ILLUMINATING DIETARY AND PHYSIOLOGICAL CHANGE IN AN INSECTIVOROUS BAT COMMUNITY EXPOSED TO ARTIFICIAL LIGHT AT NIGHT

Zachary Cravens  
_Southern Illinois University Carbondale, zcravens@gmail.com_

Follow this and additional works at: https://opensiuc.lib.siu.edu/theses

Recommended Citation  
Cravens, Zachary, "ILLUMINATING DIETARY AND PHYSIOLOGICAL CHANGE IN AN INSECTIVOROUS BAT COMMUNITY EXPOSED TO ARTIFICIAL LIGHT AT NIGHT" (2018). Theses. 2347.  
https://opensiuc.lib.siu.edu/theses/2347

This Open Access Thesis is brought to you for free and open access by the Theses and Dissertations at OpenSIUC. It has been accepted for inclusion in Theses by an authorized administrator of OpenSIUC. For more information, please contact opensiuc@lib.siu.edu.
ILLUMINATING DIETARY AND PHYSIOLOGICAL CHANGE IN AN INSECTIVOROUS BAT COMMUNITY EXPOSED TO ARTIFICIAL LIGHT AT NIGHT

by

Zachary M. Cravens

B.S. University of Illinois Urbana-Champaign, 2003

A Thesis
Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in the field of Zoology

Department of Zoology
in the Graduate School
Southern Illinois University Carbondale
May 2018
ILLUMINATING DIETARY AND PHYSIOLOGICAL CHANGE IN AN INSECTIVOROUS BAT COMMUNITY EXPOSED TO ARTIFICIAL LIGHT AT NIGHT

by

Zachary M. Cravens

A Thesis Submitted in Partial
Fulfillment of the Requirements
for the Degree of
Master of Science
In the field of Zoology

Approved by:
Dr. Justin G. Boyles, Chair
Dr. Michael W. Eichholz
Dr. F. Agustín Jimenez

Graduate School
Southern Illinois University Carbondale
April 6, 2018
AN ABSTRACT OF THE THESIS OF

ZACHARY M. CRAVENS, for the Master of Science degree in Zoology, presented on April 6, 2018 at Southern Illinois University Carbondale.

TITLE: ILLUMINATING DIETARY AND PHYSIOLOGICAL CHANGE IN AN INSECTIVOROUS BAT COMMUNITY EXPOSED TO ARTIFICIAL LIGHT AT NIGHT

MAJOR PROFESSOR: Dr. Justin G. Boyles

Global light pollution is increasing worldwide, nearly doubling over the past 25 years, and the encroachment of artificial light into remaining dark areas threatens to disturb natural rhythms of wildlife species, such as bats. Artificial light impacts the behaviour of insectivorous bats in numerous ways, including changing foraging behaviour and altering prey selection. I conducted two manipulative field experiments to investigate effects of light pollution on prey selection in an insectivorous bat community. In the first experiment, I collected fecal samples from 6 species of insectivorous bats in naturally dark and artificially lit conditions and identified prey items using molecular methods. Proportional differences of identified prey were not consistent and appear to be species specific. Red bats, little brown bats, and gray bats exhibited expected increases in moths at lit sites. Beetle-specialist big brown bats had a sizeable increase in beetle consumption around lights, while tri-colored bats and evening bats showed little change in moth consumption between experimental conditions. Dietary overlap was high between experimental conditions within each species, and dietary breadth only changed significantly
between experimental conditions in one species, the little brown bat. Our results, building on others, demonstrate that bat-insect interactions may be more nuanced than the common assertion that moth consumption increases around lights. Thus, no single policy is likely to be universally effective in minimizing effects of light pollution on foraging bats because of differences in bat and insect communities, and their interactions. Our work highlights the need for greater mechanistic understanding of bat-light interactions to predict which species will be most affected by light pollution, and to more effectively craft management strategies to minimize unnatural shifts in prey selection caused by artificial lights. In the second experiment, I again focused on changes in foraging due to light pollution by investigating expected knock-on physiological effects, which have not been studied. I measured plasma β-hydroxybutyrate concentrations from six species of insectivorous bats in naturally dark and artificially lit conditions to investigate effects of light pollution on energy metabolism. We also recorded bat calls acoustically to measure differences in activity levels between experimental conditions. Blood metabolite level and acoustic activity data suggest species-specific changes in foraging around lights. In red bats (*Lasiurus borealis*), β-hydroxybutyrate levels at lit sites were highest early in the night followed by a decrease. Acoustic data suggest pronounced peaks in activity at lit sites early in the night. In red bats on dark nights and in the other species in this community, which seem to avoid lights, β-hydroxybutyrate remained constant, or possibly increased slightly throughout the night. Taken together, our results suggest red bats actively forage around lights and may gain some energetic benefit, while other species in the community avoid lit areas and thus gain no such benefit. Our results demonstrate that artificial light may have a bifurcating effect on bat communities, whereby a few species benefit through concentrated prey resources, yet most do not. Further, this may concentrate light-intolerant species into limited dark refugia, thereby increasing competition
for depauperate insect communities, as insects are drawn to artificially lit spaces. It appears then that artificial lights change the environment in such a way as to benefit some species in insectivorous bat communities.
ACKNOWLEDGEMENTS

These studies would not have been possible without funding and logistic support from multiple partners including the Missouri Department of Conservation (especially K. Rezac, K. Womack, T. Elliott, J. Bailey, and S. Colatskie); the University of Tennessee Genomics Core Facility (especially V. Brown); the Center for Bat Research, Outreach, and Conservation at Indiana State University (especially T. Divoll); and the McCracken lab at the University of Tennessee. All capture, handling, and data collection relating to bats was approved by the Southern Illinois University Institutional Animal Care and Use Committee (IACUC) and federal, state, and local permits. Special thanks are due to J. Holub and D. Herrera for field assistance. Thanks are also due to the SIU Cooperative Wildlife Research Laboratory and Department of Zoology for teaching and research assistantships, and especially to all of the other students in the department who helped me throughout the entire process.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
</tbody>
</table>

## CHAPTERS

### CHAPTER 1

1.1 DISCLAIMER | 1

1.2 INTRODUCTION | 1

1.3 MATERIALS AND METHODS | 3

1.3.1 Study Site | 3

1.3.2 Experimental Design | 4

1.3.3 Molecular Analysis | 5

1.3.4 Data Analysis | 7

1.3.5 Statistical Analysis | 8

1.4 RESULTS | 9

1.5 DISCUSSION | 11

### CHAPTER 2

2.2 MATERIALS AND METHODS | 24

2.2.1 Study Site | 24

2.2.2 Experimental Design | 25

2.3 RESULTS | 27
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Diet overlap between the six species of insectivorous bats evaluated in this study. Observed mean values below 0.6 are generally accepted to represent biologically significant resource partitioning</td>
<td>17</td>
</tr>
<tr>
<td>1.2</td>
<td>Pairwise comparison of diet overlap between six species of insectivorous bats in experimentally lit and naturally dark experimental treatments. Values below the diagonal are the observed mean and numbers above the diagonal are the corresponding $P$ values</td>
<td>19</td>
</tr>
<tr>
<td>1.3</td>
<td>Diversity estimates between experimentally lit and naturally dark conditions in six species of insectivorous bats</td>
<td>20</td>
</tr>
<tr>
<td>2.1</td>
<td>Output from the general linear models testing the main effects of light treatment, minutes after sunset, and the interaction of treatment with minutes after sunset against $\beta$-hydroxybutyrate concentrations in the five species of insectivorous bats</td>
<td>36</td>
</tr>
<tr>
<td>2.2</td>
<td>Output from paired t-tests between activity levels in artificially lit and naturally dark conditions in six species of insectivorous bats</td>
<td>37</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The proportion of MOTUs identified in the diet of six species of insectivorous bats under experimentally lit and naturally dark conditions</td>
</tr>
<tr>
<td>1.2</td>
<td>Interpolation (rarefaction) and extrapolation of dietary species richness for each experimental condition in six species of insectivorous bats using the Chao2 estimation for incidence-based sample data. Richness is extrapolated to twice the sample size and bootstrapped 500 times</td>
</tr>
<tr>
<td>2.1</td>
<td>Changes in β-hydroxybutyrate concentrations across the night in the five species of insectivorous bats between artificially lit and naturally dark conditions. Yellow points represent values from artificially lit sites, while black points represent values from naturally dark sites. Values along the y-axis represent the range of residual values from a regression of temperature and β-hydroxybutyrate in order to account for an effect of temperature</td>
</tr>
<tr>
<td>2.2</td>
<td>Comparison of relative bat activity, summed in 15-minute intervals, across the night between experimental treatments for each bat species. Calls of common red bats are often misidentified as rarer evening bats and tricolored bats by the identification software so the patterns for these two species should be interpreted with care. Calls of the Indiana and little brown bat were combined as a single species group as they are very difficult to distinguish acoustically</td>
</tr>
<tr>
<td>2.3</td>
<td>Difference in relative abundance between treatments for each 15-minute interval, based on call data from Figure 2. Higher positive values represent greater activity at lit sites, while higher negative values indicate greater activity at unlit sites</td>
</tr>
<tr>
<td>2.4</td>
<td>Changes in β-hydroxybutyrate concentrations across the night in the five species of insectivorous bats between artificially lit and naturally dark conditions. Yellow points represent values from artificially lit sites, while black points represent values from naturally dark sites. Two outliers, one from a big brown bat and one from a red bat, have also been excluded in this figure to maintain consistency between Figure 2.1. Their removal did not change the results qualitatively</td>
</tr>
</tbody>
</table>
CHAPTER 1

ILLUMINATING PREY SELECTION IN AN INSECTIVOROUS BAT COMMUNITY
EXPOSED TO ARTIFICIAL LIGHT AT NIGHT

1.1 DISCLAIMER


1.2 INTRODUCTION

The biological world is ordered around the natural rhythm of alternating night and day. As a reliable signal over geologic time, most organisms have evolved in relation to temporal cycles of light and dark periods (Gaston et al. 2013). However, fast-paced urbanization beginning in the 20th century has led to a dramatic increase in artificial light at night (ALAN) (Hölker et al. 2010a). Global light pollution is increasing, and has nearly doubled over the past 25 years (Hölker et al. 2010a, Koen et al. In press). Currently, almost 90% of Europe and half the United States experiences light-polluted skies (Falchi et al. 2016), but those levels have remained relatively constant over the last several decades (Koen et al. In press). Conversely, developing regions with above-average species richness have experienced recent increases in light pollution extent compared to areas with low to moderate richness (Koen et al. In press). This trend will likely continue as the majority of urban growth is expected to occur near currently protected land (i.e. dark refugia) (Güneralp and Seto 2013). Encroachment of artificial light into remaining dark
areas will increasingly threaten biodiversity as 30% of vertebrates and >60% of invertebrates are nocturnal and therefore likely to be strongly impacted by ALAN (Hölker et al. 2010b).

Most bats have evolved unique behavioral and morphological adaptations (e.g. echolocation) to navigate in the absence of light (Neuweiler 1990, ter Hofstede and Ratcliffe 2016). Avoidance of lit environments is likely a significant ultimate cause of nocturnality in bats because it reduces susceptibility to predation by visual hunters, such as diurnal birds of prey (Rydell and Speakman 1995, Speakman 2001, Voigt and Lewanzik 2011). This selective pressure is strong enough that bats generally emerge from roosts just after sunset (Duverge et al. 2000), despite a pulse of insect activity just prior to sunset (Rydell et al. 1996). Therefore, bats seem to prioritize darker conditions over a higher energetic payoff under natural conditions, and the global pervasiveness of ALAN may affect this trade-off.

Artificial light at night impacts bat species in numerous ways, often leading to roost abandonment, spatial avoidance, and delayed emergence (reviewed in Stone et al. 2015, Rowse et al. 2016). Impacts on bat foraging behavior are less clear and depend on taxon-specific traits and environmental conditions. For example, clutter-adapted bats generally avoid lit conditions, whether in a consistently lit urban or semi-urban environment or in an experimentally lit environment (Stone et al. 2009, Lacoeuilhe et al. 2014, Schoeman 2016). This is likely because light-intolerant species may associate a predatory risk with lit environments (Jones and Rydell 1994). Conversely, numerous species have been observed feeding at artificial lights (Rydell 1992, Svensson and Rydell 1998, Acharya and Fenton 1999, Clare et al. 2009). Artificial light interferes with insect navigational cues, causing attraction to and unusually high densities around lights (van Langevelde et al. 2011). Higher densities alone may make aerial insects more vulnerable to predation from bats, but in some prey species, changes in behavior around lights
may also play an important role. For example, artificial light appears to interfere with highly-evolved mechanisms eared moths use to detect bat echolocation and avoid predation (Svensson and Rydell 1998, Acharya and Fenton 1999, Wakefield et al. 2015). Observations of bats foraging at lights are usually in urban or semi-urban areas (except, see Minnaar et al. 2015), where streetlights are a consistent part of the nocturnal environment. From these studies, a pattern has emerged that consumption of moths, specifically eared moths, increases at lights (Belwood and Fullard 1984, Hickey and Fenton 1990, Svensson and Rydell 1998, Minnaar et al. 2015). However, the universality of this pattern is unclear, both within and across bat communities.

We evaluated effects of light pollution on prey selection of bats at a community level. The bat community in the study area is represented by species with different wing morphologies, foraging habits, and diets, so if the general pattern of increased moth consumption around lights is found in all members of this community, the pattern is likely to be robust. To test this pattern, we manipulated naturally dark areas with a short-term artificial light treatment. We collected fecal samples from bats captured in both lit and unlit environments and used next generation sequencing of insect DNA extracted from fecal samples to measure differences in frequency of insect prey between unlit and lit conditions. We predicted bat consumption of moths (including eared moths) to increase and consumption of beetles to decrease in artificial light treatments relative to naturally dark areas.

1.3 MATERIALS AND METHODS

1.3.1 Study Site
Our study was conducted in a 15-county region of western-southwestern Missouri, USA during summer (May to August) 2016. The eastern half of the study area is within the Ozark Highlands physiographic region, which is a heavily forested landscape dominated by oak-hickory forests. To the west, the land transitions to the Osage Plains, a region historically dominated by prairie but now heavily converted to agriculture with limited forest and woodlands (Raeker et al. 2010).

1.3.2 Experimental Design

We erected temporary lights along naturally dark forest roads or streams on public lands and had two experimental conditions: unlit (control) and lit (light pollution treatment). Distance between lit and unlit sites was at least 2 km to minimize overlap in foraging ranges by individual bats, but sites were chosen with similar habitat and landscape features. At lit sites, we used 50W LED (Shenzhen Lepower Opto Electronics Co., China) producing 4200 lumens at 5500 K. Lights were elevated 3m from the ground on a metal pole and powered by a 12V lead acid battery. We used LED lighting as it is becoming more common in outdoor lighting applications as older styles, such as mercury vapor, are being phased out. We netted each survey location for three nights and ran lights for all three nights from 21:00 to 5:00. On the first two nights, we captured bats at a nearby unlit site as a control and on the third night captured bats at lit sites (Minnaar et al. 2015). Delaying capture at lit sites until the third night allowed bats to become accustomed to the lit condition, as well as provide time for them to choose to forage in the newly lit environment. We make no assumption that all bats captured at lit sites will necessarily be foraging around the lights; moreover, we expect some species may be less prone to foraging at
lights than others and therefore less likely to show dietary shifts. Nets were placed in flyways within 25m of the light in an appropriate netting location.

We netted along forested roads or streams at 20 locations throughout the summer. We held bats in cloth bags for 30-45 minutes, stored all deposited fecal pellets in 1.5 mL micro centrifuge tubes with silica beads, and assigned a unique sample ID to allow random subsampling, when necessary, for molecular analysis. Samples were kept frozen after the field season at -20 °C for 4 months before processing for DNA.

1.3.3 Molecular Analysis

We extracted DNA from 1–3 pellets of guano from each individual bat using PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Carlsbad, CA) following manufacturer’s specifications, with the minor modification of increasing the first 4°C step from 30 minutes to overnight. We discarded samples with insufficient fecal matter (<1 full pellet). Red bat (Lasiurus borealis) samples were too numerous so we subsampled by randomly selecting lit and unlit pairs from the same site. We closely followed the methods of Divoll et al. (2018). We amplified the CO1 gene with ZBJ-ArtF1c and ZBJ-ArtR2c primers (Zeale et al. 2011) modified with adapters on the 5’ end for the Illumina MiSeq platform (Illumina Corporation, San Diego, CA, USA). PCR conditions were 25 µl reactions of 1X PCR gold buffer, 2.5 mM MgCl2, 0.8 mM dNTP blend, 0.125 µl AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA), 5 µg BSA (Sigma-Aldrich, St. Louis, MI, USA), 5 µM each primer (Integrated DNA Technologies, Coralville, IA, USA), and 3 µl of fecal DNA. PCR cycling parameters were: denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec, with a final elongation step of 10 min at 72°C. Samples were processed in two batches of 95 samples each
plus a reaction blank of water in place of DNA template, which was carried through the entire process. Aerosol barrier tips were used to minimize chances of cross contamination and all steps were performed in a laminar flow hood. Amplification success was confirmed by running 5 µl of each sample on a 2% agarose gel (Sigma-Aldrich, St. Louis, MI, USA).

Initial PCR products with Illumina adapters were cleaned of unincorporated nucleotides with Agencourt AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA). The cleaned products were then amplified in a second PCR, which attaches dual indices and Illumina sequencing adapters using the Nextera XT index kit (Illumina Corporation, San Diego, CA, USA). This second-step PCR consisted of 25 µl KAPA HiFi HotStart taq (KAPA Biosystems, Wilmington, MA, USA), 5 µl each of Nextera XT index primers 1 and 2, and 5 µl of initial PCR product, brought up to 50 µl with PCR grade water. PCR cycling parameters were: denaturation at 95°C for 3 min, followed by 8 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, with a final elongation step of 5 min at 72°C.

The indexed PCR products were purified again with Agencourt AMPure XP beads. The purified, indexed products were then quantified on a Hoefer DyNA Quant 200 fluorometer (Amersham Pharmacia, Amersham, Buckinghamshire, UK) and samples were combined into 12 approximately equimolar pools to be visualized and quantified on a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples in the first run of 96 were diluted to 6 pM and the second run was diluted to 8 pM to increase yield. For each run, the diluted products were combined with PhiX control DNA (Illumina Corporation, San Diego, CA, USA) at a ratio of 20% PhiX, loaded onto a v3 600-cycle flow cell set for a paired-end read of 220 bases each, then sequenced on the Illumina MiSeq at the University of Tennessee Genomics Core (Knoxville, TN, USA).
1.3.4 Data Analysis

Sequences were analyzed using the QIIME (www.qiime.org) platform (Caporaso et al. 2010) and the workflow outlined in Divoll et al. (2018) with one additional step to only keep sequences within 10-bp of our target amplicon (see Appendix 3.1 for a flowchart of the workflow). Forward and reverse reads were joined and primer sequences were clipped. We filtered out sequences smaller than 147 bp or greater than 167 bp. Sequences were clustered into molecular operational taxonomic units (MOTUs) using the SWARM method with a resolution of 2 (Mahé et al. 2014). To account for potential OTU inflation, we excluded MOTUs that were not present at least 10 times in at least one sample. We performed filtering using a custom Python script employing the ‘pandas’ package (McKinney). We conducted further filtering of remaining MOTUs by considering within-sample MOTU occurrences <10 as potential sequencing errors and removing them. We extracted representative sequences from each MOTU cluster, based on abundance, to compare against a reference database (Divoll et al.).

The representative set of sequences (Cravens et al. 2017) was then compared to the COI database in BOLD (Ratnasingham and Hebert 2007) using the package ‘bold’ (Chamberlain 2017) in R (R Core Team 2017). We considered only the first 40 records for each representative MOTU and then filtered records with <98% similarity and country of origin outside of United States and Canada. The entire output for each representative was then separated into two groups: high quality with at least one match (≥99.36% similarity) and low quality with all matches (>98.0% but <99.36% similarity). Taxonomic identification was made based on these groupings and in all cases where there was disagreement, identification was made at the next highest level of taxonomy. In the high-quality group, matches <99.36% did not change the identification,
regardless of taxonomic divergence and in the low-quality group, variation in percent match was not considered for identification, only disagreement. As an example: a given MOTU has a 100% match from the Bold package output for the moth *Aristotelia rubidella* (family: Gelechiidae), and a 98.92% match for the moth *Hillia iris* (family: Noctuidae). Because the second match is less than 99.36% (a single base pair difference assuming 157 bp) and the first match is ≥99.36%, we identified the prey item as *Aristotelia rubidella*. If the *Hillia iris* had ≥99.36% match then, because there was disagreement at the family level, we would have identified the item only as Lepidoptera. Unique MOTUs assigned to the same taxonomy were collapsed into a single MOTU, representing one bat prey item. This may lead to certain orders being over or under split due to differences in genetic variation (Brown et al. 2015); however, this should not bias our results when measuring within species change between experimental conditions.

1.3.5 Statistical Analysis

We calculated percent frequency of occurrence of insect prey orders (number of samples containing an order divided by the total occurrences of all orders) for each bat species in both experimental conditions. Within order Lepidoptera, we also calculated percent frequency of occurrence of eared moths for each bat species as follows:

\[
\text{percent frequency of occurrence of eared moths} = \frac{\text{# of samples with eared moths}}{\text{# of eared moth occurrences in dataset}}
\]

We defined families Sphingidae, Noctuidae, Notodontidae, Geometridae, Pyralidae as eared moths, as they are known to have tympanate organs used for predator avoidance (ter Hofstede and Ratcliffe 2016). We were unable to quantify abundance of prey items given variation in insect DNA degradation as it passes through a bats intestinal tract and differences in PCR amplification. For all other analyses, we used the collapsed set of unique MOTU assumed to be bat prey species.
We used the EcoSimR 0.1.0 package (Gotelli et al. 2015) in R to determine dietary overlap among the six bat species and to assess effects of artificial light. Null models were used to determine whether extent of niche overlap was lower than would be expected by chance. We used Pianka’s (1973) measure of niche overlap and generated 1000 bootstrap randomizations of MOTU diet composition using the ‘ra3’ algorithm. We conducted this analysis including all MOTUs (all-prey analysis) as well as excluding prey only eaten by a single individual (common-prey analysis) (as per Brown et al. 2014, Clare et al. 2014a, Clare et al. 2014b). We used the iNEXT package (Hsieh et al. 2016) in R to determine extent of dietary specialization and diversity using the first three Hill numbers (or effective number of species): $q = 0$ (species richness), $q = 1$ (exponential of Shannon’s entropy index), and $q = 2$ (inverse of Simpson’s concentration index) as well as the chao2 asymptotic estimator for those numbers. Hill numbers have been increasingly used for biodiversity analysis and are preferred over other diversity indices given they are intuitive and statistically robust (Chao et al. 2014).

1.4 RESULTS

We captured 453 bats from six species (big brown bats (*Eptesicus fuscus*); red bats; gray bats (*Myotis grisescens*); little brown bats (*Myotis lucifugus*); evening bats (*Nycticeius humeralis*); and tri-colored bats (*Perimyotis subflavus*)) across both experimental conditions ($n = 297$ during unlit and $n = 151$ during lit) spanning 61 nights ($n = 42$ during unlit and $n = 19$ during lit). Light did not appear to attract new species as we captured most of the expected species based on regional species distributions, at both lit and unlit sites. We analyzed DNA from 188 fecal samples from the six species (Table 3) and recovered 71 992 648 sequencing reads. After performing bioinformatics processing, these reads were clustered and filtered down to 3078
MOTUs. Using representative sequences of the 3078 MOTUs, we identified 1129 (36.7%) with matching sequences in the BOLD database, belonging to 15 insect orders. After collapsing MOTUs with the same taxonomy, we were left with 487 unique MOTUs or unique prey items.

In general, Lepidoptera, Coleoptera, and Diptera were the most commonly identified orders and their combined proportion was relatively constant (range: ~69% to ~83%) for each bat species in both treatment groups. Specifically, Coleoptera were the most commonly identified prey for big brown and evening bats and Lepidoptera were the most common prey for red and little brown bats in both treatment groups. Diptera were the most common prey identified in the diet of gray and tri-colored bats at unlit sites, but the most common prey items at lit sites were Lepidoptera for gray bats and Coleoptera for tri-colored bats.

Based on order-level taxonomy of prey, gray bats were the only species with a significant shift between treatments ($\chi^2 = 10.11, P = 0.02$), though significance is lost after a Bonferroni correction (Figure 1). Further, this may be related to our smaller total sample size for this species. Little overall variation in prey selection was detected in any other species ($P > 0.15$). Analysis of dietary overlap values tell a similar story (results of all-prey and common-prey analyses were similar, therefore all-prey values are reported). Overlap between lit and dark treatment groups exceeded 0.6, the value at which diets are generally considered to represent biological similarity (Pianka and Pianka 1976), for all species (see Table 1). Within a species, red bats had the highest degree of overlap between lit and unlit conditions ($O_{jk} 0.906, P < 0.001$). The results were generally less conclusive when we limited our comparison of overlap values between treatment groups to prey items identified as Lepidoptera, but red bats still had a significant degree of overlap ($O_{jk} 0.9059, P < 0.001$). In general, values for dietary overlap between species pairs were lower than those found within species between treatment groups.
Further, even qualitative shifts in consumption of the two most important prey items, Coleoptera and Lepidoptera, varied across species (Figure 1). There was also no indication of the expected increase in eared-moth consumption around lights, and the only species with a significant shift in moths identified as eared moths, big brown bats ($P < 0.007$), consumed fewer eared moths in the lit treatment.

Diversity estimates showed that dietary breadth did not change substantially between experimental conditions for most species, and no clear pattern exists in the direction of change (Table 3). Only little brown and tri-colored bats had no overlap in the 95% confidence intervals for diversity accumulation curves of the first Hill number ($q = 0$) using the Chao2 estimation of incidence-based richness estimation (Figure 2). The little brown bat is the only species that had significantly higher estimated dietary diversity in lit conditions in all three diversity measures. In general, red bats had the broadest dietary diversity, while big brown bats had the narrowest, and this pattern held for each Hill number whether observed or estimated (Table 3).

1.5 DISCUSSION

We determined diet of six species of insectivorous bats to examine the impact of ALAN, at the community level, on prey selection. Contrary to expectations, no species in this community showed a significant shift in diet as seen in another study using a similar experimental design. Further, even ignoring statistical significance, our data do not support a consistent trend in shifts in dietary niche between naturally dark and experimentally lit conditions that would suggest an existing pattern we are missing due to low power. Proportional differences in identified prey appear to be species-related. Red bats, little brown bats, and gray bats followed the expected pattern at lit sites with higher moth and lower beetle consumption.
frequencies. Big brown bats are beetle specialists, and there was a substantial increase in the proportion of beetles identified under lit conditions. Evening bats and tri-colored bats showed no change in moth or beetle proportions under dark and lit conditions. There was a high degree of dietary overlap for all species between the experimental conditions ($O_{jk} > 0.719$ for all species). This may be a biological result suggesting that either bats did not choose to forage in an artificially lit condition or that bats did not select different prey in the presence of light.

Alternatively, this may be a methodological limitation as we are unable to determine true abundance of each prey item within an individual bat, so the amount of a particular prey item may change without a change in the proportion of unique prey items identified in our analysis. Additionally, dietary breadth was similar between lit and unlit sites, except for little brown and tri-colored bats. There was a high degree of overlap in the 95% confidence intervals between treatment groups in the interpolation and extrapolation curves of dietary breadth for the other four species (Figure 2). Overall, diversity and breadth estimates suggest bats were not feeding selectively on a distinct prey group in the presence of light.

Pairwise comparisons between species, within each treatment group, provide further evidence for species-specific changes in diet, as opposed to an overall pattern common to all species (Table 2). For example, the degree of overlap between big brown bats and red bats was less at lit sites ($O_{jk} = 0.345$) than unlit sites ($O_{jk} = 0.536$), suggesting increased dietary differentiation in the presence of light. Similarly, little brown bats and gray bats exhibited the greatest dietary overlap with big brown bats at unlit sites, and red bats at lit sites because of increased consumption of Lepidoptera. Finally, evening bats had a high degree of overlap with big brown bats at unlit sites ($O_{jk} = 0.705$), which is to be expected as evening bats typically prefer Coleoptera (Whitaker 1972, Feldhamer et al. 1995). However, the degree of overlap
decreases in presence of light \((O_{jk} = 0.544)\), because evening bats were not exploiting higher concentrations of beetles at lit sites as were big brown bats.

We found little evidence of increased consumption of eared moths under artificially lit treatments; in fact, eared-moth proportions decreased (although not significantly) at lit sites for most species. Conversely, Cape serotine bats \((Neoromicia capensis)\), significantly increase eared-moth consumption at experimentally manipulated lit sites in South Africa (Minnaar et al. 2015). Nearly every moth species (92.9\%) was identified as an eared moth at that study site, while the proportion of eared moths in the community we studied is likely considerably lower (Dodd et al. 2008). Our results may also be an artefact of our use of the Bold Systems database as numerous potential eared moths had multiple family level identifications and were thus only identified to the ordinal level. This may be because these moth species are not yet in the Bold Systems database or that we had sequenced degraded DNA.

Based on our current work and that of others, we propose four responses in terms of prey selection by bats around light. First, known specialists may take advantage of artificial light-induced phototaxis (van Langevelde et al. 2011) to increase prey consumption of their preferred prey. In the bat community we studied, two dietary specialists (big brown and red bats) consumed proportionally more of their preferred prey at lit sites. Big brown bats, with their powerful jaws, prefer beetles (Agosta 2002, Clare et al. 2014b), while red bats prefer softer-bodied Lepidoptera (Acharya and Fenton 1999, Clare et al. 2009). Second, some generalist species may show dietary shifts to include greater consumption of moths around lights. In our community, two generalist species (gray bat and little brown bat) exhibited such a pattern of increased moth consumption and decreased beetle consumption under artificial light. Gray bats had a 64.9\% increase in Lepidoptera prey at lit sites, the highest within-order percent increase.
Third, some species may show no shift in prey selection around lights. Two species (evening bat and tri-colored bats) exhibited little difference in the proportion of beetles and moths under the two experimental conditions. For these species, the lack of dietary change may be related to their morphology. Tri-colored bats are clutter adapted (Menzel et al. 2005), and while evening bats are not completely clutter adapted, they are weak fliers (Norberg and Rayner 1987); therefore, these bats may be avoiding the lights to avoid predation. Fourth, specialist species may decrease consumption of their preferred prey in favor of moths around lights. No species in our study showed this response, but it has been noted in Cape serotine bats, a beetle specialist that increases consumption of moths around lights (Minnaar et al. 2015).

Artificial lighting at night has varied effects on bat species and the mechanism governing behavioral responses to light is unclear. In general, species with morphological adaptations that favor faster flight in relatively uncluttered habitats are considered light-tolerant species (Rowse et al. 2016). These species often feed on positively phototactic prey around temporally stable light sources, such as streetlights (Schoeman 2016). Conversely, slower flying species with greater maneuverability to forage in and around cluttered habitats are considered light-intolerant (Rowse et al. 2016). These species are often found in lower densities in artificially lit environments and may actively avoid artificial light, although presumably light-intolerant Myotis species have been recorded near single, experimental light setups in desert environments (Fenton and Morris 1976, Bell 1980). It may be that light-intolerant species in non-desert regions are not avoiding lights, but rather the open habitat in which streetlights are found. Even light-tolerant species seem to prefer streetlights in rural areas over urban landscapes (Geggie and Fenton 1985).
The spectral composition of the LEDs used in this experiment may further explain the lack of a consistent response in our experiment. LEDs do not induce phototaxis to the same degree as other light sources, especially mercury vapor (Huemer et al. 2010, Eisenbeis and Eick 2011), likely because LEDs do not produce light in the lower UV spectrum (Stone et al. 2015). Other forms of light which lack UV light, such as high pressure sodium, also attract fewer insects (Rydell 2006). Light sources with lower insect abundance have significantly less bat activity (Blake et al. 1994); in fact, bat activity can change by as much as an order of magnitude depending on lighting technology (Rydell 1992). Interestingly, light-intolerant bats do not appear as averse to LEDs as other technologies (Lewanzik and Voigt 2017). Lower aversion may be related to UV as evidence suggests light-intolerant bats are avoiding UV light specifically (Gorresen et al. 2015). The lack of UV in LED light may change the perception by these bats leading to decreased aversion. Therefore, LED lighting may have less of a negative impact, at least with respect to foraging, for bats and their insect prey.

Numerous studies have reported bats feeding at artificial lights (Hickey and Fenton 1990, Rydell 1992, Minnaar et al. 2015, Schoeman 2016), and some of these studies have compared differences in diet with unlit sites to determine a dietary shift (Hickey and Fenton 1990, Minnaar et al. 2015). A pattern has emerged that bats generally consume more moths, and more eared moths specifically, under artificial light. In particular, much of the work on effects of artificial light on foraging bats in North America has focused on hoary, red, and Hawaiian hoary bats (Belwood and Fullard 1984, Hickey and Fenton 1990, Acharya and Fenton 1992, Hickey et al. 1996, Acharya and Fenton 1999, Jacobs 1999, Fullard 2001). Hoary and red bats are generally considered moth specialists, therefore, an increase in moth consumption around lights may be expected (and is generally supported by our results). The lack of consistent dietary change in our
study may be related to the broader range of species sampled, and suggests caution in assuming a universal response in dietary shifts around lights for all species. The oft-cited pattern, which is quickly becoming a paradigm, that ALAN leads to increases in moth consumption in insectivorous bats may not be the case for all species. Our results underscore the need for a better mechanistic understanding of interactive effects of lights on bats and their insect prey to predict which bat species will be most strongly affected by lights and to craft management plans to limit negative effects of lights on foraging bats.
Table 1.1. Diet overlap between the six species of insectivorous bats evaluated in this study. Observed mean values below 0.6 are generally accepted to represent biologically significant resource partitioning.

<table>
<thead>
<tr>
<th></th>
<th>All MOTUs</th>
<th>Common prey analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed mean</td>
<td>$P$ (Observed $\geq$ expected)</td>
</tr>
<tr>
<td>All treatments and spp.</td>
<td>0.70446</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lit treatment all spp.</td>
<td>0.60420</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Control all spp.</td>
<td>0.66239</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lit/unlit treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>big brown bat</td>
<td>0.85907</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>red bat</td>
<td>0.90643</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>gray bat</td>
<td>0.71912</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>little brown bat</td>
<td>0.81780</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>evening bat</td>
<td>0.82670</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>tri-colored bat</td>
<td>0.74422</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lepidoptera all</td>
<td>0.64481</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>treatments and spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepidoptera lit all spp.</td>
<td>0.45916</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lepidoptera control all spp.</td>
<td>0.52352</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lepidoptera lit/unlit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>big brown bat</td>
<td>0.48110</td>
<td>0.250</td>
</tr>
<tr>
<td>red bat</td>
<td>0.90586</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>gray bat</td>
<td>0.56171</td>
<td>0.570</td>
</tr>
<tr>
<td></td>
<td>All MOTUs</td>
<td>Common Prey Analysis</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td></td>
<td>Observed mean</td>
<td>( P ) (Observed ≥ expected)</td>
</tr>
<tr>
<td>evening bat</td>
<td>0.56600</td>
<td>0.083</td>
</tr>
<tr>
<td>tri-colored bat</td>
<td>0.45748</td>
<td>0.766</td>
</tr>
</tbody>
</table>
Table 1.2. Pairwise comparison of diet overlap between six species of insectivorous bats in experimentally lit and naturally dark experimental treatments. Values below the diagonal are the observed mean and numbers above the diagonal are the corresponding $P$ values.

<table>
<thead>
<tr>
<th></th>
<th>big brown bat</th>
<th>red bat</th>
<th>little brown bat</th>
<th>gray bat</th>
<th>evening bat</th>
<th>tri-colored bat</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>big brown bat</td>
<td>0.037</td>
<td>0.001</td>
<td>0.073</td>
<td>0.022</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>red bat</td>
<td>0.34471</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>little brown bat</td>
<td>0.57593</td>
<td>0.65952</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>gray bat</td>
<td>0.46833</td>
<td>0.68382</td>
<td>0.77017</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>evening bat</td>
<td>0.54435</td>
<td>0.43585</td>
<td>0.63214</td>
<td>0.59877</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>tri-colored bat</td>
<td>0.64095</td>
<td>0.54714</td>
<td>0.76412</td>
<td>0.69536</td>
<td>0.70188</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>big brown bat</th>
<th>red bat</th>
<th>little brown bat</th>
<th>gray bat</th>
<th>evening bat</th>
<th>tri-colored bat</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNLIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>big brown bat</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>red bat</td>
<td>0.53586</td>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>little brown bat</td>
<td>0.76932</td>
<td>0.6034</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>gray bat</td>
<td>0.64075</td>
<td>0.51874</td>
<td>0.81686</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>evening bat</td>
<td>0.70514</td>
<td>0.58402</td>
<td>0.76158</td>
<td>0.74654</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>tri-colored bat</td>
<td>0.54008</td>
<td>0.57741</td>
<td>0.67231</td>
<td>0.69197</td>
<td>0.77191</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.3. Diversity estimates between experimentally lit and naturally dark conditions in six species of insectivorous bats.

<table>
<thead>
<tr>
<th>Bat species</th>
<th>$n$ (samples analyzed)</th>
<th>Treatment group</th>
<th>Richness</th>
<th>$q = 1$, Shannon diversity effective # of MOTUs</th>
<th>$q = 2$, Simpson diversity effective # of MOTUs</th>
</tr>
</thead>
<tbody>
<tr>
<td>big brown bat</td>
<td>7</td>
<td>lit</td>
<td>66</td>
<td>54.40</td>
<td>85.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unlit</td>
<td>100</td>
<td>69.19</td>
<td>97.66</td>
</tr>
<tr>
<td>red bat</td>
<td>35</td>
<td>lit</td>
<td>200</td>
<td>111.89</td>
<td>164.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unlit</td>
<td>213</td>
<td>119.98</td>
<td>157.49</td>
</tr>
<tr>
<td>gray bat</td>
<td>7</td>
<td>lit</td>
<td>107</td>
<td>89.76</td>
<td>148.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unlit</td>
<td>108</td>
<td>81.69</td>
<td>134.30</td>
</tr>
<tr>
<td>little brown bat</td>
<td>9</td>
<td>lit</td>
<td>119</td>
<td>89.51</td>
<td>176.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unlit</td>
<td>150</td>
<td>87.57</td>
<td>114.84</td>
</tr>
<tr>
<td>evening bat</td>
<td>6</td>
<td>lit</td>
<td>73</td>
<td>60.29</td>
<td>101.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unlit</td>
<td>120</td>
<td>81.45</td>
<td>105.00</td>
</tr>
<tr>
<td>tri-colored bat</td>
<td>6</td>
<td>lit</td>
<td>91</td>
<td>75.31</td>
<td>146.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unlit</td>
<td>95</td>
<td>72.87</td>
<td>95.91</td>
</tr>
</tbody>
</table>

$q = 1$, Shannon diversity effective # of MOTUs = 1 - $H/q$, where $H$ is the Shannon entropy and $q$ is the order parameter.

$q = 2$, Simpson diversity effective # of MOTUs = 1/($q-1$) * $H/q$, where $H$ is the Shannon entropy and $q$ is the order parameter.
Figure 1.1. The proportion of MOTUs identified in the diet of six species of insectivorous bats under experimentally lit and naturally dark conditions.
Figure 1.2. Interpolation (rarefaction) and extrapolation of dietary species richness for each experimental condition in six species of insectivorous bats using the Chao2 estimation for incidence-based sample data. Richness is extrapolated to twice the sample size and bootstrapped 500 times.
CHAPTER 2

ILLUMINATING THE PHYSIOLOGICAL IMPLICATIONS OF ARTIFICIAL LIGHT ON AN INSECTIVOROUS BAT COMMUNITY

2.1 INTRODUCTION

Global light pollution has increased dramatically during the 20th and 21st centuries as a result of rapid urban development (Hölker et al. 2010a). Nearly 90% of Europe and over half of North America are estimated to experience light polluted skies (Falchi et al. 2016). These levels have remained relatively constant over the last several decades, while increases in light pollution extent have occurred recently in developing areas with higher than average species richness (Koen et al. In press). The use of artificial light at night (ALAN) is widespread and a major threat to biodiversity (Hölker et al. 2010b), especially for nocturnal animals such as bats that are adapted to life in dark environments (Voigt and Lewanzik 2011).

The impact of artificial lighting on bat behaviors is wide ranging and includes effects on foraging and commuting, emergence, roosting, breeding, and hibernation (as reviewed in Stone et al. 2015). Negative impacts on these behaviors could have reduced fitness costs. For instance, artificial lighting around bat roosts can lead to a delayed nightly emergence (Downs et al. 2003). By delaying emergence, bats can miss the peak in insect abundance around dusk (Jones and Rydell 1994), which could be particularly detrimental to pregnant or lactating females who have increased energetic demands (Kurta et al. 1989).

While bats, in general, appear to prefer dark environments (Lima and O'Keefe 2013), there are numerous observations of bats foraging around artificial light (Hickey et al. 1996, Acharya and Fenton 1999, Polak et al. 2011, Schoeman 2016). Concurrently, artificial light induces phototaxis in aerial insects, leading to unusually high densities around lights (van
Langevelde et al. 2011). It has been proposed that light-tolerant bats may take advantage of larger and higher densities of prey around artificial light, which could offset predation risks from flight in a lit environment (Tomassini et al. 2014). If this is the case, widespread ALAN in the nocturnal environment could significantly alter bat-insect interactions and foraging behaviors with cascading effects on the food web and ecosystem functioning (Minnaar et al. 2015, Cravens et al. 2018).

If ALAN can potentially alter foraging behaviors, we should also expect to see energetic effects on the bats. Thus, we evaluated the effect of artificial light on energy metabolism in an insectivorous bat community through plasma metabolite analysis (McGuire et al. 2009, Boyles et al. 2016). We experimentally manipulated naturally dark areas with an artificial light treatment known to cause shifts in prey selection (Minnaar et al. 2015, Cravens et al. 2018), and measured β-hydroxybutyrate levels from bats captured in both lit and unlit environments. We also measured bat activity from acoustic recordings in lit and unlit conditions. We predicted an increase in foraging intensity and activity in artificial light treatments relative to naturally dark areas.

2.2 MATERIALS AND METHODS

2.2.1 Study Site

Our study was conducted in a 9-county region of western Missouri, USA during summer (May to August) 2017. The study area lies primarily within the Ozark Highlands physiographic region, a heavily forested landscape dominated by oak-hickory forests. Along the western edge of our study area, the land begins to transition to Osage Plains, a region historically dominated
by prairie but now heavily converted to agriculture with limited forest and woodlands (Raeker et al. 2010).

2.2.2 Experimental Design

We erected temporary lights along naturally dark forest roads or streams on public lands and had two experimental conditions: unlit (control) and lit (light pollution treatment). Distance between lit and unlit sites was at least 2 km to minimize overlap in foraging ranges by individual bats, but sites were chosen with similar habitat and landscape features. At lit sites, we used 50W LED (Shenzhen Lepower Opto Electronics Co., China) producing 5600 lumens at 4000 K. We used LED lighting as it is replacing older outdoor lighting styles, such as mercury vapor. Lights were elevated 3m from the ground on a metal pole and powered by a 12V lead acid battery. We used mist nets to capture bats along forested roads or streams at 17 sites throughout the summer. We netted at each survey site for three nights and ran lights for all three nights from 21:00 to 05:00. On the first two nights, we captured bats at an unlit control site and on the third night, we captured bats at lit sites (Minnaar et al. 2015, Cravens et al.). Delaying capture at lit sites until the third night allowed bats to become accustomed to the lit condition, as well as provide time for them to choose to forage in the newly lit environment. We make no assumption that all bats captured at lit sites were necessarily foraging around the lights; moreover, we expect some species may be less prone to foraging at lights than others and therefore show less pronounced shifts in feeding rates and activity levels. Nets were placed in flyways within 25m of the light in an appropriate netting location.

We recorded bats acoustically (SM2Bat+ and SM4ZC; Wildlife Acoustics, Maynard, Massachusetts, USA) from 20:00 to 06:00 on each of three sampling nights at all lit and unlit
sites and added additional sampling nights if there was a period of rain or high winds longer than 30 minutes. We elevated detectors two meters on a metal pole away from clutter that could affect echolocation calls. We identified bat species from our acoustic detector recordings using Kaleidoscope Pro v3.1 automated identification software (Wildlife Acoustics, Maynard, Massachusetts, USA). Little brown bats (*Myotis lucifugus*) and Indiana bats (*M. sodalis*) are difficult to differentiate acoustically, so we combined calls identified as either of those species. We pooled calls identified to each species into 15-minute intervals, and report data from the second night of recording.

We measured plasma β-hydroxybutyrate concentrations from six species of insectivorous bats in naturally dark and artificially lit conditions. β-hydroxybutyrate is generally considered a fasting metabolite and increases during fasting to power metabolic processes when dietary triglycerides are low (Robinson and Williamson 1980, Jenni-Eiermann and Jenni 1991). While the mechanism is not understood, available data indicate β-hydroxybutyrate paradoxically increases in both captive and free-living bats after feeding (McGuire et al. 2009) and has thus been used as a proxy for foraging intensity (McGuire et al. 2009, Boyles et al. 2016, Sommers et al. 2017; we discuss the implications of these competing interpretations in the discussion). We removed bats from the net and collected a small (<75 µl) blood sample from the interfemoral vein by puncturing the vein with a 26-gauge needle and collecting blood with a 75-µl heparinized hematocrit tube (Fisher Scientific, Waltham, MA)(Hooper and Amelon 2014). All blood samples were collected within 10 min after removing the bat from the net. After blood collection, we centrifuged blood samples (10 min at 2,000 g; Fisher Scientific Mini Centrifuge) to obtain 10 µl of plasma which was used to quantify β-hydroxybutyrate concentration with a
handheld meter (STAT-Site M β-HB; Stanbio Laboratory, Boerne, Texas USA)(Sommers et al. 2017).

We expect foraging intensity to be highly temperature dependent because both bats and their insect prey are more active on warmer nights; thus, β-hydroxybutyrate levels are also likely temperature dependent. However, we designed our experiment specifically to determine the effect of light pollution on β-hydroxybutyrate levels. Thus, to account for temperature, we used residuals from a regression of mean nightly ambient temperature against β-hydroxybutyrate in all further analyses. We then used general linear models in program R to test the effects of treatment (lit, unlit), minutes after sunset (because the effect of the lights may change as ambient light changes), and treatment * minutes after sunset interaction for each bat species (R Core Team 2017). We used this approach because we were interested in the effect from the light treatment and not concerned with the effect of environmental and morphological variables, as these have been well established in the literature. We used a paired t-test to compare acoustic activity across the night between treatments for each bat species. We qualitatively compared temporal patterns in β-hydroxybutyrate to activity estimated with acoustic detectors for each species of bat.

2.3 RESULTS

We collected blood samples from 169 bats (n = 66 lit and n = 103 unlit) over 36 nights (n = 11 lit and n = 25 unlit) during summer 2017 (10 June – 14 August). We included samples in our statistical analysis from five species (big brown bats (Eptesicus fuscus); red bats (Lasiurus borealis); gray bats (Myotis grisescens); evening bats (Nycticeius humeralis); and tri-colored bats (Perimyotis subflavus)) and excluded little brown bats because of small sample size (n = 1). We excluded two outliers, one from a big brown bat and one from a red bat, and as their removal
did not change the results qualitatively we are reporting results with these samples excluded. We measured acoustic activity across 16 paired lit and unlit locations from 10 June – 8 August 2017. Little brown bats were detected regularly on acoustic recorders, so those data are included below.

Both blood metabolite and acoustic data suggest similar, species-specific patterns: red bats actively forage around lights and may gain some energetic benefit, while big brown and gray bats avoid lit areas and thus gain no such benefit. Specifically, the interaction between minutes after sunset and light treatment was significant in red bats ($t = 3.782, p < 0.001$); β-hydroxybutyrate levels were highest just after sunset and declined throughout the night in artificially lit sites, while at naturally dark sites, β-hydroxybutyrate levels were lowest just after sunset and increased throughout the night (Figure 1). Although activity, as indicated by acoustic recordings, was not different between treatments across the entire night ($t = 1.882, df = 39, p = 0.067$), there are two distinct periods during the night when red bats were more active at lit sites relative to unlit sites (Figure 2): one immediately after sunset corresponding to the highest β-hydroxybutyrate levels for this species, and another approximately 300 minutes after sunset. Interestingly, the only red bats we captured after approximately 250 min after sunset were around artificial lights.

Blood metabolite and acoustic data for big brown bats and gray bats indicate avoidance of lit areas. Plasma β-hydroxybutyrate levels were not significantly different between treatments, across the night, or in the treatment*minutes after sunset interaction for either big brown bats or gray bats ($p > 0.54$ in all cases). Both species were more active at unlit sites than lit sites (big brown bats: $t = 5.086, df = 39, p < 0.001$; gray bats: $t = 10.009, df = 39, p < 0.001$), which is maintained after a Bonferroni correction for multiple comparisons. Notably, when comparing the difference in activity between lit and unlit sites for each 15-minute interval throughout the night,
activity is rarely greater at lit sites for big brown bats and gray bats (Figure 3). Taken together, this strongly indicates these species are actively avoiding lit areas.

It is difficult to compare β-hydroxybutyrate levels with acoustic activity for evening and tri-colored bats. Plasma metabolite levels for evening bats were quite similar between treatments ($t = -0.017, p = 0.987$) with no significant interaction between the main effects ($t = 0.114, p = 0.911$), suggesting this species may be avoiding lit areas. Tri-colored bats may also be avoiding lit sites as their plasma metabolite levels were again very similar between treatments ($t = -1.331, p = 0.315$) and no interaction between main effects ($t = 1.251, p = 0.337$), although interpretation is limited given our small sample size. While we have no blood metabolite data for the two myotis species (Indiana bats and little brown bats), acoustic data indicate activity is significantly greater at unlit sites ($p < 0.017$).

2.4 DISCUSSION

Our data show taxon-specific effects of short-term changes in ALAN on foraging intensity and activity levels in an insectivorous bat community. Acoustic data suggest red bats actively forage around artificial lights, and β-hydroxybutyrate levels indicate they likely gain energetic benefits by doing so, regardless of the exact interpretation of β-hydroxybutyrate levels (see below). The other species in the community appear to not select artificially lit sites for foraging and showed no observable difference in β-hydroxybutyrate between experimental treatments. This is the first study, to our knowledge, demonstrating that ALAN can modify behavior sufficiently to cause knock-on effects on physiology and energetics, and because the effects are not the same on all species, ALAN might further affect competitive balance in a community.
Bats adapted for faster flight in relatively open habitats are generally considered to be light-tolerant species (Rowse et al. 2016). Red bats have a moderate aspect ratio, high wing loading, and are fast flyers with limited (relatively speaking) maneuverability (Norberg and Rayner 1987). They have been the focus of numerous studies on bats and artificial light (Acharya and Fenton 1992, Hickey et al. 1996, Acharya and Fenton 1999), and these studies have consistently shown artificial light to have an attractive effect on red bats. Conversely, slower flying species adapted for flight in cluttered habitat tend to be light-intolerant (Rowse et al. 2016) as slower flight may make bats vulnerable to predation in open, lit environments. Big brown bats and gray bats, while not necessarily clutter adapted, do have lower aspect ratios, lower wing loadings, and are slower fliers than red bats (Norberg and Rayner 1987). Our data suggests these two species, especially gray bats, avoid lit areas (Figure 2). Acoustic data suggest the other Myotis species in the community (little brown and Indiana bats) are also light adverse (Figure 2). Evening bats are not fully clutter adapted, but are weak fliers (Norberg and Rayner 1987). Although the acoustic data may be unreliable for evening bats, this species showed no change in β-hydroxybutyrate levels between treatments. Care should be taken when interpreting evening and tri-colored bat acoustic data. The Kaleidoscope software is known to commonly confuse evening and tri-colored bats with more common red bats (Ford 2017). Qualitatively, the activity patterns of these two species track closely with red bats (Figures 2 and 3), so we suspect these patterns are an artefact of the identification process, not a biological pattern. Further, evening bats did not alter diet, at least with respect to Lepidoptera and Coleoptera, in the presence of artificial light (Cravens et al. 2018), suggesting they are not actively taking advantage of prey densities around artificial light.
We recorded the highest β-hydroxybutyrate levels in red bats foraging around lights shortly after sunset, with a decrease throughout the rest of the night. There were no significant trends in β-hydroxybutyrate levels throughout the night in other species. Unlike in most other animals, β-hydroxybutyrate levels in bats appear to behave contrary to physiological norms, increasing with food intake (McGuire et al. 2009). Thus, previous papers measuring β-hydroxybutyrate levels in bats have interpreted increased β-hydroxybutyrate as an indicator of increased foraging efficiency and energy intake (McGuire et al. 2009, Boyles et al. 2016). Using this interpretation, our data suggest red bats forage heavily around lights shortly after sunset and gain energetic benefits by doing so, but do not forage around artificial light late in the night, and thus gain no energetic benefit. This is incongruous with acoustic data which indicate a second peak in red bat activity late in the night (Figure 2). Under this interpretation, some other species in this community, such as grey bats, avoid lights and thus gain no benefit of increased prey densities around lights, regardless of time of night.

Our acoustic and capture data hint that interpreting β-hydroxybutyrate levels in bats as a proxy of foraging success might be problematic, and that β-hydroxybutyrate might behave as it does in other species. Ketogenesis, or the production of β-hydroxybutyrate and other ketone bodies in the liver, occurs during periods of low food availability to provide fuel to the brain, muscles, and other organs (Flatt 1972). Thus, β-hydroxybutyrate levels increase with fasting (Jenni-Eiermann and Jenni 1991, Féry et al. 1996). Temperate-zone, insectivorous bats generally only forage on the wing for 2-8 h each night (Kurta et al. 1989). For the remainder of the daily cycle they stay in roosts and do not feed, so we might expect β-hydroxybutyrate levels to increase throughout the day, peaking immediately before the nightly foraging period. If this were
the only driver of high β-hydroxybutyrate, we would expect to see elevated levels in all species early in the night, which we do not.

Our data suggest β-hydroxybutyrate may be a better indicator of the energy source powering flight than of foraging success in bats, as it has been interpreted in the past. Although most commonly thought of as a fasting metabolite, elevated levels of ketone bodies can also be found during prolonged or intense exercise (Laffel 1999) and exercise-trained muscles oxidize β-hydroxybutyrate more efficiently than muscles from sedentary individuals (Winder et al. 1973). Thus, we might expect to see elevated β-hydroxybutyrate in bats undertaking intense foraging shortly after leaving the roost and before they can metabolize exogenous energy sources. If red bats are taking advantage of high densities of insects around lights (particularly moths) immediately after beginning foraging (Rydell et al. 1996), they may be powering flight through ketogenesis. The same reasoning may explain why β-hydroxybutyrate levels are high in little brown bats living at high latitudes, where foraging bouts are necessarily short and intense because of limited darkness (Boyles et al. 2016). The difference in β-hydroxybutyrate patterns between red bats in the two treatments in this study may relate to what the bats were doing when captured. During the artificial light treatments, red bats were likely foraging (as indicated by high, sustained acoustic activity), while red bats captured in naturally dark conditions were likely commuting to foraging areas (because they normally forage in open areas, not over roads and streams where we captured them)(Elmore et al. 2005, Menzel et al. 2005, Walters et al. 2007). The relatively lower flight costs of straight flight during commuting (Grodzinski et al. 2009, Voigt et al. 2010a, Voigt and Holderied 2012) should impose lower energetic demands, and thus less need for an upregulation of ketogenesis. Interestingly, red bats were only captured late in the night around lights, and these individuals universally had low β-hydroxybutyrate levels, although
this is based on a small sample size (Figure 1). This would suggest foraging around lights provides more energy than foraging in naturally dark areas, and as the night progresses, red bats fuel increasingly more flight through dietary proteins and fatty acids (Voigt et al. 2010b, Voigt et al. 2012). β-hydroxybutyrate levels do not decrease throughout the night in any of the species which are not selecting lit areas or in red bats at naturally dark sites. Further, both acoustic data and capture data suggest red bats are not highly active late in the night at naturally dark sites. If so, energy intake may never be high enough in the absence of artificial light, when insect densities are presumably low, for metabolized dietary sources to fully replace ketone bodies in powering flight. It is also important to note that differences in β-hydroxybutyrate between species with different diets, such as two known specialists, red bats and big brown bats, are likely not related to their consumption of different insects. The ratio of protein and lipids may differ, as red bats primarily consume moths and big brown bats beetles, however, both proteins and lipids are not immediately available as an energy source (Voigt et al. 2010b, Voigt et al. 2012). An interesting comparison would be with a species with a carbohydrate rich diet, such as a nectivorous bat (Voigt and Speakman 2007).

The production and use of ketone bodies may have other physiological benefits for bats beyond powering flight during periods of intense activity when dietary sources are not available. For example, ketogenesis inhibits lipolysis, which serves to maintain endogenous energy stores and muscle glycolysis, allowing for fattening and recharge of muscle glycogen (Féry et al. 1996, Jenni-Eiermann 2017). Thus, we might expect to see β-hydroxybutyrate used heavily to power flight during periods when storing fat is imperative, such as immediately before hibernation. In support of this prediction, some of the highest β-hydroxybutyrate levels measured in bats to date
were from little brown bats during the pre-hibernation fattening period in Ontario (McGuire et al. 2009).

The unique combination of circadian cycles, high fat diet, and an unusually expensive mode of locomotion might explain why β-hydroxybutyrate levels appear to increase with energy intake in insectivorous bats (McGuire et al. 2009). In the lab, bats are fed without foraging. However, in the field highly energetically expensive foraging occurs at the end of a daily fast when circulating triglyceride levels are low. Insectivorous, aerial-hawking bats never naturally intake energy without flying, so flight and feeding might be physiologically linked in these species, and a reliance on a low-carbohydrate diet might mean that dietary energy is not immediately available to power foraging. Feeding might signal the liver to increase ketogenesis in insectivorous bats, even without flight. An interesting test of this hypothesis would be to measure β-hydroxybutyrate levels in frugivorous or nectivorous bats, which can metabolize dietary energy almost instantly to power flight (Voigt and Speakman 2007). In these species, ketones may be less important energetic substrates for powered flight, and therefore may be less physiologically linked to flight.

Our results shed additional light on the complex interactions of the bat-insect-light system. The effect of ALAN on bat-insect interactions, from our results, appear to benefit some species, while other species may be at a disadvantage, at least with respect to bats. The potential for negative impacts, at a population level, warrant further study at a landscape scale, given the degree of artificial light in the nocturnal environment. In this bat community, red bats, with a morphological propensity for fast flight, seem to be gaining an energetic benefit by foraging around artificial lights. However, red bats may not need a competitive leg up as they are ubiquitous on the landscape and among the most common species in the region. Other species in
the area, particularly those in the genus *myotis*, are rare, and becoming rarer. This is a pattern seen in European bat communities as well, whereby species that are light-tolerant are common on the landscape and those which are light-averse tend to be rare or threatened (Stone et al. 2009, 2012, Lacoeuilhe et al. 2014). In addition to limiting spatial extent, the negative impacts of light pollution on light-intolerant species may be further compounded by decreasing prey resources in naturally dark areas where they forage (Longcore and Rich 2004, Conrad et al. 2006, Eisenbeis 2006, Groenendijk and Ellis 2011). The low capture rates (and activity levels from the acoustic data) of those species around lights would suggest that despite being caught near the light, they may just be commuting through. Thus, artificial lights may be helping common species, such as red bats, but actively hurting other, rarer species by both limiting their distribution on the landscape and concentrating insects where these species will not forage. Conservation practitioners should ensure protected lands have only necessary night lighting around infrastructure and work with lighting engineers to minimize impacts of artificial light on imperiled bat populations.
Table 2.1. Output from the general linear models testing the main effects of light treatment, minutes after sunset, and the interaction of treatment with minutes after sunset against β-hydroxybutyrate concentrations in the five species of insectivorous bats.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>big brown bat</th>
<th>red bat</th>
<th>gray bat</th>
<th>evening bat</th>
<th>tri-colored bat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>estimate</td>
<td>$P$</td>
<td>estimate</td>
<td>$P$</td>
<td>estimate</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.047</td>
<td>0.492</td>
<td>0.118</td>
<td><strong>0.001</strong></td>
<td>8.96e-03</td>
</tr>
<tr>
<td>lit treatment</td>
<td>-0.051</td>
<td>0.491</td>
<td>-0.165</td>
<td><strong>&lt;0.001</strong></td>
<td>-2.92e-02</td>
</tr>
<tr>
<td>minutes after sunset</td>
<td>-2.57e-04</td>
<td>0.464</td>
<td>-8.04e-04</td>
<td><strong>&lt;0.001</strong></td>
<td>-4.01e-05</td>
</tr>
<tr>
<td>Interaction</td>
<td>2.93e-04</td>
<td>0.476</td>
<td>0.001</td>
<td><strong>&lt;0.001</strong></td>
<td>1.69e-04</td>
</tr>
</tbody>
</table>

$n$ | 21 | 94 | 29 | 16 | 6 |
Table 2.2. Output from paired t-tests between activity levels in artificially lit and naturally dark conditions in six species of insectivorous bats

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean of difference</th>
<th>t</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>big brown bat</td>
<td>-0.164</td>
<td>-5.086</td>
<td>39</td>
<td>9.53e-06</td>
</tr>
<tr>
<td>red bat</td>
<td>0.719</td>
<td>1.882</td>
<td>39</td>
<td>0.067</td>
</tr>
<tr>
<td>gray bat</td>
<td>-1.173</td>
<td>-10.009</td>
<td>39</td>
<td>2.49e-12</td>
</tr>
<tr>
<td>evening bat</td>
<td>-0.133</td>
<td>-0.114</td>
<td>39</td>
<td>0.910</td>
</tr>
<tr>
<td>tri-colored bat</td>
<td>-0.153</td>
<td>-0.869</td>
<td>39</td>
<td>0.390</td>
</tr>
<tr>
<td>Indiana/little brown bat</td>
<td>-0.290</td>
<td>-3.179</td>
<td>39</td>
<td>0.003</td>
</tr>
</tbody>
</table>
Figure 2.1. (caption on next page)
Figure 2.1. Changes in β-hydroxybutyrate concentrations across the night in the five species of insectivorous bats between artificially lit and naturally dark conditions. Yellow points represent values from artificially lit sites, while black points represent values from naturally dark sites. Values along the y-axis represent the range of residual values from a regression of temperature and β-hydroxybutyrate in order to account for an effect of temperature.
Figure 2.2. (caption on next page)
Figure 2.2. Comparison of relative bat activity, summed in 15-minute intervals, across the night between experimental treatments for each bat species. Calls of common red bats are often misidentified as rarer evening bats and tri-colored bats by the identification software so the patterns for these two species should be interpreted with care. Calls of the Indiana and little brown bat were combined as a single species group as they are very difficult to distinguish acoustically.
Figure 2.3. Difference in relative abundance between treatments for each 15-minute interval, based on call data from Figure 2. Higher positive values represent greater activity at lit sites, while higher negative values indicate greater activity at unlit sites.
Figure 2.4. (caption on next page)
Figure 2.4. Changes in β-hydroxybutyrate concentrations across the night in the five species of insectivorous bats between artificially lit and naturally dark conditions. Yellow points represent values from artificially lit sites, while black points represent values from naturally dark sites. Two outliers, one from a big brown bat and one from a red bat, have also been excluded in this figure to maintain consistency between Figure 2.1. Their removal did not change the results qualitatively.
LITERATURE CITED


Chamberlain, S. 2017. bold: interface to Bold Systems 'API'. in R package version 0.4.0, https://CRAN.R-project.org/package=bold


APPENDICES
A.1 SUPPLEMENTARY DATA FOR CHAPTER 1

Appendix Figure A.1

Sequence processing in QIIME and python

Join paired ends

Quality filtering of sequences less than Q25

Remove sequences less than 147 or greater than 157 bp

Assign similar sequences to OTUs using 'swarm'

Trim primers

Filter OTUs not present at least 10 times in at least one sample

Filter within-sample OTUs occurrences of <10

Extract representative sequences based on abundance

Assign taxonomy in R with 'bold' package

Query BOLD for OTU matches

Trim output to top 40 matches for each OTU

Filter out records with <98% similarity and specimen from outside US and Canada

Assign similar sequences to OTUs using 'swarm'

Assign quality grouping

High quality group: matches <99.36% do not affect taxonomy

Assign taxonomy to match with ≥99.36% similarity

Low quality group: matches >98.0% but <99.36% similarity

Assign to lowest taxonomic level with identification agreement

Appendix Figure 1.1 caption on the next page
Appendix Figure 1.1 Flowchart for bioinformatics processing of Illumina MiSeq sequences. This flowchart details steps taken to ensure accurate identification of arthropods and filter out low-quality sequences (i.e. chimeras, poor database matches, etc.).
EXCLUSIVE LICENSE AGREEMENT

Date: October 27, 2017

Contributor name: Zachary Cravens

Contributor address:

Manuscript number: JAPPL-2017-00678.R1

Re: Manuscript entitled Illuminating prey selection in an insectivorous bat community, exposed to artificial light at night (the "Contribution")

for publication in Journal of Applied Ecology (the "Journal") published by John Wiley & Sons Ltd ("Wiley")

Dear Contributor(s):

Thank you for submitting your Contribution for publication. In order to expedite the editing and publishing process and enable the Owner to disseminate your Contribution to the fullest extent, we need to have this Exclusive License Agreement executed. If the Contribution is not accepted for publication, or if the Contribution is subsequently rejected, this Agreement shall be null and void.

Publication cannot proceed without a signed copy of this Agreement.
A. COPYRIGHT

1. The Contributor grants to the Owner an exclusive license of all rights of copyright in the Contribution during the full term of copyright and any extensions or renewals, including but not limited to the right to publish, republish, transmit, sell, distribute and otherwise use the Contribution in whole or in part in electronic and print editions of the Journal and in derivative works throughout the world, in all languages and in all media of expression now known or later developed, and to license or permit others to do so. For the avoidance of doubt, "Contribution" is defined to only include the article submitted by the Contributor for publication in the Journal and does not extend to any supporting information submitted with or referred to in the Contribution ("Supporting Information"). To the extent that any Supporting Information is submitted to the Journal for online hosting, the Owner is granted a perpetual, non-exclusive license to host and disseminate this Supporting Information for this purpose.

2. Reproduction, posting, transmission or other distribution or use of the final Contribution in whole or in part in any medium by the Contributor as permitted by this Agreement requires a citation to the Journal suitable in form and content as follows: (Title of Article, Contributor, Journal Title and Volume/ Issue, Copyright © [year], copyright owner as specified in the Journal, Publisher). Links to the final article on the publisher website are encouraged where appropriate.

B. RETAINED RIGHTS

Notwithstanding the above, the Contributor or, if applicable, the Contributor’s employer, retains all proprietary rights other than an exclusive license of copyright, such as patent rights, in any process, procedure or article of manufacture described in the Contribution.

C. PERMITTED USES BY CONTRIBUTOR

1. Submitted Version. The Owner licenses back the following rights to the Contributor in the version of the Contribution as originally submitted for publication (the "Submitted Version"):

   a. The right to self-archive the Submitted Version on the Contributor’s personal website, place in a not for profit subject-based preprint server or repository or in a Scholarly Collaboration Network (SCN) which has signed up to the STM article sharing principles [http://www.stm-assoc.org/stm-consultations/scn-consultation-2015/](http://www.stm-assoc.org/stm-consultations/scn-consultation-2015/)("Compliant SCNs"), or in the Contributor’s company/ institutional repository or archive. This right extends to both intranets and the Internet. The
Contributor may replace the Submitted Version with the Accepted Version, after any relevant embargo period as set out in paragraph C.2(a) below has elapsed. The Contributor may wish to add a note about acceptance by the Journal and upon publication it is recommended that Contributors add a Digital Object Identifier (DOI) link back to the Final Published Version.

b. The right to transmit, print and share copies of the Submitted Version with colleagues, including via Compliant SCNs, provided that there is no systematic distribution of the Submitted Version, e.g. posting on a listserve, network (including SCNs which have not signed up to the STM sharing principles) or automated delivery.

2. Accepted Version. The Owner licenses back the following rights to the Contributor in the version of the Contribution that has been peer-reviewed and accepted for publication, but not final (the "Accepted Version"):

a. The right to self-archive the Accepted Version on the Contributor’s personal website, in the Contributor’s company/institutional repository or archive, in Compliant SCNs, and in not for profit subject-based repositories such as PubMed Central, subject to an embargo period of 12 months for scientific, technical and medical (STM) journals and 24 months for social science and humanities (SSH) journals following publication of the Final Published Version. There are separate arrangements with certain funding agencies governing reuse of the Accepted Version as set forth at the following website: https://authorservices.wiley.com/author-resources/Journal-Authors/licensing-open-access/open-access/funder-agreements.html. The Contributor may not update the Accepted Version or replace it with the Final Published Version. The Accepted Version posted must contain a legend as follows: This is the accepted version of the following article: FULL CITE, which has been published in final form at [Link to final article]. This article may be used for non-commercial purposes in accordance with the Wiley Self-Archiving Policy [https://authorservices.wiley.com/author-resources/Journal-Authors/licensing-open-access/open-access/self-archiving.html].

b. The right to transmit, print and share copies of the Accepted Version with colleagues, including via Compliant SCNs (in private research groups only before the embargo and publicly after), provided that there is no systematic distribution of the Accepted Version, e.g. posting on a listserve, network (including SCNs which have not signed up to the STM sharing principles) or automated delivery.
3. **Final Published Version.** The Owner hereby licenses back to the Contributor the following rights with respect to the final published version of the Contribution (the "Final Published Version"):

   a. Copies for colleagues. The personal right of the Contributor only to send or transmit individual copies of the Final Published Version in any format to colleagues upon their specific request, and to share copies in private sharing groups in Compliant SCNs, provided no fee is charged, and further provided that there is no systematic external or public distribution of the Final Published Version, e.g. posting on a listserv, network or automated delivery.

   b. Re-use in other publications. The right to re-use the Final Published Version or parts thereof for any publication authored or edited by the Contributor (excluding journal articles) where such re-used material constitutes less than half of the total material in such publication. In such case, any modifications must be accurately noted.

   c. Teaching duties. The right to include the Final Published Version in teaching or training duties at the Contributor’s institution/place of employment including in course packs, e-reserves, presentation at professional conferences, in-house training, or distance learning. The Final Published Version may not be used in seminars outside of normal teaching obligations (e.g. commercial seminars). Electronic posting of the Final Published Version in connection with teaching/training at the Contributor’s company/institution is permitted subject to the implementation of reasonable access control mechanisms, such as user name and password. Posting the Final Published Version on the open Internet is not permitted.

   d. Oral presentations. The right to make oral presentations based on the Final Published Version.

4. **Article Abstracts, Figures, Tables, Artwork and Selected Text (up to 250 words).**

   a. Contributors may re-use unmodified abstracts for any non-commercial purpose. For online uses of the abstracts, the Owner encourages but does not require linking back to the Final Published Version.

   b. Contributors may re-use figures, tables, artwork, and selected text up to 250 words from their Contributions, provided the following conditions are met:

      (i) Full and accurate credit must be given to the Final Published Version.
(ii) Modifications to the figures and tables must be noted. Otherwise, no changes may be made.

(iii) The re-use may not be made for direct commercial purposes, or for financial consideration to the Contributor.

(iv) Nothing herein will permit dual publication in violation of journal ethical practices.

D. CONTRIBUTIONS OWNED BY EMPLOYER

1. If the Contribution was written by the Contributor in the course of the Contributor’s employment (as a "work-made-for-hire" in the course of employment), the Contribution is owned by the company/institution which must execute this Agreement (in addition to the Contributor’s signature). In such case, the company/institution must execute this Agreement (in addition to the Contributor’s signature). In such case, the company/institution hereby grants an exclusive license to the Owner of all rights of copyright in and to the Contribution for the full term of copyright throughout the world as specified in paragraph A above.

For company/institution-owned work, signatures cannot be collected electronically and so instead please print off this Agreement, ask the appropriate person in your company/institution to sign the Agreement as well as yourself in the space provided below, and upload the signed Agreement to the Wiley Author Services Dashboard. For production editor contact details please visit the Journal’s online author guidelines.

2. In addition to the rights specified as retained in paragraph B above and the rights granted back to the Contributor pursuant to paragraph C above, the Owner hereby grants back, without charge, to such company/institution, its subsidiaries and divisions, the right to make copies of and distribute the Final Published Version internally in print format or electronically on the Company’s internal network. Copies so used may not be resold or distributed externally. However the company/institution may include information and text from the Final Published Version as part of an information package included with software or other products offered for sale or license or included in patent applications. Posting of the Final Published Version by the company/institution on a public access website may only be done with written permission, and payment of any applicable fee(s). Also, upon payment of the applicable reprint fee, the company/institution may distribute print copies of the Final Published Version externally.

E. GOVERNMENT CONTRACTS
In the case of a Contribution prepared under U.S. Government contract or grant, the U.S. Government may reproduce, without charge, all or portions of the Contribution and may authorize others to do so, for official U.S. Government purposes only, if the U.S. Government contract or grant so requires. (U.S. Government, U.K. Government, and other government employees: see notes at end.)

F. COPYRIGHT NOTICE

The Contributor and the company/institution agree that any and all copies of the Final Published Version or any part thereof distributed or posted by them in print or electronic format as permitted herein will include the notice of copyright as stipulated in the Journal and a full citation to the Journal.

G. CONTRIBUTOR'S REPRESENTATIONS

The Contributor represents that the Contribution is the Contributor's original work, all individuals identified as Contributors actually contributed to the Contribution, and all individuals who contributed are included. If the Contribution was prepared jointly, the Contributor has informed the co-Contributors of the terms of this Agreement and has obtained their written permission to execute this Agreement on their behalf. The Contribution is submitted only to this Journal and has not been published before, has not been included in another manuscript, and is not currently under consideration or accepted for publication elsewhere. If excerpts from copyrighted works owned by third parties are included, the Contributor shall obtain written permission from the copyright owners for all uses as set forth in the standard permissions form or the Journal's Author Guidelines, and show credit to the sources in the Contribution. The Contributor also warrants that the Contribution and any submitted Supporting Information contains no libelous or unlawful statements, does not infringe upon the rights (including without limitation the copyright, patent or trademark rights) or the privacy of others, or contain material or instructions that might cause harm or injury and only utilize data that has been obtained in accordance with applicable legal requirements and Journal policies. The Contributor further warrants that there are no conflicts of interest relating to the Contribution, except as disclosed. Accordingly, the Contributor represents that the following information shall be clearly identified on the title page of the Contribution: (1) all financial and material support for the research and work; (2) identified on the title page of the Contribution: (1) all financial and material support for the research and work; (2) any financial interests the Contributor or any co-Contributors may have in companies or other entities that have an interest in the information in the Contribution or any submitted Supporting Information (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership); and (3) indication of no such financial interests if appropriate.
H. INFRINGEMENT OF COPYRIGHT

If the copyright of the Contribution is infringed, the Contributor agrees that the Owner may (without any obligation) take such steps as it considers necessary to deal with the matter. The Contributor will co-operate fully with the Owner in any infringement proceedings and agrees to execute any documents and do any acts reasonably required by the Owner for such purposes.

I. USE OF INFORMATION

The Contributor acknowledges that, during the term of this Agreement and thereafter, the Owner (and Wiley where Wiley is not the Owner) may process the Contributor's personal data, including storing or transferring data outside of the country of the Contributor's residence, in order to process transactions related to this Agreement and to communicate with the Contributor. By entering into this Agreement, the Contributor agrees to the processing of the Contributor's personal data (and, where applicable, confirms that the Contributor has obtained the permission from all other contributors to process their personal data). Wiley shall comply with all applicable laws, statutes and regulations relating to data protection and privacy and shall process such personal data in accordance with Wiley’s Privacy Policy located at: http://www.wiley.com/WileyCDA/Section/id-301465.html.

[ X ] I agree to the EXCLUSIVE LICENSE AGREEMENT as shown, consent to execution and delivery of the Exclusive License Agreement electronically and agree that an electronic signature shall be given the same legal force as a handwritten signature, and have obtained written permission from all other contributors to execute this Agreement on their behalf.

Contributor's signature (type name here): Zachary Cravens

Date: October 27, 2017

SELECT FROM OPTIONS BELOW:

[ X ] Contributor-owned work

[ ] U.S. Government work

Note to U.S. Government Employees
A contribution prepared by a U.S. federal government employee as part of the employee's official duties, or which is an official U.S. Government publication, is called a "U.S. Government work", and is in the public domain in the United States. In such case, Paragraph A.1 will not apply but the Contributor must type his/her domain in the United States. In such case, Paragraph A.1 will not apply but the Contributor must type his/her name (in the Contributor's signature line) above. Contributor acknowledges that the Contribution will be published in the United States and other countries. If the Contribution was not prepared as part of the employee's duties or is not an official U.S. Government publication, it is not a U.S. Government work.

[ ] U.K. Government work (Crown Copyright)
Note to U.K. Government Employees
For Crown Copyright this form cannot be completed electronically and should be printed off, signed in the Contributor’s signatures section above by the appropriately authorised individual and uploaded to the Wiley Author Services Dashboard. For production editor contact details please visit the Journal's online author guidelines. The rights in a contribution prepared by an employee of a UK government department, agency or other Crown body as part of his/her official duties, or which is an official government publication, belong to the Crown and must be made available under the terms of the Open Government Licence. Contributors must ensure they comply with departmental regulations and submit the appropriate authorisation to publish. If your status as a government employee legally prevents you from signing this Agreement, please contact the Journal production editor.

[ ] Other
Including Other Government work or Non-Governmental Organisation work
Note to Non-U.S., Non-U.K. Government Employees or Non-Governmental Organisation Employees
For Other Government or Non-Governmental Organisation work this form cannot be completed electronically and should be printed off, signed in the Contributor’s signatures section above by the appropriately authorised individual and uploaded to the Wiley Author Services Dashboard. For production editor contact details please visit the Journal's online author guidelines. If you are employed by the Department of Veterans Affairs in Australia, the World Bank, the World Health Organization, the International Monetary Fund, the European Atomic Energy Community, the Jet Propulsion Laboratory at California Institute of Technology, the Asian Development Bank, the Bank of International Settlements, or are a Canadian Government civil servant, please download a copy of the license agreement from https://authorservices.wiley.com/author-
resources/Journal-Authors/licensing-open-access/licensing/licensing-info-faqs.html and return it to the Journal Production Editor. If your status as a government or non-governmental organisation employee legally prevents you from signing this Agreement, please contact the Journal production editor.

Name of Government/Non-Governmental Organisation:

[ ] Company/institution owned work (made for hire in the course of employment) For "work made for hire" this form cannot be completed electronically and should be printed off, signed and uploaded to the Wiley Author Services Dashboard. For production editor contact details please visit the Journal's online author guidelines. If you are an employee of Amgen, please download a copy of the company addendum from https://authorservices.wiley.com/author-resources/Journal-Authors/licensing-open-access/licensing/licensing-info-faqs.html and return your signed license agreement along with the addendum.

Name of Company/Institution:

Authorized Signature of Employer: 

Date: 

Signature of Employee: 

Date: 

__________________________

__________________________
VITA

Zachary M. Cravens
Southern Illinois University

Zachary M. Cravens
zcravens@siu.edu; zcravens@gmail.com

University of Illinois Urbana-Champaign
Bachelor of Science, Ecology, Ethology, and Evolution, 2003

Thesis Title:
Illuminating dietary and physiological change in an insectivorous bat community exposed to artificial light at night

Special Honors and Awards:
Alan Woolf Outstanding M.S. Student, Cooperative Wildlife Research Laboratory, 2017
Top Student Presentation, Midwest Bat Working Group, 2017

Major Professor:
Justin G. Boyles

Publications: