests that prey preferences can influence foraging patterns, perhaps to a greater extent than previously thought.

The manner in which prey preferences shape foraging behaviors has important implications for the extent to which generalist predators exert top-down effects on prey communities and ecosystems. Previous studies that have characterized generalist predators in terms of biocontrol potential, responses to habitat loss, and stabilization of food webs have yielded conflicting results. In some studies, generalist predators have been shown to decrease pest populations, persist in fragmented habitat, or increase food web stability, while others have demonstrated the opposite (Prugh 2005; Ryall & Fahrig 2006; Snyder & Wise 1999; Symondson et al. 2002). As generalist predators thus have unpredictable effects on their food webs, which can also change both spatially and temporally, continuing to develop and test predictions regarding their responses to changing prey communities remains essential for both theoretical and applied ecology.

Among generalist predators, arthropodivorous vertebrates are unique as they usually consume many more prey types than obligate carnivores while maintaining much higher energy requirements than predaceous arthropods. Moreover, exclusion studies have demonstrated that arthropodivorous vertebrates can have a range of both direct and indirect effects on their respective food webs (Mooney et al. 2010). The mechanisms governing these responses are less well understood, partly due to the difficulties in defining the full suite of prey resources that are consumed by these predators. Among terrestrial arthropodivorous vertebrates, most studies relating prey preferences and prey abundance have used morphological methods that characterize predator diet composition by visual inspection of stomach contents or fecal material (Ralph et al. 1985; Whitaker et al. 2009). However, these methods are limited in taxonomic resolution and prey remains are often damaged or degraded, particularly for soft-bodied arthropods.

Newer techniques such as high-throughput amplicon sequencing (HTAS) can characterize animal diets at a much finer taxonomic resolution than other methods, providing a more comprehensive way to study the entire suite of prey resources consumed by generalist predators

(Jusino et al. 2019; Kaartinen et al. 2010; Zeale et al. 2011). Since the advent of these methods, many studies have described the diets of a wide range of taxa (Alberdi et al. 2019; Alberdi et al. 2020; Piñol et al. 2014; Pompanon et al. 2012; Wray et al. 2018). These types of studies collectively represent an important first step in describing animal diets, especially for those that use a broader diversity of prey resources than other methods are capable of detecting. For arthropodivorous vertebrates in particular, studies using molecular methods have generated a wealth of detailed data on dietary composition and dietary breadth, and relating these data with underlying prey abundance can reveal key insights into predator foraging (e.g., Arrizabalaga-Escudero et al. 2019; Vesterinen et al. 2016). As many arthropodivorous vertebrates, particularly birds and bats, are currently threatened due to habitat loss, arthropod declines, and disease, among other factors, (Nebel et al. 2010; O'Shea et al. 2016; Rioux Paquette et al. 2014; Spiller & Dettmers 2019), fully characterizing their resource requirements also represents a timely endeavor.

Bats have been the subject of benchmark studies on the use of molecular methods for characterizing the diets of generalist predators (Clare et al. 2011; Clare et al. 2014a; Clare et al. 2014b; Razgour et al. 2011), largely due to interest in the potential ecosystem services they provide in the form of agricultural pest reduction (Boyles et al. 2011; Kunz et al. 2011). The diets of two common North American bats — the little brown (*Myotis lucifugus,* Leconte 1831) and big brown bat (*Eptesicus fuscus,* Palisot de Beauvois 1796) — have been especially well described using both molecular and morphological methods, and as such, these species serve as a useful model for studying generalist arthropodivore foraging. The preferred prey of little brown and big brown bats, respectively, are frequently reported as small aquatic insects and beetles (Fenton 1980; Kurta & Baker 1990; Agosta 2002; Moosman et al. 2012), though little brown bats have been observed switching to opportunistic foraging in response to changing prey abundances (Anthony & Kunz 1977; Belwood & Fenton 1976; Burles et al. 2008). Several molecular studies on other bat species have related prey consumption with

quantitative prey abundance (Baroja et al. 2019; Krauel et al. 2018a; Krauel et al. 2018b; Weier et al. 2019), but often included select prey groups of interest rather than intensively sampled prey communities (e.g., Vesterinen et al. 2016). For little brown and big brown bats, however, dietary composition data from molecular methods have not yet been connected with underlying prey abundance information.

In this study, we characterized how prey consumption by two generalist arthropod predators, the little brown and big brown bat, changes in response to quantitative spatio-temporal variation in prey resources. We hypothesized that highly mobile generalist predators would display preferences for certain prey and that the probability of consuming most prey groups would not increase as a direct function of increasing abundance. We predicted that for both little brown and big brown bats, the probability of detecting prey in guano samples would not increase as a direct function of increasing quantitative prey availability as measured by arthropod traps. We tested these predictions by comparing HTAS dietary data from guano samples with quantitative arthropod abundance estimated from black-light traps that captured arthropods during the same time periods at locations near bat maternity roosts. From these analyses, we inferred which prey were preferred with respect to their relative abundance. As an exploratory analysis, we also assessed how dietary diversity related to underlying prey diversity and how regional or local arthropod abundance influenced dietary composition. Overall, both bat species appeared to exhibit strong preferences for certain prey groups, and the quantitative availability of most prey groups was unrelated to the probability of detection in bat guano samples.

Methods

Bat guano collection

Study sites were selected at 6 little brown and 4 big brown bat maternity roosts in southern Wisconsin (Supplementary Figure S1). These sites were located at state and county parks and privately-owned land, and were selected using the following criteria: 1) sites included a known maternity roost with bats of a visually confirmed species that consistently returned for several consecutive years; 2) sites were accessible and sampling requests were approved by land owners or managers; 3) habitat composition represented a gradient of agricultural and forest landcover; and 4) bat roosts were included in pre- and post-volancy bat count efforts conducted by volunteers as part of other state-wide monitoring efforts. Based on emergence counts from previous surveys in 2015, big brown bat colonies ranged from approximately 28–287 individuals, while little brown bat colonies ranged from approximately 89–446 individuals. Landscape conditions at these study sites included a range of habitat types, which were assessed using the USDA National Agricultural Statistics Service Cropland Data Layer (https://nassgeodata.gmu.edu/CropScape/). Within a 3km radius, the average landscape composition was 33% agricultural (ranging from 7.9 to 58%), 30% forest (ranging from 3.0 to 63%), 20% grass or pasture (ranging from 4.4 to 42%), and 11% wetland or open water (ranging from 0.15 to 40%). All sites were located near bodies of water (including small ponds, lakes, and streams of varying sizes), which are common throughout the study area.

We chose to use non-invasively collected bat guano samples collected beneath roosts to allow simultaneous sample collection at multiple sites and to avoid disturbing bats during the breeding season. Bat guano was collected weekly, with fresh pellets assumed to represent the weekly prey consumption of a bat colony at each given roost. Bat species were confirmed visually each week, and pellet identity was also confirmed based on size. Clean plastic sheets were placed under each roost for one week, with guano samples collected from late May to late August in 2015 (Julian weeks 24–35)

and mid-May to early September in 2016 (Julian weeks 23–37). Samples were initially stored at –20 °C, and then transferred to –80 °C for long-term storage. All sample collection methods were carried out in accordance with Wisconsin Department of Natural Resources guidelines, and experimental protocols were approved by the Wisconsin Natural History Inventory (NHI) Program and the University of Wisconsin-Madison.

DNA extraction, PCR, and library preparation

A subsample of 80mg (~8 pellets) was selected from each guano sample for genetic analyses. DNA was extracted from each guano subsample using a QIAGEN DNA Stool mini kit (QIAGEN, Hilden, Germany), following the manufacturer's protocols except for the following changes: 10mL ASL lysis buffer was added to 80mg guano, vortexed for 2 minutes, lysed an additional 5–10 minutes, and centrifuged at 10,000 RPM for 5 minutes before taking 1.8mL of the lysate. Additionally, 40µL of proteinase K was used per extraction instead of 10µL. Following DNA extraction, a 180 bp cytochrome oxidase C subunit 1 (COI) amplicon, the DNA barcode region generally used for arthropods, was amplified using the ANML primer pair (FWD: GGTCAACAAATCATAAAGATATTGG; REV: GGWACTAATCAATTTCCAAATCC) according to Jusino et al. (2019). This primer pair was previously validated and was shown to have less taxonomic bias than any other primers currently available for HTAS of arthropodivore diets (Jusino et al. 2019). Primers were modified for HTAS by adding a unique barcode sequence and an Ion Torrent Xpress A adapter sequence on each forward primer, and an Ion Torrent Express trP1 adapter on the reverse primer. To overcome issues with amplification, sample DNA templates were tested at full concentration, then tested at serial dilutions of 1:10, 1:20, and 1:40. For each sequencing library, a single-copy mock community of 34 known arthropod constituents was separately amplified under the same PCR conditions as a positive control (Jusino et al. 2019). Negative controls for each DNA

extraction batch and for each PCR master mix were also tested and visualized on a 2% agarose gel. These negative controls did not demonstrate visible bands.

For library preparation, all PCR products were individually purified using a Zymo Select-A-Size DNA Clean & Concentrator Kit (Zymo Research, Irvine, CA). The concentration of each purified PCR product was quantified using a Qubit dsDNA High Sensitivity Assay with a Qubit Fluorometer (Invitrogen, Carlsbad, CA). Purified PCR products were then combined in equimolar amounts for a sequencing library with a final concentration of 2000pM. Libraries were sequenced with a 400bp Hi-Q Kit on an Ion Torrent Personal Genome Machine next generation sequencing platform (PGM; ThermoFisher, Waltham, MA) with a 318 chip according to manufacturer's recommendations. A total of three libraries were sequenced consisting of approximately 72 unique barcoded samples each. Samples from different sites were processed in a randomized order and samples from both bat species were also extracted, amplified, and sequenced together in order to reduce potential batch effects (Alberdi et al. 2019).

Bioinformatics & data processing

Data from all three sequenced libraries were combined and processed cumulatively using AMPtk v1.4.0 (Palmer et al. 2018). Raw sequence data were de-multiplexed using the unique barcode index sequences, and forward and reverse primers were trimmed from the 180bp amplicon target. Measures for quality control included removal of reads shorter than 170bp or longer than 180bp and removal of samples with fewer than 4,000 reads. The DADA2 clustering algorithm (Callahan et al. 2016) was then used for de-noising and quality filtering with expected error trimming. The resulting amplicon sequence variants (ASVs) were clustered using the UCLUST algorithm employed in VSEARCH at 97% similarity to generate operational taxonomic units (OTUs) approximating species-level taxonomy (Jusino et al. 2019). Demultiplexed sequences were mapped back onto these OTU representative

sequences, and the 34-member single copy arthropod mock community was used to account for barcode switching (also referred to as index bleed). OTUs were then assigned taxonomy using the built-in curated COI database in AMPtk, and all OTUs that were not designated as arthropods or identified beyond Arthropoda were manually removed (n=153 OTUs).

Richness in bat diet was calculated as the total number of unique arthropod groups at different taxonomic levels (OTUs, species, genera, and families). To assess sufficiency of sampling intensity, accumulation curves for total family-level richness with extrapolations were created for both bat species using the R package "iNEXT" (Hsieh et al. 2016). Following taxon assignments and clustering, OTU tables were aggregated at the family, genus, and species levels. For comparison with black-light trap data, OTU tables were also aggregated into the same focal groups (Table 1). Weighted percent of occurrence (wPO, a presence/absence-based metric where read counts are converted to binary responses) and relative read abundance (RRA, a read-based metric that incorporates the total number of DNA sequence counts) were calculated following Deagle et al. 2019.

Arthropod trapping & enumeration

At each of the 10 aforementioned bat roost sites, arthropod communities were sampled weekly to quantify the available prey at each site during the same time interval when guano samples were collected. Black-light traps were used to collect night-flying arthropods that are presumed to form the majority of the prey consumed by arthropodivorous bats that usually forage by aerial hawking, such as the two species in this study. Black-light traps were placed in open areas away from main roads or paths at a distance of 50–100m from each of the bat maternity roost sites. Any vegetation surrounding the black-light trap was cleared weekly to prevent obscuration of the trap. The immediate areas of black-light trap placement included a range of vegetation such as cropland (e.g., alfalfa and corn), grassland (e.g., idle grazing land or restored prairie), or near forest edges. Traps were not placed in

forest interiors to avoid blocking or reducing the visibility of the lights. Black-light traps consisted of a 3.5 gallon polypropylene bucket with a 30cm aluminum funnel and mesh collecting bag (BioQuip Universal Light Trap, catalog number 2851). A 12-watt U-shaped bulb was affixed between three clear acrylic vanes on top of the funnel, and an aluminum lid was secured with bungee cords. An 18.6% diclorvos (2,2-dichlorovinyl dimethyl phosphate) insecticide strip (HotShot No-Pest Strip2, St. Louis, Missouri) was affixed inside the bucket. Pest strips were changed every 4 weeks to ensure equally high potency over time. Black-light traps were powered by a 12V sealed lead-acid battery, which was recharged by an attached 45-watt solar panel. Traps were turned on automatically from 20:00 through 5:00 for a consecutive 3-night period for each sampling week. Samples were collected and traps were reset weekly from mid-May to late August in 2015 and mid-May to early September in 2016.

Arthropod samples were identified by microscope in the following manner: large or noticeably unique specimens were first selected from the overall sample for identification, then the remaining sample was scanned for any specimens that were not homogenous through the entire sample, which were then also selected for separate identification. For samples containing very large numbers of individuals, the homogenous remainder was divided into a subsample for identification, then extrapolated based on the portion taken to obtain an estimate of the whole sample quantity. The selected specimens and subsamples were identified to order; and within orders, all specimens were identified to the 32 most commonly detected groups (representing 95% of all captured arthropods), with remaining rare families identified as "other Order", e.g., "other Coleoptera" (Table 1). Samples that were damaged or degraded were identified to the lowest taxonomic level possible. Enumeration of identified arthropods was conducted by visual counting with the use of a multiple unit tally counter. Arthropod identifications and DNA library preparations were performed in separate laboratories in order to avoid cross contamination.

Statistical analyses

Both read-based and presence-based taxonomy tables were used for describing dietary composition for each bat species. For statistical analyses comparing dietary differences between little brown and big brown bats, OTU tables were converted to presence/absence matrices. Interspecific dietary composition was initially assessed using a two-way ANOVA including bat species (with little brown bats as the reference group), prey order, and the interaction between bat species and prey order as independent variables, and the OTU richness within a family as the dependent variable. To test for overall trends in prey communities, differences in prey group abundances were analyzed using Welch's t-tests (with year-to-year comparisons constrained to Julian weeks 24-35 to account for differences in sampling season length).

We used binary logistic regressions, coded as generalized linear models (GLMs) with a logit link function, to test for potential relationships between the abundance of arthropod taxa and their probability of detection in bat diets, conducting separate analyses for the two bat species. The putative presence/absence of an arthropod taxa group was treated as the dependent variables, while the same arthropod taxa group and its respective abundance in given black-light trap were treated as the independent variables. Samples with arthropod abundance in excess of 10,000 individuals from the same arthropod group were excluded, and the remaining arthropod abundance data were normalized using a log base 10 transformation. A global model was structured such that the arthropod taxa group represented the main effect, while the respective abundance represented within each arthropod taxonomic group represented the interaction effect, i.e.:

$$Y=a+b_1X_1 + b_2X_1X_2 + e$$

Where Y represents the binary presence/absence of an arthropod taxa group in a sample, X_1 is the group, and X_2 is the corresponding abundance of that particular group. Collection site, Julian week, and year were also included in the global model, and were then sequentially removed from the model when terms were not statistically significant as determined by Wald tests. Araneae was chosen as the reference variable for arthropod groups because this group was neither common nor rare in black-light traps or in the diets of both bat species in this study, and the reported positive and negative effects are relative to this group. Little brown and big brown bats are typically thought to forage primarily by aerial hawking, although both display some flexibility in foraging strategies and can glean non-flying prey such as Araneae (Kurta & Baker 1990; Ratcliffe & Johnson 2003). For interaction terms, the reported slope β_{int} represents the slope of the interaction effect only, and OR represents the odds ratio of the main effect combined with the interaction.

In order to test the influence of arthropod abundance on dietary variation, we conducted a constrained ordination using the R package "vegan" (Oksanen et al. 2019). Specifically, we performed a redundancy analysis (RDA) separately for each bat species on presence/absence matrices at the family and OTU levels. RDA scores were extracted, and linear explanatory variables (including week, year, and arthropod abundances at the local and regional scales) were then fit onto the ordination as environmental vectors using the "envfit" function. For these analysis, local abundance represents the abundance of an arthropod group in a black-light sample corresponding to a guano sample from the same site and week, while regional abundance represents the mean abundance of an arthropod group aggregated across sites for each week. All analyses were conducted in R (R Core Team 2020) with additional R packages used for data processing and visualization including "dplyr", "tidyverse", "ggplot2", "reshape2", and "wesanderson" (Ram & Wickham 2018; Wickham 2020; Wickham et al. 2019; Wickham et al. 2020).

Results

A total of 105 little brown bat samples (62.5%, n=168) and 59 big brown (62.8%, n=94) bat samples were successfully amplified. A total of 1,747 arthropod OTUs were detected: 1,199 in little brown bat samples (68.6%) and 735 in big brown bat samples (42.1%). A total of 377 OTUs were detected in both bat species (21.5%). For little brown bats, 923 OTUs were identified to the family level (77.0%), 798 to the genus level (66.6%), and 618 to the species level (51.5%). For big brown bats, 540 OTUs were identified to the family level (73.5%), 496 to the genus level (67.5%), and 418 to the species level (56.9%). Between little brown and big brown bats, there was not a statistically significant difference in the number of OTUs identified at different taxonomic levels (χ^2=6, df=4, p=0.199). For both bat species, Hymenoptera and Araneae had the lowest percentages of OTUs identified beyond order, while Ephemeroptera had the highest percentages of OTUs identified beyond order (Supplementary Table S1). For the insect mock community, all 34 known arthropods were recovered and identified. Our mock community includes 2 mock members that have known sequence variants which are included in the mock (Jusino et al. 2019), and those variants cluster with the originating sequence. Three additional OTUs were also detected, for which two were identified as separate variants of a known mock community member (*Apis mellifera*), and the other was detected at very low reads (n=3 reads) and likely represents a chimeric sequence.

The most commonly detected OTUs and families for each bat species, as measured by incidence, wPO, and RRA, are reported in Tables 2 and 3. Among little brown bats, a significantly higher richness of Araneae, Diptera, Hemiptera, and Lepidoptera families, genera, and species was detected, while significantly fewer Coleopteran families, genera, and species were detected in comparison to big brown bats (Figure 1a). At the OTU level, a significantly higher richness of Araneae, Diptera, Hemiptera, and other Arthropoda and significantly lower Coleoptera richness was detected among little brown bat samples (Figure 1a). There was no statistically significant interspecific

difference in the richness of Ephemeroptera, Hymenoptera, or Trichoptera at any of the taxonomic levels. A total of 181 and 142 arthropod families were detected and identified in little brown and big brown bat samples, respectively. Using an asymptotic estimate of total family richness, 217 total families were predicted to be detected among little brown bat samples (95% CI=198, 259), and 173 families were predicted to have been detected among big brown bat samples (95% CI=156, 210; Figure 1b). For little brown bat samples at the ordinal level, Diptera had the highest mean wPO and RRA (Figure 1c). Lepidoptera had the next highest mean wPO, while Coleoptera had the next highest mean RRA. For big brown bats at the ordinal level, Coleoptera had the highest mean wPO and RRA, while Diptera had the next highest mean wPO and mean RRA (Figure 1c).

Across all black-light samples, Trichoptera had the highest mean abundance ($\bar{x}$=645.4, IQR=103.5 to 743.2), followed by Diptera: Culicidae/Chironomidae ($\bar{x}$=594.5, IQR=16 to 222), and other Coleoptera ($\bar{x}$=243.3, IQR=13.3 to 236.8; Figure 2a). The same groups also had the highest mean percentages of the total sample abundance, with Trichoptera representing 21.6% of the total sample abundance on average (IQR=10.7 to 37.2%), followed by Diptera: Culicidae/Chironomidae ($\bar{x}$=10.3, IQR=2.0 to 10.3%) and other Coleoptera ($\bar{x}$=7.9, IQR=1.7 to 10.0%) When grouped by orders, the next highest mean percentages of total sample abundance (after Trichoptera) were Coleoptera ($\bar{x}$=23.2, IQR=7.8 to 33.6%) and Diptera ($\bar{x}$=17.4, IQR=7.3 to 22.1%). Between years, there were significantly lower raw abundances of total Hemiptera ($t_{96.86}$=2.64, p=0.01), Hymenoptera ($t_{58.91}$=2.09, p=0.04), and Lepidoptera ($t_{52.44}$=4.60, p<0.001) in 2016. Qualitatively, prey communities were seldom dominated by any one particular taxonomic group (Figure 2b), although groups were highly variable overall and changed from week to week (Figure 2c).

For big brown bats, the model including collection site as variable performed significantly better than the null model (p<0.001) and was therefore retained as a predictor variable. For this analysis, 7 arthropod groups had a statistically significant positive main effect on the probability of

detection in diet and 8 arthropod groups had a statistically significant negative main effect on the probability of detection in diet (Figure 3a). None of the groups had a statistically significant interaction with its respective abundance after accounting for the main effect of arthropod group identity (Figure 3b). For little brown bats, the model including Julian week as a variable performed significantly better than the null model (p=0.007) and was therefore retained as a predictor variable. For this analysis, 3 arthropod groups had a statistically significant positive main effect on the probability of detection in diet and 17 arthropod groups has a statistically significant negative main effect on the probability of detection in diet (Figure 3a). Corixidae, other Hemiptera, other Lepidoptera, and Trichoptera had a marginally significant interaction with abundance (p=0.015, β_{int}=1.004, *OR*=0.308; p=0.020, β_{int}=0.785, *OR*=0.609; p=0.042, β_{int}=0.838, *OR*=1.96; p=0.037, β_{int}=0.438, *OR*=1.11; Figure 3b).

For big brown bats at the family level, local Coleoptera and local Lepidoptera abundance were significant vectors in the ordination (R^2=0.197, p=0.012; R^2=0.156, p=0.029), while local Hemiptera, local Hymenoptera, and regional Hemiptera were marginally significant vectors (R^2=0.122, p=0.057; R^2=0.101, p=0.100; R^2=0.124, p=0.085; Figure 4). For big brown bats at the OTU level, week and local Lepidoptera abundance were marginally significant vectors (R^2=0.161, p=0.065; R^2=0.154, p=0.079; Figure 4). For little brown bat diets at the family level, local Hymenoptera, local Trichoptera, regional Hemiptera, and regional Trichoptera abundance were significant vectors (R^2=0.121, p=0.038; R^2=0.112, p=0.029; R^2=0.108, p=0.048; R^2=0.111, p=0.047), while week, local Hemiptera, and local total abundance were marginally significant vectors (R^2=0.094, p=0.057; R^2=0.100, p=0.055; R^2=0.081, p=0.084; Figure 4). For little brown bats at the OTU level, local Coleoptera, local Diptera, local Trichoptera, local total, and regional Trichoptera abundance were significant vectors (R^2=0.323, p=0.001; R^2=0.277, p=0.001; R^2=0.170, p=0.005; R^2=0.306, p=0.001; R^2=0.146, p=0.016), while regional Diptera and regional total abundance were marginally significant vectors (R^2=0.095, p=0.078; R^2=0.104, p=0.061; Figure 4).

Discussion

The results from this study support our hypothesis that generalist predators would display preferences for certain prey, and that the local abundance of a prey group would not strongly influence the probability of its consumption. Although some less commonly consumed groups were slightly more likely to be consumed when they were more abundant, the statistical magnitude of these effects was generally small. Among both bat species, we found that prey abundance influenced community-level dietary composition, suggesting that bats do adjust their foraging patterns in response to changing prey resources, though not necessarily as a direct response to increasing quantitative abundance of a particular prey resource. As the dietary data resulting from HTAS cannot necessarily be extrapolated to represent prey quantities (Brandon-Mong et al. 2015; Clarke et al. 2014; Piñol et al. 2015), our results are not a true estimation of a functional response. Nonetheless, as described below, this study provides insights into how changes in prey abundance affect the probability of prey consumption and the overall dietary composition in two highly generalist predators.

Relating prey detection in bat diets with arthropod abundance

Quantifying prey availability is difficult for generalists that consume hundreds or even thousands of prey items. All arthropod trapping methods carry biases and do not necessarily sample arthropod communities evenly (Kirkeby et al. 2013; Kremen et al. 1993). In this study, we characterized arthropod communities by comparing the raw abundance of groups, the percentage of total sample abundance, and the intra-order proportional abundance of each group. Overall, we found that the night-flying arthropod communities in this study system were highly variable, but seldom dominated by a single arthropod group. Trichoptera were consistently abundant, as were certain groups within Diptera (namely, Culicidae/Chironomidae and other Diptera) and within Coleoptera (namely,

Carabidae, Staphylinidae, and other Coleoptera). Our results suggest that prey communities in this study system are generally not characterized by large resource pulses, at least among the taxa that are well represented by the arthropod trapping method used in this study. Despite the many challenges in relating prey abundance with generalist dietary composition, by sampling arthropod abundance near bat roosts and comparing the relative abundance of each group with the probability of its detection in guano samples, our study represents one of the most intensive efforts to associate quantitative prey information with a non-invasive HTAS-based diet study.

Perfectly sampling the entire suite of prey available to a colony of bats is impossible given large home and foraging range sizes, the diversity of available prey, and the range of different habitats those prey occupy. In this study, bats were observed flying near black-light trap locations during roost emergence counts, and as part of a separate study, passive acoustic monitoring indicated that bat foraging activity was high near black-light trap sampling locations (Chapter 3). Moreover, lactating female little brown bats have been shown to usually forage within 600m of the roost (Henry et al. 2002). Thus, we conclude that there is a reasonable a priori expectation that bat diets could track spatio-temporal variation in arthropods present at arthropod sampling locations. Nevertheless, we acknowledge that our sampling design may not fully reflect arthropod communities for bats with large foraging ranges and we suggest that future studies could also incorporate tracking efforts (perhaps in non-breeding bats or in populations that are not currently threatened) or could conduct sampling in multiple habitat types at various distances from bat roosts. We also acknowledge that black-light trap samples cannot capture the total spectrum of prey available for a highly mobile predator. The data resulting from arthropod communities as captured by black-light traps and prey communities present in diets as detected by HTAS are difficult to compare, and subsequent research efforts may consider incorporating additional HTAS analyses for prey communities. However, such studies would still require some measure of quantitative prey abundance measurement through trapping or survey

efforts, since HTAS data are semi-quantitative (Deagle et al. 2019; Jusino et al. 2019; Palmer et al. 2018). Using HTAS for both prey and diet communities would also necessitate additional measures (such as processing in separate laboratories) to avoid issues with contamination and may require further evaluation of potential amplification biases between prey community samples and fecal samples due to differences in template quality.

Classical measures of preference dictate that determining which prey are preferred requires information on both prey consumption and prey abundance, availability, or density (Chesson 1978, 1983; Rapport & Turner 1970). In this study we found that after incorporating prey abundance, the interaction between prey group identity and prey abundance was not statistically significant for most prey groups, though the magnitude of the statistical effect size of prey group identity was influenced by abundance. For example, the effect size of the highest ranked categories for both bat species based on diet information alone decreased slightly after accounting for their respective abundance (Supplementary Figure S2). Among both bat species, although several different prey groups had the largest effect sizes based on diet alone, other Diptera had the largest effect size when including abundance. These results, however, could be an artefact of either the grouping of Diptera taxa or the low abundance of Limoniidae in black-light trap samples. Alternatively, among little brown bats, both Chironomidae (in the model with diet only) and the group combining Chironomidae and Culicidae (in the model with diet and abundance) maintained large effect sizes, although the Chironomidae/Culicidae group was among the most abundant arthropod groups present in black-light trap samples. Overall, the results from this study demonstrate that while prey identity generally appears to outweigh abundance in determining the probability of detection in bat diets, incorporating some measures of background prey abundance remains important for accurately estimating the preferences of a predator.

Despite potential limitations in estimating prey availability, our results provide strong evidence that changes in local prey abundance have little effect on the probability of prey detection in bat diets. As a notable exception, other Lepidoptera (a group representing Lepidoptera not belonging to the focal groups of the study or not identified beyond the ordinal level) were more likely to be detected in little brown bat guano samples as a function of increasing abundance. Previous morphological studies have demonstrated that little brown bats can switch between opportunistic and selective foraging depending on seasonality and reproductive stage (Anthony & Kunz 1977; Belwood & Fenton 1976; Burles et al. 2008). Indeed, the focal species of this study are highly mobile predators in a complex system with many alternative prey resources, and thus their responses to changing resource availability is difficult to predict without corresponding foraging movement information such as radio-tracking data (e.g., Almenar et al. 2013). Bats have also been documented employing opportunistic foraging around objects, such as lights and even animals, that attract arthropods (Palmer et al. 2019; Rowse et al. 2016), suggesting the possibility that black-light traps may have an effect on bat foraging or on prey community sampling. It is also important to note that certain taxa (such as Ephemeroptera and Diptera: Limoniidae) were frequently detected in bat diets but seldom captured in black-light traps, likely because they are not particularly attracted to the type of trap used in this study.

While prey abundance was generally unrelated to the probability of consumption, we found that both local and regional abundance had influences on the community-level dietary composition of both bat species. These results varied slightly depending on the taxonomic levels that were assessed. For big brown bats, OTU-level dietary composition appeared to be less influenced by temporal factors or arthropod group abundance, while family-level dietary composition appeared to be more strongly influenced by the local or regional abundance of several groups. In contrast, little brown bat dietary composition was influenced by combinations of local and regional arthropod abundances, many of which were consistent at both taxonomic levels. The effect of Julian week, in contrast, appeared to

have an effect only on the family-level community composition. These results suggest that although big brown bats may tend to consume prey that are intrinsically less variable in quantitative abundance, temporal changes may be more influential than local-scale spatial changes in prey availability.

Our findings demonstrate that little brown and big brown bats can adjust their foraging strategies in response to changes in prey communities, but that the probability of detecting prey in their diets does not increase directly as a function of quantitative prey availability and likely involves complex behaviors related to prey preferences. Despite interspecific differences in total dietary composition, both bat species displayed strong preferences for particular prey. These patterns are consistent with previous studies suggesting that these bat species are usually not limited by prey availability and do not compete directly with each other, likely due to their physiological differences and high dispersal abilities (Kunz 1973; Barclay & Brigham 1991; Moosman et al. 2012). We also found that arthropod predators such as spiders, predatory beetles, and lacewings, were somewhat common in the diets of both bat species. In combination with their apparent selectivity, foraging at a high trophic level suggests that these bat species could have both consumptive and nonconsumptive effects on arthropod communities, which may consequently alter prey behavior or otherwise complicate the relationship between prey availability and prey consumption. The patterns observed in this study may also be influenced by some degree of individual-level specialization (Bolnick et al. 2002), as both little brown and big brown bats tend to have large maternity colonies (Fenton 1980; Kurta & Baker 1990), and the sampling design of this study represents colony-level diet composition. Overall, our results provide additional evidence that selective predation among generalists may be more common than previously thought, particularly among predators that are highly mobile and that forage in species-rich systems.

Implications for HTAS studies on predators of arthropods

The diets of both bat species contained many taxonomic groups, but Diptera and Coleoptera had the highest OTU, species, genus, and family richness among little brown and big brown bat guano samples, respectively. A higher taxonomic richness of prey items was detected in little brown bat diets in comparison with big brown bat diets, with accumulation curves indicating that sample sizes in this study were sufficient for drawing comparisons between bat species. These results are generally consistent with previous studies that used both molecular and morphological methods (e.g., Agosta 2002; Anthony & Kunz 1977; Belwood & Fenton 1976; Burles et al. 2008; Clare et al. 2014a; Clare et al. 2014b). Notably for both bat species, the percentage of OTUs identified to the species, genus, and family levels were highly variable within different arthropod orders. For example, while Diptera: Chironomidae had the highest richness of OTUs, this family is highly speciose and well represented in reference databases. Despite ever-increasing database building efforts, arthropods still tend to have fewer reference sequences identified beyond the ordinal level, and often retain incomplete or unresolved taxonomy (Hebert et al. 2016; Stork 2018). Thus, using HTAS for dietary studies in a highly generalist predator that consumes prey from underrepresented taxonomic groups represents a unique challenge from several perspectives.

While the taxonomic richness of prey items can serve as a proxy of underlying functional or genetic diversity, read-based and presence-based metrics (e.g., RRA and wPO, respectively) are also frequently used for characterizing dietary composition. In this study, weighted presence-based and read-based measures were generally consistent, with a few notable exceptions. For example, Lepidoptera tended to have a mean RRA that was much lower than the mean wPO for both bat species. Similarly, the mean RRA for Diptera tended to be lower than the mean wPO for big brown bats. These differences may be attributed to biases inherent to occurrence-based metrics, which can potentially overestimate the importance of food items consumed in low quantities and can be highly

sensitive to contamination issues (Deagle et al. 2019; Lamb et al. 2019). In contrast, we found that among big brown bat guano samples an OTU assigned to *Potamyia flava* (Trichoptera: Hydropsychidae) had a mean RRA that was more than three times higher than the OTU with the next highest mean RRA. The same OTU was also detected among little brown bat guano samples, but did not have an unusually high mean RRA, and other members of the mock community in the order Trichoptera did not have unusually high reads (Supplementary Figure S3). Additionally, an evaluation of the primer set used in this study showed that other frequently used primers (ZBJ, COI L/H) did not detect *P. flava* (Jusino et al. 2019). The high mean RRA of this prey item among big brown bat guano samples could also be driven by instances where few total prey items were detected. However, other studies have noted that Trichoptera, which often emerge en masse, may be particularly desirable to bats (Whitaker 2004). In the context of the mock community and ecological background information, the read-based metrics associated with *Potamyia flava* in big brown bat diets could potentially reflect some degree of biomass within guano samples. Although read-based metrics can be highly sensitive to recovery and PCR biases, and as such, their value remains only semi-quantitative (Deagle et al. 2019; Jusino et al. 2019; Palmer et al. 2018), these results nonetheless demonstrate the utility of mock communities for comparing and contextualizing both read-based and presence-based metrics.

When comparing our results with previous morphological and molecular studies, the importance of defining taxonomic levels was readily apparent. For example, we found that for both bat species, the OTU with the highest raw incidence did not belong to the family with the highest raw incidence. Similarly, for big brown bats the OTU with the highest mean wPO and mean RRA at the OTU level corresponded with the highest family-level mean RRA, but not with the family-level mean wPO. In contrast, for little brown bats, the OTU with the highest mean RRA did not correspond with the highest family-level mean RRA or wPO. These results suggest that, in addition to differences between richness-based, read-based, and presence-based metrics, considering the taxonomic level of

prey detected in dietary samples can also influence the interpretation of HTAS data. Strategies such as aggregating prey categories at higher taxonomic levels or assigning trait-based functional analyses may provide better approximations of prey resource states (e.g. Arrizabalaga-Escudero et al. 2019), as the high resolution of most OTU-based prey categories likely do not correspond with how prey are actually distinguished by predators.

Comparing dietary composition with prey availability in many highly generalist species, including arthropodivorous vertebrates, remains challenging, particularly when connecting the different data types resulting from both molecular methods and capture-based studies. However, as this study demonstrates, the most frequently consumed prey and the preferred prey are not necessarily the same, and some measure of underlying prey availability must be quantified in order to accurately determine when predation is selective. The need for improved practices among DNA barcoding for dietary studies has been highlighted by several recent papers (e.g., Zinger et al. 2019, Jusino et al. 2019, Alberdi et al. 2019). However, comparatively fewer studies have provided guidelines for the interpretation of data in terms of understanding ecological processes. While we encourage the use of robust positive controls — such as mock communities — as a solution for parameterizing the biases inherent to molecular methods, we also emphasize the serious need for considering how the resulting data can be interpreted in order to fit within an ecological framework.

Acknowledgements

The authors thank Brenna Decker, Erin Green, Emma Pelton, Michael Stocking for designing and building the solar powered black-light traps. We thank Mike & Jane Balch, Rhonda Enge, Marci & Jim Hess, Elaine Horn, Gary Jansen, Phillip Kerl, and Elaine Swanson for their contributions to sample collection for this project. We thank John Arthur, Kathleen Gruentzel, Heather Kaarakka, Darren Marsh, and J. Paul White for assistance coordinating sampling at state and county parks. We

also thank three anonymous reviewers for their helpful comments and assistance with improving early versions of this manuscript. The authors dedicate this paper to Ronald Leslie Kerl, a University of Wisconsin alumnus and community scientist who participated in this project and devoted his life to science education.

References

Agosta, S. J. (2002). Habitat use, diet and roost selection by the big brown bat (Eptesicus fuscus) in North America: a case for conserving an abundant species. *Mammal Review*, *32*(3), 179-198.

Alberdi, A., Aizpurua, O., Bohmann, K., Gopalakrishnan, S., Lynggaard, C., Nielsen, M., & Gilbert, M. T. P. (2019). Promises and pitfalls of using high-throughput sequencing for diet analysis. *Molecular Ecology Resources*, *19*(2), 327-348.

Alberdi, A., Razgour, O., Aizpurua, O., Novella-Fernandez, R., Aihartza, J., Budinski, I., ... & Russo, D. (2020). DNA metabarcoding and spatial modelling link diet diversification with distribution homogeneity in European bats. *Nature Communications*, *11*(1), 1-8.

Almenar, D., Aihartza, J., Goiti, U., Salsamendi, E., & Garin, I. (2013). Hierarchical patch choice by an insectivorous bat through prey availability components. *Behavioral Ecology and Sociobiology*, *67*(2), 311-320.

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.

Arrizabalaga-Escudero, A., Merckx, T., García-Baquero, G., Wahlberg, N., Aizpurua, O., Garin, I., ... & Aihartza, J. (2019). Trait-based functional dietary analysis provides a better insight into the foraging ecology of bats. *Journal of Animal Ecology*, *88*(10), 1587-1600.

Barclay, R. M., & Brigham, R. M. (1991). Prey detection, dietary niche breadth, and body size in bats: why are aerial insectivorous bats so small?. *The American Naturalist*, *137*(5), 693-703.

Baroja, U., Garin, I., Aihartza, J., Arrizabalaga-Escudero, A., Vallejo, N., Aldasoro, M., & Goiti, U. (2019). Pest consumption in a vineyard system by the lesser horseshoe bat (*Rhinolophus hipposideros*). *PloS one*, *14*(7), e0219265.

Baudrot, V., Perasso, A., Fritsch, C., Giraudoux, P., & Raoul, F. (2016). The adaptation of generalist predators' diet in a multi-prey context: Insights from new functional responses. *Ecology*, *97*(7), 1832-1841.

Belwood, J. J., & Fenton, M. B. (1976). Variation in the diet of Myotis lucifugus (Chiroptera: Vespertilionidae). *Canadian Journal of Zoology*, *54*(10), 1674-1678.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist*, *161*(1), 1-28.

Boyles, J. G., Cryan, P. M., McCracken, G. F., & Kunz, T. H. (2011). Economic importance of bats in agriculture. *Science*, *332*(6025), 41-42.

Brandon-Mong, G. J., Gan, H. M., Sing, K. W., Lee, P. S., Lim, P. E., & Wilson, J. J. (2015). DNA metabarcoding of insects and allies: an evaluation of primers and pipelines. *Bulletin of Entomological Research*, *105*(6), 717-727.

Burles, D. W., Brigham, R. M., Ring, R. A., & Reimchen, T. E. (2008). Diet of two insectivorous bats, *Myotis lucifugus* and *Myotis keenii*, in relation to arthropod abundance in a temperate Pacific Northwest rainforest environment. *Canadian Journal of Zoology*, *86*(12), 1367-1375.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581-583.

Charney, N., & Record, S. (2015) Jost diversity measures for community data. *Package 'vegetarian.*' R package version 1.2.

Chesson, J. (1978). Measuring preference in selective predation. *Ecology*, *59*(2), 211-215.

Chesson J (1983) The estimation and analysis of preference and its relatioship to foraging models. *Ecology, 64*(5), 1297-1304.

Chesson, P. L. (1984). Variable predators and switching behavior. Theoretical Population Biology, 26(1), 1-26.

Clare, E. L., Barber, B. R., Sweeney, B. W., Hebert, P. D. N., & Fenton, M. B. (2011). Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Molecular Ecology*, *20*(8), 1772-1780.

Clare, E. L., Symondson, W. O., Broders, H., Fabianek, F., Fraser, E. E., MacKenzie, A., ... & Menzies, A. K. (2014a). The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. *Molecular Ecology*, *23*(15), 3618-3632.

Clare, E. L., Symondson, W. O., & Fenton, M. B. (2014b). An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). *Molecular Ecology*, *23*(15), 3633-3647.

Clarke, L. J., Soubrier, J., Weyrich, L. S., & Cooper, A. (2014). Environmental metabarcodes for insects: in silico PCR reveals potential for taxonomic bias. *Molecular Ecology Resources*, *14*(6), 1160-1170.

Dale, B. W., Adams, L. G., & Bowyer, R. T. (1994). Functional response of wolves preying on barren-ground caribou in a multiple-prey ecosystem. *Journal of Animal Ecology*, 644-652.

Deagle, B. E., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., ... & Eveson, J. P. (2019). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data?. *Molecular Ecology*, *28*(2), 391-406.

Fenton, M. B. (1980). *Myotis lucifugus*. *Mammalian Species*, 142, 1-8.

Hebert, P. D., Ratnasingham, S., Zakharov, E. V., Telfer, A. C., Levesque-Beaudin, V., Milton, M. A., ... & DeWaard, J. R. (2016). Counting animal species with DNA barcodes: Canadian insects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1702), 20150333.

Henry, M., Thomas, D. W., Vaudry, R., & Carrier, M. (2002). Foraging distances and home range of pregnant and lactating little brown bats (*Myotis lucifugus*). *Journal of Mammalogy*, *83*(3), 767-774.

Holling, C.S. (1959a) The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. *The Canadian Entomologist*, *91*(5), 293-320.

Holling, C.S. (1959b) Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist, 91*(7), 385-398.

Hsieh, T., Ma, K., Chao, A. (2016) iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution, 7*(12), 1451-1456.

Jusino, M. A., Banik, M. T., Palmer, J. M., Wray, A. K., Xiao, L., Pelton, E., ... & Lindner, D. L. (2019). An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. *Molecular Ecology Resources*, *19*(1), 176-190.

Kaartinen, R., Stone, G. N., Hearn, J., Lohse, K., & Roslin, T. (2010). Revealing secret liaisons: DNA barcoding changes our understanding of food webs. *Ecological Entomology*, *35*(5), 623-638.

Kirkeby, C., Græsbøll, K., Stockmarr, A., Christiansen, L. E., & Bødker, R. (2013). The range of attraction for light traps catching *Culicoides* biting midges (Diptera: Ceratopogonidae). *Parasites & Vectors*, *6*(1), 67.

Krauel, J. J., Ratcliffe, J. M., Westbrook, J. K., & McCracken, G. F. (2018a). Brazilian free-tailed bats (Tadarida brasiliensis) adjust foraging behaviour in response to migratory moths. *Canadian Journal of Zoology*, *96*(6), 513-520.

Krauel, J. J., Brown, V. A., Westbrook, J. K., & McCracken, G. F. (2018b). Predator–prey interaction reveals local effects of high-altitude insect migration. *Oecologia*, *186*(1), 49-58.

Kremen, C., Colwell, R. K., Erwin, T. L., Murphy, D. D., Noss, R. A., & Sanjayan, M. A. (1993). Terrestrial arthropod assemblages: their use in conservation planning. *Conservation Biology*, 796-808.

Krey, K. L., Blubaugh, C. K., Chapman, E. G., Lynch, C. A., Snyder, G. B., Jensen, A. S., ... & Snyder, W. E. (2017). Generalist predators consume spider mites despite the presence of alternative prey. *Biological Control*, *115*, 157-164.

Kunz, T. H. (1973). Resource utilization: temporal and spatial components of bat activity in central Iowa. *Journal of Mammalogy*, *54*(1), 14-32.

Kunz, T.H., Braun de Torrez, E., Bauer, D., Lobova, T., Fleming, T.H. (2011) Ecosystem services provided by bats. *Annals of the New York Academy of Sciences, 1223*(1), 1-38.

Kurta, A., & Baker, R. H. (1990). *Eptesicus fuscus*. *Mammalian Species*, (356), 1-10.

Lamb, P. D., Hunter, E., Pinnegar, J. K., Creer, S., Davies, R. G., & Taylor, M. I. (2019). How quantitative is metabarcoding: A meta-analytical approach. *Molecular Ecology*, *28*(2), 420-430.

Manly, B. F. J., Miller, P., & Cook, L. M. (1972). Analysis of a selective predation experiment. *The American Naturalist*, *106*(952), 719-736.

Mooney, K. A., Gruner, D. S., Barber, N. A., Van Bael, S. A., Philpott, S. M., & Greenberg, R. (2010). Interactions among predators and the cascading effects of vertebrate insectivores on arthropod communities and plants. *Proceedings of the National Academy of Sciences*, *107*(16), 7335-7340.

Moosman, 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.

Murdoch, W. W., & Oaten, A. (1975). Predation and population stability. In *Advances in Ecological Research* (Vol. 9, pp. 1-131). Academic Press.

Nebel, S., Mills, A., McCracken, J., & Taylor, P. (2010). Declines of aerial insectivores in North America follow a geographic gradient. *Avian Conservation and Ecology*, *5*(2).

Novak, M., Wolf, C., Coblentz, K. E., & Shepard, I. D. (2017). Quantifying predator dependence in the functional response of generalist predators. *Ecology Letters*, *20*(6), 761-769.

O'Shea, T. J., Cryan, P. M., Hayman, D. T., Plowright, R. K., & Streicker, D. G. (2016). Multiple mortality events in bats: a global review. *Mammal Review*, *46*(3), 175-190.

Oksanen, Jari, F. Guillaume Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. (2019). *Vegan: Community Ecology Package*. R package version 2.5-6.

Oksanen, T., Oksanen, L., Schneider, M., & Aunapuu, M. (2001). Regulation, cycles and stability in northern carnivore-herbivore systems: back to first principles. *Oikos*, *94*(1), 101-117.

Palmer, J. M., Jusino, M. A., Banik, M. T., & Lindner, D. L. (2018). Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. *PeerJ*, *6*, e4925.

Palmer, M. S., Krueger, J., & Isbell, F. (2019). Bats join the ranks of oxpeckers and cleaner fish as partners in a pest-reducing mutualism. *Ethology*, *125*(3), 170-175.

Panzacchi, M., Linnell, J. D. C., Odden, J., Odden, M., & Andersen, R. (2008). When a generalist becomes a specialist: patterns of red fox predation on roe deer fawns under contrasting conditions. *Canadian Journal of Zoology*, *86*(2), 116-126.

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*(4), 819-830.

Pinol, J., San Andrés, V., Clare, E. L., Mir, G., & Symondson, W. O. C. (2014). A pragmatic approach to the analysis of diets of generalist predators: The use of next-generation sequencing with no blocking probes. *Molecular Ecology Resources*, *14*(1), 18-26.

Pompanon, F., Deagle, B. E., Symondson, W. O., Brown, D. S., Jarman, S. N., & Taberlet, P. (2012). Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology*, *21*(8), 1931-1950.

Preston, D. L., Henderson, J. S., Falke, L. P., Segui, L. M., Layden, T. J., & Novak, M. (2018). What drives interaction strengths in complex food webs? A test with feeding rates of a generalist stream predator. *Ecology*, *99*(7), 1591-1601.

Pringle, R. M. (2020) Untangling Food Webs. In *Unsolved Problems in Ecology* (pp. 225-238). Princeton University Press.

Prugh, L. R. (2005). Coyote prey selection and community stability during a decline in food supply. *Oikos, 110*(2), 253-264.

Pulliam, H. R. (1974). On the theory of optimal diets. *The American Naturalist, 108*(958*),* 59-74.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ralph, C. P., Nagata, S. E., & Ralph, C. J. (1985). Analysis of droppings to describe diets of small birds. *Journal of Field Ornithology*, 165-174.

Ram, K., & Wickham, H. (2018). *wesanderson: A Wes Anderson palette generator*. R package version 0.3.6.

Rapport, D. J., & Turner, J. E. (1970). Determination of predator food preferences. *Journal of Theoretical Biology*, *26*(3), 365-372.

Ratcliffe, J. M., & Dawson, J. W. (2003). Behavioural flexibility: the little brown bat, *Myotis lucifugus*, and the northern long-eared bat, *M. septentrionalis*, both glean and hawk prey. *Animal Behaviour*, *66*(5), 847-856.

Razgour, O., Clare, E. L., Zeale, M. R., Hanmer, J., Schnell, I. B., Rasmussen, M., ... & Jones, G. (2011). High-throughput sequencing offers insight into mechanisms of resource partitioning in cryptic bat species. *Ecology and Evolution*, *1*(4), 556-570.

Rioux Paquette, S., Pelletier, F., Garant, D., & Bélisle, M. (2014). Severe recent decrease of adult body mass in a declining insectivorous bird population. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1786), 20140649.

Ritger, A. L., Fountain, C. T., Bourne, K., Martín-Fernández, J. A., & Pierotti, M. E. (2020). Diet choice in a generalist predator, the invasive lionfish (*Pterois volitans/miles*). *Journal of Experimental Marine Biology and Ecology*, *524*, 151311.

Roubinet, E., Jonsson, T., Malsher, G., Staudacher, K., Traugott, M., Ekbom, B., & Jonsson, M. (2018). High redundancy as well as complementary prey choice characterize generalist predator food webs in agroecosystems. *Scientific Reports*, *8*(1), 1-10.

Rowse, E. G., Lewanzik, D., Stone, E. L., Harris, S., Jones, G. (2016). Dark matters: the effects of artificial lighting on bats. In *Bats in the Anthropocene: Conservation of Bats in a Changing World* (pp. 187-213). Springer.

Ryall, K. L., & Fahrig, L. (2006). Response of predators to loss and fragmentation of prey habitat: a review of theory. *Ecology*, *87*(5), 1086-1093.

Sacks, B. N., & Neale, J. C. (2002). Foraging strategy of a generalist predator toward a special prey: coyote predation on sheep. *Ecological Applications*, *12*(1), 299-306.

Schoener, T. W. (1971). Theory of feeding strategies. *Annual Review of Ecology and Systematics, 2*(1), 369-404.

Sih, A., & Christensen, B. (2001). Optimal diet theory: when does it work, and when and why does it fail?. *Animal Behaviour*, *61*(2), 379-390.

Snyder, W. E., & Wise, D. H. (1999). Predator interference and the establishment of generalist predator populations for biocontrol. *Biological Control*, *15*(3), 283-292.

Spiller, K. J., & Dettmers, R. (2019). Evidence for multiple drivers of aerial insectivore declines in North America. *The Condor*, *121*(2), duz010.

Stork, N. E. (2018). How many species of insects and other terrestrial arthropods are there on Earth?. *Annual Review of Entomology*, *63*, 31-45.

Symondson, W. O. C., Sunderland, K. D., & Greenstone, M. H. (2002). Can generalist predators be effective biocontrol agents?. *Annual Review of Entomology*, *47*(1), 561-594.

Thompson, R. M., Brose, U., Dunne, J. A., Hall Jr, R. O., Hladyz, S., Kitching, R. L., ... & Tylianakis, J. M. (2012). Food webs: reconciling the structure and function of biodiversity. *Trends in Ecology & Evolution*, *27*(12), 689-697.

Vesterinen, E. J., Ruokolainen, L., Wahlberg, N., Peña, C., Roslin, T., Laine, V. N., ... & Lilley, T. M. (2016). What you need is what you eat? Prey selection by the bat *Myotis daubentonii*. *Molecular Ecology*, *25*(7), 1581-1594.

Weier, S. M., Moodley, Y., Fraser, M. F., Linden, V. M., Grass, I., Tscharntke, T., & Taylor, P. J. (2019). Insect pest consumption by bats in macadamia orchards established by molecular diet analyses. *Global Ecology and Conservation*, *18*, e00626.

Whitaker, J. O. (2004). Prey selection in a temperate zone insectivorous bat community. *Journal of Mammalogy*, *85*(3), 460-469.

Whitaker, J.O., McCracken, G.F., Siemers, B.M. (2009). Food habits analysis of insectivorous bats. In *Ecological and Behavioral Methods for the Study of Bats* (pp. 567-592). The Johns Hopkins University Press.

Whitney, T. D., Sitvarin, M. I., Roualdes, E. A., Bonner, S. J., & Harwood, J. D. (2018). Selectivity underlies the dissociation between seasonal prey availability and prey consumption in a generalist predator. *Molecular Ecology*, *27*(7), 1739-1748.

Wickham, H. (2020). *reshape2: Flexibly reshape data: a reboot of the reshape package*. R package version 1.4.4.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D. A., François, R., ... & Kuhn, M. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686.

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., & Woo, K. (2020). *Package 'ggplot2'. Create Elegant Data Visualisations Using the Grammar of Graphics*. R package version 3.3.2.

Wickham, H., Francois, R., Henry, L., & Müller, K. (2020). *dplyr: A Grammar of Data Manipulation*. R package version 1.0.0.

Woo, K. J., Elliott, K. H., Davidson, M., Gaston, A. J., & Davoren, G. K. (2008). Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. *Journal of Animal Ecology*, *77*(6), 1082-1091.

Wray, A. K., Jusino, M. A., Banik, M. T., Palmer, J. M., Kaarakka, H., White, J. P., ... & Peery, M. Z. (2018). Incidence and taxonomic richness of mosquitoes in the diets of little brown and big brown bats. *Journal of Mammalogy*, *99*(3), 668-674.

Zeale, M. R., Butlin, R. K., Barker, G. L., Lees, D. C., & Jones, G. (2011). Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources*, *11*(2), 236-244.

Zinger, L., Bonin, A., Alsos, I. G., Bálint, M., Bik, H., Boyer, F., ... & De Barba, M. (2019). DNA metabarcoding—Need for robust experimental designs to draw sound ecological conclusions. *Molecular Ecology*, *28*(8), 1857-1862.

Table 1: Focal families for black-light and guano samples.

order	subgroup	common name
Araneae	none	spiders
Coleoptera	Carabidae	ground beetles
Coleoptera	Coccinellidae: Harmonia	lady beetles
Coleoptera	Elateridae	click beetles
Coleoptera	Hydrophilidae	water scavenger beetles
Coleoptera	Scarabaeidae	scarab beetles
Coleoptera	Scarabaeidae: Phyllophaga	June beetles
Coleoptera	Silphidae	carrion beetles
Coleoptera	Staphylinidae	rove beetles
Diptera	Culicidae & Chironomidae	mosquitoes & midges
Diptera	Muscidae	house flies
Diptera	Sarcophagidae	flesh flies
Diptera	Syrphidae	hover flies
Diptera	Tachinidae	tachinid flies
Hemiptera	Cicadellidae	leafhoppers
Hemiptera	Corixidae	water boatmen
Hemiptera	Miridae	plant bugs
Hymenoptera	Braconidae	braconid wasps
Hymenoptera	Formicidae	ants
Hymenoptera	Ichneumonidae	ichneumonid wasps
Lepidoptera	Arctiidae	tiger moths
Lepidoptera	Geometridae	geometr moths
Lepidoptera	Lasiocampidae	lappet moths
Lepidoptera	micromoth	micromoths
Lepidoptera	Noctuidae	owlet moths
Lepidoptera	Sphingidae	sphinx moths
Neuroptera	Chrysopidae & Hemerobiidae	lacewings
Opiliones	none	harvestmen
Orthoptera	none	grasshoppers, crickets, katydids
Parasitiformes	none	ticks & mites
Plecoptera	none	stoneflies
Trichoptera	none	caddisflies

Table 2a. Top arthropod OTUs detected in guano samples, MYLU

OTU ID	order	family	genus	species	incidence	mean wPO	mean RRA
OTU12	Ephemeroptera	Caenidae	*Caenis*	*C. amica*	42	0.014	0.059
OTU13	Diptera	Chironomidae	*Procladius*		30	0.010	0.035
OTU20	Hemiptera	Corixidae	*Trichocorixa*	*T. borealis*	27	0.009	0.021
OTU41	Diptera	Psychodidae	*Psychoda*	*P. alternata*	25	0.008	0.006
OTU7	Diptera	Chironomidae	*Coelotanypus*		22	0.008	0.027
OTU143	Diptera	Chironomidae			20	0.007	0.006
OTU15	Lepidoptera	Depressariidae	*Agonopterix*	*A. robiniella*	20	0.008	0.050
OTU158	Lepidoptera	Tortricidae	*Acleris*	*A. semipurpurana*	19	0.005	0.002
OTU2	Diptera	Chironomidae	*Tanypus*		19	0.011	0.046
OTU145	Ephemeroptera	Caenidae	*Caenis*	*C. amica*	18	0.006	0.005
OTU55	Diptera	Chironomidae	*Cryptochironomus*		18	0.005	0.006
OTU22	Diptera	Chironomidae	*Chironomus*		17	0.006	0.015
OTU30	Hymenoptera				17	0.007	0.019
OTU1460	Hymenoptera	Apidae	*Apis*	*A. mellifera*	16	0.005	0.001
OTU503	Lepidoptera	Tortricidae	*Proteoteras*	*P. crescentana*	16	0.003	0.000
OTU120	Hymenoptera				15	0.004	0.004
OTU150	Diptera	Limoniidae	*Geranomyia*		15	0.004	0.002
OTU385	Diptera	Chironomidae	*Glyptotendipes*	*G. meridionalis*	15	0.004	0.000
OTU476	Diptera				15	0.004	0.000
OTU654	Hymenoptera	Apidae	*Apis*	*A. mellifera*	15	0.005	0.000

Table 2b. Top arthropod OTUs detected in guano samples, EPFU

OTU ID	order	family	genus	species	incidence	mean wPO	mean RRA
OTU9	Coleoptera	Elateridae	*Melanotus*	*M. similis*	25	0.017	0.064
OTU1	Trichoptera	Hydropsychidae	*Potamyia*	*P. flava*	22	0.024	0.194
OTU21	Coleoptera	Elateridae	*Hemicrepidius*	*H. memnonius*	19	0.011	0.025
OTU193	Coleoptera	Carabidae	*Agonum*	*A. placidum*	16	0.008	0.002
OTU1314	Coleoptera	Elateridae	*Hemicrepidius*	*H. memnonius*	14	0.007	0.001
OTU123	Coleoptera	Carabidae	*Notiobia*	*N. terminata*	13	0.006	0.002
OTU148	Coleoptera	Carabidae	*Harpalus*	*H. pensylvanicus*	13	0.006	0.005
OTU282	Coleoptera	Carabidae	*Harpalus*		13	0.007	0.001
OTU204	Coleoptera				12	0.006	0.001
OTU3	Diptera	Limoniidae			12	0.008	0.051
OTU429	Hymenoptera	Ichneumonidae	*Enicospilus*		12	0.006	0.000
OTU629	Coleoptera	Cantharidae	*Rhagonycha*	*R. lignosa*	12	0.006	0.000
OTU1445	Diptera				11	0.005	0.000
OTU255	Diptera	Tipulidae	*Nephrotoma*	*N. ferruginea*	11	0.009	0.001
OTU34	Diptera	Sciaridae			11	0.007	0.007
OTU38	Megaloptera	Corydalidae	*Chauliodes*	*C. pectinicornis*	11	0.005	0.046
OTU993	Coleoptera	Elateridae	*Hemicrepidius*	*H. memnonius*	11	0.005	0.000
OTU30	Hymenoptera				10	0.006	0.011
OTU20	Hemiptera	Corixidae	*Trichocorixa*	*T. borealis*	9	0.007	0.016
OTU23	Coleoptera	Pyrochroidae	*Dendroides*	*D. canadensis*	9	0.007	0.015

Table 3. Ranking of top families detected in guano by different measures

MYLU

order	family	richness	order	family	incidence	order	family	mean wPO	order	family	mean RRA
Diptera	Chironomidae	156	Diptera	Chironomidae	96	Diptera	Chironomidae	0.065	Diptera	Chironomidae	0.218
Diptera		86	Diptera		71	Hymenoptera		0.045	Hymenoptera		0.071
Hymenoptera		54	Hymenoptera		63	Diptera		0.038	Ephemeroptera	Caenidae	0.067
Lepidoptera	Tortricidae	50	Lepidoptera	Tortricidae	62	Lepidoptera	Tortricidae	0.033	Lepidoptera	Depressariidae	0.059
Lepidoptera		44	Diptera	Limoniidae	59	Lepidoptera		0.030	Diptera	Limoniidae	0.043
Coleoptera		40	Lepidoptera		55	Diptera	Limoniidae	0.030	Lepidoptera	Tineidae	0.038
Diptera	Limoniidae	34	Ephemeroptera	Caenidae	47	Ephemeroptera	Caenidae	0.027	Trichoptera	Hydropsychidae	0.033
Hemiptera	Miridae	25	Coleoptera		46	Coleoptera	Elateridae	0.026	Diptera		0.033
Lepidoptera	Gelechiidae	25	Coleoptera	Elateridae	43	Coleoptera		0.026	Diptera	Psychodidae	0.032
Diptera	Ceratopogonidae	22	Lepidoptera	Gelechiidae	43	Diptera	Psychodidae	0.023	Hemiptera	Corixidae	0.030
Coleoptera	Elateridae	20	Hemiptera	Miridae	43	Lepidoptera	Gelechiidae	0.023	Coleoptera	Elateridae	0.029
Hemiptera		19	Diptera	Psychodidae	39	Hemiptera	Miridae	0.021	Diptera	Drosophilidae	0.026
Diptera	Cecidomyiidae	18	Hemiptera	Corixidae	35	Hemiptera	Corixidae	0.019	Diptera	Chaoboridae	0.025
Araneae		17	Araneae		33	Araneae		0.019	Lepidoptera	Gelechiidae	0.021
Hymenoptera	Ichneumonidae	16	Lepidoptera	Depressariidae	30	Lepidoptera	Depressariidae	0.017	Ephemeroptera	Heptageniidae	0.020
Diptera	Tipulidae	15	Diptera	Tipulidae	30	Ephemeroptera	Heptageniidae	0.016	Lepidoptera	Tortricidae	0.019
Diptera	Muscidae	13	Diptera	Culicidae	29	Diptera	Tipulidae	0.016	Trichoptera	Leptoceridae	0.017
Coleoptera	Carabidae	12	Diptera	Drosophilidae	28	Lepidoptera	Geometridae	0.015	Coleoptera		0.017
Diptera	Culicidae	12	Lepidoptera	Geometridae	28	Diptera	Culicidae	0.015	Diptera	Muscidae	0.011
Lepidoptera	Geometridae	12	Ephemeroptera	Heptageniidae	28	Diptera	Drosophilidae	0.014	Diptera	Cecidomyiidae	0.010

EPFU

order	family	richness	order	family	incidence	order	family	mean wPO	order	family	mean RRA
Coleoptera		54	Coleoptera		39	Coleoptera		0.045	Trichoptera	Hydropsychidae	0.195
Lepidoptera		43	Coleoptera	Carabidae	39	Coleoptera	Carabidae	0.041	Coleoptera	Elateridae	0.119
Diptera		38	Diptera	Limoniidae	37	Coleoptera	Elateridae	0.040	Diptera	Limoniidae	0.107
Diptera	Chironomidae	34	Diptera		36	Trichoptera	Hydropsychidae	0.040	Coleoptera	Scarabaeidae	0.065
Hymenoptera		34	Coleoptera	Elateridae	36	Diptera		0.039	Megaloptera	Corydalidae	0.061
Coleoptera	Carabidae	28	Lepidoptera		32	Diptera	Limoniidae	0.039	Ephemeroptera	Heptageniidae	0.051
Diptera	Limoniidae	27	Trichoptera	Hydropsychidae	29	Lepidoptera		0.037	Hymenoptera		0.046
Lepidoptera	Tortricidae	25	Hymenoptera		28	Diptera	Chironomidae	0.031	Diptera	Sepsidae	0.031
Coleoptera	Elateridae	22	Diptera	Chironomidae	25	Hymenoptera		0.030	Diptera	Chironomidae	0.030
Coleoptera	Cerambycidae	19	Coleoptera	Scarabaeidae	25	Coleoptera	Scarabaeidae	0.027	Lepidoptera	Tineidae	0.019
Coleoptera	Scarabaeidae	19	Lepidoptera	Tortricidae	25	Ephemeroptera	Heptageniidae	0.027	Hemiptera	Miridae	0.018
Diptera	Tipulidae	16	Diptera	Tipulidae	22	Lepidoptera	Tortricidae	0.025	Coleoptera		0.017
Hemiptera	Miridae	13	Coleoptera	Cerambycidae	21	Diptera	Tipulidae	0.021	Coleoptera	Pyrochroidae	0.017
Hymenoptera	Ichneumonidae	13	Hymenoptera	Ichneumonidae	21	Coleoptera	Cerambycidae	0.021	Hemiptera	Corixidae	0.016
Lepidoptera	Gelechiidae	12	Ephemeroptera	Heptageniidae	19	Coleoptera	Pyrochroidae	0.019	Coleoptera	Carabidae	0.015
Coleoptera	Pyrochroidae	11	Coleoptera	Pyrochroidae	19	Hymenoptera	Ichneumonidae	0.018	Diptera		0.014
Araneae		9	Hemiptera	Miridae	17	Coleoptera	Hydrophilidae	0.017	Diptera	Psychodidae	0.014
Coleoptera	Hydrophilidae	9	Lepidoptera	Gelechiidae	16	Hemiptera	Miridae	0.017	Lepidoptera	Tortricidae	0.013
Ephemeroptera	Heptageniidae	9	Coleoptera	Cantharidae	15	Diptera	Sciaridae	0.016	Coleoptera	Cerambycidae	0.012
Hemiptera		9	Coleoptera	Hydrophilidae	14	Lepidoptera	Tineidae	0.014	Lepidoptera	Lasiocampidae	0.012

Figure Legends

Figure 1. Characterization of bat diets using HTAS. A) Comparison of within-order richness at family, genus, species, and OTU taxonomic levels. Black bar represents the median, boxes represent the interquartile range (IQR), whiskers represent minimum and maximum values, and shades indicate the taxonomic level for each major arthropod order. B) Interpolated and extrapolated accumulation curves for family-level taxonomic richness. Solid lines represent interpolation, dotted lines represent extrapolation, and colors indicate bat species. C) Density distribution of relative read abundance, with colors indicating major arthropod orders. Transparent colors represent RRA, a read-based metric of relative abundance within a sample, while solid colors represent wPO, a presence-based metric of relative abundance within a sample. EPFU=big brown bat, MYLU=little brown bat.

Figure 2. Characterizing arthropod prey communities using black-light traps. A) Log_{10} abundance of focal arthropod groups in black-light traps. Black bar represents the median, boxes represent the interquartile range (IQR), whiskers represent minimum and maximum values, and colors indicate major arthropod orders. B) Density distribution of the percentage of total sample abundance for major arthropod orders as a percent of the total arthropod abundance in black-light trap samples. C) Black-light trap intra-ordinal community composition by Julian week in years 2015 & 2016. Colors represent major arthropod orders and groups and the shades of each color represents lower-level taxonomic groups within each category.

Figure 3. Relationships between bat diets & local arthropod prey abundance and diversity. A) Binary logistic regression main effects of arthropod group identity as predictors of the probability of detection (presence/absence) of arthropod prey in bat guano samples. Points indicate the estimate, lines indicate the 95% confidence interval. The dotted line indicates zero, such that confidence intervals non-

overlapping with zero suggest statistically meaningful model terms. Closed circles indicate overlap with zero, open triangles indicate non-overlap with zero. B) Binary logistic regression interaction effects between arthropod group identity and quantitative arthropod abundance as predictors of the probability of detection in guano samples after accounting for the main effect of group identity. Points indicate the estimate, lines indicate the 95% confidence interval. The dotted line indicates zero, such that confidence intervals non-overlapping with zero suggest statistically meaningful model terms. Closed circles indicate overlap with zero, open triangles indicate non-overlap with zero.

Figure 4. Influences of temporal variables and arthropod abundance on the composition of bat diets. Local abundance represents the abundance of arthropod groups at a site in a particular week and year, while regional abundances represent the abundance of arthropod groups at all sites in a particular week and year. A) Redundancy analysis (RDA) plots based on family-level presence/absence matrices, with overlaid statistically significant and marginally significant environmental vectors. B) RDA plots based on OTU level presence/absence matrices, with overlaid statistically significant and marginally significant environmental vectors. Bold text indicates environmental vectors with $p<0.05$, while regular text indicates environmental vectors with $p<0.10$. Point symbols represent distinct sampling sites. EPFU=big brown bat, MYLU=little brown bat.

Supplementary Figure S1. Map of study sites. EPFU=big brown bat, MYLU=little brown bat.

Supplementary Figure S2. Binary logistic regression main effects of arthropod group identity as predictors of the probability of detection (presence/absence) of arthropod prey in bat guano samples, without incorporating arthropod abundance information. Points indicate the estimate, lines indicate

the 95% confidence interval. The dotted line indicates zero, such that confidence intervals non-overlapping with zero suggest statistically meaningful model terms.

Supplementary Figure S3. Heatmap of arthropod mock communities across three sequencing runs. RRA=relative read abundance.

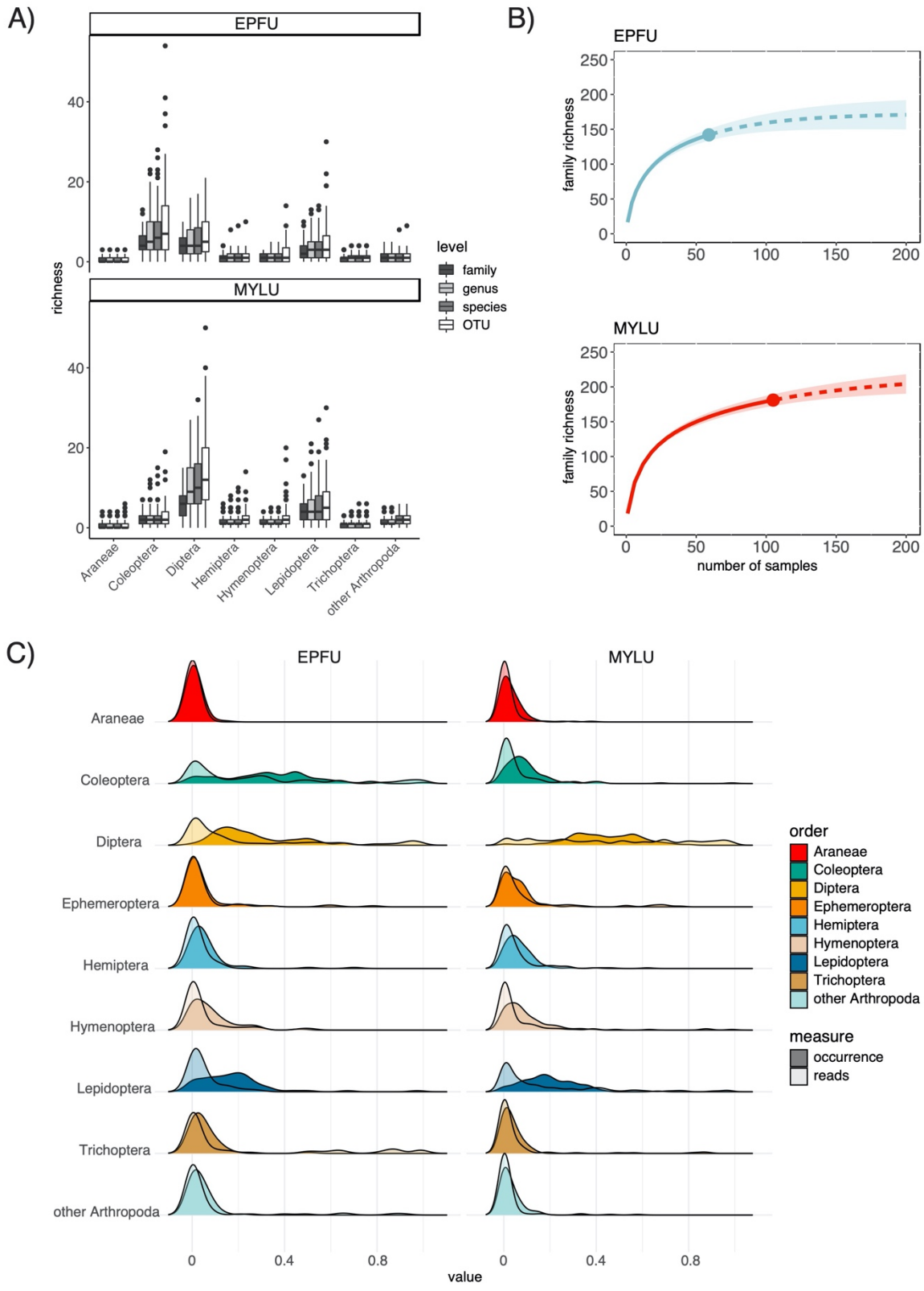

A)
EPFU
MYLU
richness
level
family
genus
species
OTU
Araneae
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Trichoptera
other Arthropoda
B)
EPFU
MYLU
family richness
number of samples
C)
EPFU
MYLU
order
measure
occurrence
reads
value

Figure 1

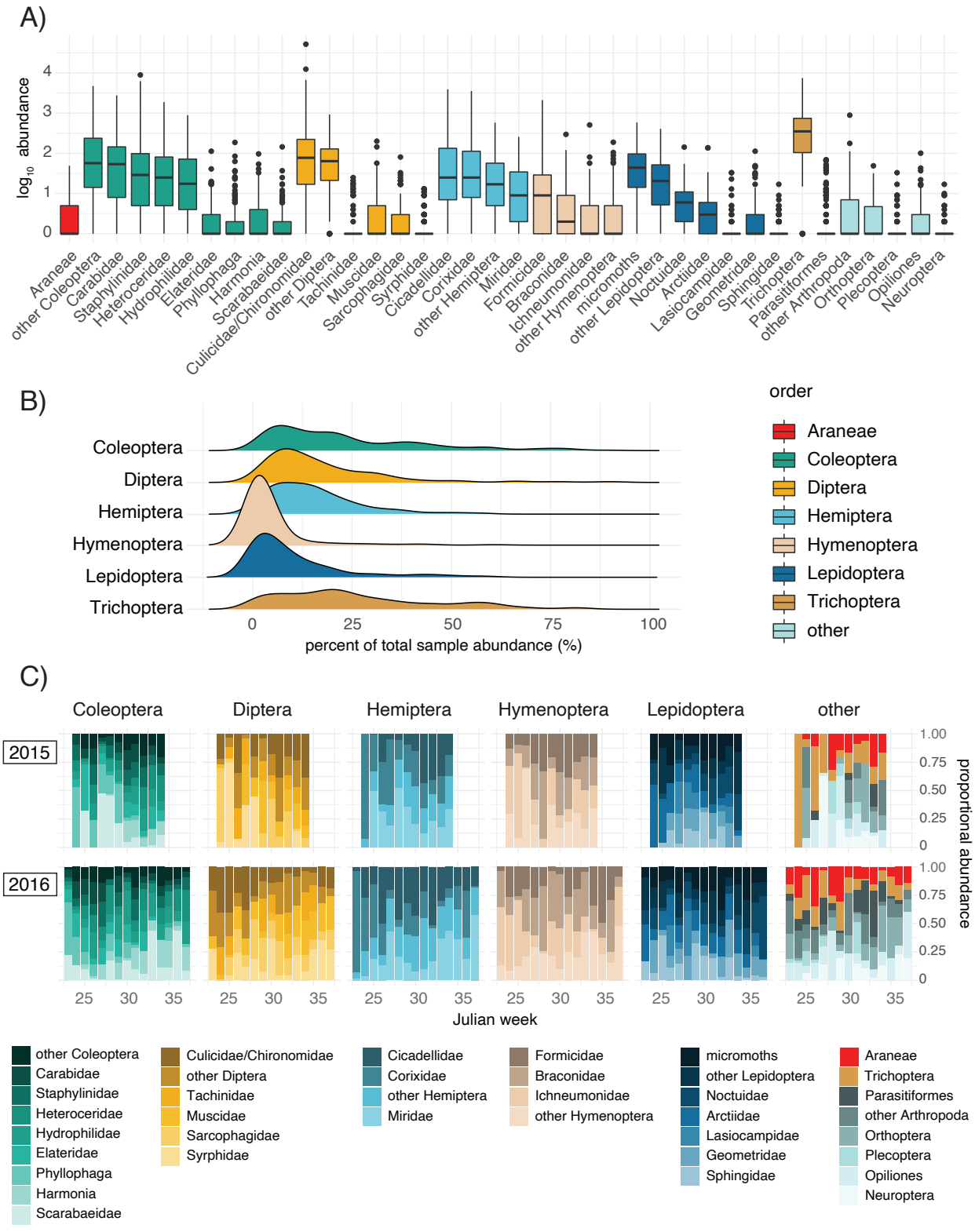

A)
log10 abundance
0
1
2
3
4
Araneae
other Coleoptera
Carabidae
Staphylinidae
Heteroceridae
Hydrophilidae
Elateridae
Phyllophaga
Harmonia
Scarabaeidae
Culicidae/Chironomidae
other Diptera
Tachinidae
Muscidae
Sarcophagidae
Syrphidae
Cicadellidae
Corixidae
other Hemiptera
Miridae
Formicidae
Braconidae
Ichneumonidae
other Hymenoptera
micromoths
other Lepidoptera
Noctuidae
Arctiidae
Lasiocampidae
Geometridae
Sphingidae
Trichoptera
Parasitiformes
other Arthropoda
Orthoptera
Plecoptera
Opiliones
Neuroptera
B)
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Trichoptera
0
25
50
75
100
percent of total sample abundance (%)
order
Araneae
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Trichoptera
other
C)
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
other
2015
2016
1.00
0.75
0.50
0.25
0
proportional abundance
25
30
35
Julian week
other Coleoptera
Carabidae
Staphylinidae
Heteroceridae
Hydrophilidae
Elateridae
Phyllophaga
Harmonia
Scarabaeidae
Culicidae/Chironomidae
other Diptera
Tachinidae
Muscidae
Sarcophagidae
Syrphidae
Cicadellidae
Corixidae
other Hemiptera
Miridae
Formicidae
Braconidae
Ichneumonidae
other Hymenoptera
micromoths
other Lepidoptera
Noctuidae
Arctiidae
Lasiocampidae
Geometridae
Sphingidae
Araneae
Trichoptera
Parasitiformes
other Arthropoda
Orthoptera
Plecoptera
Opiliones
Neuroptera

Figure 2

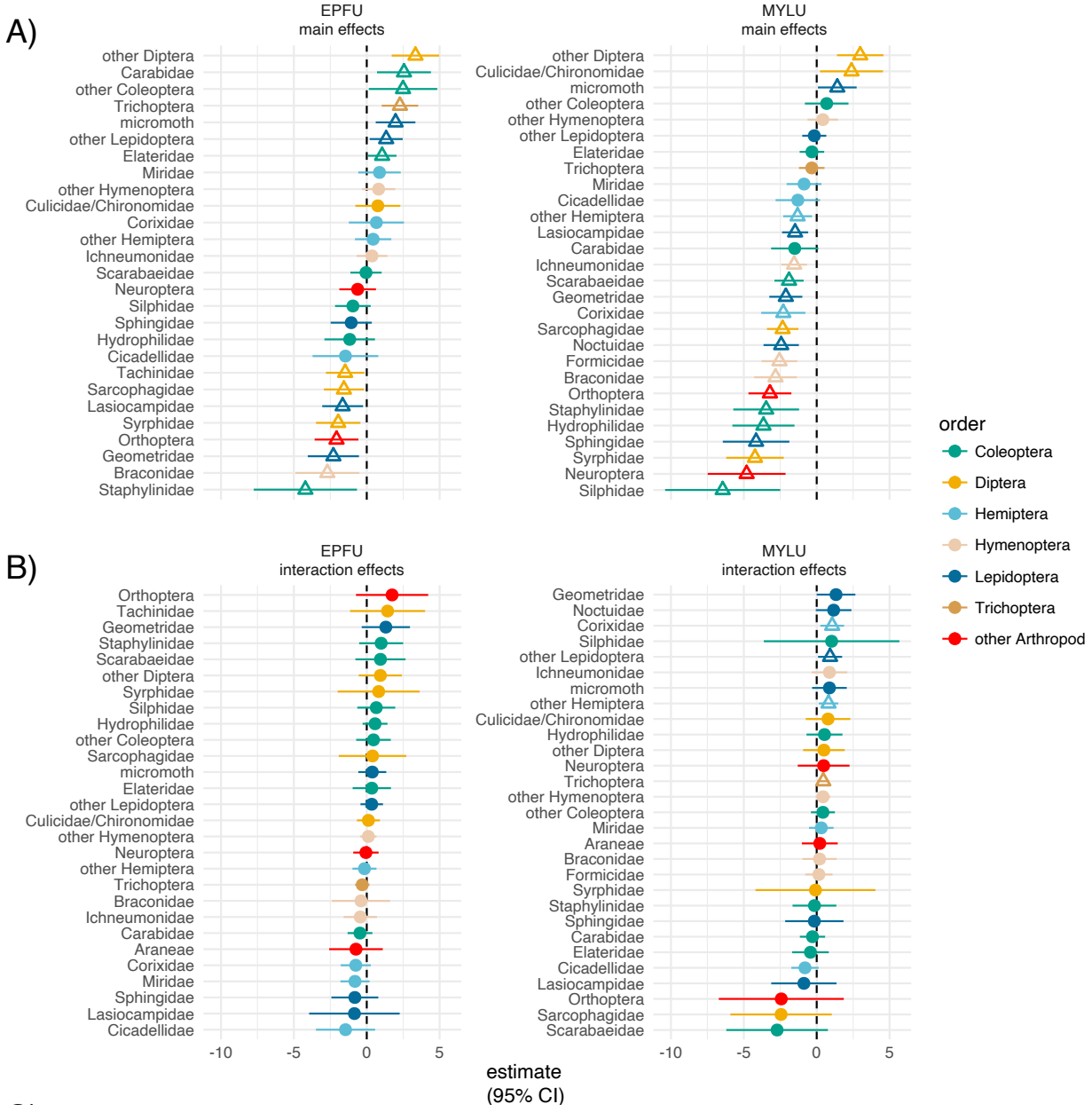

A)
EPFU
main effects
other Diptera
Carabidae
other Coleoptera
Trichoptera
micromoth
other Lepidoptera
Elateridae
Miridae
other Hymenoptera
Culicidae/Chironomidae
Corixidae
other Hemiptera
Ichneumonidae
Scarabaeidae
Neuroptera
Silphidae
Sphingidae
Hydrophilidae
Cicadellidae
Tachinidae
Sarcophagidae
Lasiocampidae
Syrphidae
Orthoptera
Geometridae
Braconidae
Staphylinidae
MYLU
main effects
other Diptera
Culicidae/Chironomidae
micromoth
other Coleoptera
other Hymenoptera
other Lepidoptera
Elateridae
Trichoptera
Miridae
Cicadellidae
other Hemiptera
Lasiocampidae
Carabidae
Ichneumonidae
Scarabaeidae
Geometridae
Corixidae
Sarcophagidae
Noctuidae
Formicidae
Braconidae
Orthoptera
Staphylinidae
Hydrophilidae
Sphingidae
Syrphidae
Neuroptera
Silphidae
order
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Trichoptera
other Arthropod
B)
EPFU
interaction effects
Orthoptera
Tachinidae
Geometridae
Staphylinidae
Scarabaeidae
other Diptera
Syrphidae
Silphidae
Hydrophilidae
other Coleoptera
Sarcophagidae
micromoth
Elateridae
other Lepidoptera
Culicidae/Chironomidae
other Hymenoptera
Neuroptera
other Hemiptera
Trichoptera
Braconidae
Ichneumonidae
Carabidae
Araneae
Corixidae
Miridae
Sphingidae
Lasiocampidae
Cicadellidae
MYLU
interaction effects
Geometridae
Noctuidae
Corixidae
Silphidae
other Lepidoptera
Ichneumonidae
micromoth
other Hemiptera
Culicidae/Chironomidae
Hydrophilidae
other Diptera
Neuroptera
Trichoptera
other Hymenoptera
other Coleoptera
Miridae
Araneae
Braconidae
Formicidae
Syrphidae
Staphylinidae
Sphingidae
Carabidae
Elateridae
Cicadellidae
Lasiocampidae
Orthoptera
Sarcophagidae
Scarabaeidae
-10
-5
0
5
estimate
(95% CI)

Figure 3

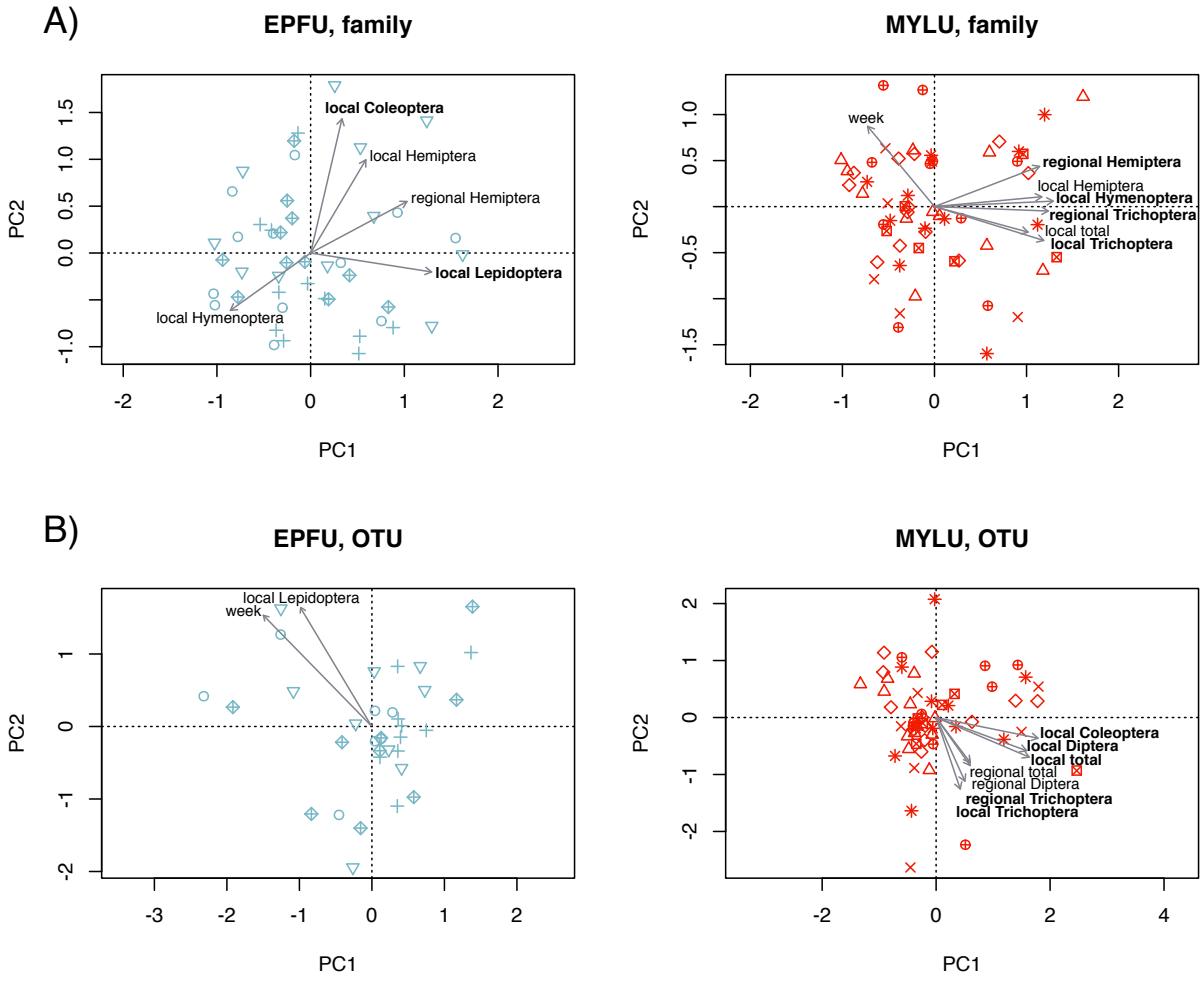
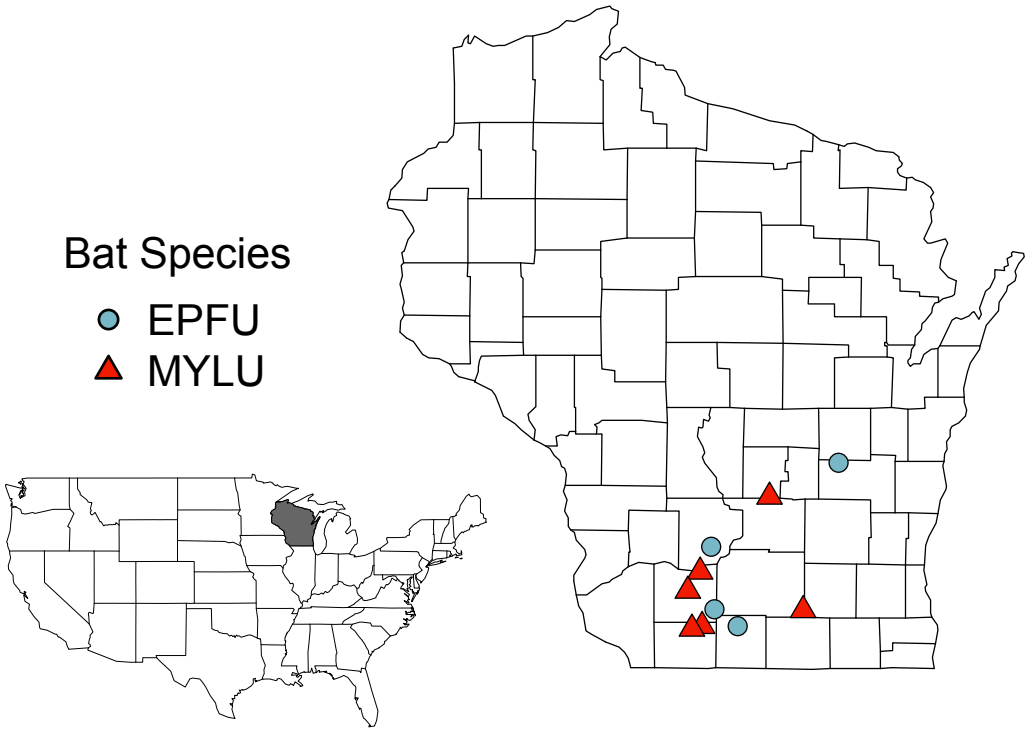

A)
EPFU, family
local Coleoptera
local Hemiptera
regional Hemiptera
local Lepidoptera
local Hymenoptera
PC1
PC2
MYLU, family
week
regional Hemiptera
local Hemiptera
local Hymenoptera
regional Trichoptera
local total
local Trichoptera
PC1
PC2
B)
EPFU, OTU
local Lepidoptera
week
PC1
PC2
MYLU, OTU
local Coleoptera
local Diptera
local total
regional total
regional Diptera
regional Trichoptera
local Trichoptera
PC1
PC2

Figure 4

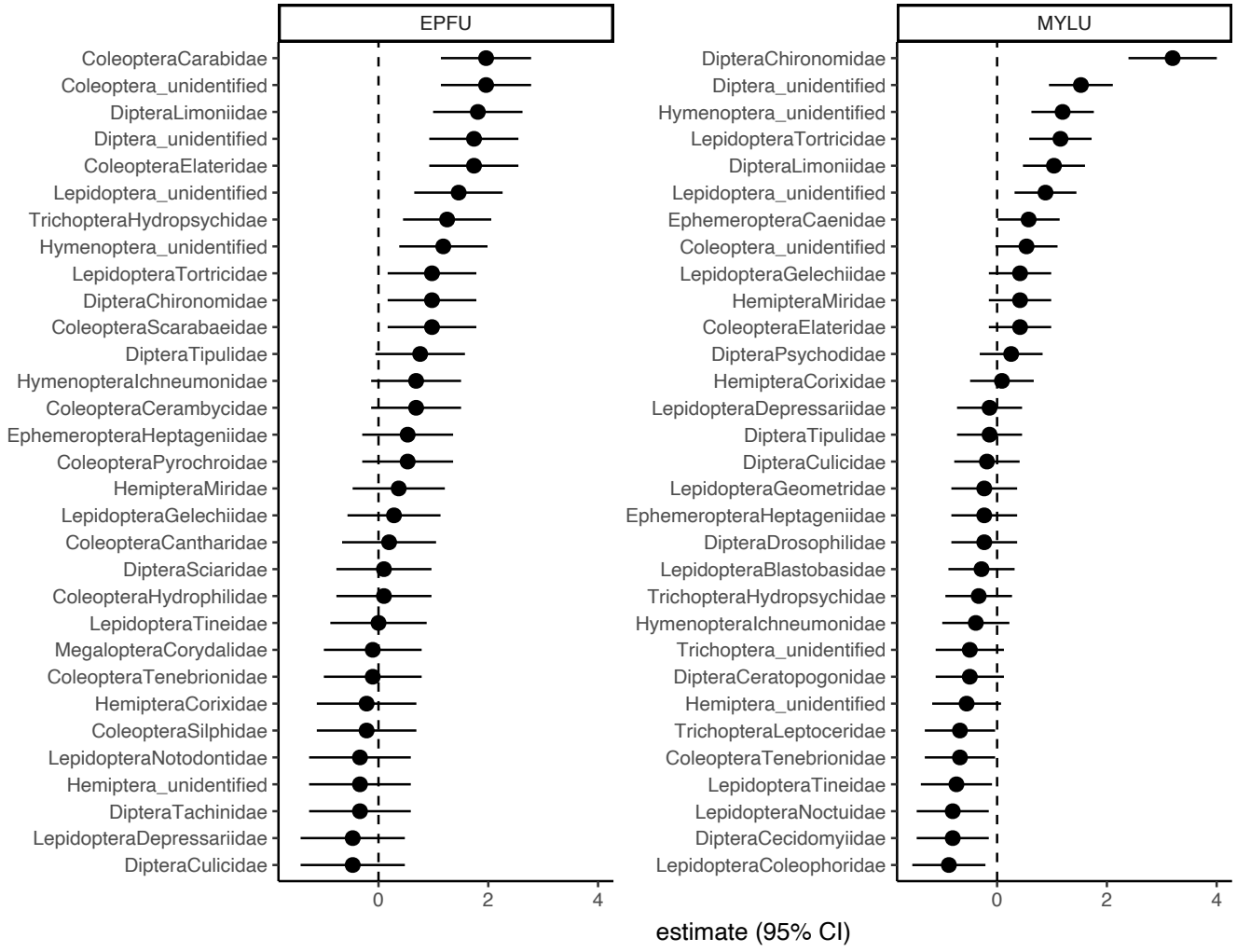
Supplementary Materials

Supplementary Table S1: Total OTUs detected with number and percentage identified at each taxonomic level.

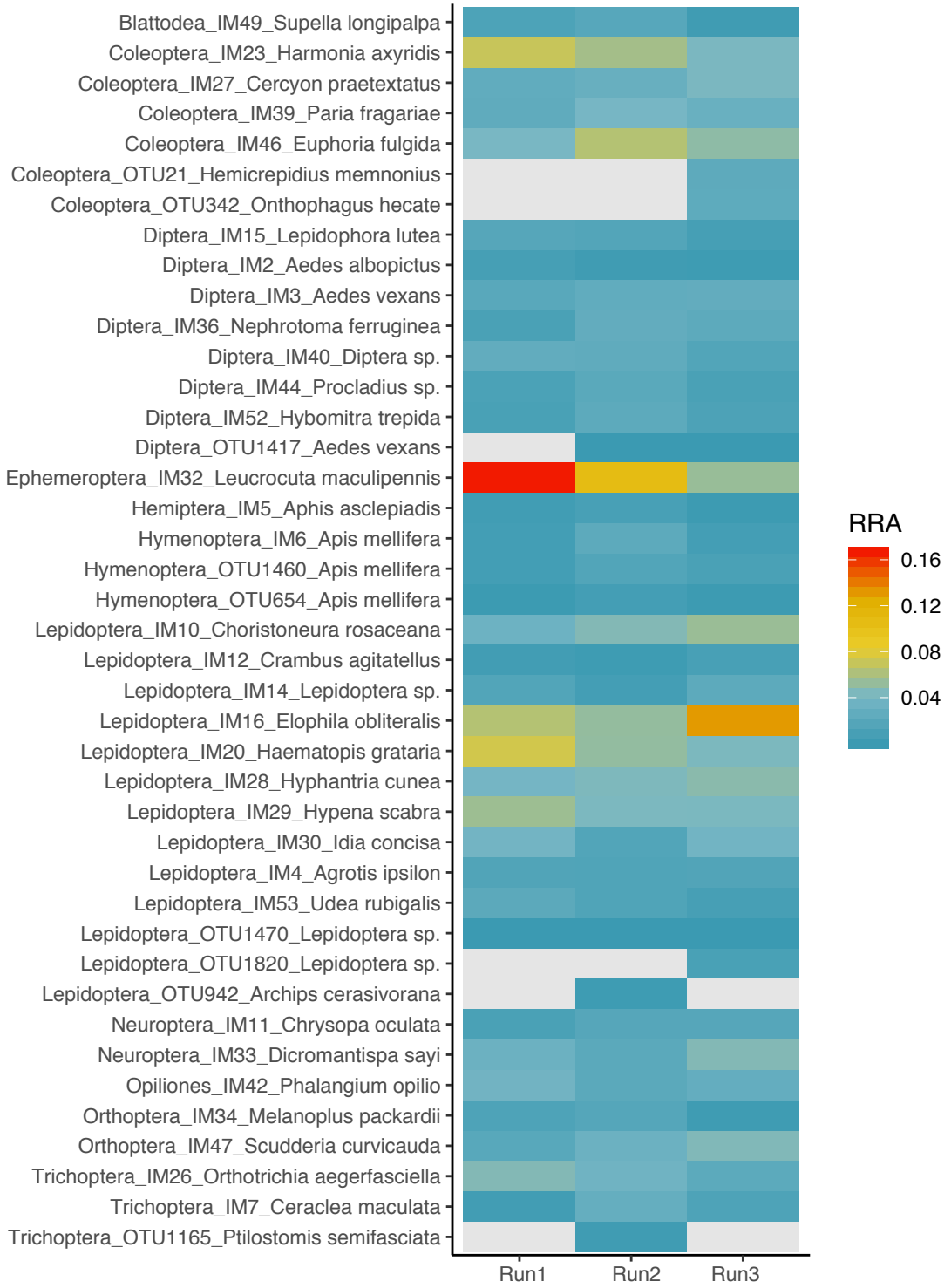
Bat Species	Arthropod order	OTUs	family	%	genus	%	species	%
MYLU								
	Araneae	37	20	54.1%	20	54.1%	20	54.1%
	Coleoptera	138	98	71.0%	94	68.1%	83	60.1%
	Diptera	498	412	82.7%	306	61.4%	185	37.1%
	Ephemeroptera	25	22	88.0%	22	88.0%	21	84.0%
	Hemiptera	81	62	76.5%	57	70.4%	48	59.3%
	Hymenoptera	92	38	41.3%	32	34.8%	20	21.7%
	Lepidoptera	258	214	82.9%	210	81.4%	192	74.4%
	Trichoptera	33	25	75.8%	25	75.8%	25	75.8%
	other Arthropoda	37	32	86.5%	32	86.5%	24	64.9%
EPFU		OTUs	family	%	genus	%	species	%
	Araneae	16	7	43.8%	7	43.8%	7	43.8%
	Coleoptera	212	158	74.5%	156	73.6%	142	67.0%
	Diptera	194	156	80.4%	122	62.9%	83	42.8%
	Ephemeroptera	15	14	93.3%	14	93.3%	14	93.3%
	Hemiptera	44	35	79.5%	34	77.3%	27	61.4%
	Hymenoptera	54	20	37.0%	18	33.3%	9	16.7%
	Lepidoptera	153	110	71.9%	109	71.2%	104	68.0%
	Trichoptera	19	17	89.5%	17	89.5%	17	89.5%
	other Arthropoda	28	23	82.1%	19	67.9%	15	53.6%



Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3

CHAPTER 2: SHIFTS IN BAT ISOTOPIC NICHES REFLECT HISTORICAL LAND-USE CHANGES

Amy K. Wray[1], Claudio Gratton[2], and M. Zachariah Peery[1]

[1]*Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, Madison, WI, USA*

[2]*Department of Entomology, University of Wisconsin-Madison, Madison, WI, USA*

Citation: Wray, A. K., Gratton, C., and Peery, M. Z. Shifts in bat isotopic niches reflect historical land-use changes. *Oecologia*, in review.

Abstract

Agricultural intensification has been linked with population declines and reduced diversity among arthropods and their predators. Despite conservation and habitat management implications, the effects of land-use change on the trophic relationships between nocturnal arthropod predators and their prey have seldom been described. To assess how arthropodivorous bats may have shifted their diets in response to land-use changes, we compared bulk $\delta^{13}C$ and $\delta^{15}N$ values from little brown and big brown bats collected in the Upper Midwestern region of the United States from 1898–2019. We also related isotopic niches with climate and landcover variables as an attempt to explain the mechanisms underlying changes in arthropodivorous bat diets over time. We found that interspecific isotopic niche overlap increased from 26% to 47% between historical and contemporary time periods. These changes were largely driven by shifts among contemporary big brown bats, which were isotopically more similar to little brown bats than to historical conspecifics. Variance in stable isotope values was partly explained by the prevalence of grasses, forest, or open water, but not by the prevalence of agricultural cover (e.g., corn crops) on the landscape. Our results suggest that the isotopic niches of arthropodivorous bats have converged over time, which may reflect homogenization of prey resources driven by land-use changes. These findings highlight the importance of investigating how the loss of habitat diversity and corresponding declines in prey resources influence aerial arthropodivore populations, particularly as they decline from a myriad of other stressors.

Introduction

Ecosystems worldwide have experienced rapid changes due to anthropogenic activities. Understanding how practices such as increasingly intensive agriculture and the homogenization of habitats influence food webs is important for understanding the mechanisms underlying changes in

biological communities (Matson et al. 1997; Tilman et al. 2002; Tscharntke et al. 2005). Recent studies have also raised alarms regarding declines in arthropod populations, which are attributed to a range of causes including habitat loss, pesticide use, and climate change (Hallmann et al. 2017; Sánchez-Bayo & Wyckhuys 2019). Agricultural intensification and declines in prey availability, in turn, have been linked to population declines and reduced functional diversity among arthropod predators (Benton et al. 2002; Nebel et al. 2010; Spiller & Dettmers 2019). While some long-term datasets have provided insights regarding changes in arthropod communities over time, these types of data are unavailable for many regions and the influence of these changes on arthropod predators is less well understood, particularly within the nocturnal arthropod food web.

The dietary niches of top predators reflect many of the underlying ecological processes in their respective food webs. Spatial and temporal variability in dietary niches can reflect shifts in interspecific competition, niche expansion, or niche compression in response to changing habitat and prey resources (Azevedo et al. 2006; Brickner et al. 2014; Manlick et al. 2017; Carbonell Ellgutter et al. 2020). In other systems, some predators have expanded their diets to include different, human-associated food resources in order to meet energy requirements (Murray et al. 2015; Kirby et al. 2016; Moss et al. 2016). Collectively, these studies demonstrate how quantifying dietary niche breadth and overlap among predators can signal their level of adaptability, while also providing insights for the development of predator-focused ecosystem restoration efforts (Ritchie et al. 2012). Previous work has often focused on apex consumers, which have displayed well-documented cascading effects in their respective food webs (Estes et al. 2011). Nevertheless, vertebrate arthropodivores also function as top predators in their food webs and can initiate trophic cascades (Mooney et al. 2010). For vertebrate arthropod predators such as bats and birds, narrower dietary niches have been associated with increased extinction risk (Julliard et al. 2004; Boyles & Storm 2007). However, the spatiotemporal variability of the dietary niches of vertebrate arthropod predators have been less frequently

investigated, despite the potential for using this information to clarify the mechanisms underlying population declines among these taxa.

Several studies on vertebrate arthropodivores reflect the influences of human-modified landscapes on dietary niches, but most have focused on birds (Stanton et al. 2016; Michelson et al. 2018). Approximately 70% of all bats are arthropodivores, which, in comparison to most arthropodivorous birds, are typically nocturnal and consume an entirely different suite of arthropod prey (Fenton & Fleming 1976). Studies on the contemporary diets of many common bat species have demonstrated the influences of habitat on bat diets by using both molecular and morphological methods (Agosta, 2002; Whitaker, 2004; Clare et al. 2014a; Clare et al. 2014b). While agricultural intensification has generally demonstrated negative effects on bat activity, abundance, and species richness (Racey & Entwistle 2005; Williams-Guillén et al. 2016), certain arthropodivorous bat species not only persist in agriculturally-dominated habitats, but actually exploit agriculturally-associated prey and thereby provide valuable ecosystem services (Williams-Guillén et al. 2008; Kunz et al. 2011; McCracken et al. 2012). Nonetheless, comparatively little is known about the historical diets of arthropodivorous bats or how their contemporary foraging patterns may be constrained by the availability of prey resources.

Stable isotope analyses have successfully been used to measure changes in animal foraging following anthropogenic habitat modification and to compare historical, contemporary, and even paleontological dietary composition using carbon and nitrogen isotopic ratios (Chamberlain et al. 2005; Blight et al. 2015). In terrestrial habitats, $\delta^{13}C$ values in animal tissues generally reflect the relative contributions of C_3 and C_4 plants at the base of the food web, with plants such as corn and other C_4 grasses having distinctly higher values of $\delta^{13}C$ in comparison to other vegetation types (Ben-David & Flaherty 2012; Layman et al. 2012). Values of $\delta^{15}N$ also reflect underlying resources as well as changes in trophic levels, with higher $\delta^{15}N$ values roughly indicative of a higher trophic position (Ben-David

& Flaherty 2012; Layman et al. 2012). Isotopic values can be analyzed in multivariate space, and in doing so can approximate the Eltonian functional niche of a consumer. Isotopic niche metrics are not necessarily equivalent to trophic niches and do not necessarily reflect changes in the identity of the prey consumed by a predator (Hette-Tronquart, 2019). However, changes in isotopic values and isotopic niches can reflect large-scale changes in prey or in the plant materials consumed by prey, which percolate up through the food web and are ultimately incorporated into the tissues of predator in question and are therefore useful in quantifying changes in foraging patterns over time (Peterson & Fry 1987; Marshall et al. 2019).

In order to assess whether and to what extent arthropodivorous bats may have shifted their dietary niches in the recent past, we compared bulk carbon and nitrogen isotopic ratios from little brown (*Myotis lucifugus,* Leconte 1831) and big brown bat (*Eptesicus fuscus,* Palisot de Beauvois 1796) hair collected in the Upper Midwestern region of North America over a 121-year period. We hypothesized that bat diets would reflect historical shifts in vegetation and would become more interspecifically similar over time. Due to the increasingly intensive corn-dominated agriculture in the study area, we predicted that $\delta^{13}C$ values in bat tissues would increase and that interspecific isotopic niche overlap would increase over time. We also compared stable isotope values against climate and landcover variables in order to ascertain the potential mechanisms contributing to these changes.

Methods

Study area

We sampled museum specimens and bat carcasses from part of the Upper Midwestern region of North America including Wisconsin, Illinois, and Iowa, between the time periods of 1898-2019. Prior to European occupation that began in the 1850s, Illinois, and Iowa, and southern Wisconsin were

dominated primarily by savanna, prairie, and deciduous forests, while areas north of the floristic tension zone in Wisconsin were dominated by coniferous forest, mixed deciduous-coniferous forest, and coniferous savanna (Iverson, 1988; Rhemtulla et al. 2007, 2009; Gallant et al. 2011). By the 1930s, much of the native vegetation in the southern part of this region was converted to agricultural land, with coniferous forest and savanna in the northern portion of Wisconsin largely converted to deciduous and mixed deciduous-coniferous forests (Rhemtulla et al. 2007). From the mid-1900s through the present, agricultural production (especially corn production) also became increasingly intensive (Kucharik & Ramankutty 2005).

Sample collection & preparation

Hair was sampled from 32 little brown and 47 big brown bat carcasses previously collected by the USGS National Wildlife Health Center and the Wisconsin Department of Natural Resources from 2009–2019. Within this sample set, bone fragments were collected from 24 little brown and 40 big brown bat carcasses. Hair samples were also collected from 66 little brown and 26 big brown bat museum specimens from the University of Wisconsin-Madison Zoological Museum, the Field Museum, and the National Museum of Natural History, which were originally collected from 1898–2008. Historical groups are defined as specimens collected between 1898–1973, while contemporary groups are defined as those collected between 1990–2019. From 1973–1990, few museum specimens were available (possibly related to the 1973 moratorium on bat banding in the United States), thus providing a natural split for the two groups (Ellison 2008). For little brown bats, samples collected prior to 1935 were also compared with samples collected after 1993 to assess the potential influence of sample grouping methods and to account for land-use changes that had already occurred by the mid-1930s.

To remove surface contaminants, hair samples were washed in a 2:1 chloroform:methanol solution with rinsing repeated three times. Bone fragments were decalcified by soaking in HCl for 48 hours. Lipids were extracted from the remaining bone collagen by rinsing with ddH_2O, followed by soaking in 2:1 chloroform:methanol for 12 hours, with the 2:1 solution replaced 3x and soaking repeated. Tissues were then dried in an oven at 60°C for 48 hours and homogenized using sterile dissection scissors. Homogenized samples were loaded into tin capsules at dry quantities ranging from 0.4–1.2 mg ($\bar{x}$=0.8mg) for hair keratin and 0.4–2.0mg ($\bar{x}$=1.4mg) for bone collagen. Sample material was sent to the University of New Mexico for $\delta^{13}C$ and $\delta^{15}N$ analysis on a Costech 4010 elemental analyzer (Valencia, CA) coupled with a Thermo Scientific Delta V mass spectrometer (Waltham, MA). Carbon and nitrogen stable isotope abundances are expressed as parts per mil using $\delta X\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000$ (Ben-David & Flaherty 2012) relative to the international standards Vienna Peedee belemnite and atmospheric nitrogen, respectively. To correct for changes in carbon baselines due to emissions (i.e., the Suess effect, see Verburg 2007), $\delta^{13}C$ values were corrected as per Farmer & Leonard 2011.

Landcover & climate data

To compare the potential influence of local landcover on stable isotope values, landcover composition data were derived for samples collected in Wisconsin with specific locality information that corresponded with available landcover data layers. For these analyses, 27 historical samples (little brown bats collected 1903–1938) and 52 contemporary samples (little brown and big brown bats collected 2008–2019) met the aforementioned criteria. Historical landcover data were derived from a digitized version of Finley's original vegetation of Wisconsin map, which was compiled from public land surveys conducted from 1832–1866 (Finely, 1976; see Schulte & Mladenoff 2001 for further details). Contemporary landcover composition data were derived from Wiscland 2.0, which represents

30m resolution landcover in the state of Wisconsin (https://dnr.wi.gov/maps/WISCLAND.html). Buffers were placed around sample collection location and the corresponding landcover was extracted within each buffer. We chose buffers with a 5km radius to account for the maximum foraging distances for both bat species and to capture the broader-scale landcover influences on arthropod communities. In order to visualize landcover changes and to compare between sample collection localities in historical and contemporary time periods, we aggregated vegetation classes into similar broad-scale categories and calculated the percentage of total landcover for each category within the designated 5km buffer area (Fig. 1, Table S1a–b). To assess the potential influence of collection year and corresponding climate variables, which can influence isotopic baselines of vegetation, climate data for total annual precipitation and average annual temperature were also derived for the Upper Midwestern region from the United States Climate Divisional Database via the National Ocean and Atmospheric Administration Statewide Time Series Dataset (https://www.ncdc.noaa.gov/cag/national/time-series).

Statistical analysis

Unpaired group means for isotope values were compared using a 95% confidence interval with 5,000 bootstrap resamples calculated using the R package "dabestr" (Ho et al. 2019). Metrics such as isotopic niche breadth can be measured in bivariate δ-space, which allow for the estimation of inter- and intra-specific dietary niche breadth and overlap (Newsome et al. 2007; Flaherty & Ben-David 2010). To assess different metrics of isotopic niche breadth, we used total area (TA), standard ellipse area (SEA), corrected standard ellipse area (SEAc), and 95% maximum likelihood ellipse area. We also estimated niche overlap by using maximum likelihood fitted ellipses with the overlap prediction interval scaled to 95%. All isotopic niche metrics were calculated using the R package "SIBER" (Jackson et al. 2011).

Due to low resolution in the historical vegetation data layer, we separated data into categories to compare samples collected in areas dominated by forest with samples collected in areas dominated by prairie and savanna. One sample collection area was evenly covered by both landcover types and was therefore excluded. Stable isotope values for these groups were compared using unpaired group mean differences. We also estimated historical and contemporary interspecific habitat overlap of landcover using the "nicheoverlap" function in the R package "indicspecies" (De Caceres & Jansen 2016), for which we included all available landcover variables from the Finley map (n=16 categories, Table S1a) and from the Wiscland 2.0 level 2 data layer (n=14 categories, Table S1c).

We assessed the importance of landcover and climate variables as predictors of stable isotope values using separate generalized linear models. For landcover models, which included only the subset of samples for which specific locality information was available (n=52), we used bat species and the interaction between bat species and the percentage of cool-season (C_3) grass, warm-season (C_4) grass, continuous corn, developed/urban area, forest, open water, pasture, and wetland as predictor variables (Table S2). For climate models, which included all samples for which the zone of collection were available (n=163), we used bat species and the interaction between bat species and year, Julian week, collection zone (i.e., north or south of the floristic tension zone), average annual temperature, and total annual precipitation as predictor variables. Both response and continuous predictor variables were standardized based on the mean and standard deviation, calculated in the R package "effsize" (Torchiano 2020). We tested all variables for correlation and found that year, Julian week, and precipitation had Pearson's correlation coefficients r>0.5, so these terms were restricted from occurring in the same models. To avoid potential issues with variance-inflation, landcover models were also restricted to a maximum of four terms. We compared submodels using Akaike's information criteria with a small sample size bias correction term (AICc). Model averaging and calculation of effect sizes with 95% confidence intervals were performed using the R package "MuMIn" (Bartoń, 2015).

Results

For little brown bats, $\delta^{13}C$ values from historical samples were higher than contemporary samples but $\delta^{15}N$ values did not differ between periods, whereas historical big brown bat samples had lower values $\delta^{13}C$ and $\delta^{15}N$ in comparison to contemporary samples (Table 1a). These patterns were also consistent on a continuous time scale, with $\delta^{13}C$ values increasing for little brown bats (β=0.018, p<0.001) and both $\delta^{13}C$ and $\delta^{15}N$ values decreasing for big brown bats (β=0.012, p=0.09; β=–0.019, p=0.005, respectively) over time (Fig. 2a).

For little brown bat samples, both historical and contemporary ellipse area metrics were larger than for big brown bat samples (Fig. 2b). Between time periods, ellipse area metrics were similar for little brown bat samples, while contemporary big brown bat samples had larger ellipse area metrics than historical big brown bat samples (Table 1b). For little brown bats the 95% ML ellipse overlap between historical and contemporary samples was 78.24%, while for big brown bats the 95% ML ellipse overlap between historical and contemporary samples was 32.72%. Interspecifically, the 95% ML ellipse overlap was 25.94% for historical samples and 47.10% for contemporary samples.

For both bat species, bone collagen had higher $\delta^{13}C$ values ($\bar{x}_{bone}$=–22.4 ± 1.82‰; $\bar{x}_{hair}$= –23.9 ± 2.02‰) and $\delta^{15}N$ values ($\bar{x}_{bone}$=11.0 ± 2.46‰; $\bar{x}_{hair}$= 9.52 ± 2.34‰) in comparison to hair keratin. The mean (± SD) tissue-to-tissue differences in $\delta^{13}C$ for little brown and big brown bats were 1.72 ± 1.12‰ and 1.26 ± 0.93‰, respectively. The mean tissue-to-tissue differences in $\delta^{15}N$ for little brown and big brown bats were 1.83 ± 0.97‰ and 1.25 ± 0.91‰, respectively, which likely represent tissue-specific differences in fractionation. Ellipse area metrics for little brown bat samples were larger for hair than for bone, while ellipse area metrics for big brown bat samples were similar for hair and bone (Table 1c). The 95% ML ellipse overlap between little brown and big brown bats were 37.1% and 43.1% for bone and hair samples, respectively (Figure 2c).

For little brown bat samples collected prior to 1940, samples collected in areas historically dominated by savanna and prairie were on average higher by 2.6‰ for $\delta^{15}N$ values in comparison to samples collected in areas dominated by forest (95% CI=1.18, 3.87‰). For these samples, $\delta^{13}C$ values were not significantly different between habitat types (Unpaired mean difference=0.586‰, 95% CI=–1.09, 3.24‰). For contemporary samples with available locality information, the interspecific overlap in habitat resources was 93.4% (95% CI=86.3, 98.3%), whereas the interspecific overlap in habitat resources for the same sites in the historical time period was 85.3% (95% CI=59.8, 97.5%). All of the top contemporary landcover models for $\delta^{13}C$ included the percentage of C_4 grass, with other competing models (determined by ΔAIC) including combinations of the percentage of C_3 grass, pasture, and corn (Fig. 3a, Table 2). The top contemporary landcover models for $\delta^{15}N$ included the percentage of forest and open water, with competing models including combinations of the percentage of pasture, urban/developed areas, and wetland (Fig. 3a, Table 2). The top climate models for both $\delta^{13}C$ and $\delta^{15}N$ included year and the zone of collection, with competing models for $\delta^{15}N$ including year or week (Fig. 3b, Table 2). For both landcover and climate models, the standardized effect sizes differed by bat species (Fig. 3).

Discussion

Historical shifts in animal diets have been previously investigated to assess their potential for adapting to a changing landscape. While other studies have used stable isotopes to study bats in the context of niche overlap, migration, and paleontological diet (e.g., Cryan et al. 2004; Wurster et al. 2010; Broders et al. 2014), our study represents the first attempt to connect recent historical shifts in bat diets, as inferred by stable isotopes, with temporal, climatic, and landcover variables that potentially explain the mechanisms underlying the observed changes. Overall, our results indicate that little brown and big brown bat isotopic niches have converged over time and further analyses suggest that the observed

shifts in bat dietary niches are at least partially driven by anthropogenically-associated changes in landcover.

The prevalence and production intensity of corn, a C_4 grass, increased dramatically over time in our study area. Since C_4 grasses have distinct isotopic signatures that are higher in $\delta^{13}C$ in comparison to other vegetation types, we expected that $\delta^{13}C$ values in tissues from arthropodivorous bats would also increase over time. However, while $\delta^{13}C$ values among contemporary little brown bats were higher than historical little brown bats, $\delta^{13}C$ values among contemporary big brown bats were lower than historical big brown bats. For little brown bats on a local scale, higher values of $\delta^{13}C$ were not associated with the percentage of corn, but rather, with the percentage of other C_4 grasses. The C_4 grass variable used in this study, which primarily includes native warm-season grass species but can also include various native forbs, may therefore contribute to higher dietary diversity among little brown bats, despite corn constituting a larger percentage of the total area. These results also suggest that the loss of historical savanna and prairie habitats, which include both C_3 and C_4 plants and are well documented to support diverse arthropod assemblages (Whiles & Charlton 2006), may be related to the increasing homogenization of bat isotopic niches over time. This interpretation is consistent with previous studies, which have shown that even in areas where bats do feed on agriculturally-associated arthropods, native vegetation provides more consistent prey resources (Davidai et al. 2015). As such, the observed interspecific convergence in $\delta^{13}C$ values may reflect an increasing reliance on isotopically similar prey associated with remnant natural vegetation that is less common in this area.

Our study demonstrates that broader isotopic niches are not necessarily associated with greater flexibility. While narrower in comparison to little brown bats, the isotopic niches of big brown bats shifted more substantially over time. Common prey items for big brown bats typically include predaceous arthropods, such as staphylinid and carabid beetles (Agosta 2002; Clare et al. 2014b), which may suggest that the observed decline in $\delta^{15}N$ values over time represents some degree of trophic

downgrading. These results are consistent with a previous study that demonstrated DDT-related severe declines in beetle populations from the 1940s through the 1960s were reflected in declining $\delta^{15}N$ values present in chimney swift feathers (Nocera et al. 2012). In our study, higher percentage of forest corresponded with lower $\delta^{15}N$ values in big brown bats. Nitrogen deposition from anthropogenic inputs is also associated with higher $\delta^{15}N$ values among aquatic and riparian arthropods in this study region, although the overall effects of shifting $\delta^{15}N$ baselines are associated with decreasing $\delta^{15}N$ on a broader scale (Diebel & Vander Zanden 2009; Holtgrieve et al. 2011). Therefore, forest habitat may be associated with lower nitrogen deposition, which could further explain the relationships between increasing percentage of forest and decreasing $\delta^{15}N$ values for big brown bats as well as the relationships between increasing percentage of open water and increasing $\delta^{15}N$ values for little brown bats.

We attempted to further explain the mechanism behind changes in isotopic niche breadth and overlap in little brown and big brown bats by connecting isotope values with corresponding climate and temporal variables. Higher annual precipitation was associated with slightly higher values of $\delta^{13}C$ among little brown bats, which is consistent with previous studies that have documented little brown bat exploitation of aquatic insects and dietary composition associations with precipitation (Whitaker 2004; Moosman et al. 2012). However, seasonal or spatial variables such as the zone of collection, year, and Julian week were generally better predictors of the isotope signatures present in bat tissues than climate variables such as precipitation and temperature. These results are likely related to the climate of the Upper Midwest region, which experienced decreasing annual precipitation from the late 1800s through the 1930s followed by an increase through the present, but typically includes frequent summer rainfall (Andresen et al. 2012). While the effects of climate and vegetation are interrelated and therefore remain difficult to disentangle, these results further support our conclusion that the observed

shifts in bat isotopic niches are more immediately related to changes in vegetation, rather than to shifts in isotopic baselines.

By comparing stable isotope values from different tissues from the same individuals, we demonstrate that seasonal isotopic niches (represented by hair keratin) and long-term isotopic niches (represented by bone collagen) were reasonably consistent for both bat species. For big brown bats, the isotopic niche breadth estimated from hair keratin and bone collagen were similar. For little brown bats, the isotopic niche breadth from hair keratin was slightly broader, suggesting that this species may have some degree of seasonal isotopic variation in their diet. In comparison to the interspecific niche overlap calculated from bone samples, the interspecific niche overlap calculated from hair samples was higher by 6%, suggesting that calculating niche overlap from hair samples may slightly overestimate longer-term interspecific niche overlap. The tissue-specific differences in $\delta^{13}C$ and $\delta^{15}N$ values from this study were also similar to previously published reports (O'Connell et al. 2001; O'Regan et al. 2008). While sampling of historical bone tissue for small-bodied species is overly destructive and therefore impractical for the use of museum specimens, the observed similarity in niche breadth and overlap present in hair keratin and bone collagen from the same individuals suggests that at the population level, hair keratin can still adequately reflect niche breadth and overlap as inferred from bulk stable isotope values.

Multiple recent studies on other predators have demonstrated the effects of human-mediated land-use changes on isotopic niches (e.g., Hobart et al. 2019; Manlick et al. 2019). For arthropodivores and other highly generalist species, studies using bulk stable isotopes can be more difficult since mixing models may be impossible or unreasonable to develop if prey are not isotopically differentiable. Methodological limitations related to baselines shifts in atmospheric $\delta^{13}C$ and $\delta^{15}N$ values also influence how stable isotope data should be interpreted (Casey & Post 2011; Shipley & Matich 2020). While modern changes in $\delta^{13}C$ baselines due to carbon emissions have been fairly well quantified and

can be corrected for mathematically, baseline shifts of $\delta^{15}N$ values, which can be affected by nitrogen deposition as well as other processes such fire and grazing, are less straightforward. With newer tools such as compound-specific stable isotope analyses, it may be possible to better understand changes in the trophic ecology of arthropodivores (e.g., Campbell et al. 2017). Nonetheless, we propose that future studies using stable isotopes as well as other methods such as DNA-based diet analysis continue to consider how contemporary patterns in bat foraging may be constrained by anthropogenically-driven habitat changes in the recent past.

The result from this study have several key implications for applied ecology. In temperate areas, the acquisition of adequate food resources is particularly crucial for winter survival in hibernating bats, and body condition has also been shown to influence white-nose syndrome survival (Speakman & Racey 1989; Jonasson & Willis 2011; Cheng et al. 2019). For little brown bats and other small-bodied hibernating bats, which suffer particularly high mortality from white-nose syndrome (Frick et al. 2010; Frank et al. 2014), understanding how reliance on arthropods in a homogenous landscape, presumably with lower abundance and diversity of prey, may influence survival and reproduction also remains an important future question in bat conservation. In addition to reflecting underlying arthropod communities, characterizing changes in arthropodivorous bat diets over time therefore has clear conservation implications for the support of arthropods and their predators. For example, our results suggest that the loss of landcover diversity is related to increasingly interspecific dietary niche overlap, which warrants future investigation into the potential benefits of restoring historically prevalent habitat such as savanna and prairie. Although it has been previously shown that natural habitats promote bat activity in several agriculturally-dominated systems (e.g., Kelly et al. 2016; Kahnonitch et al. 2016; Olimpi & Philpott 2018), further defining region- and species-specific habitat and prey requirements may provide actionable conservation management solutions for mitigating bat population declines. Since aerial arthropodivores, including bats and birds, face many conservation

challenges on a global scale, continuing to investigate the effects of legacies of land-use change will lead to a better understanding of factors that constrain contemporary populations of these threatened taxa.

Acknowledgements

The authors thank The Field Museum, the Smithsonian National Museum of Natural History, the University of Wisconsin-Madison Zoological Museum, the USGS National Wildlife Health Center, and the Wisconsin Department of Natural Resources for providing samples. Specifically, we thank Anne Ballmann, Nicole Edmison, Adam Ferguson, Paula Holahan, Heather Kaarakka, Darrin Lunde, Bruce Patterson, and J. Paul White for their assistance with sample acquisition. We thank Monika Shea and David Mladenoff for providing the Finley 1976 data layer. We also thank Brendan Hobart, Phil Manlick, and Jonathan Pauli for valuable discussions contributing to the development of this study.

References

Agosta, S. J. (2002). Habitat use, diet and roost selection by the big brown bat (*Eptesicus fuscus*) in North America: a case for conserving an abundant species. *Mammal Review*, *32*(3), 179-198.

Andresen J., Hilberg, S., & Kunkel, K. (2012). Historical climate and climate trends in the Midwestern USA. *US National Climate Assessment Midwest Technical Input Report*:1-18.

Azevedo, F., Lester, V., Gorsuch, W., Lariviere, S., Wirsing, A., & Murray, D. (2006). Dietary breadth and overlap among five sympatric prairie carnivores. *Journal of Zoology, 269*(1), 127-135.

Bartoń, K. (2015). *MuMIn: Model selection and model averaging based on information criteria (AICc and alike)*. R package version 1.15.1.

Ben-David, M., & Flaherty, E. A. (2012). Stable isotopes in mammalian research: a beginner's guide. *Journal of Mammalogy*, *93*(2), 312-328.

Benton, T .G., Bryant, D. M., Cole, L., & Crick, H. Q. (2002). Linking agricultural practice to insect and bird populations: a historical study over three decades. *Journal of Applied Ecology, 39*(4), 673-687.

Blight, L. K., Hobson, K. A., Kyser, T. K., & Arcese, P. (2015). Changing gull diet in a changing world: a 150-year stable isotope (δ13C, δ15N) record from feathers collected in the Pacific Northwest of North America. *Global Change Biology, 21*(4), 1497-1507.

Boyles, J. G., & Storm, J. J. (2007). The perils of picky eating: dietary breadth is related to extinction risk in insectivorous bats. *PLoS One, 2*(7), e672.

Brickner, K. M., Grenier, M. B., Crosier, A. E., & Pauli, J. N. (2014). Foraging plasticity in a highly specialized carnivore, the endangered black-footed ferret. *Biological Conservation*, 169, 1-5.

Broders, H. G., Farrow, L. J., Hearn, R. N., Lawrence, L. M., & Forbes, G. J. (2014). Stable isotopes reveal that little brown bats have a broader dietary niche than northern long-eared bats. *Acta Chiropterologica, 16*(2), 315-325.

Campbell, C. J., Nelson, D. M., Ogawa, N. O., Chikaraishi, Y., & Ohkouchi, N. (2017). Trophic position and dietary breadth of bats revealed by nitrogen isotopic composition of amino acids. *Scientific Reports, 7*(1), 1-9.

Carbonell Ellgutter, J. A., Ehrich, D., Killengreen, S. T., Ims, R. A., & Unnsteinsdóttir, E. R. (2020). Dietary variation in Icelandic arctic fox (*Vulpes lagopus*) over a period of 30 years assessed through stable isotopes. *Oecologia,* 192, 403-414.

Casey, M. M., & Post, D. M. (2011). The problem of isotopic baseline: reconstructing the diet and trophic position of fossil animals. *Earth-Science Reviews, 106*(1-2), 131-148.

Chamberlain, C.P., Waldbauer, J. R., Fox-Dobbs, K., Newsome, S. D., Koch, P. L., Smith, D. R., Church, M. E., Chamberlain, S. D., Sorenson, K. J., & Risebrough, R. (2005). Pleistocene to recent dietary shifts in California condors. *Proceedings of the National Academy of Sciences, 102*(46), 16707-16711.

Cheng, T. L., Gerson, A., Moore, M. S., Reichard, J. D, DeSimone, J., Willis, C. K., Frick, W. F., & 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., Symondson, W. O., Broders, H., Fabianek, F., Fraser, E. E., MacKenzie, A., Boughen, A., Hamilton, R., Willis, C. K., Martinez-Nuñez, F., & Menzies, A. K. (2014a). The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. *Molecular Ecology, 23*(15), 3618-3632.

Clare, E. L., Symondson, W. O. C., & Fenton, M. B. (2014b). An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). *Molecular Ecology, 23*(15), 3633-3647.

Cryan, P. M., Bogan, M. A., Rye, R. O., Landis, G. P., & Kester, C. L. (2004). Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. *Journal of Mammalogy 85*(5), 995-1001.

Davidai, N., Westbrook, J. K., Lessard, J. P., Hallam, T. G., & McCracken, G. F. (2015). The importance of natural habitats to Brazilian free-tailed bats in intensive agricultural landscapes in the Winter Garden region of Texas, United States. *Biological Conservation,* 190, 107-114.

De Caceres, M., & Jansen, F. (2016). *Indicspecies: Relationship between species and groups of sites.* R package version 1.7.6.

Diebel, M. W., & Vander Zanden, M. J. (2009). Nitrogen stable isotopes in streams: effects of agricultural sources and transformations. *Ecological Applications, 19*(5), 1127-1134.

Ellison, L. (2008). Summary and analysis of the US Government bat banding program: *U.S. Geological Survey Open-File Report 2008*, 1363:1-117.

Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., Carpenter, S. R., Essington, T. E., Holt, R. D., Jackson, J. B., & Marquis, R. J. (2011). Trophic downgrading of planet Earth. *Science, 333*(6040), 301-306

Farmer, R., & Leonard, M. (2011). Long-term feeding ecology of Great Black-backed Gulls (*Larus marinus*) in the northwest Atlantic: 110 years of feather isotope data. *Canadian Journal of Zoology 89*(2), 123-133

Fenton, M. B., & Fleming, T. (1976). Ecological interactions between bats and nocturnal birds. *Biotropica*, 104-110

Finley, R. (1976). Original vegetation cover of Wisconsin, compiled from US General Land Office notes. University of Wisconsin-Extension, Map (1: 500 000), Madison, Wisconsin.

Flaherty, E. A., & Ben-David, M. (2010). Overlap and partitioning of the ecological and isotopic niches. *Oikos, 119*(9), 1409-1416.

Frank, C. L., Michalski, A., McDonough, A. A., Rahimian, M., Rudd, R. J., & Herzog, C. (2014). The resistance of a North American bat species (*Eptesicus fuscus)* to white-nose syndrome (WNS). *PLoS One, 9*(12), e113958.

Frick, W. F., Pollock, J. F., Hicks, A. C., Langwig, K. E., Reynolds, D. S., Turner, G. G., Butchkoski, C. M., & Kunz, T. H. (2010). An emerging disease causes regional population collapse of a common North American bat species. *Science, 329*(5992), 679-682.

Gallant, A. L., Sadinski, W., Roth, M. F., & Rewa, C. A. (2011). Changes in historical Iowa land cover as context for assessing the environmental benefits of current and future conservation efforts on agricultural lands. *Journal of Soil and Water Conservation, 66*(3), 67A-77A.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., & Goulson, D. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PloS One, 12*(10), e0185809.

Hette-Tronquart, N. (2019). Isotopic niche is not equal to trophic niche. *Ecology Letters, 22*(11), 1987-1989.

Ho, J., Tumkaya, T., Aryal, S., Choi, H., & Claridge-Chang, A. (2019). Moving beyond P values: data analysis with estimation graphics. *Nature Methods, 16*(7), 565-566.

Hobart, B. K., Jones, G. M., Roberts, K. N., Dotters, B. P., Whitmore, S. A., Berigan, W. J., Raphael, M. G., Keane, J. J., Gutiérrez, R. J., & Peery, M. Z. (2019). Trophic interactions mediate the response of predator populations to habitat change. *Biological Conservation*, 238, 108217.

Holtgrieve, G. W., Schindler, D. E., Hobbs, W. O., Leavitt, P. R., Ward, E. J., Bunting, L., Chen, G., Finney, B. P., Gregory-Eaves, I., Holmgren, S., & Lisac, M. J. (2011). A coherent signature of anthropogenic nitrogen deposition to remote watersheds of the northern hemisphere. *Science, 334*(6062), 1545-1548.

Iverson, L. R. (1988). Land-use changes in Illinois, USA: the influence of landscape attributes on current and historic land use. *Landscape Ecology, 2*(1), 45-61.

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology, 80*(3), 595-602.

Jonasson, K. A., & Willis, C. K. (2011). Changes in body condition of hibernating bats support the thrifty female hypothesis and predict consequences for populations with white-nose syndrome. *PLoS One, 6*(6), e21061.

Julliard, R., Jiguet, F., & Couvet, D. (2004). Common birds facing global changes: what makes a species at risk? *Global Change Biology, 10*(1), 148-154.

Kahnonitch, I., Lubin, Y., & Korine, C. (2018). Insectivorous bats in semi-arid agroecosystems−effects on foraging activity and implications for insect pest control. *Agriculture, Ecosystems & Environment,* 261, 80-92.

Kelly, R. M., Kitzes, J., Wilson, H., & Merenlender, A. (2016). Habitat diversity promotes bat activity in a vineyard landscape. *Agriculture, Ecosystems & Environment*, 223, 175-181.

Kirby, R., Alldredge, M. W., & Pauli, J. N. (2016). The diet of black bears tracks the human footprint across a rapidly developing landscape. *Biological Conservation*, 200, 51-59.

Kucharik, C. J., & Ramankutty, N. (2005). Trends and variability in US corn yields over the twentieth century. *Earth Interactions, 9*(1), 1-29.

Kunz, T. H., de Torrez, E. B., Bauer, D., Lobova, T., & Fleming, T. H. (2011). Ecosystem services provided by bats. *Annals of the New York Academy of Sciences, 1223*(1), 1-38.

Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., Matich, P., Rosenblatt, A. E., Vaudo, J. J., Yeager, L. A., & Post, D. M. (2012). Applying stable

isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews, 87*(3), 545-562.

Manlick, P. J., Petersen, S. M., Moriarty, K. M., & Pauli, J. N. (2019). Stable isotopes reveal limited Eltonian niche conservatism across carnivore populations. *Functional Ecology, 33*(2), 335-345.

Manlick, P. J., Woodford, J. E., Zuckerberg, B., & Pauli, J. N. (2017). Niche compression intensifies competition between reintroduced American martens (*Martes americana*) and fishers (*Pekania pennanti*). *Journal of Mammalogy, 98*(3), 690-702.

Marshall, H. H., Inger, R., Jackson, A. L., McDonald, R. A., Thompson, F. J., & Cant, M. A. (2019). Stable isotopes are quantitative indicators of trophic niche. *Ecology Letters, 22*(11), 1990-1992.

Matson, P. A., Parton, W. J., Power, A., & Swift, M. (1997). Agricultural intensification and ecosystem properties. *Science, 277*(5325), 504-509.

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.

Michelson, C. I., Clark, R. G., & Morrissey, C. A. (2018). Agricultural land cover does not affect the diet of Tree Swallows in wetland-dominated habitats. *The Condor: Ornithological Applications, 120*(4), 751-764.

Mooney, K. A., Gruner, D. S., Barber, N. A., Van Bael, S. A., Philpott, S. M., & Greenberg, R. (2010). Interactions among predators and the cascading effects of vertebrate insectivores on arthropod communities and plants. *Proceedings of the National Academy of Sciences, 107*(16), 7335-7340.

Moosman, 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.

Moss, W. E., Alldredge, M. W., Logan, K. A., & Pauli, J. N. (2016). Human expansion precipitates niche expansion for an opportunistic apex predator (*Puma concolor*). *Scientific Reports,* 6, 39639.

Murray, M., Cembrowski, A., Latham, A. D., Lukasik, V. M., Pruss, S., & St Clair, C. C. (2015). Greater consumption of protein-poor anthropogenic food by urban relative to rural coyotes increases diet breadth and potential for human–wildlife conflict. *Ecography, 38*(12), 1235-1242.

Nebel, S., Mills, A., McCracken, J., & Taylor, P. (2010). Declines of aerial insectivores in North America follow a geographic gradient. *Avian Conservation and Ecology, 5*(2), 1.

Newsome, S. D., Martinez del Rio, C., Bearhop, S., & Phillips, D. L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and the Environment, 5*(8), 429-436.

NOAA. (2018). National Centers for Environmental Information, Climate at a Glance: Divisional Time Series.

Nocera, J. J., Blais, J. M., Beresford, D. V., Finity, L. K., Grooms, C., Kimpe, L. E., Kyser, K., Michelutti, N., Reudink, M. W., & Smol, J. P. (2012). Historical pesticide applications coincided with an altered diet of aerially foraging insectivorous chimney swifts. *Proceedings of the Royal Society B: Biological Sciences, 279*(1740), 3114-3120.

O'Connell ,T. C., Hedges, R. E., Healey, M., & Simpson, A. (2001). Isotopic comparison of hair, nail and bone: modern analyses. *Journal of Archaeological Science, 28*(11), 1247-1255.

Olimpi, E. M., & Philpott, S. M. (2018). Agroecological farming practices promote bats. *Agriculture, Ecosystems & Environment,* 265, 282-291.

O'Regan, H. J., Chenery, C., Lamb, A. L., Stevens, R. E., Rook, L., & Elton, S. (2008). Modern macaque dietary heterogeneity assessed using stable isotope analysis of hair and bone. *Journal of Human Evolution, 55*(4), 617-626.

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics, 18*(1), 293-320.

Racey, P. A., & Entwistle, A. C. (2005). Conservation Ecology of Bats. In *Bat Ecology* (pp 680-722). University of Chicago Press.

Rhemtulla, J. M., Mladenoff, D. J., & Clayton, M. K. (2007). Regional land-cover conversion in the US upper Midwest: magnitude of change and limited recovery (1850–1935–1993). *Landscape Ecology, 22*(1), 57-75.

Rhemtulla, J. M., Mladenoff, D. J., & Clayton, M. K. (2009). Legacies of historical land use on regional forest composition and structure in Wisconsin, USA (mid-1800s–1930s–2000s). *Ecological Applications, 19*(4), 1061-1078.

Ritchie, E. G., Elmhagen, B., Glen, A. S., Letnic, M., Ludwig, G., & McDonald, R. A. (2012). Ecosystem restoration with teeth: what role for predators? *Trends in Ecology & Evolution, 27*(5), 265-271.

Sánchez-Bayo, F., & Wyckhuys, K.A. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, 232, 8-27.

Schulte, L. A., & Mladenoff, D. J. (2001). The original US public land survey records: their use and limitations in reconstructing presettlement vegetation. *Journal of Forestry, 99*(10), 5-10.

Shipley, O. N., & Matich, P. (2020). Studying animal niches using bulk stable isotope ratios: an updated synthesis. *Oecologia,* 193, 27-51.

Speakman, J. R., & Racey, P. A. (1989). Hibernal ecology of the pipistrelle bat: energy expenditure, water requirements and mass loss, implications for survival and the function of winter emergence flights. *Journal of Animal Ecology, 58*(3), 797-813.

Spiller, K. J., & Dettmers, R. (2019). Evidence for multiple drivers of aerial insectivore declines in North America. *The Condor, 121*(2), duz010.

Stanton, R. L., Morrissey, C. A., & Clark, R. G. (2016). Tree Swallow (*Tachycineta bicolor*) foraging responses to agricultural land use and abundance of insect prey. *Canadian Journal of Zoology, 94*(9), 637-642.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature, 418*(6898), 671-677.

Torchiano, M. (2020). *Effsize: Efficient effect size estimate.* R package version 0.8.0.

Tscharntke, T., Klein, A. M., Kruess, A., Steffan-Dewenter, I., & Thies, C. (2005). Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. *Ecology Letters, 8*(8), 857-874.

Verburg, P. (2007). The need to correct for the Suess effect in the application of δ13C in sediment of autotrophic Lake Tanganyika, as a productivity proxy in the Anthropocene. *Journal of Paleolimnology, 37*(4), 591-602.

Whiles, M. R., & Charlton, R. E. (2006). The ecological significance of tallgrass prairie arthropods. *Annual Review of Entomology, 51,* 387-412.

Whitaker, J. O. (2004). Prey selection in a temperate zone insectivorous bat community. *Journal of Mammalogy, 85*(3), 460-469

Williams-Guillén, K., Olimpi, E., Maas, B., Taylor, P. J., & Arlettaz, R. (2016). Bats in the anthropogenic matrix: challenges and opportunities for the conservation of Chiroptera and their ecosystem services in agricultural landscapes. In *Bats in the Anthropocene: Conservation of bats in a changing world* (pp 151-186). Springer.

Williams-Guillén, K., Perfecto, I., & Vandermeer, J. (2008). Bats limit insects in a neotropical agroforestry system. *Science, 320*(5872), 70.

Wisconsin Department of Natural Resources. (2016). Wisconsin Wiscland 2.0 Land Cover Database Levels 1-4, 2016.

Wurster, C. M., McFarlane, D. A., Bird, M. I., Ascough, P., & Athfield, N. B. (2010). Stable isotopes of subfossil bat guano as a long-term environmental archive: insights from a Grand Canyon cave deposit. *Journal of Cave and Karst Studies, 72*(2), 111-121.

Table 1a. Unpaired mean difference and 95% confidence intervals for bat hair from different time periods.

	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
	$\bar{x}_{contemp} - \bar{x}_{hist}$	lower CI	upper CI	$\bar{x}_{contemp} - \bar{x}_{hist}$	lower CI	upper CI
EPFU, contemporary vs. historical (pre-1973)	–0.93	–1.71	–0.08	–1.25	–1.99	–0.51
MYLU, contemporary vs. historical (pre-1973)	1.27	0.38	2.15	–0.64	–1.56	0.29
MYLU, contemporary vs. historical (pre-1935)	1.52	0.53	2.52	–0.34	–1.29	0.63

Table 1b. Area metrics for hair (carcasses and museum specimens)

	n	TA	SEA	SEAc	95% ML ellipse area
EPFU, historical	19	17.25	6.36	6.74	40.35
EPFU, contemporary	54	38.30	9.60	9.79	58.59
MYLU, historical	65	77.40	15.64	15.89	95.15
MYLU, contemporary	33	45.39	15.08	15.56	93.18

Table 1c. Area metrics for paired hair/bone samples from the same individuals (carcasses only)

	n	TA	SEA	SEAc	95% ML ellipse area
EPFU, bone	40	38.89	10.45	10.72	64.21
EPFU, hair	40	34.11	9.44	9.69	57.99
MYLU, bone	24	26.69	9.76	10.20	61.09
MYLU, hair	24	38.85	14.26	14.91	89.26

Table 2. Model averaging results for the top 5 models for each analysis.

Landcover models (*n*=52)	logLik	AIC*c*	Δ	weight
$\delta^{13}C$ ~ species+species:C3 grass+species:C4 grass	-58.07	132.69	0	0.2
$\delta^{13}C$ ~ species+species:C4 grass	-60.82	132.94	0.25	0.18
$\delta^{13}C$ ~ species+species:corn+species:C4 grass	-58.2	132.94	0.25	0.18
$\delta^{13}C$ ~ species+species:pasture+species:C4 grass	-59.3	135.14	2.45	0.06
$\delta^{13}C$ ~ species+species:forest+species:C4 grass	-59.63	135.81	3.12	0.04
$\delta^{15}N$ ~ species+species:open water+species:pasture+species:forest	-49.32	120.92	0	0.34
$\delta^{15}N$ ~ species+species:open water+species:developed+species:forest	-50.32	122.93	2.01	0.12
$\delta^{15}N$ ~ species+species:open water+species:forest+species:wetland	-50.52	123.32	2.4	0.1
$\delta^{15}N$ ~ species+species:open water+species:forest	-53.71	123.96	3.05	0.07
$\delta^{15}N$ ~ species+species:forest+species:wetland+species:C4 grass	-50.87	124.02	3.11	0.07

Climate & temporal models (*n*=163)	logLik	AIC*c*	Δ	weight
$\delta^{13}C$ ~ species+species:year+species:zone	-188.7	392.12	0	0.7
$\delta^{13}C$ ~ species+species:precip+species:zone	-190.51	395.74	3.63	0.11
$\delta^{13}C$ ~ species+species:temp+species:year+species:zone	-188.52	396.22	4.11	0.09
$\delta^{13}C$ ~ species+species:year	-193.43	397.25	5.13	0.05
$\delta^{13}C$ ~ species+species:precip+species:temp+species:zone	-190.22	399.61	7.49	0.02
$\delta^{15}N$ ~ species+species:zone	-200.64	411.66	0	0.35
$\delta^{15}N$ ~ species+species:year+species:zone	-198.8	412.32	0.66	0.26
$\delta^{15}N$ ~ species+species:week+species:zone	-199.5	413.72	2.06	0.13
$\delta^{15}N$ ~ species+species:precip+species:zone	-199.92	414.55	2.89	0.08
$\delta^{15}N$ ~ species+species:temp+species:zone	-200.11	414.94	3.28	0.07

Figure Legends

Figure 1. Historical and contemporary landcover composition in Wisconsin, USA. Map inset shows the location of the total study area within the continental United States, with Wisconsin shaded in dark gray and Iowa and Illinois in light gray. (a) Historical vegetation from 1832-1866, adapted from Finley 1976. Open circles on map indicate 5km buffers surrounding the collection sites for little brown bat samples collected prior to 1940 (*n*=27 samples). (b) Contemporary vegetation from 2011–2019, adapted from Wiscland 2.0. Open circles on map indicate landcover within 5km buffers surrounding the collection sites for little brown and big brown bat samples collected from 2008-2019 (*n*=52 samples). The bar plots below each map shows the ranges and median values of major landcover categories within buffers, separated by bat species. EPFU=big brown bat, MYLU=little brown bat.

Figure 2. Stable isotope values present in bat tissues. (a) Changes in $\delta^{13}C$ and $\delta^{15}N$ values over time for hair keratin from bat specimens collected in Wisconsin, Iowa, and Illinois, USA, 1898-2019 (*n*=171). (b) 95% maximum likelihood ellipses for historical (*n*=84) and contemporary (*n*=87) hair keratin. (c) 95% maximum likelihood ellipses for contemporary bone collagen and hair keratin. Gray lines indicate tissue sample pairs from the same individual specimens (*n*=64). EPFU=big brown bat, MYLU=little brown bat.

Figure 3. Influences of landcover, climate, and temporal variables as predictors of observed variation in $\delta^{13}C$ and $\delta^{15}N$ values from bat hair samples. (a) Landcover models for the subset of contemporary samples with available specific collection locality information (*n*=52). (b) Climate and temporal models for historical and contemporary samples with available year, Julian week, and collection zone information (*n*=163). Standardized effect sizes represent the model-averaged slope and 95% confidence interval associated with each variable. Open triangles indicate non-overlap with zero,

closed circles indicate overlap with zero. Purple indicates positive mean effect size and red indicates negative mean effect size. EPFU=big brown bat, MYLU=little brown bat.

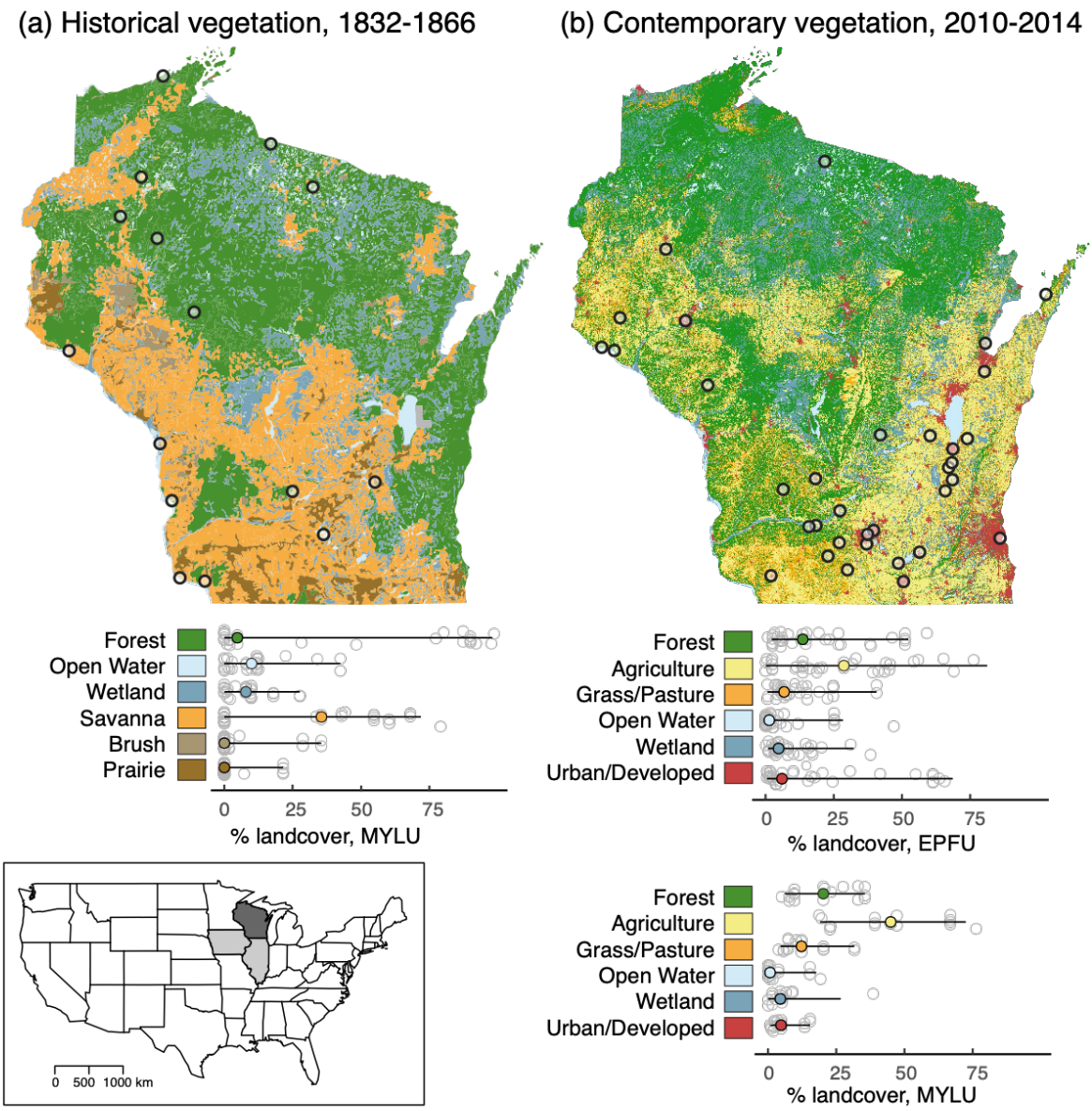

(a) Historical vegetation, 1832-1866
(b) Contemporary vegetation, 2010-2014
Forest
Open Water
Wetland
Savanna
Brush
Prairie
0
25
50
75
% landcover, MYLU
Forest
Agriculture
Grass/Pasture
Open Water
Wetland
Urban/Developed
0
25
50
75
% landcover, EPFU
0 500 1000 km
Forest
Agriculture
Grass/Pasture
Open Water
Wetland
Urban/Developed
0
25
50
75
% landcover, MYLU

Figure 1

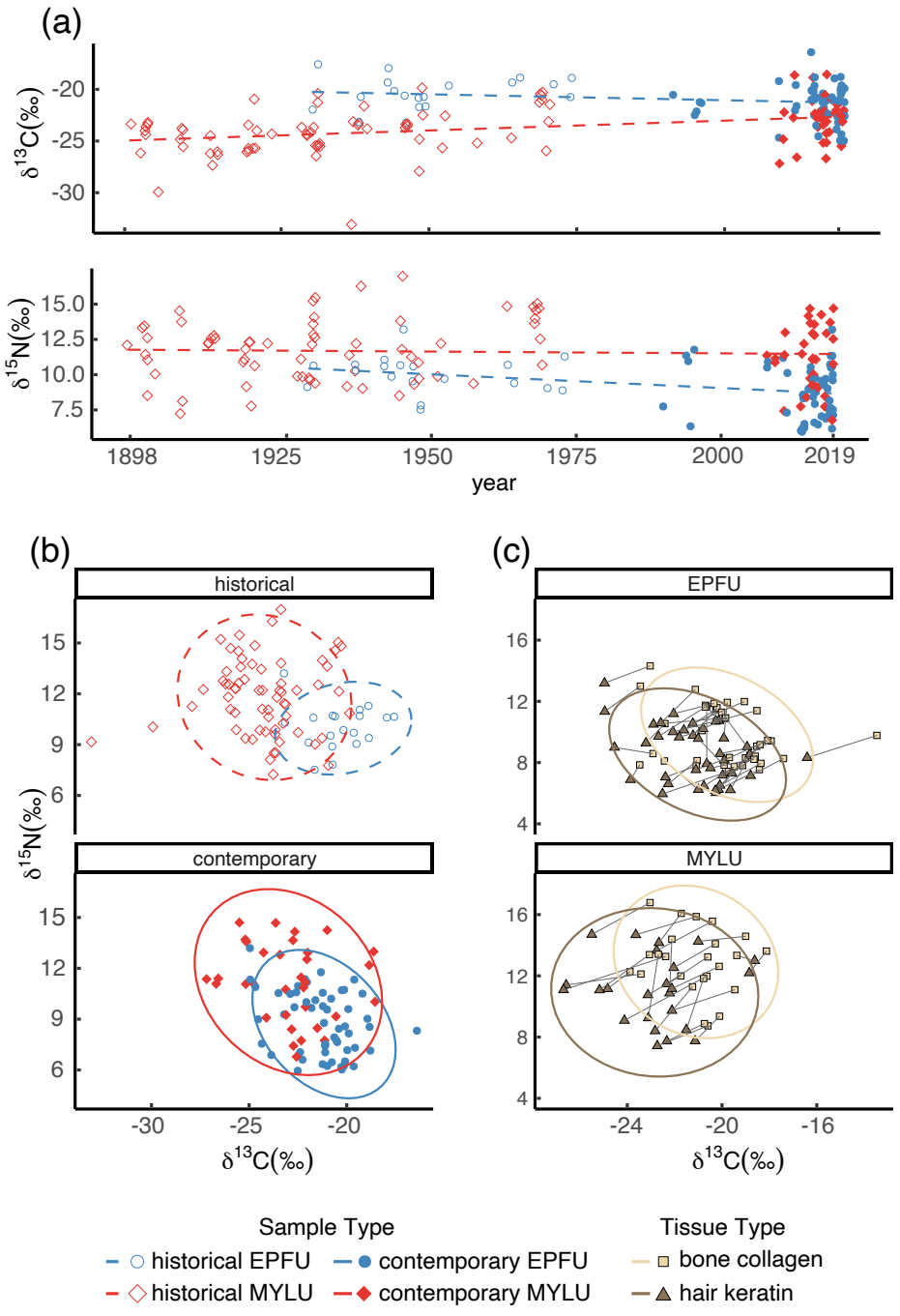

(a)
δ13C(‰)
-20
-25
-30
δ15N(‰)
15.0
12.5
10.0
7.5
1898
1925
1950
1975
2000
2019
year
(b)
historical
contemporary
δ15N(‰)
15
12
9
6
-30
-25
-20
δ13C(‰)
(c)
EPFU
MYLU
16
12
8
4
-24
-20
-16
δ13C(‰)
Sample Type
historical EPFU
contemporary EPFU
historical MYLU
contemporary MYLU
Tissue Type
bone collagen
hair keratin

Figure 2

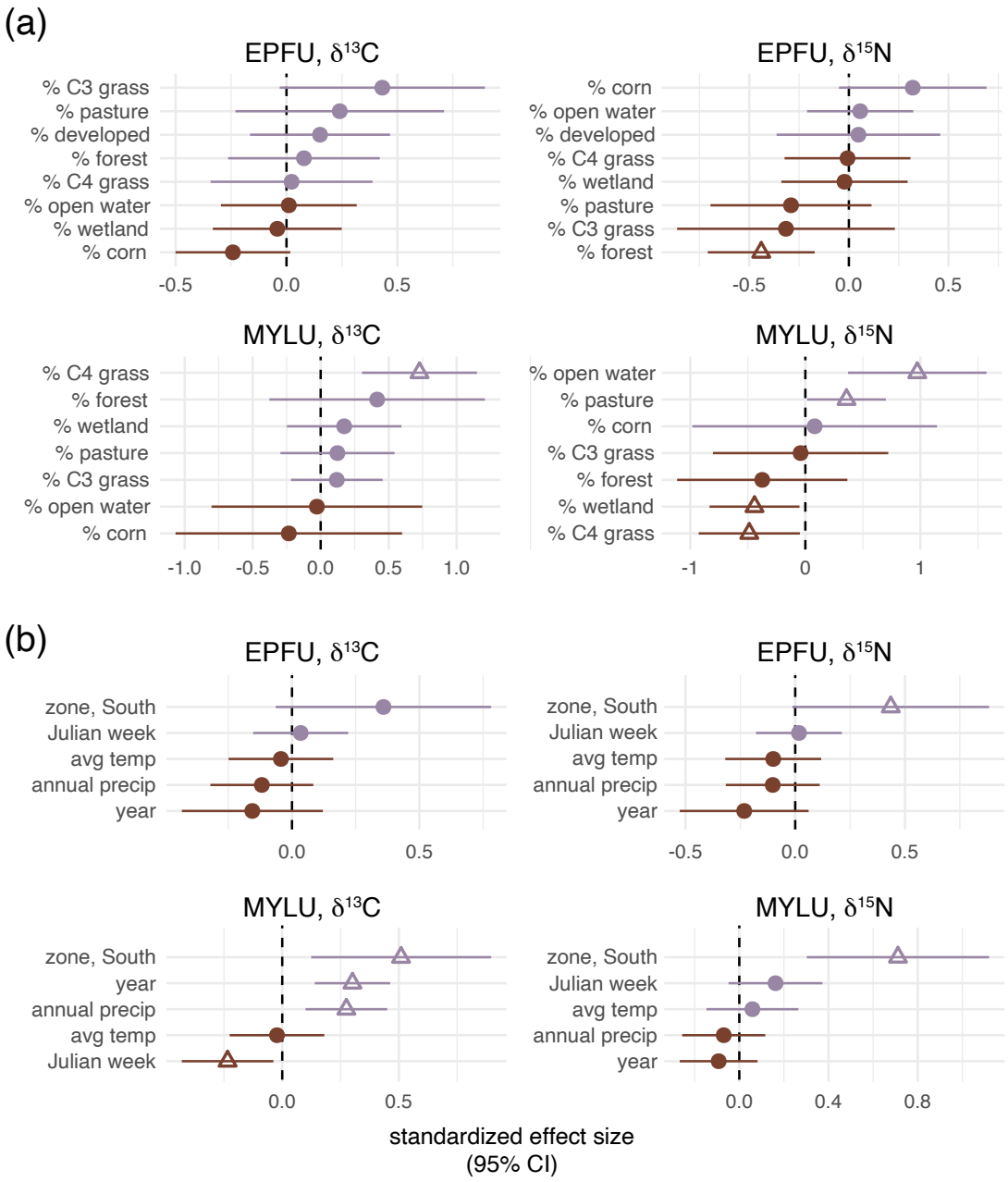

(a)
EPFU, δ13C
% C3 grass
% pasture
% developed
% forest
% C4 grass
% open water
% wetland
% corn
-0.5
0.0
0.5
EPFU, δ15N
% corn
% open water
% developed
% C4 grass
% wetland
% pasture
% C3 grass
% forest
-0.5
0.0
0.5
MYLU, δ13C
% C4 grass
% forest
% wetland
% pasture
% C3 grass
% open water
% corn
-1.0
-0.5
0.0
0.5
1.0
MYLU, δ15N
% open water
% pasture
% corn
% C3 grass
% forest
% wetland
% C4 grass
-1
0
1
(b)
EPFU, δ13C
zone, South
Julian week
avg temp
annual precip
year
0.0
0.5
EPFU, δ15N
zone, South
Julian week
avg temp
annual precip
year
-0.5
0.0
0.5
MYLU, δ13C
zone, South
year
annual precip
avg temp
Julian week
0.0
0.5
MYLU, δ15N
zone, South
Julian week
avg temp
annual precip
year
0.0
0.4
0.8
standardized effect size
(95% CI)

Figure 3

Supplementary Materials

Table S1. Historical & contemporary landcover categories used to compare habitat changes between time periods.

(a). Historical landcover categories: Finley 1976

Variable	Class ID	Definition
Forest	1	White spruce, balsam fir, tamarack, white cedar, white birch, aspen
	2	Beech, hemlock, sugar maple, yellow birch, white pine, red pine
	3	Hemlock, sugar maple, yellow birch, white pine, red pine
	4	Sugar maple, yellow birch, white pine, red pine
	7	Aspen, white birch, pine
	8	Beech, sugar maple, basswood, red oak white oak, black oak
	9	Sugar maple, basswood, red oak, white oak, black oak
Open Water	97	Open water
Wetland	14	Swamp conifers: white cedar, black spruce, tamarack, hemlock
	15	Lowland hardwoods: willow, soft maple, box elder, ash, elm, cottonwood, river birch
	16	Marsh and sedge meadow, wet prairie, lowland shrubs
Savanna	6	Jack pine, scrub (Hill's), oak forests & barrens
	10	Oak: white oak, black oak, bur oak
	11	Oak openings: bur oak, white oak, black oak
Brush	13	Brush
Prairie	12	Prairie

(b). Contemporary landcover categories: Wiscland 2.0, Level 1

Variable	Class ID	Definition
Forest	4000	Upland area with woody perennial plants, the trees reaching a mature height of >6 feet with definite crown (closure of ≥10%).
Agriculture	2000	Land under cultivation for food or fiber.
Grass/Pasture	3000	Non-cultivated herbaceous vegetation dominated by perennial grasses.
Open Water	5000	Areas of water with no vegetation present.
Wetland	6000	Water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, with wet soils.
Urban/Developed	1000	Structures/areas associated with intensive human activity and land use.

(c). Contemporary landcover categories: Wiscland 2.0, Level 2

Variable	Class ID	Definition
Developed, High	1100	≥50% solid impervious cover of man-made materials.
Developed, Low	1200	≥25% but <50% solid impervious cover of man-made materials.
Forage Grassland	3100	Perennial herbaceous vegetation used for livestock forage/grazing.
Idle Grassland	3200	Perennial herbaceous vegetation NOT for livestock forage/grazing.
Coniferous Forest	4100	Canopies with distinct crown closure ≥67% coniferous tree group. Broad-leaved deciduous species group <33% of canopy. Examples: jack pine, red pine, white spruce, hemlock, tamarack.
Deciduous Forest	4200	Canopies with distinct crown closure ≥67% broad-leaved deciduous tree group. Coniferous species group <33% of canopy. Examples: aspen, oak, maple, birch, balsam poplar.
Mixed Forest	4300	Canopy with distinct crown closure, ≤67% coniferous or deciduous.
Open Water	5000	Areas of water with no vegetation present.
Aquatic Herbaceous	6100	Floating herbaceous plants growing entirely on or in a water body and covering ≥30% of the area.
Wet Meadow	6200	Herbaceous plants above the surface of water or wet soil covering ≥30%.
Lowland Scrub/Shrub	6300	≥30% woody vegetation, <20 feet tall, tree cover <10%, in wetland areas.
Forested Wetland	6400	Wetlands dominated by woody perennial plants, canopy cover >10%, trees reaching a mature height ≥20 feet, and covering ≥30% of the area.
Barren	7000	Land of limited ability to support life, <33% vegetation or other cover.
Shrubland	8000	Vegetation with a persistent woody stem, low growth of ≤20 feet, coverage ≥33%, <10% tree cover interspersed.

Table S2. Summary & accuracy assessment of Wiscland 2.0 variables used in landcover model.

Class ID	Level	Variable	Definition	Precision (%)	Recall (%)	F (1.0)	AUC
1000	1	Urban/Developed	Structures/areas associated with intensive human activity and land use.	90.7	97.8	0.94	0.99
2120	3	Continuous Corn	Corn grain or corn silage grown every year in a 6-year rotation.	NA	NA	NA	NA
3120	3	Pasture	Lands covered by herbaceous vegetation, primarily perennial grasses, used for grazing livestock. Kentucky bluegrass is the most common pasture grass, but many other grass species are grazed. Forbs (red and white clover, yarrow, dandelion, common and giant ragweed, common mullein, wild carrot, thistles) may be present.	76.4	85.9	0.81	0.93
3210	3	Cool-season grass (C3)	Lands covered primarily by grasses and forbs, >80% of grasses are cool-season varieties, and <5% shrubs/woody vegetation cover. May be fields planted for wildlife/conservation purposes or old/idle fields.	55.0	56.8	0.56	0.78
3220	3	Warm-season grass (C4)	Lands covered primarily by grasses and forbs, >80% of grasses are native warm-season varieties, and <5% shrubs/woody vegetation cover. Fields may be heavily grass-dominated, or can contain forbs. Common grasses: big and little bluestem, switchgrass, indiangrass, side-oats grama. Common native forbs: yellow coneflower, bee balm, spiderwort, oxeye and round-headed bush clover.	62.3	90.1	0.74	0.95
4000	1	Forest	Upland area with woody perennial plants, the trees reaching a mature height of >6 feet with definite crown (closure of ≥10%).	84.2	95.0	0.89	0.95
5000	1	Open Water	Areas of water with no vegetation present.	98.7	97.4	0.98	0.99
6000	1	Wetland	Water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, with wet soils.	98.2	91.7	0.95	0.95

CHAPTER 3: BAT DECLINES FROM WHITE-NOSE SYNDROME REVEAL TOP-DOWN INFLUENCES ON ARTHROPODS

Amy K. Wray[1,] M. Zachariah Peery[1], Jade M. Kochanski[2], Emma Pelton[2,3], Daniel L. Lindner[4], and Claudio Gratton[2]

[1]Department of Forest & Wildlife Ecology, University of Wisconsin-Madison, Madison, WI, USA; [2]Department of Entomology, University of Wisconsin-Madison, Madison, WI, USA; [3]Xerces Society for Invertebrate Conservation, Portland, OR, USA; [4]Center for Forest Mycology Research, Northern Research Station, USDA Forest Service, Madison, WI, USA

ABSTRACT

Widespread declines of predators provide a powerful way to understand their influences on prey populations. In North America, white-nose syndrome has resulted in precipitous declines in hibernating bat populations, raising the question of whether these declines relieve top-down pressure and manifest in changes in arthropod abundance. The severe decline of little brown bats (*Myotis lucifugus*), in particular, provides a natural predator-removal experiment which may clarify the influences of bats on arthropod prey. During the summer period from 2015–2018, we performed intensive arthropod black-light trapping surveys, passive ultrasonic acoustic monitoring, and roost emergence counts at little brown and big brown bat (*Eptesicus fuscus*) maternity roosts in Wisconsin, USA. From 2015 to 2018, little brown bat populations at maternity roosts declined by 95%, with corresponding high-frequency acoustic activity declining by 79.1%. In comparison, big brown bat populations declined by 37.8%, but corresponding low-frequency acoustic activity did not decline. Total arthropod abundance declined consistently over the four-year period, which included declines within all major arthropod orders. Based on linear mixed effects models, certain groups of arthropods declined less over time at sites with bat maternity roosts compared to sites far from roosts with less bat activity. These results suggest that white-nose syndrome-related population declines in bats release predation pressure on some of their arthropod prey, although in this study the effects of bat declines did not outweigh the declining trend in arthropod abundance. Overall, this study provides evidence that bats have measurable top-down effects on arthropod abundance.

Significance statement

Our study is the first to examine the top-down ecological consequences of disease-related bat declines on the nocturnal arthropod food web. While we expected that arthropods would increase following bat declines, we found substantial decreases in arthropod abundance across all major orders over the

study period. However, total arthropod abundance and the abundance of certain arthropod groups declined less over time at sites near little brown bat roosts in years after little brown bat population declines. Collectively, these results demonstrate that disease-related declines in arthropodivorous bat populations have ecological consequences that include arthropod prey release on a local scale.

Introduction

Infectious diseases that impact predators can affect biological communities and ecosystems indirectly through trophic cascades (Ostfeld and Holt 2004; Collinge et al. 2008). While disease-related mortality events among some predators have demonstrated cascading effects on their respective ecosystems (Lindström et al. 1994; Montecino-Latorre et al. 2016), the effects of disease-related mortality in highly generalist predators is comparatively less well understood. Unlike specialist taxa with strong prey linkages, the effects of generalist predator loss on biological communities may be weaker and less predictable (Polis and Strong 1996; Jiang & Morin 2005). Nevertheless, despite their wider dietary niche breadth and reticulate trophic linkages, generalist predators can have important, yet complex, top-down effects on prey populations in some systems (Symondson et al. 2002; Finke & Denno 2005; Mooney et al. 2010). For example, exclusion experiments involving generalist predators have demonstrated increases in prey herbivory despite apparently weak interactions between predators and their prey (Carter & Rypstra 1995; Moran & Hurd 1997; Strong et al. 2000). In contrast, other studies have shown that the exclusion of generalist predators can lead to decreases in prey herbivory via the release of other predators (Snyder & Ives 2001; Finke & Denno 2003; Hunt-Joshi et al. 2005). Biases inherent in common methods for studying predator-prey relationships, such as those associated with exclusion experiments, food-web modeling, and determination of diet composition can also be difficult to disentangle within increasingly complex systems [e.g., Williams et al. (2002); Braley et al. (2010); Perillo et al. (2015)]. Thus, the ecological consequences of generalist predator loss — whether

due to disease or other factors — remain largely uncertain and difficult to predict without empirical evidence.

While disease-related losses in biodiversity can have conservation, public health, and economic consequences (Keesing et al. 2010; Estes et al. 2011; Dirzo et al. 2014), disease-related predator declines also provide novel opportunities for understanding the trophic effects of predators (Trewby et al. 2007). As infectious diseases often affect predator populations quickly, they can generate ecosystem-level changes on time scales that are tractable to assess. Additionally, when the disease in question is specific to a single host or a similar group of hosts, the effects of the exclusion of one predator (or a group of predators) can be detected without necessarily being confounded by environmental variables or by the unintentional exclusion of other predators. Indeed, several recent wildlife disease outbreaks, including infectious cancer in Tasmanian devils, white-nose syndrome (WNS) in North American bats, and wasting disease in sea stars meet these criteria (Bates et al. 2009; Frick et al. 2010; Hollings et al. 2016). Natural experiments using disease-related declines can also be affected by other factors such as changes in foraging behaviors or nutritional demands (Fenton and Rands 2006; Venesky et al. 2009). Nonetheless, treating disease-related population declines as a natural exclusion experiment for investigating top-down trophic effects, particularly among complex systems and/or generalist predators, offers unique circumstances for studying the influences of predators in their respective food webs.

The emergence and spread of white-nose syndrome (WNS) in North America currently threaten hibernating bats on a continent-wide scale (Frick et al. 2010). WNS results from infection with the fungus *Pseudogymnoascus destructans,* which causes torpid bats to increase their arousal, metabolism, and rates of evaporative water loss, thereby depleting energy and fluid reserves (Reeder et al. 2012; Willis 2015; McGuire et al. 2017). Since the initial detection of WNS in 2006, millions of bat deaths have been reported (Dzal et al. 2011). Despite habits that are often generalist and that include consumption

of predaceous arthropods, previous studies have shown that arthropodivores, including bats, can suppress arthropod herbivory, reduce plant damage, and indirectly increase the biomass of vegetation (Mooney et al. 2010). In agricultural areas, arthropodivorous bats in particular can limit herbivory and thereby increase crop yields via predation of crop pests (Kalka et al. 2008; McCracken et al. 2012). The natural pest suppression provided by bats has been valued nationally at $3.7–53 billion per year (Boyles et al. 2011). Though WNS has already led to local extirpation events across much of eastern North America (Frick et al. 2015), the potential loss of these ecosystem services and other top-down effects of arthropod predator declines have not been thoroughly assessed. Predicted declines among bat populations in key agricultural areas, such as the Upper Midwestern region of the United States, provide a particularly useful study system for answering questions related to the role of disease as a cause of rapid predator population declines and the potential top-down effects of generalist predator declines on complex prey communities.

Leveraging expected WNS-induced declines in bat populations in Wisconsin, we hypothesized that bats as generalist predators exert top-down effects on populations of their prey and thus shape arthropod communities. WNS was detected in Wisconsin in 2014 and consequent declines in bat populations were predicted to occur over the following 4–5 years (Wilder et al. 2011). Little brown bats (*Myotis lucifugus*, Leconte 1831) have been documented to experience severe mortality from white-nose syndrome, often in excess of 90%, whereas big brown bats (*Eptesicus fuscus*, Palisot de Beauvois 1796) are typically more resistant with much lower mortality rates (Frick et al. 2010; Frank et al. 2014). As such, we designed a quasi Before-After-Control-Impact study (sensu Green 1979) to test our hypothesis by comparing arthropod abundance with little brown and big brown bat activity throughout the summers of 2015–2018 (Figure 1). Specifically, we quantified the effects of expected bat declines on annual arthropod abundance by conducting intensive arthropod sampling at paired subsites near and far from large bat maternity roosts, with high and comparatively lower bat activity,

respectively (Figure 1A). We predicted that as little brown bat populations declined substantially from WNS, the abundance of their most common prey items would increase at roost subsites relative to paired control subsites with lower bat activity (Figure 1B & 1C). In contrast, we predicted that since big brown bat populations were expected to decline to a much lesser extent, changes in the abundance of prey consumed by big brown bats would be relatively similar between roost subsites and paired control subsites with lower bat activity.

Results and Discussion

Changes in bat roost size and acoustic activity between 2015–2018 followed our predictions that declines in little brown bat populations and corresponding high-frequency acoustic activity would be substantial, while declines in big brown bat populations and corresponding low-frequency activity would be comparatively less severe. Roost counts for little brown bats declined by 95.0% from an average of 232.8 bats per roost in 2015 (ranging from 118–409 bats) to an average of 11.6 bats per roost in 2018 (ranging from 0–50 bats), with two roosts abandoned (Figure 2A). Acoustic surveys confirmed the results of visual observations at roosts, as the mean number of high-frequency pulses declined by 79.1% ($p<0.001$) while the number of low-frequency pulses did not change significantly (Figure 2B). Observed rates of high-frequency activity declines were consistent with a previous study in New York state, which found a 78% decline in little brown bat acoustic activity (Dzal et al. 2011). Since its initial detection in Grant County, Wisconsin in 2014, white-nose syndrome was considered to have spread through the most of the southern portion of the state by 2015, and by 2016 was specifically confirmed or suspected in the counties in which 9 of 10 study sites were located (Lorch et al. 2016; Survey 2020). The observed declines in this study can therefore be largely attributed to effects from white-nose syndrome, which particularly afflicts little brown bats as well as other high-frequency echolocating bats in the study area, such as tri-colored and northern long-eared bats, although big

brown bats can also be affected to a lesser extent (Frick et al. 2010; Frank et al. 2014). Roost counts for big brown bats declined by 37.8% from an average of 152.8 bats per roost in 2015 (ranging from 22–428 bats) to an average of 95 bats per roost in 2018 (ranging from 30–180 bats; Figure 2A), but trends were influenced by declines at a single large roost and may therefore be partially related to bat movement rather than disease. Overall, the observed trends in this study are consistent with previous studies in eastern North America and highlight the escalating losses of hibernating bats as WNS continues to spread across the continent (Frick et al. 2010; Frick et al. 2015).

We found that arthropod abundance did not increase over time but rather, showed declining trends in all major arthropod orders, with total arthropod abundance declining by nearly 50% between 2015 and 2018 ($p<0.001$; Table 1). Trichoptera, Coleoptera, and Diptera were the most abundant orders and tended to decline less over time, whereas less abundant orders such as Lepidoptera, Hymenoptera, and Hemiptera declined more substantially ($p<0.001$ for all major arthropod orders; Table 1). In light of several recent studies raising alarms regarding arthropod declines resulting from a variety of factors such as insecticide use, climate change, and habitat disturbances (Hallmann et al. 2017; Sánchez-Bayo and Wyckhuys 2019; Seibold et al. 2019), the observed declining trend in abundance could represent a true decline in arthropods. However, arthropod populations tend to fluctuate cyclically, and the 4-year length of our study period may not capture the full cycle of shifts in arthropod abundance (Thomas et al. 2019; Montgomery et al. 2020). While declines were detected among all major orders between 2015 and 2018, not all groups declined consistently between years, and some groups such as Coleoptera experienced increases between certain years. As such, these trends may reflect some artefact of cyclical arthropod population fluctuations (Shortall et al. 2009). Intensive weekly sampling of arthropod communities conducted in this study could also have influenced the observed trends, though we assumed that these effects would be consistent between control and treatment subsites. Nonetheless, using paired sampling subsites with similar landscape

composition, weather, and land treatment practices at control and treatment subsites provides a study framework such that the primary difference between treatment and control subsites is related to the presence of large bat maternity roosts and corresponding higher bat activity, which we also confirmed via passive acoustic monitoring. As such, our study represents one of the most thorough attempts to characterize the relationships between bat activity and arthropod abundance.

Declining little brown bat activity and abundance due to WNS appeared to influence yearly changes in arthropod abundance. Mean total arthropod abundance were reasonably similar at control and treatment subsites in each of the four years (Figure 3A). However, after accounting for site-level variance using linear mixed effects models, some groups of arthropods were more abundant at subsites with little brown bat roosts relative to control subsites with lower levels of bat activity as disease-related bat declines progressed (Figure 3B). For several arthropod groups, post hoc tests indicated significant pairwise differences between treatment and control sites, which tended to occur during the latter years of the study after little brown bats declined in abundance (Figure 3B). Specifically, total arthropod abundance was higher at little brown bat treatment subsites in comparison to paired control subsites in 2016, and this trend was largely driven by higher abundance among Diptera, especially Chironomidae/Culicidae, which were also higher at little brown bat treatment subsites in 2017 (Figure 3B). Chironomid midges are among the most common prey of little brown bats (Belwood & Fenton 1976; Anthony & Kunz 1977; Clare et al. 2011, Clare et al. 2014a), and as such, their relative increases at little brown bat treatment subsites following bat declines may indicate a release of predation pressure. The lack of difference between control and treatment subsites in 2018 suggests that these trends do not necessarily persist in the longer term or may be irregular between years, which could be due to the presence of other predators capable of capitalizing on increases in local arthropod abundances. At little brown bat treatment subsites, total Hymenoptera were also lower in abundance in 2015 but were similar between treatment and control subsites in later years, while micromoths were

lower in abundance in 2018 and Noctuidae were lower in abundance in 2015 and 2017 (Figure 3B). Although little brown bats consume hymenopterans such as flying ants and Braconid wasps (which were the most common hymenopterans captured in black-light traps), as well as moths in the family Noctuidae, these groups are not typically among the arthropods most frequently detected in little brown bat diets and are not generally considered preferred prey (Anthony & Kunz 1977; Clare et al. 2011). In contrast, micromoths are more frequently consumed by little brown bats in certain regions (Whitaker & Lawhead 1992), but the lower abundance of micromoths in 2018 does not follow the prediction of increasing prey abundance in response to disease-related little brown bat declines. As such, while the trends observed in Diptera and specifically in Chironomidae/Culicidae at little brown bat sites may reflect a causative relationship between bat declines and changing arthropod abundance, the mechanisms underlying the observed differences in other arthropod groups are less clear. Overall, these results provide evidence that on a local scale, WNS-related declines among little brown bats appear to have top-down influences leading to an increase in the relative abundance of their most common prey.

Although big brown bat roost sizes did not decline, and low-frequency acoustic activity did not decline to the same extent as little brown bats, we also observed temporal changes in the differences between big brown bat control and treatment subsites that may suggest potential complex mechanisms underlying the influences of bats on arthropod abundance. For big brown bats, post hoc tests from linear mixed effects models demonstrated total arthropod abundance was higher at treatment subsites in comparison to paired control subsites in 2018, a trend largely driven by Hemiptera and Trichoptera, which were also more abundant at big brown bat treatment subsites in 2017 (Figure 3B). Micromoths and were less abundant at big brown bat treatment subsites in 2015, while Noctuidae were less abundant in 2017 (Figure 3B). However, for instances where big brown bat control and treatment subsites differed, the overlap between the confidence intervals from other years

remained similar, which suggests that the subsite-specific differences did not change drastically over time. Since big brown bats consume micromoths, the lack of difference in micromoth abundance between big brown bat control and treatment subsites in later years may reflect a similar indirect release of predation pressure, although the observed trend among Noctuidae is less clear since these moths are seldom reported in the diets of big brown bats (Agosta 2002; Clare et al. 2014b). Although little brown and big brown bats typically consume different prey, big brown bats have been previously reported to consume more dipteran and lepidopteran prey in regions with higher interspecific competition (Moosman et al. 2012), and one study suggested that big brown bats are at least partially capable of expanding their dietary niches following WNS-related declines in little brown bat populations (Morningstar et al. 2019). Although study sites included in the analyses met the a priori assumptions of having higher bat activity in the respective frequency group for the species of bat present at a given treatment subsite, background bat activity also remained high at all subsites. We observed increases in low-frequency acoustic activity at three of the five little brown bat sites, which may further suggest that big brown bats or other low-frequency echolocating bats increased their activity at sites formerly dominated by little brown bats and other high-frequency echolocating bats (Supplementary Figure S1). Due to the flexible foraging strategies of big brown bats, combined with their propensity to travel long distances while foraging (Brigham 1991; Henry et al. 2002), the influences of big brown bats on arthropod abundance following disease-related declines in other species are likely complex and may involve indirect interactions that are difficult to quantify. Despite the difficulties associated with detecting changes in prey abundance and relating these changes with declines in some bat species as well as changes in the behaviors of other bat species, this study provides evidence that WNS in bats can have top-down effects on arthropod abundance that involve direct prey release, as in the case of little brown bats, and potentially other effects such as altered foraging patterns as in the case of big brown bats.

Complex interactions between generalist predators and their prey are difficult to characterize due to methodological limitations. For example, acoustic autoclassification software lacks the ability to distinguish between multiple bats echolocating and a single bat echolocating frequently, while accurately identifying calls of different species also remains challenging (Lemen et al. 2015). In our study area little brown and big brown bats are by far the most frequently captured, but the true population sizes of all bat species in the area are not well known (Huebschman 2019). While estimates of low-frequency acoustic activity may not be directly attributable to big brown bats and likely include other low-frequency echolocating bats that are present throughout the study area, the emergence counts conducted as part of this study suggest that big brown bats are likely the most abundant species at their respective study subsites. Additionally, our study is not a true exclusion experiment, as background bat activity remained present at all subsites since other bat species occur in the area, and other predators such as arthropodivorous birds are also present. As volant organisms, bats are also capable of travelling long distances to reach foraging habitats, potentially as a way to exploit temporal changes in arthropod abundance (Brigham 1991). As such, linking the effects of disease-related bat declines with local-scale differences in arthropod abundance at sites near and far from bat roosts remains challenging. Like all arthropod survey methods, black-light trap sampling methods carry biases in terms of which arthropods are most attracted, and as such do not perfectly reflect the abundance of all arthropod taxa (Kremen et al. 1993; Kirkeby et al. 2013). Nonetheless, this study represents one of the most comprehensive attempts to characterize the influences of bats on arthropod abundance and provides a framework for future studies that may seek to evaluate the trophic consequences of disease-related bat declines.

The effects of predators on prey communities have been well documented in many systems, but few studies have focused on vertebrate predators of arthropods. In some systems, the loss or decline of predators due to disease has been shown to directly result in prey community changes, such

as increases in prey abundance or shifts in trophic control (Ostfeld & Holt 2004; Wilmers et al. 2006; Hollings et al. 2016). Though arthropodivorous bats are small-bodied, the total biomass of arthropods consumed by bats is substantial (Morrison & Lindell 2012), and the loss of millions of bats from WNS thus represents a biomass displacement that could have regional effects on the nocturnal arthropod food web. For example, Boyles et al. (2011) estimated that the loss of one million little brown bats would represent between 660 and 1320 fewer metric tons of insects consumed each year. Due to the amounts of arthropods collectively consumed by bat species assemblages, rapid declines in bat populations could have other unexpected top-down consequences. Both little brown and big brown bats forage at a high trophic level, consuming predatory beetles, spiders, lacewings, and parasitoid wasps, among other arthropods (Chapter 1). As such, their influences on arthropod abundance are likely complex, and bat declines may not directly lead to prey release, but rather could influence arthropod abundance via nonconsumptive effects or release of other predators, such as predatory arthropods or other aerial arthropodivores. Though the effects of arthropod predator declines remain difficult to quantify, with many other factors such as climate change, pesticide use, and habitat loss as potential confounding variables, considering the ecosystem impacts of bat declines as well as declines among other aerial arthropodivores remains a timely endeavor.

Conclusions

We found that the effects of bat declines interacted with yearly arthropod declines, most notably with total arthropod abundance declining less at little brown bat roost sites in the first year following WNS-related declines. In light of the evidence of substantial little brown bat declines, the relative increases in Diptera — the most common prey of little brown bats —in particular suggests that the observed differences between treatment and control subsites in the later years of the study may be related to some level of predation pressure release. The results from big brown bat treatment subsites also

suggest that top-down effects of bat predation on arthropods could involve changes in foraging patterns among bat species that are less severely afflicted by WNS. However, the question of whether and to what extent big brown bats (or other bat species) are capable of filling the trophic niche of little brown bats remains largely unanswered. Additionally, the influence of arthropod abundance on bat survival remains unknown. While body condition has been directly related to survival from white-nose syndrome (Jonasson & Willis 2011; Cheng et al. 2019), few studies have examined how arthropod prey availability may influence the ability of bats to acquire adequate nutrition prior to hibernation. These questions may be particularly important for bats at northern latitudes, where hibernation periods tend to be much longer, although connectivity between summer roost sites and hibernacula have also seldom been examined for many bat species. Considering the observed arthropod declines in this study and in other broader-scale studies, further investigation of potential comorbidities between prey abundance, body condition, and WNS severity may be warranted. We also suggest that future studies examining the influence of bats on arthropod abundance may consider adopting a broader spatial and temporal scale or incorporate bat movement and capture data.

Methods

Study species

Little brown and big brown bats are among the most common bat species in North America (Fenton 1980; Kurta & Baker 1990). Little brown bats are high-frequency echolocators that tend to be generalist in their foraging habits, mostly consuming aquatic insects — particularly those with swarming behaviors (such as chironomid midges) — though they also consume terrestrial prey (including moths, true bugs, beetles, and spiders) and may adjust their foraging behaviors depending on availability (Buchler 1976; Whitaker & Lawhead 1992; Clare et al. 2014a). In contrast, big brown bats are low-frequency echolocators and are often referred to as "beetle specialists" but are well known

to consume a variety of other arthropods such as flies, caddisflies, true bugs, and moths (Kunz & Whitaker 1983; Agosta 2002; Clare et al. 2014b). We selected little brown bats as a focal study species because, prior to WNS-related declines, they were the most abundant bat species in the study region (Huebschman 2019). We selected big brown bats as a second focal study species because they are also abundant but were expected to decline less from WNS (based on previous population trends observed in the eastern region of North America, e.g., Frick et al. 2015), thus allowing for a comparative analysis of the effects of disease-related bat declines in differentially afflicted species. Both little brown and big brown bats also frequently form large maternity roosts within human-built structures, which allows for ease of detection and monitoring between years.

Study area

Study sites were selected at 10 bat maternity roosts in southern Wisconsin (Figure 4A). Each site consisted of a subsite located 50–100m away from a known bat maternity roost (hereafter referred to as a "treatment"), and an additional subsite located 3–10km from each respective bat roost (hereafter referred to as a "control"). To confirm the identity of bat species at each treatment subsite presence and to estimate the abundance and population trends, roost emergence counts were conducted during Julian weeks 24–25 and weeks 29–30, roughly corresponding to the pre- and post-volancy periods for bat pups for each year of the study. Roost counts were conducted by standard visual surveys with observers stationed at bat emergence points at sundown prior to bat emergence, with counts continued until no more bats emerged or until the sky was too dark to distinguish bats visually. These surveys were conducted as part of a state-wide community science-based monitoring initiative, and counts were averaged between each survey time period.

For each bat roost subsite, paired control subsites were selected such that immediate landscape composition was similar to treatment subsites but with comparatively lower levels of bat activity due

to the distance outside of the expected core foraging areas of each maternity roost, which was then confirmed via acoustic activity indexing (see below). Landscape composition for paired sites were compared by assessing major landcover categories derived from the USDA CropScape Cropland Data Layer (https://nassgeodata.gmu.edu/CropScape/). To capture the surrounding landcover composition within relevant ranges of bat foraging distances, we placed 3km buffers around each subsite and extracted the corresponding landcover data (Figure 4B). Based on a paired t-test, there was no statistically significant difference in landcover percentage between control and treatment subsites (p>0.05 for all categories).

Black-light trapping for arthropod communities

Arthropod communities at each of the 20 subsites were sampled weekly. Black-light traps were used to collect night-flying moths and other arthropods that are presumed to form the majority of prey in the diet of arthropodivorous bats. Black-light trapping protocols followed Chapter 1, with traps turned on automatically from 20:00 through 5:00 for a consecutive 3-night period (Thursday–Saturday) during each sampling week. Samples were collected and traps were reset weekly from late May to late August in 2015 and mid-May to early September in 2016–2018. A total of 864 black-light trap samples were collected from 2015–2018.

Insect and arthropod samples were identified and counted (or estimated via subsampling and extrapolation) by microscope, also following Chapter 1. Briefly, arthropods were identified to order; and within orders, all specimens were identified to the 43 most commonly detected groups (representing 95% of all captured arthropods), with remaining rare families identified as "other Order", e.g., "other Coleoptera". Samples that were damaged or degraded were identified to the lowest taxonomic level possible. Overall, we captured, identified, and enumerated a total of 2,003,493 arthropods. The most commonly captured arthropod orders were Coleoptera, Diptera, Hemiptera,

Hymenoptera, Lepidoptera, and Trichoptera, hereafter referred to as major arthropod orders (Figure 4C). On average, the most abundant arthropod groups were Trichoptera (caddisflies, $\bar{x}$ =641.2, IQR=46.5–668), followed by Diptera (flies): Chironomidae and Culicidae (midges and mosquitoes, $\bar{x}$=495.8, IQR=10–172.5) and other Coleoptera (beetles, $\bar{x}$ =177.6, IQR=10–177; Figure 4C). Over the course of the study (2015–2018) there was an overall decline in mean total arthropod abundance by 48.9% (Table 1, Figure 4D). This trend also held true for all major orders, with declines between 2015 and 2018 ranging from –15.7% for total Diptera to –92.7% for total Hymenoptera (Table 1, Figure 4D).

Indexing bat activity with passive acoustic recording

Bat activity was indexed by deploying Songmeter SM3ZC passive acoustic recording devices (Wildlife Acoustics, Maynard, MA) at all treatment and control subsites (*n*=20 subsites). Recordings occurred nightly from sunset to sundown every day of each week during the summer period (late May-early September), yielding a total of 6,245 recording nights. Raw acoustic data were processed in batches using Kaleidoscope PRO version 4.1.0 (Wildlife Acoustics, Maynard, MA). Autoclassification identifications used the built-in Bats of North America database version 4.1.0, with region set to Wisconsin. This includes classification for the seven bat species found throughout the state: the big brown (*Eptesicus fuscus*), Eastern red (*Lasiurus borealis*), hoary (*Lasiurus cinereus*), silver-haired (*Lasionycteris noctivagans*), little brown (*Myotis lucifugus*), Northern long-eared (*Myotis septentrionalis*), and tri-colored bat (*Perimyotis subflavus*). Signal parameters were set between 8–120 kHz and 2–500 ms, with a maximum inter-syllable gap of 500 ms and a minimum of 2 pulses.

Post-processing, data batches were combined and cleaned in R Studio version 1.3.1056 (R Core Team, 2020). To avoid noise contamination and potential bat attraction effects, we removed all days during which black-light traps were on (Thursday, Friday, and Saturday). Next, we aggregated

data into high and low-frequency groups to account for the inherent shortcomings of autoclassification software (Lemen et al. 2015). For this dataset, 88.6% of high-frequency group pulses were initially autoclassified as little brown bat calls, while 54.2% of low-frequency were initially autoclassified as big brown bat pulses. Hoary bats accounted for 41% of autoclassified low-frequency pulses, although this species is seldom captured in the state. Although hoary bats are likely underrepresented by capture surveys due to their high-altitude foraging habits, they may be overrepresented by acoustic surveys due to their lower-frequency calls, which can be detected from greater distances than other bat calls, or due to the high variability of their calls which can be confused with other species (O'Farrell et al. 2000; Huebschman 2019). Nonetheless, while estimates of low-frequency acoustic activity may not be directly attributable to big brown bats and include other low-frequency echolocating bats that are present throughout the study area, corresponding emergence counts suggest that big brown bats are likely the most abundant species at their respective study subsites. As such, pulses identified as hoary, silver-haired, and big brown bats were combined into the low-frequency group category, while pulses identified as little brown, Eastern red, little brown, Northern long-eared, and tri-colored bats were combined into the high-frequency group category. These groupings yielded a final total of 1,460,540 identified low-frequency pulses and 2,109,930 identified high-frequency pulses. The number of high-frequency and low-frequency pulses were then aggregated by subsite, year, and Julian week, with the arithmetic mean taken to account for uneven sampling nights

Statistical analyses

As an evaluation of the a priori assumption that subsites close to roosts (i.e., "treatment") had higher bat activity than subsites far from roosts ("controls"), we compared the amount of acoustic activity at paired subsites for the frequency category corresponding to the type of bat roost present (i.e., high-

frequency activity for little brown bat sites and low-frequency activity for big brown bat sites). We tested for differences at each paired treatment and control subsites using Wilcoxon signed-rank tests, which indicated that 9 out of 10 sites had significantly higher overall bat activity for the expected frequency group at the treatment subsite in comparison to the control subsite (Supplementary Figure S2). One site (site D) had higher low-frequency bat activity at the control subsite in comparison to the treatment subsite and was therefore excluded from all subsequent analyses involving comparisons between control and treatment subsites (Supplementary Figure S2). To determine how bat activity changed over time, yearly differences between the total number of high-frequency and low-frequency pulses were also assessed using unpaired Wilcoxon rank sum tests.

We used linear mixed effects models with count data transformed using a log(x+1) transformation to preserve information present among zero values in order to assess whether arthropod abundance differed at treatment versus control sites over time. Since arthropod count data for most groups had high variance, this approach was chosen for its robustness under a wide range of conditions (Ives 2015). Samples with total arthropod abundance in the lowest 0.5 percentile, which had fewer than 63 individual arthropods present (n=44 samples), were excluded from analyses since these low sample abundances could indicate partial trap failure, tampering or sample removal by terrestrial predators, or poor weather conditions, resulting in 740 total remaining samples included in analyses. Separate models were created for total arthropod abundance, the abundance of major arthropod orders, and the abundance of five arthropod groups that were among the most common in black-light traps and which are known to occur in bat diets. These five groups included Chironomidae/Culicidae (Diptera), Carabidae (Coleoptera), Corixidae (Hemiptera), micromoths (Lepidoptera), and Noctuidae (Lepidoptera). For all models, fixed effects included year, bat species, (i.e., whether sites were classified as little brown or big brown bat sites), treatment (i.e., the identity of a subsite as near versus far from a known bat maternity roost), and the interactions between year, bat

species, and treatment. To account for repeated sampling, we used site and the time period of sampling (i.e., the week and year of sample collection) nested within a site as random intercepts. Since yearly trends were not necessarily linear, we treated year as a factor variable. Model assumptions (linearity, homogeneity of variance, normal distribution of residuals) were also tested and we confirmed that these assumptions were met (model outputs are available in Supplementary Table S1). We then used post hoc Tukey tests for each model to determine pairwise contrasts between different levels of fixed predictor variables, specifically, testing for differences between paired control and treatment subsites for each bat species within each year. Statistical analyses were performed and 95% confidence intervals for coefficients were calculated using the R packages "lmerTest","lme4", "lsmeans" and "emmeans" (Bates et al. 2007; Kuznetsova et al. 2017; Lenth 2018; Lenth et al. 2018).

Additional tools used for data processing and data visualization included the R packages "dplyr", "ggplot2", "maps", "reshape2", "tidyverse", and "wesanderson" (Ram & Wickham 2018; Wickham 2019; Wickham et al. 2020; Wickham et al. 2020a,b) and SankeyMATIC (Bogart 2018).

Acknowledgements

The authors thank Brenna Decker, Erin Green, and Michael Stocking for designing and building the solar powered black-light traps. We thank Mike & Jane Balch, Rhonda & Danny Enge, Jeanne Foster, Leigh Hale, Marci & Jim Hess, Elaine Horn, Gary Jansen, Phillip & Ronald Kerl, Doug Kiel, Jeremy Lynch, Pat Tesar, Elaine Swanson, Jimmy Voegeli, and Jean Warrior for permitting sampling at their properties. We also thank John Arthur, Bruce Folley, Kathleen Gruentzel, Heather Kaarakka, Darren Marsh, Thomas Meyer, and J. Paul White for assistance coordinating sampling at state and county parks.

References

Agosta, S. J. (2002). Habitat use, diet and roost selection by the big brown bat (Eptesicus fuscus) in North America: a case for conserving an abundant species. *Mammal Review, 32*(3), 179-198.

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.

Bates, A. E., Hilton, B. J., & Harley, C. D. (2009). Effects of temperature, season and locality on wasting disease in the keystone predatory sea star Pisaster ochraceus. *Diseases of Aquatic Organisms, 86*(3), 245-251.

Bates, D., Sarkar, D., Bates, M. D., & Matrix, L. (2007). *The lme4 package*. R package version 2.1.74.

Belwood, J., & Fenton, M. (1976). Variation in the diet of *Myotis lucifugus* (Chiroptera: Vespertilionidae). *Canadian Journal of Zoology, 54*(10), 1674-1678.

Bogart, S. (2018). SankeyMATIC: A Sankey diagram builder for everyone.

Boyles, J. G., Cryan, P. M., McCracken, G. F., & Kunz, T. H. (2011). Economic importance of bats in agriculture. *Science, 332*(6025), 41-42.

Braley, M., Goldsworthy, S. D., Page, B., Steer, M., & Austin, J. J. (2010). Assessing morphological and DNA-based diet analysis techniques in a generalist predator, the arrow squid Nototodarus gouldi. *Molecular Ecology Resources, 10*(3), 466-474.

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.

Buchler, E. R. (1976). Prey selection by *Myotis lucifugus* (Chiroptera: Vespertilionidae). *The American Naturalist, 110*(974), 619-628.

Carter, P. E., & Rypstra, A. L. (1995). Top-down effects in soybean agroecosystems: spider density affects herbivore damage. *Oikos*, 433-439.

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., Barber, B., Sweeney, B., Hebert, P., & Fenton, M. B. (2011). Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Molecular Ecology, 20*(8), 1772-1780.

Clare, E. L., Symondson, W. O., Broders, H., Fabianek, F., Fraser, E. E., MacKenzie, A., . . . Martinez-Nuñez, F. (2014a). The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. *Molecular Ecology, 23*(15), 3618-3632.

Clare, E. L., Symondson, W. O., & Fenton, M. B. (2014b). An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). *Molecular Ecology, 23*(15), 3633-3647.

Collinge, S. K., Ray, C., & Cully Jr, J. F. (2008). Effects of disease on keystone species, dominant species, and their communities. In *Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems* (pp. 129-144). Princeton University Press.

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J., & Collen, B. (2014). Defaunation in the Anthropocene. *Science, 345*(6195), 401-406.

Dzal, Y., McGuire, L. P., Veselka, N., & Fenton, M. B. (2011). Going, going, gone: the impact of white-nose syndrome on the summer activity of the little brown bat (*Myotis lucifugus*). *Biology Letters, 7*(3), 392-394.

Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., . . . Jackson, J. B. (2011). Trophic downgrading of planet Earth. *Science, 333*(6040), 301-306.

Fenton, A., & Rands, S. (2006). The impact of parasite manipulation and predator foraging behavior on predator–prey communities. *Ecology, 87*(11), 2832-2841.

Fenton, M. B. (1980). *Myotis lucifugus*. *Mammalian Species*(142), 1-8.

Finke, D. L., & Denno, R. F. (2003). Intra-guild predation relaxes natural enemy impacts on herbivore populations. *Ecological Entomology, 28*(1), 67-73.

Finke, D. L., & Denno, R. F. (2005). Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecology Letters, 8*(12), 1299-1306.

Frank, C. L., Michalski, A., McDonough, A. A., Rahimian, M., Rudd, R. J., & Herzog, C. (2014). The resistance of a North American bat species (*Eptesicus fuscus*) to white-nose syndrome (WNS). *PLoS One, 9*(12), e113958.

Frick, W. F., Pollock, J. F., Hicks, A. C., Langwig, K. E., Reynolds, D. S., Turner, G. G., . . . Kunz, T. H. (2010). An emerging disease causes regional population collapse of a common North American bat species. *Science, 329*(5992), 679-682.

Frick, W. F., Puechmaille, S. J., Hoyt, J. R., Nickel, B. A., Langwig, K. E., Foster, J. T., . . . Haarsma, A. J. (2015). Disease alters macroecological patterns of North American bats. *Global Ecology and Biogeography, 24*(7), 741-749.

Green, R. H. (1979). *Sampling Design and Statistical Methods for Environmental Biologists*. John Wiley & Sons.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., . . . Hörren, T. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One, 12*(10), e0185809.

Henry, M., Thomas, D. W., Vaudry, R., & Carrier, M. (2002). Foraging distances and home range of pregnant and lactating little brown bats (*Myotis lucifugus*). *Journal of Mammalogy, 83*(3), 767-774.

Hollings, T., Jones, M., Mooney, N., & McCallum, H. (2016). Disease-induced decline of an apex predator drives invasive dominated states and threatens biodiversity. *Ecology, 97*(2), 394-405.

Huebschman, J. J. (2019). Bats in southwest Wisconsin during the era of white-nose syndrome. *Northeastern Naturalist, 26*(1), 168-182.

Hunt-Joshi, T. R., Root, R. B., & Blossey, B. (2005). Disruption of weed biological control by an opportunistic mirid predator. *Ecological Applications, 15*(3), 861-870.

Ives, A. R. (2015). For testing the significance of regression coefficients, go ahead and log-transform count data. *Methods in Ecology and Evolution, 6*(7), 828-835.

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.

Jonasson, K. A., & Willis, C. K. (2011). Changes in body condition of hibernating bats support the thrifty female hypothesis and predict consequences for populations with white-nose syndrome. *PLoS One, 6*(6).

Kalka, M. B., Smith, A. R., & Kalko, E. K. (2008). Bats limit arthropods and herbivory in a tropical forest. *Science, 320*(5872), 71-71.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., . . . Mitchell, C. E. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature, 468*(7324), 647.

Kirkeby, C., Græsbøll, K., Stockmarr, A., Christiansen, L. E., & Bødker, R. (2013). The range of attraction for light traps catching *Culicoides* biting midges (Diptera: Ceratopogonidae). *Parasites & vectors, 6*(1), 67.

Kremen, C., Colwell, R., Erwin, T., Murphy, D., Noss, R. a., & Sanjayan, M. (1993). Terrestrial arthropod assemblages: their use in conservation planning. *Conservation Biology, 7*(4), 796-808.

Kunz, T. H., & Whitaker, J. O. (1983). An evaluation of fecal analysis for determining food habits of insectivorous bats. *Canadian Journal of Zoology, 61*(6), 1317-1321.

Kurta, A., & Baker, R. H. (1990). *Eptesicus fuscus*. *Mammalian Species,* (356), 1-10.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software, 82*(13), 1-26.

Lemen, C., Freeman, P. W., White, J. A., & Andersen, B. R. (2015). The problem of low agreement among automated identification programs for acoustical surveys of bats. *Western North American Naturalist*, 218-225.

Lenth, R. (2018). Package 'lsmeans'. *The American Statistician, 34*(4), 216-221.

Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2018). *Emmeans: Estimated marginal means, aka least-squares means.* R package version 1.1.3.

Lindström, E. R., Andrén, H., Angelstam, P., Cederlund, G., Hörnfeldt, B., Jäderberg, L., . . . Swenson, J. E. (1994). Disease reveals the predator: sarcoptic mange, red fox predation, and prey populations. *Ecology, 75*(4), 1042-1049.

Lorch, J. M., Palmer, J. M., Lindner, D. L., Ballmann, A. E., George, K. G., Griffin, K., . . . Anderson, C. D. (2016). First detection of bat white-nose syndrome in western North America. *MSphere, 1*(4).

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.

McGuire, L. P., Mayberry, H. W., & Willis, C. K. (2017). White-nose syndrome increases torpid metabolic rate and evaporative water loss in hibernating bats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 313*(6), R680-R686.

Montecino-Latorre, D., Eisenlord, M. E., Turner, M., Yoshioka, R., Harvell, C. D., Pattengill-Semmens, C. V., . . . Gaydos, J. K. (2016). Devastating transboundary impacts of sea star wasting disease on subtidal asteroids. *PLoS One, 11*(10), e0163190.

Montgomery, G. A., Dunn, R. R., Fox, R., Jongejans, E., Leather, S. R., Saunders, M. E., . . . Wagner, D. L. (2020). Is the insect apocalypse upon us? How to find out. *Biological Conservation, 241*, 108327.

Mooney, K. A., Gruner, D. S., Barber, N. A., Van Bael, S. A., Philpott, S. M., & Greenberg, R. (2010). Interactions among predators and the cascading effects of vertebrate insectivores on arthropod communities and plants. *Proceedings of the National Academy of Sciences, 107*(16), 7335-7340.

Moosman, 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.

Moran, M. D., & Hurd, L. (1997). A trophic cascade in a diverse arthropod community caused by a generalist arthropod predator. *Oecologia, 113*(1), 126-132.

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).

Morrison, E. B., & Lindell, C. A. (2012). Birds and bats reduce insect biomass and leaf damage in tropical forest restoration sites. *Ecological Applications, 22*(5), 1526-1534.

O'Farrell, M. J., Corben, C., & Gannon, W. L. (2000). Geographic variation in the echolocation calls of the hoary bat (Lasiurus cinereus). *Acta Chiropterologica, 2*(2), 185-196.

Ostfeld, R. S., & Holt, R. D. (2004). Are predators good for your health? evaluating evidence for top-down regulation of zoonotic disease reservoirs. *Frontiers in Ecology and the Environment, 2*(1), 13-20.

Perillo, A. C., Kucharik, C. J., Meehan, T. D., Serbin, S. P., Singh, A., Townsend, P. A., . . . Gratton, C. (2015). Use of insect exclusion cages in soybean creates an altered microclimate and differential crop response. *Agricultural and Forest Meteorology, 208*, 50-61.

Polis, G. A., & Strong, D. R. (1996). Food web complexity and community dynamics. *The American Naturalist, 147*(5), 813-846.

Ram, K., & Wickham, H. (2018). *wesanderson: A Wes Anderson palette generator*. R package version 0.3.6.

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

Sánchez-Bayo, F., & Wyckhuys, K. A. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation, 232*, 8-27.

Seibold, S., Gossner, M. M., Simons, N. K., Blüthgen, N., Müller, J., Ambarlı, D., . . . Habel, J. C. (2019). Arthropod decline in grasslands and forests is associated with landscape-level drivers. *Nature, 574*(7780), 671-674.

Shortall, C. R., Moore, A., Smith, E., Hall, M. J., Woiwod, I. P., & Harrington, R. (2009). Long-term changes in the abundance of flying insects. *Insect Conservation and Diversity, 2*(4), 251-260.

Snyder, W. E., & Ives, A. R. (2001). Generalist predators disrupt biological control by a specialist parasitoid. *Ecology, 82*(3), 705-716.

Strong, A. M., Sherry, T. W., & Holmes, R. T. (2000). Bird predation on herbivorous insects: indirect effects on sugar maple saplings. *Oecologia, 125*(3), 370-379.

Symondson, W., Sunderland, K., & Greenstone, M. (2002). Can generalist predators be effective biocontrol agents? *Annual Review of Entomology, 47*(1), 561-594.

Thomas, C., Jones, T. H., & Hartley, S. E. (2019). "Insectageddon": a call for more robust data and rigorous analyses. *Global Change Biology*.

Trewby, I. D., Wilson, G. J., Delahay, R. J., Walker, N., Young, R., Davison, J., . . . McDonald, R. A. (2007). Experimental evidence of competitive release in sympatric carnivores. *Biology letters, 4*(2), 170-172.

United States Geological Survey. (2020). White-nose Syndrome occurrence map. Available online at: www.whitenosesyndrome.org/where-is-wns. Accessed 12 November 2020.

Venesky, M. D., Parris, M. J., & Storfer, A. (2009). Impacts of *Batrachochytrium dendrobatidis* infection on tadpole foraging performance. *EcoHealth, 6*(4), 565-575.

Whitaker, J. O., & Lawhead, B. (1992). Foods of *Myotis lucifugus* in a maternity colony in central Alaska. *Journal of Mammalogy*, 646-648.

Wickham, H. (2020). *reshape2: Flexibly reshape data: a reboot of the reshape package*. R package version 1.4.4.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D. A., François, R., . . . Hester, J. (2019). Welcome to the Tidyverse. *Journal of Open Source Software, 4*(43), 1686.

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., & Woo, K. (2020a). *Package 'ggplot2'. Create Elegant Data Visualisations Using the Grammar of Graphics*. R Package version 3.3.2.

Wickham, H., Francois, R., Henry, L., & Müller, K. (2020b). *dplyr: A Grammar of Data Manipulation*. R package version 1.0.0.

Wilder, A. P., Frick, W. F., Langwig, K. E., & Kunz, T. H. (2011). Risk factors associated with mortality from white-nose syndrome among hibernating bat colonies. *Biology Letters, 7*(6), 950-953.

Williams, R. J., Berlow, E. L., Dunne, J. A., Barabási, A.-L., & Martinez, N. D. (2002). Two degrees of separation in complex food webs. *Proceedings of the National Academy of Sciences, 99*(20), 12913-12916.

Willis, C. K. (2015). Conservation physiology and conservation pathogens: white-nose syndrome and integrative biology for host–pathogen systems. *Integrative and Comparative Biology, 55*(4), 631-641.

Wilmers, C. C., Post, E., Peterson, R. O., & Vucetich, J. A. (2006). Predator disease out-break modulates top-down, bottom-up and climatic effects on herbivore population dynamics. *Ecology Letters, 9*(4), 383-389.

Table 1. Arthropod abundance, Julian weeks 25-34

group	year	total	$\bar{x}$	$\bar{x}_{t2} - \bar{x}_{t1}$	%	p	$\bar{x}_{t4} - \bar{x}_{t1}$	%	p
total Arthropoda	2015	442760	3513.97						
	2016	498931	2585.13	-928.83	-26.4%	<0.001			
	2017	356695	1981.64	-603.50	-23.3%	0.05			
	2018	326821	1795.72	-185.92	-9.4%	0.20	-1718.25	-48.9%	<0.001
total Coleoptera	2015	129007	1023.87						
	2016	158279	820.10	-203.77	-19.9%	<0.001			
	2017	76551	425.28	-394.82	-48.1%	<0.001			
	2018	117219	644.06	218.78	51.4%	0.18	-379.80	-37.1%	<0.001
total Diptera	2015	69552	552.00						
	2016	122647	635.48	83.48	15.1%	<0.001			
	2017	99203	551.13	-84.35	-13.3%	0.16			
	2018	84698	465.37	-85.75	-15.6%	<0.001	-86.63	-15.7%	<0.001
total Lepidoptera	2015	29978	237.92						
	2016	17718	91.80	-146.12	-61.4%	<0.001			
	2017	14044	78.02	-13.78	-15.0%	<0.001			
	2018	10003	54.96	-23.06	-29.6%	<0.001	-182.96	-76.9%	<0.001
total Hemiptera	2015	86862	689.38						
	2016	57138	296.05	-393.33	-57.1%	<0.001			
	2017	31695	176.08	-119.97	-40.5%	0.05			
	2018	24687	135.64	-40.44	-23.0%	<0.001	-553.74	-80.3%	<0.001
total Hymenoptera	2015	30667	243.39						
	2016	7985	41.37	-202.02	-83.0%	0.02			
	2017	4775	26.53	-14.85	-35.9%	0.08			
	2018	3236	17.78	-8.75	-33.0%	<0.001	-225.61	-92.7%	<0.001
total Trichoptera	2015	95068	754.51						
	2016	132908	688.64	-65.87	-8.7%	0.08			
	2017	129362	718.68	30.04	4.4%	0.09			
	2018	86048	472.79	-245.89	-34.2%	0.04	-281.72	-37.3%	<0.001

Figure Legends

Figure 1. Conceptual diagram. A) Study site design, with passive acoustic monitors and black-light traps located near and far from bat roosts in order to quantify bat acoustic activity and arthropod abundance, respectively. B) Predictions for bat population declines following detection of white-nose syndrome (WNS) in Wisconsin in 2014. C) Predictions for arthropod abundance at treatment and control subsites following the expected declines in bat populations. EPFU=big brown bat, MYLU=little brown bat. Diagram not to scale.

Figure 2. Bat population size, acoustic activity estimations, and total arthropod abundance. A) Spatially-arranged changes in roost size by year, determined as the mean value of pre- and post-volancy roost emergence counts. EPFU=big brown bat, MYLU=little brown bat. B) Total nightly identified pulses at all study sites, 2015–2018. High-frequency group (HFG) corresponds to little brown bats, low-frequency group (LFG) corresponds to big brown bats. Bars indicate median values, while boxplots indicate the interquartile range and whiskers indicate the full range of values. Half-violin plots indicate the distribution of observed values. Points are jittered for visibility. EPFU=big brown bat, MYLU=little brown bat.

Figure 3. A) Total arthropod abundance and changes over time for bat roost subsites and paired control subsites. Closed shapes represent treatment subsites, while open shapes represent control subsites. Colored points and lines indicate mean values for all subsites in total, while gray points and lines indicate mean values for each subsite. Points indicate the mean abundance within a subsite type in a given year, while bars indicate one standard deviation from the mean. Points are jittered for visibility. B) Results from linear mixed effects models, demonstrating the estimate and 95% confidence intervals of pairwise contrasts (between treatments and controls) from post hoc testing. EPFU

indicates paired treatment and control subsites with a big brown bat roost, MYLU indicates paired treatment and control subsites with a little brown bat roost. Shaded gray areas indicate years after WNS-related little brown bat declines were detected. Closed circles indicate overlap with zero, while open triangles indicate nonoverlap with zero which suggests a statistically meaningful effect. EPFU=big brown bat, MYLU=little brown bat.

Figure 4. Summary of study site characteristics. A) Map of maternity roost locations in Wisconsin, USA, with lines indicating county boundaries. Inset shows position of study area within the continental United States (with Wisconsin shaded in gray). B) Landcover composition comparisons between paired treatment and control subsites at a 3km scale. C) Sankey diagram of the total abundance of arthropods identified by microscope. D) Yearly abundance of total arthropods and major arthropod orders, 2015–2018. Bars indicate the median values, while boxplots indicate the interquartile range and whiskers indicating the full range of values. Half-violin plots indicate the distribution of observed values. EPFU=big brown bat, MYLU=little brown bat.

Supplementary Figure S1. Mean weekly identified pulses at all paired study sites, 2015–2018. High-frequency group (HFG) corresponds to little brown bats, low-frequency group (LFG) corresponds to big brown bats. Bars indicate median values, while boxplots indicate the interquartile range and whiskers indicate the full range of values. Points are jittered for visibility. EPFU=big brown bat, MYLU=little brown bat.

Supplementary Figure S2. Validating site assumptions of higher acoustic activity at treatment subsites. EPFU=big brown bat, MYLU=little brown bat.

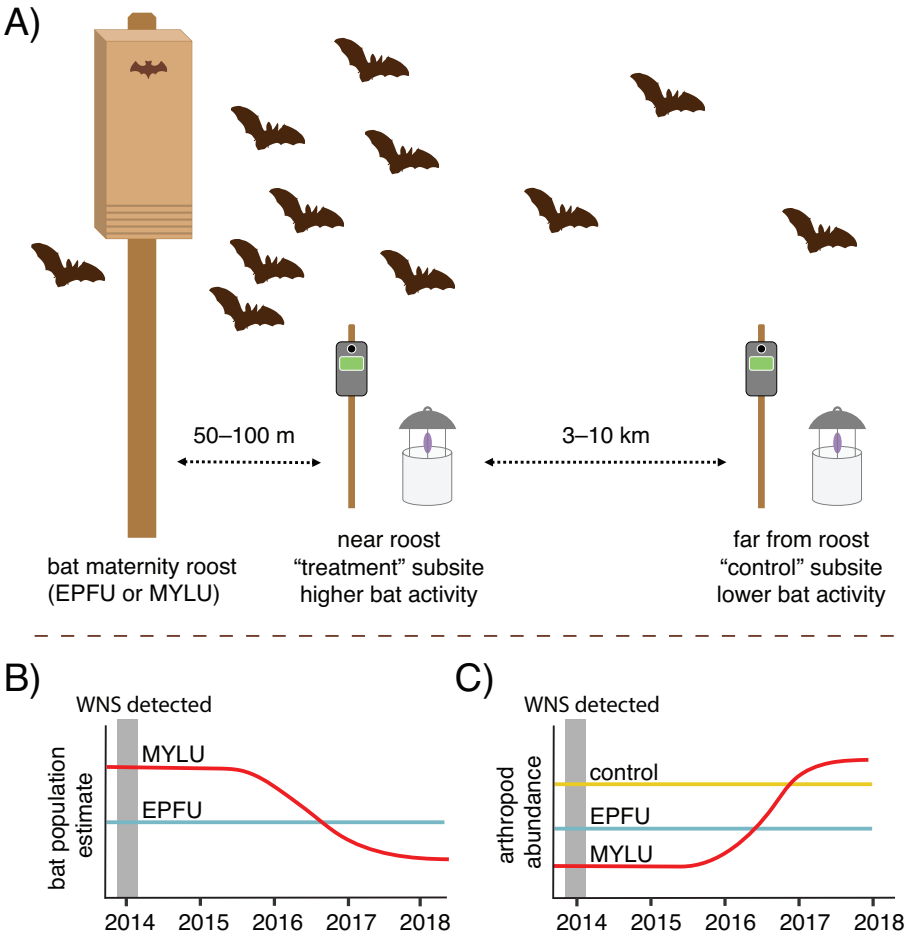

A)
50–100 m
3–10 km
bat maternity roost
(EPFU or MYLU)
near roost
“treatment” subsite
higher bat activity
far from roost
“control” subsite
lower bat activity
B)
WNS detected
bat population estimate
MYLU
EPFU
2014
2015
2016
2017
2018
C)
WNS detected
arthropod abundance
control
EPFU
MYLU
2014
2015
2016
2017
2018

Figure 1

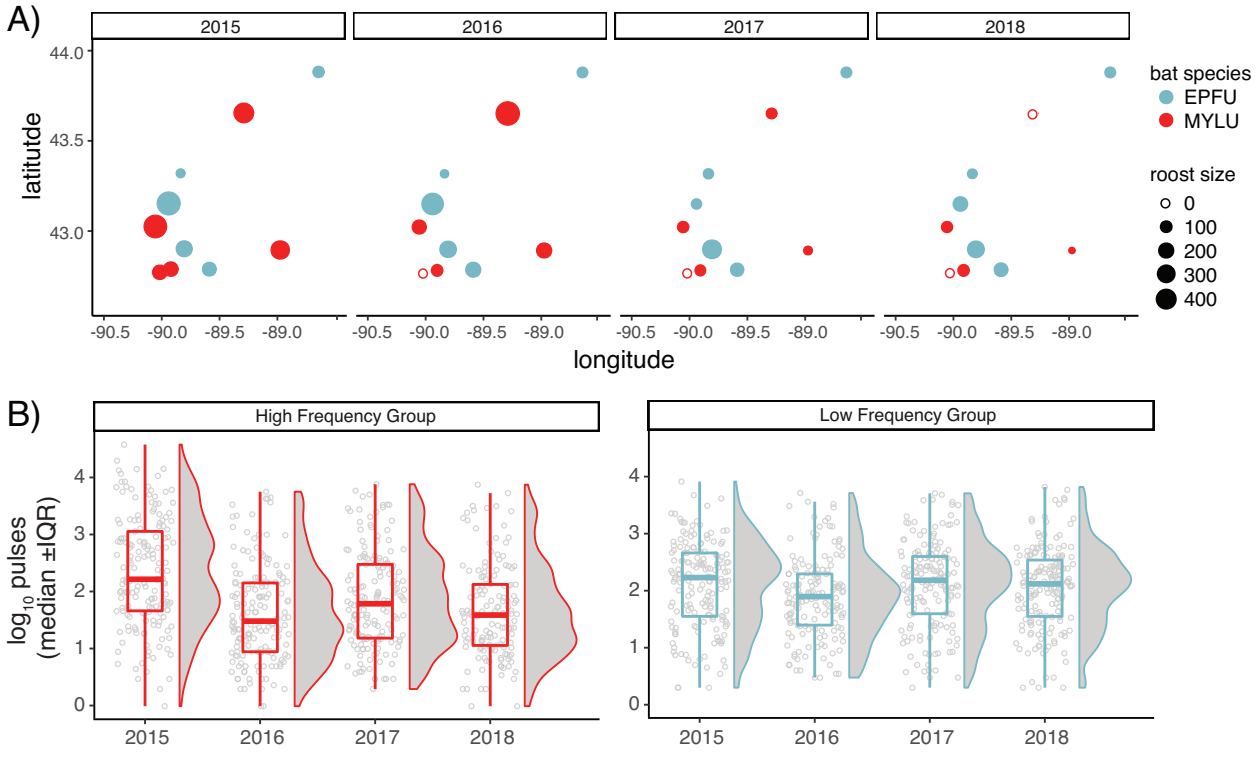

A)
2015
2016
2017
2018
44.0
43.5
43.0
latitutde
-90.5
-90.0
-89.5
-89.0
longitude
bat species
EPFU
MYLU
roost size
0
100
200
300
400
B)
High Frequency Group
Low Frequency Group
log10 pulses
(median ±IQR)
4
3
2
1
0
2015
2016
2017
2018

Figure 2

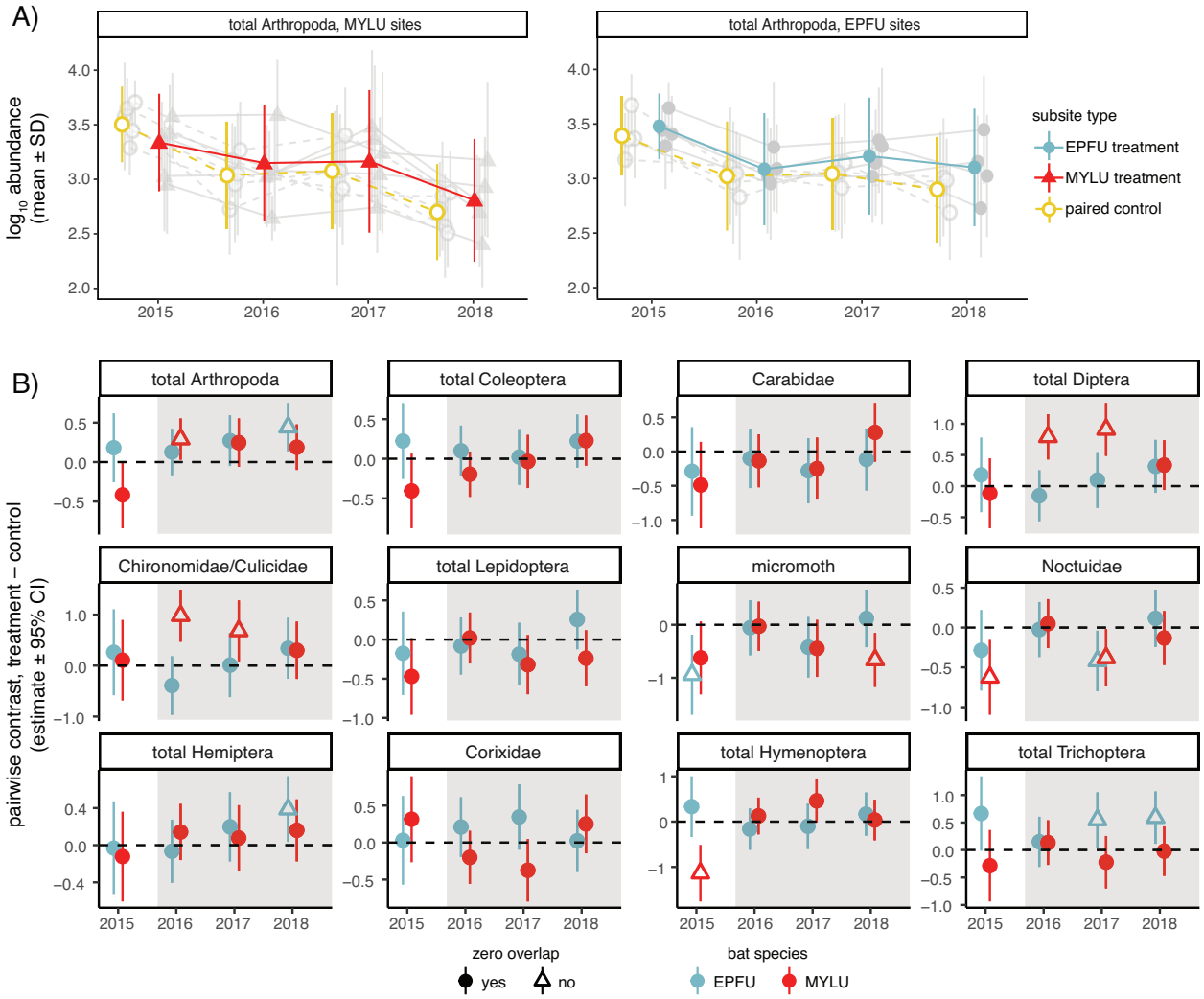

A)
total Arthropoda, MYLU sites
total Arthropoda, EPFU sites
log10 abundance (mean ± SD)
subsite type
EPFU treatment
MYLU treatment
paired control
B)
total Arthropoda
total Coleoptera
Carabidae
total Diptera
Chironomidae/Culicidae
total Lepidoptera
micromoth
Noctuidae
total Hemiptera
Corixidae
total Hymenoptera
total Trichoptera
pairwise contrast, treatment – control (estimate ± 95% CI)
zero overlap
yes
no
bat species
EPFU
MYLU

Figure 3

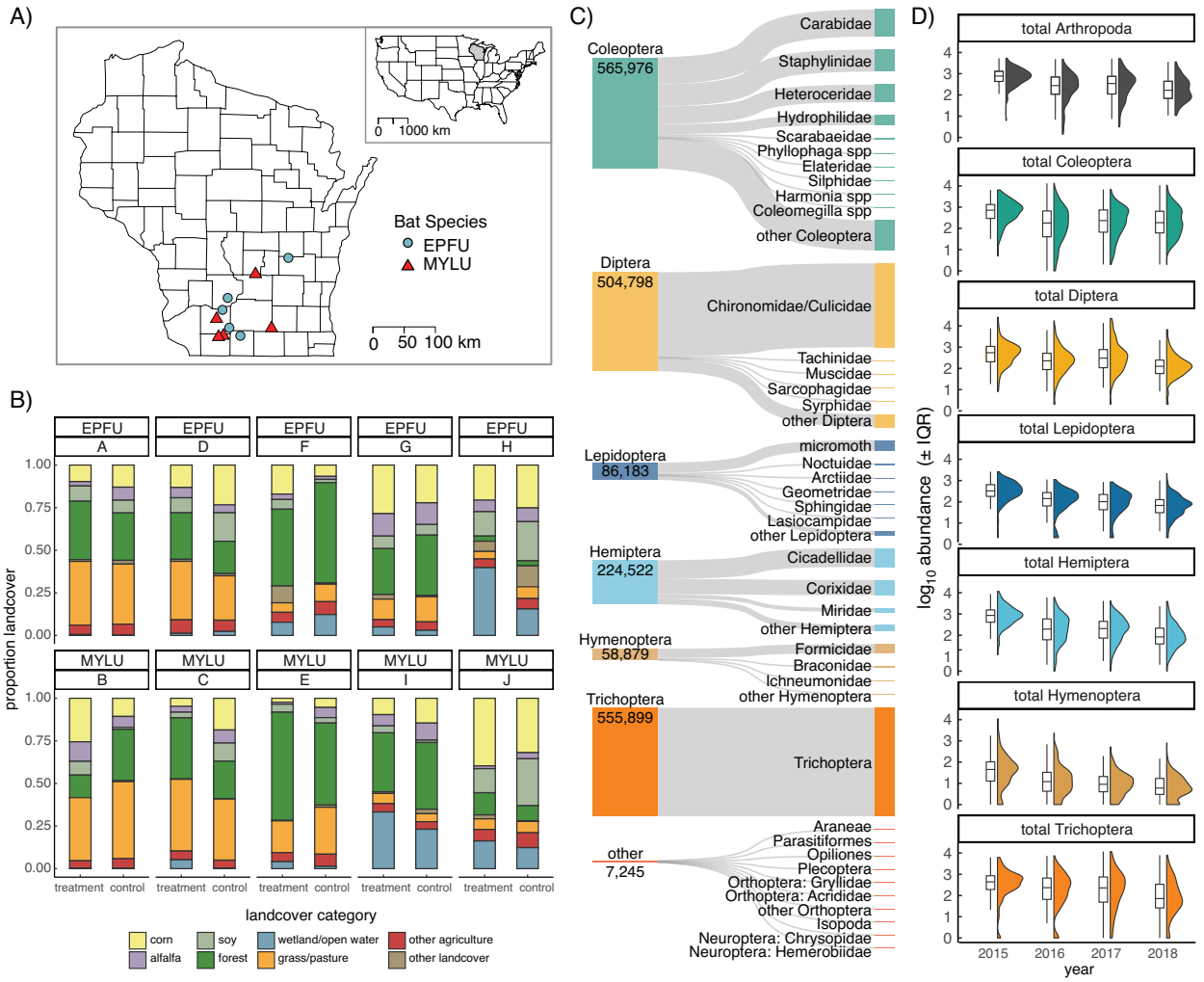

A)
0 1000 km
Bat Species
EPFU
MYLU
0 50 100 km
B)
EPFU
A
EPFU
D
EPFU
F
EPFU
G
EPFU
H
MYLU
B
MYLU
C
MYLU
E
MYLU
I
MYLU
J
proportion landcover
1.00
0.75
0.50
0.25
0.00
treatment
control
landcover category
corn
soy
wetland/open water
other agriculture
alfalfa
forest
grass/pasture
other landcover
C)
Coleoptera
565,976
Carabidae
Staphylinidae
Heteroceridae
Hydrophilidae
Scarabaeidae
Phyllophaga spp
Elateridae
Silphidae
Harmonia spp
Coleomegilla spp
other Coleoptera
Diptera
504,798
Chironomidae/Culicidae
Tachinidae
Muscidae
Sarcophagidae
Syrphidae
other Diptera
Lepidoptera
86,183
micromoth
Noctuidae
Arctiidae
Geometridae
Sphingidae
Lasiocampidae
other Lepidoptera
Hemiptera
224,522
Cicadellidae
Corixidae
Miridae
other Hemiptera
Hymenoptera
58,879
Formicidae
Braconidae
Ichneumonidae
other Hymenoptera
Trichoptera
555,899
Trichoptera
other
7,245
Araneae
Parasitiformes
Opiliones
Plecoptera
Orthoptera: Gryllidae
Orthoptera: Acrididae
other Orthoptera
Isopoda
Neuroptera: Chrysopidae
Neuroptera: Hemerobiidae
D)
total Arthropoda
total Coleoptera
total Diptera
total Lepidoptera
total Hemiptera
total Hymenoptera
total Trichoptera
log10 abundance (± IQR)
0
1
2
3
4
2015
2016
2017
2018
year

Figure 4

Supplementary Materials

Table S1. Coefficients for linear mixed effects models. Statistically significant model terms highlighted in bold.

	Est	SE	df	*t* value	*p*
Arthropoda					
Intercept	7.812	0.250	71.63	31.249	<0.001
year2	**-0.813**	**0.267**	**632.77**	**-3.041**	**0.002**
year3	**-0.741**	**0.275**	**620.97**	**-2.689**	**0.007**
year4	**-1.082**	**0.270**	**618.99**	**-4.011**	**<0.001**
MYLU	0.242	0.337	72.76	0.718	0.475
tx	0.182	0.219	372.96	0.828	0.408
year2:MYLU	-0.268	0.356	645.06	-0.753	0.452
year3:MYLU	-0.266	0.375	648.96	-0.710	0.478
year4:MYLU	-0.708	0.365	640.74	-1.943	0.052
year2:tx	-0.054	0.264	363.23	-0.205	0.838
year3:tx	0.089	0.273	361.90	0.327	0.744
year4:tx	0.261	0.268	363.05	0.974	0.331
MYLU:tx	-0.597	0.305	418.53	-1.956	0.051
year2:MYLU:tx	**0.761**	**0.363**	**395.22**	**2.095**	**0.037**
year3:MYLU:tx	0.572	0.378	392.43	1.513	0.131
year4:MYLU:tx	0.343	0.371	394.73	0.923	0.357
Coleoptera					
Intercept	6.760	0.331	42.49	20.441	<0.001
year2	**-1.245**	**0.323**	**598.40**	**-3.851**	**<0.001**
year3	**-0.891**	**0.334**	**586.90**	**-2.670**	**0.008**
year4	**-0.925**	**0.327**	**584.80**	**-2.826**	**0.005**
MYLU	-0.512	0.444	42.52	-1.153	0.255
tx	0.224	0.241	362.70	0.930	0.353
year2:MYLU	0.302	0.430	613.00	0.702	0.483
year3:MYLU	-0.110	0.452	617.60	-0.243	0.808
year4:MYLU	-0.293	0.441	608.80	-0.666	0.506
year2:tx	-0.125	0.290	354.50	-0.430	0.667
year3:tx	-0.202	0.298	353.70	-0.676	0.500
year4:tx	-0.001	0.293	354.70	-0.003	0.998
MYLU:tx	-0.629	0.338	402.30	-1.864	0.063
year2:MYLU:tx	0.335	0.400	382.90	0.836	0.404
year3:MYLU:tx	0.573	0.417	380.40	1.375	0.170
year4:MYLU:tx	0.635	0.409	382.50	1.553	0.121
Carabidae					
Intercept	5.162	0.345	219.54	14.944	<0.001
year2	**-1.371**	**0.415**	**619.26**	**-3.305**	**0.001**
year3	**-0.760**	**0.428**	**608.09**	**-1.777**	**0.076**
year4	**-1.209**	**0.419**	**606.32**	**-2.885**	**0.004**
MYLU	-0.351	0.465	223.10	-0.755	0.451
tx	-0.289	0.325	372.33	-0.890	0.374
year2:MYLU	-0.009	0.553	632.84	-0.016	0.987
year3:MYLU	-0.327	0.581	637.23	-0.563	0.573
year4:MYLU	-0.288	0.566	629.37	-0.509	0.611
year2:tx	0.189	0.391	363.53	0.484	0.629
year3:tx	0.009	0.403	361.91	0.021	0.983
year4:tx	0.174	0.397	362.88	0.438	0.661
MYLU:tx	-0.200	0.454	415.36	-0.440	0.660
year2:MYLU:tx	0.164	0.539	393.63	0.305	0.761
year3:MYLU:tx	0.231	0.561	391.07	0.412	0.681
year4:MYLU:tx	0.595	0.551	392.94	1.080	0.281
Diptera					
Intercept	5.240	0.352	29.88	14.880	<0.001
year2	-0.207	0.312	690.26	-0.662	0.508
year3	0.184	0.320	680.49	0.575	0.565
year4	**-1.059**	**0.314**	**679.11**	**-3.378**	**0.001**
MYLU	0.391	0.476	30.73	0.822	0.417
tx	0.181	0.301	379.89	0.599	0.549
year2:MYLU	-0.722	0.417	695.67	-1.731	0.084
year3:MYLU	**-0.969**	**0.439**	**696.86**	**-2.206**	**0.028**
year4:MYLU	-0.529	0.426	691.64	-1.242	0.215
year2:tx	-0.335	0.364	367.35	-0.920	0.358
year3:tx	-0.083	0.376	365.10	-0.222	0.824
year4:tx	0.136	0.369	366.55	0.368	0.713
MYLU:tx	-0.295	0.413	432.94	-0.716	0.474
year2:MYLU:tx	**1.241**	**0.495**	**403.35**	**2.507**	**0.013**
year3:MYLU:tx	**1.108**	**0.516**	**400.23**	**2.149**	**0.032**
year4:MYLU:tx	0.316	0.506	402.74	0.625	0.532

	Est	SE	df	*t* value	*p*
micromoth					
Intercept	4.101	0.328	85.23	12.499	<0.001
year2	**-0.750**	**0.355**	**723.76**	**-2.115**	**0.035**
year3	**-0.731**	**0.363**	**718.98**	**-2.011**	**0.045**
year4	**-1.382**	**0.356**	**718.52**	**-3.887**	**<0.001**
MYLU	0.734	0.446	89.38	1.645	0.103
tx	**-0.942**	**0.380**	**404.09**	**-2.478**	**0.014**
year2:MYLU	-0.652	0.475	724.87	-1.373	0.170
year3:MYLU	-0.811	0.500	724.04	-1.623	0.105
year4:MYLU	-0.689	0.485	722.61	-1.422	0.155
year2:tx	0.886	0.461	390.68	1.923	0.055
year3:tx	0.518	0.476	387.10	1.089	0.277
year4:tx	**1.063**	**0.467**	**388.57**	**2.274**	**0.024**
MYLU:tx	0.318	0.514	456.79	0.618	0.537
year2:MYLU:tx	-0.290	0.621	425.41	-0.467	0.641
year3:MYLU:tx	-0.340	0.648	422.38	-0.525	0.600
year4:MYLU:tx	**-1.101**	**0.635**	**424.29**	**-1.734**	**0.084**
Noctuidae					
Intercept	1.932	0.217	191.51	8.907	0.000
year2	-0.193	0.255	703.76	-0.755	0.450
year3	-0.460	0.262	695.51	-1.756	0.080
year4	**-0.704**	**0.256**	**694.40**	**-2.747**	**0.006**
MYLU	0.183	0.295	198.66	0.620	0.536
tx	-0.285	0.255	388.62	-1.120	0.263
year2:MYLU	-0.239	0.341	708.07	-0.699	0.485
year3:MYLU	0.024	0.359	708.53	0.067	0.947
year4:MYLU	-0.171	0.349	704.75	-0.490	0.625
year2:tx	0.261	0.308	375.84	0.846	0.398
year3:tx	-0.134	0.318	372.86	-0.421	0.674
year4:tx	0.401	0.312	374.27	1.283	0.200
MYLU:tx	-0.340	0.348	442.24	-0.978	0.329
year2:MYLU:tx	0.413	0.418	411.71	0.989	0.323
year3:MYLU:tx	0.379	0.436	408.55	0.870	0.385
year4:MYLU:tx	0.093	0.427	410.72	0.218	0.828
Hemiptera					
Intercept	6.013	0.240	311.29	25.085	<2.00E-16
year2	**-1.110**	**0.294**	**651.08**	**-3.771**	**<0.001**
year3	**-1.281**	**0.303**	**640.37**	**-4.225**	**<0.001**
year4	**-2.126**	**0.297**	**638.71**	**-7.162**	**<0.001**
MYLU	0.071	0.324	317.97	0.220	0.826
tx	-0.031	0.253	380.20	-0.124	0.902
year2:MYLU	-0.161	0.393	662.24	-0.410	0.682
year3:MYLU	-0.198	0.413	665.93	-0.478	0.632
year4:MYLU	-0.231	0.402	659.06	-0.575	0.566
year2:tx	-0.034	0.305	369.84	-0.112	0.911
year3:tx	0.229	0.315	367.62	0.727	0.468
year4:tx	0.420	0.310	368.74	1.356	0.176
MYLU:tx	-0.091	0.351	428.57	-0.261	0.794
year2:MYLU:tx	0.299	0.418	403.11	0.715	0.475
year3:MYLU:tx	-0.029	0.436	400.21	-0.066	0.947
year4:MYLU:tx	-0.136	0.428	402.21	-0.319	0.750
Corixidae					
Intercept	4.464	0.393	36.81	11.372	<0.001
year2	**-1.397**	**0.370**	**630.93**	**-3.772**	**<0.001**
year3	**-1.355**	**0.382**	**619.12**	**-3.550**	**<0.001**
year4	**-1.573**	**0.374**	**617.09**	**-4.205**	**<0.001**
MYLU	-0.789	0.528	37.23	-1.494	0.144
tx	0.031	0.301	373.60	0.102	0.919
year2:MYLU	0.893	0.494	643.29	1.807	0.071
year3:MYLU	0.977	0.519	647.17	1.882	0.060
year4:MYLU	0.743	0.505	638.80	1.469	0.142
year2:tx	0.179	0.363	364.02	0.494	0.622
year3:tx	0.315	0.374	362.85	0.842	0.401
year4:tx	-0.007	0.368	364.01	-0.019	0.985
MYLU:tx	0.284	0.420	418.42	0.678	0.498
year2:MYLU:tx	-0.695	0.499	395.64	-1.392	0.165
year3:MYLU:tx	-1.004	0.520	392.87	-1.933	0.054
year4:MYLU:tx	-0.056	0.510	395.23	-0.110	0.913

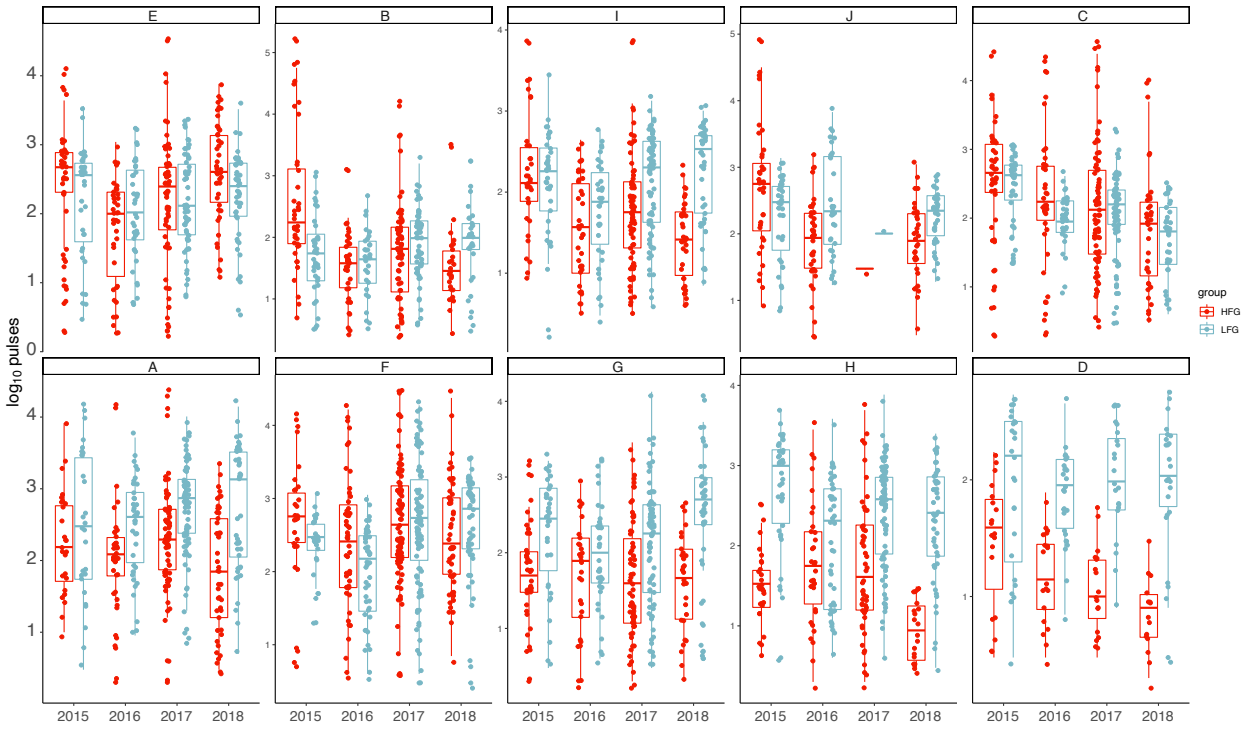
Table S1 (continued)

Chironomidae					
Intercept	3.983	0.501	29.67	7.956	0.000
year2	0.146	0.443	685.56	0.330	0.742
year3	**0.964**	**0.455**	**675.11**	**2.121**	**0.034**
year4	**-1.101**	**0.445**	**673.61**	**-2.474**	**0.014**
MYLU	0.370	0.676	30.49	0.547	0.589
tx	0.263	0.424	369.99	0.619	0.536
year2:MYLU	-0.664	0.592	691.58	-1.122	0.262
year3:MYLU	-1.056	0.623	692.99	-1.696	0.090
year4:MYLU	-0.574	0.605	687.28	-0.950	0.343
year2:tx	-0.655	0.513	357.56	-1.277	0.203
year3:tx	-0.252	0.529	355.38	-0.477	0.634
year4:tx	0.077	0.520	356.82	0.148	0.883
MYLU:tx	-0.157	0.582	423.09	-0.269	0.788
year2:MYLU:tx	**1.529**	**0.697**	**393.56**	**2.192**	**0.029**
year3:MYLU:tx	0.829	0.727	390.42	1.140	0.255
year4:MYLU:tx	0.117	0.713	392.96	0.164	0.870

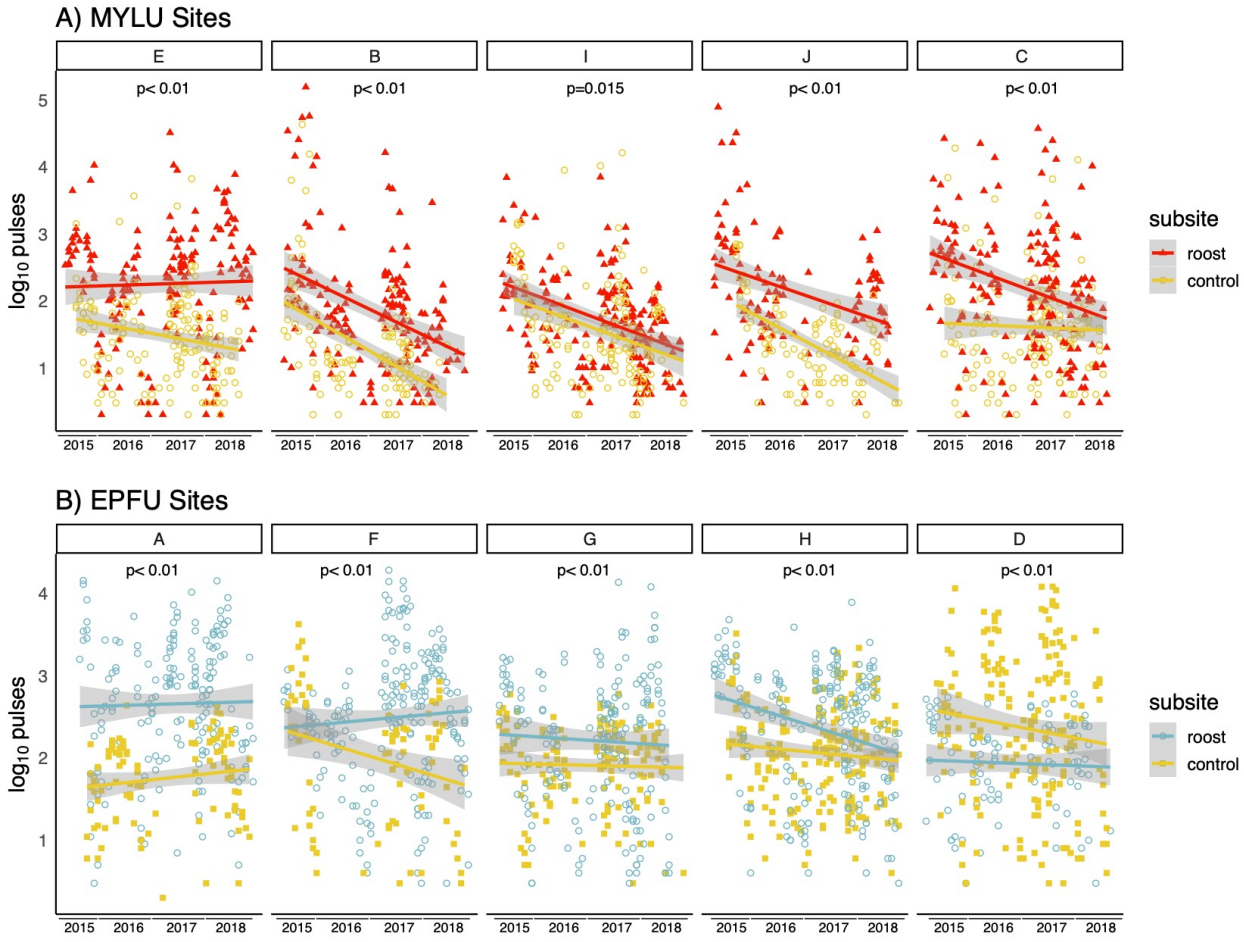
Hymenoptera					
Intercept	2.940	0.315	82.40	9.326	<0.001
year2	-0.160	0.341	699.32	-0.469	0.639
year3	**-0.708**	**0.351**	**690.68**	**-2.020**	**0.044**
year4	**-1.209**	**0.343**	**689.50**	**-3.524**	**<0.001**
MYLU	**1.616**	**0.428**	**85.76**	**3.776**	**<0.001**
tx	0.332	0.337	389.37	0.986	0.325
year2:MYLU	**-1.653**	**0.457**	**703.82**	**-3.618**	**<0.001**
year3:MYLU	**-1.543**	**0.481**	**704.54**	**-3.210**	**0.001**
year4:MYLU	**-1.388**	**0.467**	**700.28**	**-2.975**	**0.003**
year2:tx	-0.496	0.407	376.65	-1.217	0.224
year3:tx	-0.434	0.420	374.01	-1.033	0.302
year4:tx	-0.165	0.413	375.45	-0.401	0.689
MYLU:tx	**-1.468**	**0.460**	**442.65**	**-3.192**	**0.002**
year2:MYLU:tx	**1.759**	**0.553**	**412.57**	**3.183**	**0.002**
year3:MYLU:tx	**2.029**	**0.576**	**409.44**	**3.524**	**0.000**
year4:MYLU:tx	**1.338**	**0.565**	**411.78**	**2.368**	**0.018**

Lepidoptera					
Intercept	4.916	0.258	51.00	19.028	<0.001
year2	**-0.627**	**0.259**	**715.41**	**-2.421**	**0.016**
year3	**-1.037**	**0.265**	**709.27**	**-3.908**	**<0.001**
year4	**-1.620**	**0.260**	**708.56**	**-6.238**	**<0.001**
MYLU	0.431	0.351	53.17	1.230	0.224
tx	-0.175	0.268	402.05	-0.652	0.515
year2:MYLU	-0.445	0.347	717.54	-1.284	0.199
year3:MYLU	-0.296	0.365	717.32	-0.811	0.417
year4:MYLU	-0.261	0.354	714.83	-0.738	0.461
year2:tx	0.093	0.325	388.82	0.286	0.775
year3:tx	-0.012	0.335	385.73	-0.035	0.972
year4:tx	0.431	0.329	387.20	1.311	0.191
MYLU:tx	-0.296	0.364	455.17	-0.812	0.417
year2:MYLU:tx	0.397	0.439	424.26	0.906	0.366
year3:MYLU:tx	0.161	0.457	421.20	0.353	0.725
year4:MYLU:tx	-0.199	0.449	423.35	-0.444	0.658

Trichoptera					
Intercept	5.344	0.343	139.33	15.579	<0.001
year2	0.051	0.395	652.05	0.129	0.897
year3	-0.207	0.407	640.89	-0.508	0.612
year4	**-0.819**	**0.399**	**639.09**	**-2.054**	**0.040**
MYLU	**0.927**	**0.463**	**142.68**	**2.001**	**0.047**
tx	0.664	0.341	381.89	1.950	0.052
year2:MYLU	-0.647	0.528	662.64	-1.225	0.221
year3:MYLU	-0.361	0.555	666.00	-0.651	0.515
year4:MYLU	**-1.068**	**0.540**	**658.73**	**-1.978**	**0.048**
year2:tx	-0.514	0.411	371.39	-1.252	0.211
year3:tx	-0.117	0.424	369.61	-0.276	0.783
year4:tx	-0.076	0.416	370.82	-0.181	0.856
MYLU:tx	**-0.950**	**0.472**	**430.10**	**-2.013**	**0.045**
year2:MYLU:tx	**0.933**	**0.563**	**404.83**	**1.658**	**0.098**
year3:MYLU:tx	0.181	0.586	401.92	0.309	0.758
year4:MYLU:tx	0.341	0.576	404.19	0.592	0.554



Supplementary Figure S1



Supplementary Figure S2

CHAPTER 4: BIG BROWN BATS DO NOT FILL THE FUNCTIONAL ROLE OF LITTLE BROWN BATS FOLLOWING DISEASE-RELATED DECLINES

Amy K. Wray[1], Claudio Gratton[2], Michelle A. Jusino[3,4], Jing Jamie Wang[1], Jade M. Kochanski[2], Jon Palmer[3], Mark T. Banik[3], Daniel L Lindner[3], and M. Zachariah Peery[1]

[1]Department of Forest & Wildlife Ecology, University of Wisconsin-Madison, Madison, WI, USA; [2]Department of Entomology, University of Wisconsin-Madison, Madison, WI, USA; [3]Center for Forest Mycology Research, Northern Research Station, USDA Forest Service, Madison, WI, USA; [4]Department of Biology, William & Mary, Williamsburg, VA, USA

ABSTRACT

Understanding the potential for persisting species to fill the role of extirpated or declining species remains a central focus of restoration ecology. In North America, bats are currently threatened on a continent-wide scale by the emergence and spread of white-nose syndrome (WNS), a devastating disease resulting from infection with the fungus (*Pseudogymnoascus destructans*). WNS was initially detected in Wisconsin in March 2014 and was predicted to cause precipitous declines among Wisconsin bats — all of which are arthropodivores — within the subsequent four to five years. We tested whether big brown bats, which exhibit some resistance to WNS, can partially fill the functional role of other bat species following WNS-related declines. Specifically, we performed high-throughput amplicon sequencing of little brown (*Myotis lucifugus*) and big brown bat (*Eptesicus fuscus*) guano samples collected at maternity roosts from 2015–2018 and compared changes in diet composition before versus after WNS-related bat population declines. Overall, we found that both little brown and big brown bat dietary niches contracted following substantial WNS-related declines in little brown bat populations, but interspecific overlap did not change. We also found that following bat population declines, the incidence and taxonomic richness of agricultural pest taxa detected in diet samples decreased for both bat species. The results of this study demonstrate that big brown bats do not necessarily expand their dietary niches following population declines among other bat species, which suggests that the functional role of little brown bats is ecologically distinct. As such, this study emphasizes the importance of developing conservation strategies to mitigate population declines among bat species that are severely impacted by WNS as well as among other declining generalist predators.

Introduction

Species tend to diverge and specialize over long periods of time, but generalist habits can be an asset in eras dominated by change. On an evolutionary scale, specialization can lead to speciation, yet the persistence of generalism can buffer species against extinction and facilitate persistence (Dennis et al. 2011; Loxdale et al. 2011). Generalism has been associated with reduced extinction risk, and species with flexible resource requirements and are often better able to adapt to the rapidly changing environments of the Anthropocene (Boyles & Storm, 2007; Colles et al. 2009; Purvis et al. 2000). As certain species decline, the question of whether other species can serve as ecological replacements to maintain interaction networks has also been raised (Parker et al. 2010; Rubenstein et al. 2006; Tylianakis et al. 2010). In some examples, ecological replacement has included intentional introductions for ecosystem restoration (e.g., Wingate 1982; Hunter et al. 2013), while other studies have documented unintentional introductions or range expansions that involve one species filling the vacant trophic niches left behind by another (e.g., Nowak 2002; Carlsson et al. 2010; Tompkins et al. 2003). In light of global predator declines and recent emphases on the role of predators in restoration ecology (e.g., Estes et al. 2011; Ritchie et al. 2012), the question of whether and to what extent surviving predators may be capable of filling the vacant functional roles of extirpated predators remains largely unanswered. As such, assessing the equivalency of similar predator species therefore represents an important first step for conservation strategies focused on preserving ecosystem functioning.

Vertebrate arthropodivores are declining globally due to a myriad of factors, including habitat loss and degradation, disease, and declines in arthropod prey (Nebel et al. 2010; Nocera et al. 2012; O'Shea et al. 2016; Spiller & Dettmers, 2019). Given that vertebrate arthropodivores have been shown to have top-down influences on prey, the effects of declining populations may, consequently, have cascading effects on their respective food webs (Mooney et al. 2010). Declines among aerial vertebrate

arthropodivores, such as birds and bats, have been of particular interest due to their high energy requirements that necessitate consumption of large quantities of arthropods (Boyles & Willis 2010; Kelly et al. 2013; Nyffeler et al. 2018), which may result in disproportionate influences on the arthropod food web. Some aerial arthropodivores also function as natural enemies of arthropod pests, thereby providing valuable ecosystem services (Boyles et al. 2011; Kunz et al. 2011; Maas et al. 2016). Though many aerial arthropodivores are currently in decline, others persist and even appear to be expanding their ranges (McCracken et al. 2018; Williams-Guillén et al. 2016). Despite their frequent generalist foraging habits, aerial insectivores can display strong levels of dietary niche partitioning (Kent & Sherry 2020; Patterson et al. 2003), and therefore, fill distinct ecological roles that may not be easily replaced by other sympatric species. However, understanding whether persisting taxa are able to compensate for the functional roles of other declining populations has seldom been determined among aerial arthropodivores, largely due to methodological challenges associated with characterizing resource requirements prior to population declines.

Anatomical and physiological constraints often limit the foraging of generalist predators. In contrast to most systems where larger body size is competitively advantageous, among arthropodivorous bats small body size provides a competitive advantage by increasing agility during flight, which in turn means that a smaller bat can consume small prey items that a larger bat cannot catch, while also being able to capture larger prey (Aguirre et al. 2003; Barclay & Brigham 1991). Indeed, certain bat species are capable of consuming prey larger than their own body size and will occasionally land and consume the preferred parts of the prey while discarding the rest (O'Shea & Vaughan 1977; Santana & Cheung 2016). Since arthropodivorous bats largely rely on echolocation while hunting, certain echolocation frequencies are also adapted for acquiring specific prey, and echolocation may play a role in limiting dietary niche breadth (Arbour et al. 2019). For example, bats that use lower-frequency echolocation are typically more adept at capturing larger prey, while bats that

use higher-frequency echolocation are better at capturing smaller prey (Denzinger & Schnitzler 2013; Jones & Holderied 2007). Such physiological constraints are generally considered to be more important determinants of bat trophic niches than direct competition with other bats in similar guilds (Schoeman & Jacobs 2011). As such, the morphological constraints that shape bat foraging strategies casts further doubt on the potential for ecological equivalency among species with differing morphology.

In North America, many hibernating bat species have undergone precipitous declines due to white-nose syndrome (WNS), a fungal disease first detected in 2006 (Frick et al. 2016). These population declines present a unique circumstance under which fundamental ecological questions about the role of arthropodivorous bats as predators may be answered. In eastern North America where WNS has been present for over a decade, little brown bats have declined in excess of 90%, leaving some regions locally extirpated (Frick et al. 2016). Comparatively, other common bat species such as big brown bats experience infection from white-nose syndrome yet have declined with much less severity (Frank et al. 2014; Frick et al. 2010). Big brown bats are often noted to persist in human-dominated habitats, readily utilizing built structures such as bat houses and barns (Voigt et al. 2016). Additional studies have shown that in the past century, big brown bat dietary niches have shifted substantially, with a convergence upon the niches of other species such as little brown bats (Chapter 2). These observed patterns, combined with their apparent resistance of big brown bats to white-nose syndrome, raises the question of whether big brown bats are capable of partially or fully adopting the open niche spaces left behind by declines among other arthropodivorous bats.

In this study, we quantified changes in dietary composition and niche overlap in two generalist arthropodivorous bat species following the rapid, WNS-induced decline of one species and the persistence of another. Specifically, we tested whether declines in little brown bats would lead to niche expansion among big brown bats by comparing changes in overall diet composition and prevalence

of known agricultural pests as measured using high-throughput amplicon sequencing (HTAS) methods. We hypothesized that due to physiological constraints, big brown bats would be unable to adopt the dietary niche space formerly occupied by little brown bats. Under this hypothesis, we predicted that big brown bats would not expand their dietary niche to include more small-bodied arthropods following declines in little brown bats resulting from WNS. We also predicted that following declines in little brown bats, the incidence and taxonomic richness of agricultural pest taxa detected in big brown bat guano samples would not increase. Overall, our hypotheses were partially supported, and we found that prior to WNS-related bat declines, the dietary niches of little brown and big brown bats already overlapped somewhat, and while niche breadth decreased for both bat species, interspecific niche overlap did not change. We also found that following WNS-related declines, both bat species consumed a lower richness and incidence of agricultural pest taxa.

Methods

Bat guano collection & detection of arthropod DNA

We collected bat guano samples weekly at 5 little brown and 5 big brown bat roosts, including one site with a little brown bat colony present in a bat house and a big brown bat colony present in a nearby barn. We collected guano by placing a clean plastic sheet under each roost for one week, with samples collected weekly at all sites for the duration of the summer from 2015–2018 (late May–late August). Following collection, samples were stored on ice during transport and subsequently kept at –80°C for long-term storage. Additional samples were collected by citizen scientists at 3 or 4 time periods during the summer from 2015–2018, yielding an additional two little brown and two big brown roost sites (Figure 1A). These samples were initially stored at –20°C, then shipped overnight on ice and kept at –80°C for long-term storage. The identity of bat species was confirmed by directly observing bats and by comparing guano pellet size at the time of collection. The number of bats per

roost was also estimated via emergence counts conducted as part of the Wisconsin Bat Program's Great Wisconsin Bat Count. These counts occurred at least twice per year in the early and late summer, which approximately corresponds to the pre- and post-volancy reproductive periods. Approximately 30 minutes before sunset, volunteers positioned themselves near bat roosts and counted bats as they emerged. In 2015, little brown bat colonies had an average of 210 bats per roost (ranging from 94–409 bats), while big brown bat colonies had an average of 137 bats per roost (ranging from 21–428 bats). By 2018, little brown bat colonies declined to an average of 19 bats per roost (ranging from 0–50 bats), while big brown bat colonies declined to an average of 76 bats per roost (ranging from 26–180 bats). Overall, the total average number of little brown bats per year was 1,015.7 in the first two years, and 119.9 in the second two years, representing a decline of –88.2%. The total average number of big brown bats per year was 928.7 in the first two years, and 542.3 in the second two years, representing a decline of –41.6%. All sample collection and animal observation methods were carried out in accordance with guidelines of the Wisconsin Department of Natural Resources and the American Society of Mammalogists (Sikes 2016). Experimental protocols were approved by the Wisconsin Natural History Inventory (NHI) Program and the University of Wisconsin-Madison College of Agricultural and Life Sciences (CALS) Animal Care and Use Committee (ACUC).

Sequencing arthropod COI isolated from bat guano

DNA extraction, PCR, and high throughput amplicon sequencing followed Jusino et al. 2019 with the same modifications presented in Chapter 1. Briefly, DNA was extracted from bat guano using a Qiagen DNA Stool mini kit (Qiagen Inc. Germantown, Maryland), with a 180-bp region of the COI subunit c amplified using PCR with ANML primers (Jusino et al., 2019). Thermocycler parameters followed Hebert et al. 2003, with the exception of the final extension at 72°C increased from 5 to 7 minutes. A mock community of 34 known arthropod constituents was also amplified under the same

conditions as a positive control (Jusino et al. 2019). PCR products were purified using a Zymo Select-a-Size kit (Zymo, Irvine, California), and five total equimolar libraries were constructed with approximately 72 samples per library. Negative and positive controls were included, and samples were processed in a randomized order to reduce potential batch processing biases. Sequencing was performed on an Ion Torrent Personal Genome Machine platform (PGM; ThermoFisher Scientific, Inc., Santa Fe, New Mexico) according to the manufacturer's recommendations with an Ion PGM 318v2 chip. Raw sequence data were then processed using AMPtk (Palmer, Jusino, Banik, & Lindner, 2018). This data processing procedure includes de-multiplexing using unique barcode index sequences, stripping of forward and reverse primers, and quality filtering and denoising with the DADA2 algorithm (Callahan et al. 2016). Operational taxonomic units were clustered at 97% similarity, generating an OTU table of demultiplexed sequencing reads. Taxonomy was then assigned using the built-in COI database in AMPtk v1.4.2 (Palmer et al. 2018), which resulted in a total of 1,786 OTUs. All OTUs were identified to phylum, class, and order, with 87.4% identified to family, 74.8% identified to genus, and 56.9% identified to species. Following taxonomy assignment, we removed all OTUs that were not identified as insects or arachnids, (n=36 OTUs), as well ectoparasites including Mesostigmata, Trombidiformes, Sarcoptiformes, and Siphonatera, which do not represent prey items (n=87 OTUs).

Statistical analyses

Post-processing, OTU tables were converted into weighted percent occurrence (wPO), a presence-based metric, and relative read abundance (RRA), a read-based metric, following Deagle et al. (2019). Within major arthropod orders (Araneae, Coleoptera, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Lepidoptera, and Trichoptera), we compared mean values of wPO and RRA in samples collected between 2015–2016 with samples collected between 2017–2018 using separate Wilcoxon

rank-sum tests for each measure and for each bat species. We then aggregated OTU tables at the family level for the estimation of niche metrics. We calculated niche breadth as Levin's adjusted niche breadth, B_a, and calculated niche overlap as Pianka's measure of niche overlap, O_{jk}, which provides a symmetrical estimate of the niche overlap between two species (Hurlbert 1978; Levins 1968; MacArthur & Levins 1967). To visualize diet communities in multivariate space, we performed non-metric multidimensional scaling (NMDS) using the metaMDS function with a modification of the raupcrick function as described by Chase et al. 2011, which were performed separately for presence/absence matrices at the OTU and family levels. For both levels, we excluded any taxon groups that were not detected at least 5 times in order to reduce the influences of infrequently detected diet items. To assess whether the bat diet communities differed by species, time period, collection site, and Julian week, we used nonparametric PERMANOVA tests performed by the "adonis" function with 999 replicates and assessed the influences of dispersion using the "betadisper" function to separately test each predictor variable. We also searched taxonomy tables for certain arthropod taxa that are known agricultural pests in the study area. To compare incidence and taxonomic richness of pest taxa detected in samples collected between 2015–2016 with samples collected between 2017–2018, we used chi-squared tests and Welch's t-tests, respectively, which were conducted separately for each bat species. All analyses were performed in R, with multivariate analyses conducted using the R package "vegan" (Oksanen et al. 2013). Additional packages used for data processing and visualization include "dplyr", "ggplot2", "tidyverse", "wesanderson", and "reshape2 (Ram and Wickham 2018; Wickham 2020; Wickham et al. 2019; Wickham et al. 2020).

Results

A total of 173 little brown and 142 big brown bat samples were successfully amplified. Overall, 1,663 arthropod prey OTUs were detected representing 19 orders, 221 families, 703 genera, and 891 species.

A total of 1,334 OTUs were found among little brown bats and 865 OTUs were found among big brown bats, of which 536 OTUs were shared between both. The most commonly detected prey families for little brown bats, as measured by incidence, were Diptera: Chironomidae, followed by Lepidoptera: Tortricidae and Diptera: Limoniidae. For big brown bats, the most commonly detected prey families were Coleoptera: Elateridae, Diptera: Limoniidae, and unidentified Coleoptera (Supplementary Table S1). The most common prey, as measured by wPO and RRA, were fairly consistent, except for big brown bats where Trichoptera: Hydropsychidae had a much higher RRA than wPO (Supplementary Table S1).

We found that little brown and big brown bat dietary composition were distinct, but intraspecific dietary composition did not change substantially between pre- and post-WNS time periods for either bat species. Little brown bats diets contained a higher richness of Diptera, Lepidoptera, and Hemiptera, and big brown bat diets contained a higher richness of Coleoptera, which is consistent with previous studies (e.g., Chapter 1). The RRA and wPO within arthropod orders differed by bat species but remained similar between time periods within each bat species (Figure 1B, Supplementary Table S2). In the later time period, significantly more Diptera and fewer Hemiptera and Hymenoptera were detected in big brown bat diets, though these effects differed between wPO and RRA measurements (Figure 1B, Supplementary Table S2). For little brown bats, significantly more Diptera and more Trichoptera, as well as fewer Hemiptera and fewer Ephemeroptera were detected after declines from WNS, though these effects differed between wPO and RRA (Figure 1B, Supplementary Table S2). The arthropod families most commonly detected for each species also remained similar between time periods, as ranked by wPO (Table 1).

In general, little brown and big brown bats showed differences in dietary niche breadth, but the observed patterns did not change substantially between the pre- and post-WNS time periods. Little brown bats displayed higher niche breadth in comparison to big brown bats, and total interspecific

niche overlap was 0.281. For both bat species, niche breadth from samples collected in 2015–2016 was higher than niche breadth from samples collected in 2017–2018. Little brown bat niche breadth decreased by 24.2% between time periods, while big brown bat niche breadth decreased by 34.6% between time periods. Interspecific niche overlap was similar between time periods, increasing by only 2.3% from 0.281 to 0.288 in the post-WNS time period. NMDS plots also showed that diet composition differed more between species than between time periods. PERMANOVA demonstrated that species was the best predictor of variation at the family and OTU levels (Figure 2A, Table 3). At both the family and OTU levels, Julian week was also a significant predictor of variation, while collection site and time period were also significant predictors at the OTU level (Figure 2A, Table 3). At both the family and OTU levels, there were significant differences in multivariate dispersion between bat species and between collection sites, and at the OTU level there were also significant differences in multivariate dispersion between time periods and Julian week (Figure 2A, Table 3). These results suggest that while bat species identity was the most important factor in determining diet community structure and dispersion, other factors were also influential, particularly at the OTU level.

Several agricultural pests were detected in bat guano samples, which were most commonly detected in little brown bat samples collected prior to WNS-related declines. Cumulatively, we detected at least one agricultural pest taxa in 45.1% of little brown bat samples and in 33.8% of big brown samples. For little brown bats, this percentage decreased from 53.3% to 15.8% between the two time periods, while for big brown bats this percentage decreased from 43.2% to 23.6% between the two time periods. The most commonly detected agricultural pest taxa for little brown bats was *Agrotis ipsilon*, which was detected in 13% of all samples, while the most commonly detected agricultural pest taxa for big brown bats was *Phyllophaga anxia*, which was detected in 8% of all samples. For both bat species, the incidence of most individual pest taxa also declined between time periods

(Figure 2B). The difference in the number of samples with at least one agricultural pest taxa present between time periods was statistically significant for little brown bats (χ^2=15.4, df=1, p<0.001) and for big brown bats (χ^2=5.31, df=1, p=0.021). Little brown bats had an average of 0.54 pest taxa per sample (ranging from 0–4 pest taxa per sample), while big brown bats had an average of 0.45 pest taxa per sample (ranging from 0–3 pest taxa per sample). For little brown bats, the average richness of pest taxa per sample was significantly higher in the first time period ($\bar{x}$=0.89±0.17) in comparison to the second time period ($\bar{x}$=0.18±0.15, $t_{136.61}$=6.19, p<0.001). For big brown bats, the average richness of pest taxa per sample was also significantly higher in the first time period ($\bar{x}$=0.61±0.19) in comparison to the second time period ($\bar{x}$=0.29±0.15, $t_{133.18}$=2.61, p=0.01).

Discussion

Overall, our findings suggest that big brown bats cannot function as ecological replacements for rapidly declining little brown bats. In this study, we tested whether the effects of bat population declines influenced changes in dietary composition, interspecific niche overlap, and the amount of agricultural pest taxa consumed. We found that both little brown and big brown population sizes declined, with little brown bats declining to a much greater extent than big brown bats, which followed our a priori assumptions. In the time period after WNS-related bat population declines, the dietary composition of bats did not change substantially, nor did dietary overlap increase, although the niche breadth of both bat species decreased. Though little brown and big brown bats exhibited some niche overlap prior to WNS-related declines, the lack of changes in dietary composition and niche overlap suggests that little brown and big brown bats are complementary predators with differing dietary niches. Additionally, the observed decline in niche breath may suggest that both bat species display some degree of individual specialization (Bolnick et al., 2002, 2003), with the individuals within a population consuming different prey resources that ultimately contribute to a broader population-

level dietary niche. These results are consistent with one previous study which also suggested that big brown bat dietary niche breadth may be driven by individual specialization (Cryan et al. 2012). Although there is previous evidence of within-population dietary variation in arthropodivorous bats (Johnston & Fenton 2001), the contributions of individuals to population-level dietary breadth has seldom been fully explored.

While niche breadth decreased for both bat species, niche overlap did not change. In fact, niche overlap was somewhat high (0.281) in the first time period prior to WNS-related declines in bat populations. These results are also consistent with a previous study using stable isotopes (Chapter 2), which demonstrated that little brown and big brown bat isotopic niches have converged over the past century, with contemporary isotopic niches overlapping by 47%. The observed level of overlap suggests that little brown and big brown bats share some similar resources, but that the quantity of similar resources consumed did not necessarily change in response to WNS-related declines. One previous study, which compared pre- and post-WNS dietary composition based on stomach contents of bats collected at different sites, found that overlap in dietary composition did increase following WNS-related bat declines (Morningstar et al. 2019). However, in light of the observed variation between sites and between weeks, particularly at the OTU level, as well as the known high spatial and temporal turnover in the diets of both bat species (Wray et al. in revision), the comparison of individual bat diets may not be appropriate if collected at different sites or during different time periods (e.g., pre- and post-WNS). Additionally, the aforementioned study also concluded that increasing niche overlap may suggest increasing interspecific competition between bat species. However, just as co-occurrence does not necessarily imply ecological interactions (Blanchet et al. 2020), increasing niche overlap does not necessarily imply increasing competition. Indeed, the principle of competition relies on the supposition that resources shared by two species must be limiting in order for competition to occur, and coexistence has been shown to persist under many cases where the assumptions of

competitive exclusion are not met (Chase et al. 2002; Holt 1977). As such, our results suggest that WNS-related declines in little brown bats likely do not lead to increases in niche overlap in this study area, but rather demonstrates that interspecific niche overlap between little brown and big brown bats has remained fairly consistent in recent history despite rapid changes in population size and increasing dietary overlap over the past century.

While big brown bats share some prey resources with little brown bats, the results of this study suggest that they do not readily adopt the assumed open dietary niche space left behind following little brown bat declines. These results support our hypothesis as well as previous research which suggests that big brown bat foraging is limited by intrinsic factors such as body size and echolocation. Indeed, little brown and big brown bats diverged from each other more than 20 million years ago (Lack & Van Den Bussche 2010) and are differentially adapted for foraging on different prey types. In this study, we did not quantify the influences of prey availability, although other studies have detected declining arthropod abundance in this region (Chapter 3) and globally (Hallmann et al. 2017; Sánchez-Bayo & Wyckhuys 2019; Seibold et al. 2019). Previous studies have shown that these bat species maintain strong prey preferences independently of changing prey availability (Wray et al. in revision), and the lack of increasing niche overlap following little brown bat declines also suggests that prey availability is unlikely to be a limiting factor in determining bat dietary niche overlap. Nonetheless, other factors, such as habitat or roost availability, could represent limiting factors where the larger body size of big brown bats may provide a competitive advantage over smaller-bodied species such as little brown bats (Agosta 2002). For example, in this study we observed complete roost abandonment at two little brown bat roost sites by 2018, and we posit that if these roosts were later adopted by big brown bats, it is unlikely that little brown bats could reoccupy them upon population recovery. As such, further exploration into the potential competition between little brown and big brown bats for

roost space or other habitat requirements are warranted and may potentially be more important than limitations due to food resources.

This study is the first to characterize the functional role of bats as predators with the goal of assessing whether widespread, flexible, and comparatively successful sympatric species have the potential to serve as ecological replacements for other declining species. While big brown bats likely cannot fill the trophic role of little brown bats, possibly due to morphological constraints, other bat species may be more similar to the little brown bat and could more effectively serve as ecological replacements. However, most of these more similar species, such as other species in the genus *Myotis*, also experience severe declines due to WNS. (Frick et al. 2016). Species that are not affected by WNS, such as migratory bats, are also less abundant and do not cluster in large colonies in this study region (Huebschman 2019), and as such may not influence prey communities in the same manner. While other studies have demonstrated the successful reintroductions of extirpated predators leading to restoration of ecosystem functioning (e.g., Mittelbach et al. 1995; Ripple & Beschta 2012), such efforts often rely on the possibility of ex situ conservation strategies such as captive breeding or translocation — none of which have ever successfully been implemented with little brown bats or other bat species severely affected by WNS (Davy & Whitear 2016). As such, it is unlikely that the functional role of the little brown bat can be restored either naturally or through conservation intervention. Further, several other bat species have been severely impacted by WNS and are expected to face extirpation in many regions (Frick et al. 2010; Thogmartin et al. 2013). The growing body of evidence regarding the function of arthropodivorous bats as ecologically important predators thus raises concerns regarding potential top-down consequences of WNS-related bat declines. Overall, the findings of this study highlight the importance of continuing to support little brown bat recovery, while also emphasizing the need for conservation of bats and other aerial arthropodivores in general due to the probability that each species serves a unique ecological role that cannot necessarily be filled by another.

Acknowledgements

The authors thank thank Pen & Lynn Ackley, Mike & Jane Balch, Rhonda Enge, Marci & Jim Hess, Elaine Horn, Gary Jansen, Ron & Phillip Kerl, Liese Pfeifer, Cathy Prescott, Tom & Linda Ross, Elaine Swanson, and Mimi Wuest for their contributions to sample collection for this project. We thank John Arthur, Kathleen Gruentzel, Heather Kaarakka, Darren Marsh, and J. Paul White for assistance coordinating sampling at state and county parks.

References

Agosta, S. J. (2002). Habitat use, diet and roost selection by the big brown bat (Eptesicus fuscus) in North America: a case for conserving an abundant species. *Mammal Review, 32*(3), 179-198.

Aguirre, L., Herrel, A., Van Damme, R., & Matthysen, E. (2003). The implications of food hardness for diet in bats. *Functional Ecology, 17*(2), 201-212.

Arbour, J. H., Curtis, A. A., & Santana, S. E. (2019). Signatures of echolocation and dietary ecology in the adaptive evolution of skull shape in bats. *Nature Communications, 10*(1), 1-13.

Barclay, R. M. R., & Brigham, R. M. (1991). Prey Detection, Dietary Niche Breadth, and Body Size in Bats - Why Are Aerial Insectivorous Bats So Small. *American Naturalist, 137*(5), 693-703.

Blanchet, F. G., Cazelles, K., & Gravel, D. (2020). Co-occurrence is not evidence of ecological interactions. *Ecology Letters*, *23*(7), 1050-1063.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2002). The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist, 161*(1), 1-28.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist, 161*(1), 1-28.

Boyles, J. G., Cryan, P. M., McCracken, G. F., & Kunz, T. H. (2011). Economic importance of bats in agriculture. *Science, 332*(6025), 41-42.

Boyles, J. G., & Storm, J. J. (2007). The perils of picky eating: dietary breadth is related to extinction risk in insectivorous bats. *PLoS One, 2*(7), e672.

Boyles, J. G., & Willis, C. K. (2010). Could localized warm areas inside cold caves reduce mortality of hibernating bats affected by white-nose syndrome? *Frontiers in Ecology and the Environment, 8*(2), 92-98.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*(7), 581.

Carlsson, N. O., Jeschke, J. M., Holmqvist, N., & Kindberg, J. (2010). Long-term data on invaders: when the fox is away, the mink will play. *Biological Invasions, 12*(3), 633-641.

Chase, J. M., Abrams, P. A., Grover, J. P., Diehl, S., Chesson, P., Holt, R. D., . . . Case, T. J. (2002). The interaction between predation and competition: a review and synthesis. *Ecology Letters, 5*(2), 302-315.

Chase, J. M., Kraft, N. J., Smith, K. G., Vellend, M., & Inouye, B. D. (2011). Using null models to disentangle variation in community dissimilarity from variation in α-diversity. *Ecosphere, 2*(2), 1-11.

Colles, A., Liow, L. H., & Prinzing, A. (2009). Are specialists at risk under environmental change? Neoecological, paleoecological and phylogenetic approaches. *Ecology Letters, 12*(8), 849-863.

Cryan, P. M., Stricker, C. A., & Wunder, M. B. (2012). Evidence of cryptic individual specialization in an opportunistic insectivorous bat. *Journal of Mammalogy, 93*(2), 381-389.

Davy, C., & Whitear, A. (2016). Feasibility and pitfalls of ex situ management to mitigate the effects of an environmentally persistent pathogen. *Animal Conservation, 19*(6), 539-547.

Deagle, B. E., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., . . . Eveson, J. P. (2019). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology, 28*(2), 391-406.

Dennis, R. L., Dapporto, L., Fattorini, S., & Cook, L. M. (2011). The generalism–specialism debate: the role of generalists in the life and death of species. *Biological Journal of the Linnean Society, 104*(4), 725-737.

Denzinger, A., & Schnitzler, H. U. (2013). Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Frontiers in Physiology, 4*, 164.

Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., . . . Jackson, J. B. (2011). Trophic downgrading of planet Earth. *Science, 333*(6040), 301-306.

Frank, C. L., Michalski, A., McDonough, A. A., Rahimian, M., Rudd, R. J., & Herzog, C. (2014). The resistance of a North American bat species (*Eptesicus fuscus*) to white-nose syndrome (WNS). *PLoS One, 9*(12), e113958.

Frick, W. F., Pollock, J. F., Hicks, A. C., Langwig, K. E., Reynolds, D. S., Turner, G. G., . . . Kunz, T. H. (2010). An emerging disease causes regional population collapse of a common North American bat species. *Science, 329*(5992), 679-682.

Frick, W. F., Puechmaille, S. J., & Willis, C. K. R. (2016). White-Nose Syndrome in Bats. In *Bats in the Anthropocene: Conservation of Bats in a Changing World* (pp 245-262). Springer.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., . . . Hörren, T. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One, 12*(10), e0185809.

Hebert, P. D., Cywinska, A., & Ball, S. L. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B: Biological Sciences, 270*(1512), 313-321.

Holt, R. D. (1977). Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology, 12*(2), 197-229.

Huebschman, J. J. (2019). Bats in southwest Wisconsin during the era of white-nose syndrome. *Northeastern Naturalist, 26*(1), 168-182.

Hunter, E. A., Gibbs, J. P., Cayot, L. J., & Tapia, W. (2013). Equivalency of Galápagos giant tortoises used as ecological replacement species to restore ecosystem functions. *Conservation Biology, 27*(4), 701-709.

Hurlbert, S. H. (1978). The measurement of niche overlap and some relatives. *Ecology, 59*(1), 67-77.

Johnston, D. S., & Fenton, M. B. (2001). Individual and population-level variability in diets of pallid bats (Antrozous pallidus). *Journal of Mammalogy, 82*(2), 362-373.

Jones, G., & Holderied, M. W. (2007). Bat echolocation calls: adaptation and convergent evolution. *Proceedings of the Royal Society B: Biological Sciences, 274*(1612), 905-912.

Jusino, M. A., Banik, M. T., Palmer, J. M., Wray, A. K., Xiao, L., Pelton, E., . . . Zachariah Peery, M. (2019). An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. *Molecular Ecology Resources, 19*, 176-190.

Kelly, J. F., Bridge, E. S., Frick, W. F., & Chilson, P. B. (2013). Ecological energetics of an abundant aerial insectivore, the Purple Martin. *PLoS One, 8*(9), e76616.

Kent, C. M., & Sherry, T. W. (2020). Behavioral niche partitioning reexamined: Do behavioral differences predict dietary differences in warblers? *Ecology*, e03077.

Kunz, T. H., Braun de Torrez, E., Bauer, D., Lobova, T., & Fleming, T. H. (2011). Ecosystem services provided by bats. *Annals of the New York Academy of Sciences, 1223*(1), 1-38.

Lack, J. B., & Van Den Bussche, R. A. (2010). Identifying the confounding factors in resolving phylogenetic relationships in Vespertilionidae. *Journal of Mammalogy, 91*(6), 1435-1448.

Levins, R. (1968). *Evolution in changing environments: Some Theoretical Explorations*: Princeton University Press.

Loxdale, H. D., Lushai, G., & Harvey, J. A. (2011). The evolutionary improbability of 'generalism'in nature, with special reference to insects. *Biological Journal of the Linnean Society, 103*(1), 1-18.

Maas, B., Karp, D. S., Bumrungsri, S., Darras, K., Gonthier, D., Huang, J. C. C., . . . Michel, N. L. (2016). Bird and bat predation services in tropical forests and agroforestry landscapes. *Biological Reviews, 91*(4), 1081-1101.

MacArthur, R., & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist, 101*(921), 377-385.

McCracken, G. F., Bernard, R. F., Gamba-Rios, M., Wolfe, R., Krauel, J. J., Jones, D. N., . . . Brown, V. A. (2018). Rapid range expansion of the Brazilian free-tailed bat in the southeastern United States, 2008–2016. *Journal of Mammalogy, 99*(2), 312-320.

Mittelbach, G. G., Turner, A. M., Hall, D. J., Rettig, J. E., & Osenberg, C. W. (1995). Perturbation and resilience: a long-term, whole-lake study of predator extinction and reintroduction. *Ecology, 76*(8), 2347-2360.

Mooney, K. A., Gruner, D. S., Barber, N. A., Van Bael, S. A., Philpott, S. M., & Greenberg, R. (2010). Interactions among predators and the cascading effects of vertebrate insectivores on arthropod communities and plants. *Proceedings of the National Academy of Sciences, 107*(16), 7335-7340.

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).

Nebel, S., Mills, A., McCracken, J., & Taylor, P. (2010). Declines of aerial insectivores in North America follow a geographic gradient. *Avian Conservation and Ecology, 5*(2).

Nocera, J. J., Blais, J. M., Beresford, D. V., Finity, L. K., Grooms, C., Kimpe, L. E., . . . Smol, J. P. (2012). Historical pesticide applications coincided with an altered diet of aerially foraging insectivorous chimney swifts. *Proceedings of the Royal Society B: Biological Sciences, 279*(1740), 3114-3120.

Nowak, R. M. (2002). The original status of wolves in eastern North America. *Southeastern Naturalist, 1*(2), 95-130.

Nyffeler, M., Şekercioğlu, Ç. H., & Whelan, C. J. (2018). Insectivorous birds consume an estimated 400–500 million tons of prey annually. *The Science of Nature, 105*(7-8), 47.

O'Shea, T. J., Cryan, P. M., Hayman, D. T. S., Plowright, R. K., & Streicker, D. G. (2016). Multiple mortality events in bats: a global review. *Mammal Review, 46*(3), 175-190.

O'Shea, T. J., & Vaughan, T. A. (1977). Nocturnal and seasonal activities of the pallid bat, Antrozous pallidus. *Journal of Mammalogy, 58*(3), 269-284.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R., . . . Wagner, H. (2013). *Package 'vegan'*. R package version 2.9.

Palmer, J. M., Jusino, M. A., Banik, M. T., & Lindner, D. L. (2018). Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. *PeerJ, 6*, e4925.

Parker, K. A., Seabrook-Davison, M., & Ewen, J. G. (2010). Opportunities for nonnative ecological replacements in ecosystem restoration. *Restoration Ecology, 18*(3), 269-273.

Patterson, B. D., Willig, M. R., & Stevens, R. D. (2003). Trophic strategies, niche partitioning, and patterns of ecological organization. In *Bat Ecology (*pp. 536-557). University of Chicago Press.

Purvis, A., Gittleman, J. L., Cowlishaw, G., & Mace, G. M. (2000). Predicting extinction risk in declining species. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 267*(1456), 1947-1952.

Ram, K., & Wickham, H. (2018). *wesanderson: A Wes Anderson palette generator*. R package version 0.3.6.

Ripple, W. J., & Beschta, R. L. (2012). Trophic cascades in Yellowstone: the first 15 years after wolf reintroduction. *Biological Conservation, 145*(1), 205-213.

Ritchie, E. G., Elmhagen, B., Glen, A. S., Letnic, M., Ludwig, G., & McDonald, R. A. (2012). Ecosystem restoration with teeth: what role for predators? *Trends in Ecology & Evolution, 27*(5), 265-271.

Rubenstein, D. R., Rubenstein, D. I., Sherman, P. W., & Gavin, T. A. (2006). Pleistocene Park: Does re-wilding North America represent sound conservation for the 21st century? *Biological Conservation, 132*(2), 232-238.

Sánchez-Bayo, F., & Wyckhuys, K. A. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation, 232*, 8-27.

Santana, S. E., & Cheung, E. (2016). Go big or go fish: morphological specializations in carnivorous bats. *Proceedings of the Royal Society B: Biological Sciences, 283*(1830), 20160615.

Schoeman, M. C., & Jacobs, D. S. (2011). The relative influence of competition and prey defences on the trophic structure of animalivorous bat ensembles. *Oecologia, 166*(2), 493-506.

Seibold, S., Gossner, M. M., Simons, N. K., Blüthgen, N., Müller, J., Ambarlı, D., . . . Habel, J. C. (2019). Arthropod decline in grasslands and forests is associated with landscape-level drivers. *Nature, 574*(7780), 671-674.

Sikes, R. S. (2016). 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy, 97*(3), 663-688.

Spiller, K. J., & Dettmers, R. (2019). Evidence for multiple drivers of aerial insectivore declines in North America. *The Condor, 121*(2), duz010.

Thogmartin, W. E., Sanders-Reed, C. A., Szymanski, J. A., McKann, P. C., Pruitt, L., King, R. A., . . . Russell, R. E. (2013). White-nose syndrome is likely to extirpate the endangered Indiana bat over large parts of its range. *Biological Conservation, 160*, 162-172.

Tompkins, D., White, A. R., & Boots, M. (2003). Ecological replacement of native red squirrels by invasive greys driven by disease. *Ecology Letters, 6*(3), 189-196.

Tylianakis, J. M., Laliberté, E., Nielsen, A., & Bascompte, J. (2010). Conservation of species interaction networks. *Biological Conservation, 143*(10), 2270-2279.

Voigt, C. C., Phelps, K. L., Aguirre, L. F., Schoeman, M. C., Vanitharani, J., & Zubaid, A. (2016). Bats and buildings: the conservation of synanthropic bats. In *Bats in the Anthropocene: Conservation of Bats in a Changing World* (pp. 427-462): Springer.

Williams-Guillén, K., Olimpi, E., Maas, B., Taylor, P. J., & Arlettaz, R. (2016). Bats in the anthropogenic matrix: challenges and opportunities for the conservation of Chiroptera and their ecosystem services in agricultural landscapes. In *Bats in the Anthropocene: Conservation of Bats in a Changing World* (pp. 151-186). Springer.

Wickham, H. (2020). *reshape2: Flexibly reshape data: a reboot of the reshape package*. R package version 1.4.4.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D. A., François, R., ... & Kuhn, M. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686.

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., & Woo, K. (2020). *Package 'ggplot2'. Create Elegant Data Visualisations Using the Grammar of Graphics*. R package version 3.3.2.

Wickham, H., Francois, R., Henry, L., & Müller, K. (2020). *dplyr: A Grammar of Data Manipulation*. R package version 1.0.0.

Wingate, D. B. (1982). Successful reintroduction of the yellow-crowned night-heron as a nesting resident on Bermuda. *Colonial Waterbirds*, 104-115.

Wray, A. K., Peery, M. Z, Jusino, M. A., Kochanski, J. M., Banik, M. T., Palmer, J. M., Lindner, D. L., & Gratton, C. Predator preferences shape the diets of arthropodivorous bats more than quantitative local prey abundance. *Molecular Ecology,* in revision.

Table 1. Top 20 prey items of big brown and little brown bats, ranked by weighted percent occurrence (wPO) for each time period. Changes in top prey are highlighted in bold.

EPFU, 2015–2016			EPFU, 2017–2018		
order	family	mean wPO	order	family	mean wPO
Coleoptera	Elateridae	0.0527	Trichoptera	Hydropsychidae	0.089
Trichoptera	Hydropsychidae	0.0524	Coleoptera	Scarabaeidae	0.070
Coleoptera	Carabidae	0.0498	Coleoptera	Elateridae	0.070
Coleoptera	unidentified	0.0469	Diptera	Limoniidae	0.064
Diptera	Limoniidae	0.0459	Coleoptera	Carabidae	0.044
Lepidoptera	Tortricidae	0.0376	Coleoptera	unidentified	0.044
Ephemeroptera	Heptageniidae	0.0366	Megaloptera	Corydalidae	0.043
Diptera	Chironomidae	0.0330	Diptera	Chironomidae	0.040
Coleoptera	Scarabaeidae	0.0305	Lepidoptera	Tortricidae	0.036
Coleoptera	Pyrochroidae	0.0269	Coleoptera	Pyrochroidae	0.030
Coleoptera	Cerambycidae	0.0262	Diptera	unidentified	0.024
Hemiptera	Miridae	0.0241	Diptera	Tipulidae	0.023
Diptera	Tipulidae	0.0222	Coleoptera	Hydrophilidae	0.021
Coleoptera	Hydrophilidae	0.0213	**Hemiptera**	**Cicadellidae**	**0.019**
Diptera	unidentified	0.0196	Coleoptera	Cerambycidae	0.017
Hymenoptera	Ichneumonidae	0.0180	Hemiptera	Miridae	0.015
Megaloptera	Corydalidae	0.0167	**Diptera**	**Culicidae**	0.014
Lepidoptera	**Tineidae**	**0.0148**	Coleoptera	Tenebrionidae	0.014
Trichoptera	**Leptoceridae**	**0.0135**	Ephemeroptera	Heptageniidae	0.012
Coleoptera	Tenebrionidae	0.0128	Hymenoptera	Ichneumonidae	0.012

MYLU, 2015–2016			MYLU, 2017–2018		
order	family	mean wPO	order	family	mean wPO
Diptera	Chironomidae	0.0744	Diptera	Chironomidae	0.086
Lepidoptera	Tortricidae	0.0387	Lepidoptera	Tortricidae	0.049
Diptera	Limoniidae	0.0337	Coleoptera	Elateridae	0.044
Coleoptera	Elateridae	0.0337	Trichoptera	Hydropsychidae	0.043
Diptera	unidentified	0.0285	Diptera	Culicidae	0.032
Lepidoptera	Gelechiidae	0.0268	Lepidoptera	Gelechiidae	0.031
Ephemeroptera	**Caenidae**	**0.0259**	Lepidoptera	Depressariidae	0.031
Hemiptera	**Miridae**	**0.0251**	Diptera	unidentified	0.028
Trichoptera	Hydropsychidae	0.0240	**Araneae**	**unidentified**	**0.025**
Trichoptera	**Leptoceridae**	**0.0207**	**Coleoptera**	**Scarabaeidae**	**0.025**
Hemiptera	**Corixidae**	**0.0193**	**Coleoptera**	**Dermestidae**	**0.024**
Diptera	Tipulidae	0.0184	Diptera	Tipulidae	0.023
Ephemeroptera	Heptageniidae	0.0182	**Araneae**	**Theridiidae**	**0.021**
Lepidoptera	Depressariidae	0.0176	**Coleoptera**	**unidentified**	**0.021**
Diptera	Culicidae	0.0175	Diptera	Limoniidae	0.019
Diptera	**Psychodidae**	**0.0166**	**Diptera**	**Chaoboridae**	**0.019**
Hymenoptera	**Ichneumonidae**	**0.0155**	Ephemeroptera	Heptageniidae	0.018
Diptera	**Cecidomyiidae**	**0.0154**	Coleoptera	Carabidae	0.018
Lepidoptera	**Tineidae**	**0.0148**	**Diptera**	**Tachinidae**	**0.017**
Coleoptera	Carabidae	0.0140	**Lepidoptera**	**Crambidae**	**0.015**

Table 2. Family-level niche breadth and overlap for little brown and big brown bats.

time	*B*, MYLU	B_a, MYLU	*B*, EPFU	B_a, EPFU	overlap, O_{jk}
2015–2018	51.3252	0.2207	35.8268	0.1527	0.2813
2015–2016	52.1028	0.2241	42.0538	0.1801	0.2811
2017–2018	39.7123	0.1698	27.8405	0.1177	0.2876

Table 3. PERMANOVA and Betadisper test results.

Family-level		PERMANOVA					Betadisper	
term	Df	Sums Of Sqs	Mean Sqs	*F*	R^2	*p*	*F*	*p*
Species	1	2.42	2.416	13.97	0.052	0.01	15.89	<0.001
Site	12	2.83	0.236	1.36	0.061	0.14	1.89	0.04
Time	1	0.29	0.286	1.65	0.006	0.24	0.01	0.92
Week	1	0.68	0.682	3.94	0.015	0.03	0.99	0.47
Residuals	233	40.3	0.173		0.866			
Total	248	46.52			1			

OTU-level		PERMANOVA					Betadisper	
term	Df	Sums Of Sqs	Mean Sqs	*F*	R^2	*p*	*F*	*p*
Species	1	10.12	10.124	62.14	0.117	0.01	16.4	<0.001
Site	13	32.25	2.481	15.22	0.371	0.01	3.17	<0.001
Time	1	2.20	2.202	13.51	0.025	0.01	3.98	0.047
Week	1	2.00	2.004	12.30	0.023	0.01	2.90	<0.001
Residuals	247	40.25	0.163		0.464			
Total	263	86.82			1			

Figure Legends

Figure 1. Characterization of study sites and bat dietary composition. A) Map of study locations with points indicating the relative size of roosts. Inset shows location of study sites within the continental United States. B) Ordinal level dietary composition between time periods for each bat species. EPFU=big brown bat, MYLU=little brown bat.

Figure 2. Changes in bat dietary composition over time. A) NMDS plot of family-level and OTU-level dietary communities with 80% confidence interval ellipses. Solid lines indicate the first time period (2015–2016), while dashed lines indicate the second time period (2017–2018). Shapes indicate bat species. B) Heatmap of agricultural pest taxa detected in bat guano samples. Values indicate the percentage of guano samples for which each agricultural pest was detected. EPFU=big brown bat, MYLU=little brown bat.

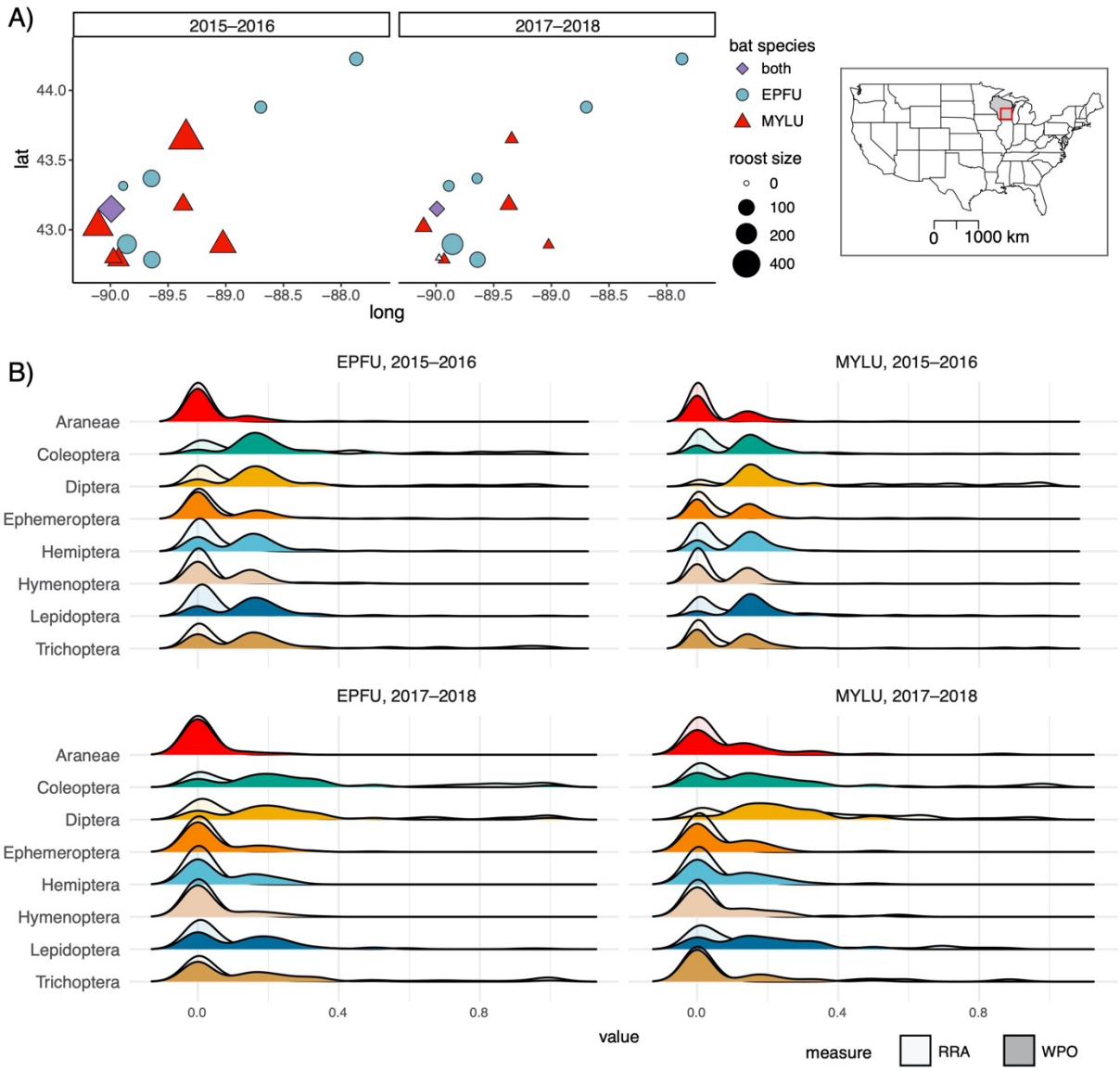

A)
2015–2016
2017–2018
lat
long
bat species
both
EPFU
MYLU
roost size
0
100
200
400
0
1000 km
B)
EPFU, 2015–2016
MYLU, 2015–2016
EPFU, 2017–2018
MYLU, 2017–2018
Araneae
Coleoptera
Diptera
Ephemeroptera
Hemiptera
Hymenoptera
Lepidoptera
Trichoptera
value
measure
RRA
WPO

Figure 1

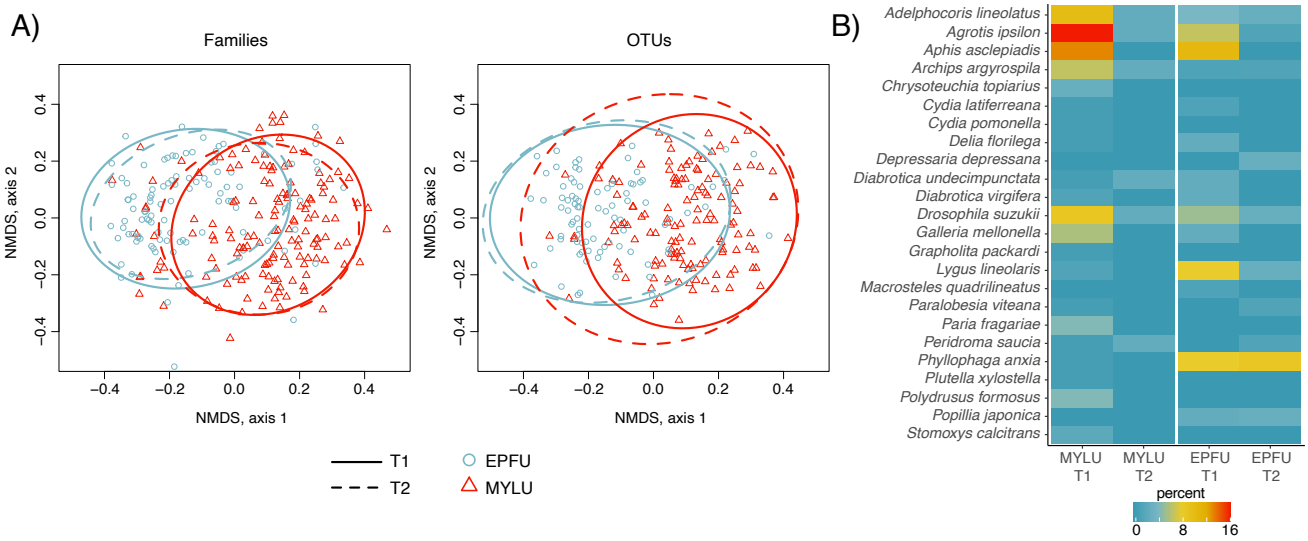

A)
Families
OTUs
NMDS, axis 1
NMDS, axis 2
−0.4
−0.2
0.0
0.2
0.4
T1
T2
EPFU
MYLU
B)
Adelphocoris lineolatus
Agrotis ipsilon
Aphis asclepiadis
Archips argyrospila
Chrysoteuchia topiarius
Cydia latiferreana
Cydia pomonella
Delia florilega
Depressaria depressana
Diabrotica undecimpunctata
Diabrotica virgifera
Drosophila suzukii
Galleria mellonella
Grapholita packardi
Lygus lineolaris
Macrosteles quadrilineatus
Paralobesia viteana
Paria fragariae
Peridroma saucia
Phyllophaga anxia
Plutella xylostella
Polydrusus formosus
Popillia japonica
Stomoxys calcitrans
MYLU T1
MYLU T2
EPFU T1
EPFU T2
percent
0
8
16

Figure 2

Supplementary Materials

Supplementary Table S1. Top 20 prey families by incidence, RRA, and wPO, 2015–2018

MYLU

order	family	incidence	mean RRA	mean wPO
Diptera	Chironomidae	138	0.2132	0.0770
Lepidoptera	Tortricidae	93	0.0369	0.0410
Diptera	Limoniidae	78	0.0359	0.0305
Coleoptera	Elateridae	75	0.0574	0.0360
Diptera	unidentified	71	0.0133	0.0284
Lepidoptera	Gelechiidae	58	0.0153	0.0278
Ephemeroptera	Caenidae	53	0.0439	0.0221
Hemiptera	Miridae	51	0.0106	0.0216
Lepidoptera	Depressariidae	50	0.0494	0.0204
Trichoptera	Hydropsychidae	49	0.0830	0.0281
Diptera	Culicidae	46	0.0279	0.0207
Diptera	Tipulidae	44	0.0029	0.0193
Hemiptera	Corixidae	41	0.0215	0.0170
Trichoptera	Leptoceridae	40	0.0157	0.0168
Ephemeroptera	Heptageniidae	39	0.0165	0.0182
Diptera	Psychodidae	37	0.0281	0.0146
Hymenoptera	Ichneumonidae	34	0.0017	0.0134
Coleoptera	unidentified	32	0.0022	0.0136
Coleoptera	Carabidae	31	0.0132	0.0148
Diptera	Ceratopogonidae	30	0.0010	0.0102

EPFU

order	family	incidence	mean RRA	mean wPO
Coleoptera	Elateridae	83	0.1600	0.0609
Diptera	Limoniidae	71	0.1000	0.0547
Coleoptera	unidentified	70	0.0099	0.0453
Coleoptera	Carabidae	67	0.0176	0.0472
Trichoptera	Hydropsychidae	65	0.1963	0.0701
Coleoptera	Scarabaeidae	56	0.0751	0.0493
Lepidoptera	Tortricidae	54	0.0301	0.0368
Diptera	Chironomidae	45	0.0628	0.0364
Coleoptera	Pyrochroidae	43	0.0201	0.0284
Diptera	Tipulidae	42	0.0044	0.0226
Coleoptera	Cerambycidae	36	0.0070	0.0219
Hemiptera	Miridae	35	0.0103	0.0196
Coleoptera	Hydrophilidae	33	0.0256	0.0211
Ephemeroptera	Heptageniidae	32	0.0435	0.0249
Megaloptera	Corydalidae	30	0.0576	0.0292
Hymenoptera	Ichneumonidae	27	0.0040	0.0151
Diptera	unidentified	26	0.0050	0.0218
Coleoptera	Tenebrionidae	21	0.0032	0.0135
Hemiptera	Cicadellidae	21	0.0004	0.0130
Coleoptera	Silphidae	21	0.0012	0.0100

Supplementary Table S2. Differences in ordinal-level dietary composition between time periods. Statistically significant results, as determined by Wilcoxon rank sum tests, highlighted in bold.

measure	order	bat species	$\bar{x}_{2015–2016}$	$\bar{x}_{2017–2018}$	*p*
RRA	Araneae	EPFU	0.0083	0.0061	0.2561
wPO	Araneae	EPFU	0.0332	0.0195	0.2287
RRA	Coleoptera	EPFU	0.3121	0.3402	0.9050
wPO	Coleoptera	EPFU	0.1796	0.2308	0.1017
RRA	Diptera	EPFU	0.2160	0.2595	0.7765
wPO	**Diptera**	**EPFU**	**0.1546**	**0.2200**	**0.0369**
RRA	Ephemeroptera	EPFU	0.0745	0.0283	0.1632
WPO	Ephemeroptera	EPFU	0.0673	0.0500	0.3386
RRA	**Hemiptera**	**EPFU**	**0.0353**	**0.0062**	**0.0007**
wPO	**Hemiptera**	**EPFU**	**0.1179**	**0.0673**	**0.0044**
RRA	**Hymenoptera**	**EPFU**	**0.0344**	**0.0096**	**0.0033**
wPO	**Hymenoptera**	**EPFU**	**0.0782**	**0.0407**	**0.0070**
RRA	Lepidoptera	EPFU	0.0542	0.0915	0.4805
wPO	Lepidoptera	EPFU	0.1474	0.1294	0.3697
RRA	Trichoptera	EPFU	0.1953	0.2005	0.3657
wPO	Trichoptera	EPFU	0.1216	0.1361	0.6359
RRA	Araneae	MYLU	0.0137	0.0404	0.1692
wPO	Araneae	MYLU	0.0592	0.0909	0.3413
RRA	Coleoptera	MYLU	0.0974	0.1991	0.4254
wPO	Coleoptera	MYLU	0.1410	0.1532	0.9912
RRA	Diptera	MYLU	0.4473	0.3307	0.0691
wPO	**Diptera**	**MYLU**	**0.1846**	**0.2216**	**0.0325**
RRA	**Ephemeroptera**	**MYLU**	**0.0826**	**0.0257**	**0.0304**
wPO	Ephemeroptera	MYLU	0.0879	0.0579	0.0671
RRA	**Hemiptera**	**MYLU**	**0.0511**	**0.0313**	**0.0029**
wPO	**Hemiptera**	**MYLU**	**0.1243**	**0.0792**	**0.0051**
RRA	Hymenoptera	MYLU	0.0277	0.0522	0.2605
wPO	Hymenoptera	MYLU	0.0840	0.0673	0.0983
RRA	Lepidoptera	MYLU	0.1667	0.1754	0.4127
wPO	Lepidoptera	MYLU	0.1586	0.1716	0.8280
RRA	**Trichoptera**	**MYLU**	**0.1011**	**0.1189**	**0.0461**
wPO	Trichoptera	MYLU	0.0916	0.0694	0.0821