ABSTRACT

Title of Dissertation:	EVALUATING RELATIONSHIPS BETWEEN BATS AND LANDSCAPE CHARACTERISTICS IN A WHITE-NOSE SYNDROME POSITIVE REGION: DOES URBANIZATION PLAY AN IMPORTANT ROLE?
	Shannon P. Browne, Doctor of Philosophy, 2021
Dissertation directed by:	Assistant Professor, Jennifer M. Mullinax, Department of Environmental Science and Technology

White-nose Syndrome (WNS) is a cascading disease process, initiated by a skin fungus, killing most cave bat populations across North America. WNS severity differs with the ecology of both the pathogen and the bat species host. This study aimed to identify the habitat features most related to cave bat species by surveying cave bats and habitat features across the urban-rural gradient in Maryland. Our understanding of cave bat ecology in Maryland has changed recently due to technological advancements, though prominent gaps still exist in the urbanized Piedmont and Coastal Plain provinces. As such, a novel urban definition was created to allow research sites to well represent each level of the gradient, and sites were surveyed in random order during four seasons from 2015 to 2017. We found unique

results when analyzing predator-prey correlations at weekly scales as well as nightly generalized linear mixed models (GLMM) of habitat usage. At both temporal scales, big brown (*Eptesicus fuscus*) and tri-colored bat (*Perimyotis subflavus*) activity were higher at urban than suburban sites. While analyzing predatory-prey correlations at the weekly level, big brown bat activity was highly related to their preferred prey counts at urban sites, while tri-colored bat activity correlated to their preferred prey counts at rural sites. Tri-colored bat activity also occurred at sites during seasons that supported less activity by big brown bats. When analyzing habitat features at the nightly level using GLMMs, in suburbia, big brown bat activity appeared most related to their preferred prey and taller trees. Meanwhile, tri-colored bat activity still held a strong relationship with prey at rural sites across multiple seasons. Big brown bats may select suburbia for roosting and foraging while tri-colored bats may select rural settings for foraging. Interestingly, prey were most abundant at rural sites, indicating rural settings may support higher habitat quality along the gradient. Possible reasons for the seemingly spatial and temporal partitioning by these two species included differing resource availability and some form of niche partitioning. Repeated acoustic surveys and telemetry studies across spatial and temporal scales are recommended to aid bats in greatest need during WNS.

EVALUATING RELATIONSHIPS BETWEEN BATS AND LANDSCAPE CHARACTERISTICS IN A WHITE-NOSE SYNDROME POSITIVE REGION: DOES URBANIZATION PLAY AN IMPORTANT ROLE?

by

Shannon P. Browne

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Advisory Committee:
Professor Jennifer M. Mullinax, Chair
Gerald S. Wilkinson
J. Edward Gates
William W. Bowerman
Lowell W. Adams

Dedication

I dedicate this dissertation to my entire family, especially my husband and Team SccS, to represent everything we've accomplished over the last decade. Spencer, you have taught me (late in life) the true meaning of family and dedication. We've survived cancer treatments, multiple surgeries, numerous trips to the E.R., a global pandemic, and a major house renovation to make more room for our joyfully expanding family. And we're still running 5Ks together and speaking to each other. Go Team SccS! Now, let's hope all this work doesn't lead to a 3,000 page dissertation that no one will ever look at (Chidi Anagonye, The Good Place)!!!!

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Table 17.—We defined three distinct levels of urbanization (Urban, Suburban, and Rural) within our urban-rural gradient in the Mid-Atlantic region using U.S. Census Bureau human population size data and percentages of imperviousness, forested, and open landscape. We first set a range for percent impervious surface. Then, we required a minimum amount of land devoid of impervious surfaces and required specific amounts of forest and open land classes ensuring potential sites maintain heterogeneity and avoid biasing our results.

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Table 19.—Competing GLMM models for cave bat activity were compared for each urbanization level. Models were ranked based on AICc. Each global model consisted of habitat variables correlated (rho > 0.3) to individual species in each urbanization level and were fitted with season and year as fixed effects. Forward section was used to create simplified global models for comparison. Best performing models were compared to null models. All models were set with site as a random effect. Final models were reviewed for effects from interactions and zero inflation and adjusted when necessary. Pseudo R^2 results (CR^2 and MR^2) were provided for best performing models. Species include big brown (EPFU) and tri-colored bat (PESU). Model codes include: Global (global models contained all correlated independent variables), Inter (models fitted for interactions), Null (models containing only random effect of site), Simple (global models simplified using forward selection to retain only significant variables), ZIF (the zero-inflated component of the model was fitted with variables when needed). Final variables included in best performing models were Coleoptera counts (COLE), Diptera counts (DIPT), season (SEAS), temperature (TEMP), tree height (TREE), and year (YEAR).

Table 20. Coefficient estimates for each species' best performing generalized linear mixed models (GLMM) along the urban-rural gradient. All models contained site as a random effect. Asterisk * denotes variable holding statistical significant ($P < 0.05$) within the model. Species codes include EPFU (big brown bat) and PESU (tri-colored bat). Variables adjacent to and immediate following ZIF indicate results for the zero inflated component of the GLMM model.

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Autumn) across the urban-rural gradient. Insects were collected each week at each site. Coleoptera counts and total insect counts are displayed below among our four seasons across the urban-rural gradient. Big brown bat activity (A) appears high in Urban sites (blue) during late summer and fall while their preferred prey (C) appear higher during these seasons at Rural sites (red). Tri-colored bat activity (B) appears high during late summer at Rural sites (red) while total insect counts (D) were also highest during late summer at Rural sites.

Chapter 1: Updated ecology of six cave bats occurring in the eastern US

ABSTRACT

Many cave bat populations in eastern North America significantly declined due to White-nose Syndrome (WNS). WNS affects bat species differently, due in part to their unique ecology. Our understanding of their ecology has changed in recent years due to technological and analytical advancements. These advancements helped us learn many details about their diet and habitat use; however, knowledge has been shared through a narrow scope, throughout multiple journals, spanning several decades, making it difficult to synthesize. We selected the state of Maryland as our case study to identify one state's foundational knowledge of cave bat species' ecology, then reviewed the status of bat research before and after WNS' arrival in that state to identify patterns and research gaps. We then broadened our search to learn the most current diet preference and habitat use of these species in the eastern United States. In our review of Maryland, we noted most investigations occurred in the western, mountainous regions and before WNS' arrival, leaving a gap in knowledge about cave bat ecology in the urbanized, Piedmont, and Coastal Plain regions of the state plus any changes in their ecology that may have resulted from WNS. During our broader review, we noticed many species' diets and habitat usage changed within their regions, possibly due to differing resource availability at the local scale, cascading effects from WNS, or both. We also noticed research regarding certain habitat features for some species was still lacking. Our findings indicate further investigation into diet and habitat after WNS has become well established in each local region is warranted to improve management recommendations for bat species of concern, especially along the urban-rural gradient.

KEY WORDS

Eptesicus fuscus, *Myotis leibii*, *Myotis lucifugus*, *Myotis septentrionalis*, *Myotis sodalis*, *Perimyotis subflavus*, White-nose Syndrome

INTRODUCTION

Multiple cave bat species in North America are in peril due to White-nose Syndrome (WNS). WNS is an often fatal disease caused by the psychrophilic fungus *Pseudogymnoascus destructans* (Pd). Pd decimated cave bat populations starting in Howes Cave in New York in 2006, then radiated across much of North America to now 39 of the United States and 7 Canadian provinces (Hoyt et al. 2021). WNS has been so devastating to little brown bats (*Myotis lucifugus)*, formerly one of the most common species in North America, that Frick et al. (2010) predicted a significant risk of regional extirpation. Dobony et al. (2011) observed little brown bat population declines as well; however, they also witnessed survival and reproduction of previously infected bats during recaptures, allowing for cautious optimism for this significantly impacted species. Bat species management should be firmly grounded with the understanding that Pd infections affect bat species differently (Cheng et al. 2021) based on the ecology of the pathogen and the bat species host (Hoyt et al. 2021), thus highlighting the importance of mastering the ecology of each unique bat species.

The following cave bat species occurring in eastern North America have been confirmed for Pd infection and lesions: big brown (*Eptesicus fuscus*), gray (*Myotis grisescens*), Eastern small-footed (*Myotis leibii*), little brown, Northern long-eared (*Myotis septentrionalis*), Indiana (*Myotis sodalis*), and tri-colored bat (*Perimyotis subflavus*; Hoyt et al. 2021). Currently, the United States Fish and Wildlife Service (USFWS) list gray and Indiana bats as endangered,

Northern long-eared as threatened, and little brown and tri-colored bats are under review in the candidate or petition process (USFWS 2020). In the eastern portion of the US, these species occur in USFWS' Northeast Region (CT, DC, DE, MA, MD, ME, NH, NJ, NY, PA, RI, VA, VT, WV) and Southeast Region (AL, AR, FL, GA, KY, LA, MS, NC, PR, SC, TN, and USVI) though Pd has not been detected as far south as Florida (Hoyt et al. 2021). We selected to review Maryland as our case study as this state is located in the middle of two USFWS regions and supports six of the seven eastern US cave bat species. Additionally, the Maryland Department of Natural Resources (MDDNR) confirmed the presence of Pd early in the outbreak (MDDNR 2011) and therefore has been monitoring cave bat populations for over a decade.

MDDNR lists all bat species hibernating in the state (MDDNR 2016*a*) on their State Wildlife Action Plan as Species of Greatest Conservation Need (SGCN) according to the following rank: Eastern small-footed, little brown, Northern long-eared, Indiana, and tri-colored as "highly state rare" (S1) and big brown as "more common" (S5; MDDNR 2016*b*). Their ecology was included in the most comprehensive published text regarding Maryland mammals over 50 years ago (Paradiso 1969). This guide provided morphological measurements from individuals captured from bat banding studies and other surveys conducted within the state, distribution across North America, general diet, hibernacula locations, reproductive cycles, and roost type for species when available. While this guide was considered comprehensive at the time of publication, newer technology such as bat acoustic detection, molecular DNA analysis of diet, and radio telemetry has better informed our current knowledge regarding the ecology of cave bats. Since 1969, research has been published in multiple peer-reviewed journals focusing on the ecology of bat species that hibernate in Maryland, expanding our knowledge about their home range size, nightly foraging distance, specific habitat preferences, insect orders consumed,

roost tree species, and more. We reviewed peer-reviewed literature published since 1969 to update our ecological knowledge of these species within the state of Maryland and also compared the status of their research before and after WNS' arrival in this state in order to identify research patterns and gaps. We then broadened our literature review to learn the newest results of these species' diet and habitat use in the Northeast and Southeast regions to reach our paper's overall objective of updating the ecology of cave bat species occurring in the eastern United States.

METHODS

We performed a literature review using Web of Science including the following topic keywords: bats, diet, distance, *Eptesicus fuscus*, habitat, home range, Maryland, *Myotis leibii*, *Myotis lucifugus*, *Myotis septentrionalis*, *Myotis sodalis*, *Perimyotis subflavus* (plus its former name *Pipistrellus subflavus*), and urban. For one species (*Myotis leibii*), we allowed the keyword range to be substituted for the keyword home range to glean more results. We retained all peer-reviewed journal articles published from January 1, 1970 through September 11, 2021 involving cave bat research that occurred in the state of Maryland. We then conducted a second broader literature review to include the most current results for these cave bat species in North America by removing the keyword Maryland and reported results on research in the Northeast and Southeast regions of the United States. We selected the following US states (plus Washington, DC) to represent the Northeast region: CT, DE, MA, MD, ME, NH, NJ, NY, PA, RI, VA, VT, WV. The Southeast region consisted of the following US states: AL, AR, FL, GA, KY, LA, MS, NC, SC, TN. Literature review results from these states included mean ± SD or ± SE if it was published.

RESULTS

Literature review - Maryland. We documented and reviewed a total of 23 journal articles during our case study literature review, with 20 studying big brown (87 %), 16 discussing tri-colored (70 %), 14 covering little brown (61 %), 10 on Northern long-eared (44 %), 8 covering Eastern small-footed (35 %), and 7 involving Indiana bats (30 %). Two studies (9 %) did not distinguish between *Myotis* species. Research focused on habitat (n=12, 52 %), summer roosts (n = 4, 17 %), toxin levels (n = 3, 13 %), diets (n = 2, 9 %), viruses (n = 2, 9 %), and one study (4 %) was a single species ecology literature review. Most of the research was conducted pre-WNS (n = 18, 78 %), three were post-WNS (13 %), and two (9 %) spanned years before and after WNS' arrival in the state. Of these 23 studies, five involved urbanization (22 %; Table 1).

Literature review - Expanded. We expanded our review to include results of these six bat species between 1970 - 2021 and found a total of 928 journal articles, with 272 covering big brown (29 %), 237 discussing little brown (26 %), 142 studying Northern long-eared (15 %), 134 investigating tri-colored (14 %), 118 covering Indiana (13 %), and 25 involving Eastern small-footed bats (3 %). Most of the research focused on habitat (n = 345, 38 %) and roosts used (n = 332, 36 %), while 84 focused on diets (9 %), 77 (8 %) on distances traveled (i.e., nightly, between habitat features), 70 on urban usage (8 %), and 20 on home range size (2 %; Table 2). We synthesized the most recent results regarding each species' summer roost types, roost tree species used, home range size, nightly foraging distance, diet, foraging strategy, and WNS threat level (Table 3).

Big brown bat – Abundance and Distribution. This large-bodied species was documented by Paradiso (1969) as being distributed in eastern North America from Quebec, ON to Nuevo Leon, MX and commonly found in Maryland's Piedmont and Coastal Plain provinces though

was classified as uncommon in all other provinces. Since 1969 but prior to WNS, their population sizes in other Maryland provinces may have increased as supported by numerous studies (Agosta et al. 2003, Agosta and Morton 2003, Griffith and Gates 1985, Johnson et al. 2008, Lookingbill et al. 2010). This synurbic (urban dwelling) species was documented in many Northeastern and Southeastern urban areas: Athens, GA (Menzel et al. 2001), Boston, MA (Johnson and Gates 2019), Greensboro, NC (Li et al. 2020, Parker et al. 2019), New York City, NY (Parkins and Clark 2015), Providence, RI (Richardson et al. 2021), Washington, DC (Deeley et al. 2021*a*, Johnson et al. 2008, Lookingbill et al. 2010), and multiple cities in NC (Li and Kalcounis-Rueppell 2018, Schimpp et al. 2018). Recently, Evans et al. (2021) found their abundance at sites in the Southeast increased with more developed land cover and activity increased each year post-WNS' arrival, indicating they may be thriving in urbanized areas after the establishment of WNS. Recently, big brown bats were found at an additional urban site inside Washington, DC compared to pre-WNS surveys there (Deeley et al. 2021*a*), further supporting this idea. The reported increase in abundance may be related to this species having the lowest WNS threat level (medium) compared to the other hibernating species discussed here (Cheng et al. 2021).

Big brown bat – Ecology. Big brown bats are well known for exhibiting great flexibility of diet, use of habitat types, and roosting locations. Studies continued to document their generalist habits after the establishment of WNS in multiple areas of the Northeast and Southeast regions (Deeley et al. 2021*a*, O'Keefe et al. 2019, Johnson et al. 2021). Their generalist nature may partially be due to their wing morphology. Their relatively low wing loading (mass per area), high aspect ratio (wingspan squared per area), and short and pointed wingtips should allow for slow maneuverable flight while their long wingspan should confine them to open, uncluttered

areas (Norberg and Rayner 1987) thus leading to their foraging strategy classification of open-space hawking (Denzinger and Schnitzler 2013, Norberg and Rayner 1987).

A literature review of big brown bats in Maryland and throughout North America reported summer roosts included anthropogenic sources such as bat boxes, buildings, mines, storm sewers, tunnels, and woodpiles in addition to caves, rock crevices, and trees of *Fagus, Pinus, Populus, Pseudotsuga, Quercus, Sequoia,* and *Thuja* species (Agosta 2002). Drake et al. (2020) characterized big brown bats as dead tree roost generalists occupying 87 % of the eastern temperate forest region and reported their average (mean ± SD) tree roost characteristics as a diameter breast height (DBH) of 26.4 ± 5.1 cm, tree height of 13.9 ± 3.7 m, roost height of 7.7 ± 2.8 m, canopy cover of 61 ± 4.9 %, deciduous species composition of 27.7 %, dead tree composition of 86.4 %, with no roosts in actual foliage.

Menzel et al. (2001) reported big brown females in the Southeast averaged (mean ± SE) 2,906 ± 1,129.8 ha for home range size, foraged a maximum distance of 5 km, and changed foraging habitat usage during each stage of pregnancy (pine, upland hardwood, and bottomland hardwood during pregnancy; bottomland hardwood during parturition; upland and bottomland hardwood during early lactation; pine forest during late lactation). They concluded that usage across the urban-rural gradient in the Southeast changed during each stage of pregnancy. During parturition, big brown females selected low moderate levels of development (26 - 50 %) then selected higher levels of development (51 - 75 %) during both early and late lactation time periods (Menzel et al. 2001). They were reported using highest levels of development (76 – 100 %) only during pregnancy and avoided lowest levels (0 – 25 %) during all pup-rearing time periods (Menzel et al. 2001). In the Northeastern US, home range and foraging distance information was not identified, so we expanded results to include more regions for comparison.

In the Midwest, Duchamp et al. (2004) reported reproductive female home range size averaged (mean ± SE) 270 ± 49 ha (2.7 ± 0.49 km²) and non-reproductive female home range averaged 1903 ± 558 ha (19.03 ± 5.58 km²). Brigham (1991) reported their nightly travel distance averaged (mean ± SE) 1.8 ± 0.1 km with a maximum 4.4 km in British Columbia while they averaged 0.9 ± 0.09 km each night in Ontario.

A study in Maryland and Pennsylvania found big brown bat diet consisted of (in descending order) Coleoptera, Hemiptera, Hymenoptera, Diptera, Neuroptera, and Lepidoptera (Agosta and Morton 2003) while another study at sites in the Northeast and Southeast did not report Hymenoptera but did report Trichoptera (Dodd et al. 2014). A study in the far Northeast reported slightly different diet composition and diversity (in descending order) of Coleoptera, Lepidoptera, Hymenoptera, Trichoptera, Hemiptera, Diptera, Neuroptera, Orthoptera, Odonata, and Arachnida (Thomas et al. 2012).

Eastern small-footed bat – Abundance and Distribution. This species' range was documented along eastern North America from Quebec, ON, through MD, to NC (Paradiso 1969). Relatively little was reported about this species likely because of their small size, elusive behavior (Johnson and Gates 2008*a*), and patchy distribution (Best and Jennings 1997). Additionally, these obligate hibernators prefer to live solitarily, making them very challenging to study (Johnson and Gates 2008*a*). Interestingly, after the establishment of WNS, their abundances may have slightly increased in the Southeast (Hoyt et al. 2021, O'Keefe et al. 2019), and they were the most active species during acoustic surveys in the Northeast (Reynolds 2017). Their sensitivity to WNS is not clear as their WNS threat level was not included by Cheng et al. (2021) while a previous report indicated they may not be as sensitive to WNS compared to other species (Turner et al. 2011).

Eastern small-footed bat – Ecology. According to a 2006 literature review, their summer roosts occurred included bridges, buildings, mines, tunnels, caves, cliffs, rock crevices, and tree bark (Thompson 2006), though all other articles from our review omitted trees. A study in the Southeast region reported they roosted in barns, boulders, and cliff faces and remained in roosts an average (mean ± SE) of 3.3 ± 0.9 days (Loeb and Jodice 2018). A study in the Northeast region (Johnson et al. 2011a) reported roosting exclusively in rocks and changing roosts an average (mean ± SE) of every 1.1 ± 0.04 days for males and 1.1 ± 0.06 days for females. Additionally, Johnson et al. (2011a) reported roosts for Eastern small-footed males were within 458 ± 16.7 m of water while closer (226 ± 31.2 m) for females. They speculated that this difference was possibly due to energy conservation during dehydrating lactation time periods. A different study in the Northeast found summer roosts in sunny rocky outcrops in shale barrens (Johnson and Gates 2008a), and two recent studies indicated summer activity was likely restricted to locations near their winter hibernacula (Reynolds 2017, Deeley et al. 2021*a*).

In the Northeastern US, Eastern Small-footed bats' spent the majority of their time in forested areas (93.6 %, Johnson et al. 2009a). Given their average wingspan, low to high aspect ratio, average to low wing loading, short and rounded wing tips, and average, broad wings, they are built to fly slowly in forests (Norberg and Rayner 1987). Their foraging strategy has been described as narrow-space hawking, or capturing prey while flying (Denzinger and Schnitzler 2013, Norberg and Rayner 1987) and possibly gleaning, meaning sweeping prey off vegetation surfaces (Johnson et al. 2009a). Home range and travel distance were not reported in the Southeast, but a study in the Northeast reported home range sizes ranged from 10.2 - 99.7 ha while nightly travel averaged (mean ± SE) 189.5 ± 64 m to 897.8 ± 31.9 m (Johnson et al. 2009a). Recently in the Southeast region, their presence and captures were reported across many

types of habitats, excluding urban habitats (Fagan et al. 2018, O'Keefe et al. 2019). Outside our regions, in Ontario, Canada, their activity did not change over open field, edge, forest, or water habitats (Mayberry et al. 2020). Habitat use post-WNS indicated they may be more of a habitat generalist than originally predicted, they may be surviving WNS better than other hibernating species, or both. Even so, with their rarity, proximity to hibernacula, and small home range size, it is understandable that they were not reported inside urban areas. We located one article post WNS that reported their recent presence in Maryland via acoustic activity but only on the rural side of the urban-rural gradient closest to their known hibernacula in the state (Deeley et al. 2021*a*) supporting the idea that they likely would not inhabit urban settings. Lastly, diet analyses in the Northeast and Southeast revealed Lepidoptera, Coleoptera, Diptera, Neuroptera, Hemiptera, Hymenoptera, Trichoptera, and Araneae (Dodd et al. 2014, Johnson et al. 2012). A study in the far Northeast confirmed most of those insect orders except Neuroptera and additionally reported Ephemeroptera, Plecoptera, and Orthoptera (Thomas et al. 2012).

Little brown bat – Abundance and Distribution. This species ranged across most of the United States and Canada and was reported as abundant in all parts of Maryland (Paradiso 1969). This gregarious species was known to form colonies of several thousand individuals and readily roost in anthropogenic structures in the Northeast and Southeast regions (Paradiso 1969). One model indicated that during additional anthropogenic disturbances, little brown bat populations increased winter survivability when they hibernated in larger numbers (Boyles and Brack 2009). However, after WNS was established, studies reported varying responses to different types or amounts of anthropogenic features. Several studies reported their activity decreased with increasing developed land cover (Evans et al. 2021, Nocera et al. 2020). Outside of our regions, little brown bat occupancy was highest closer to roads (Pauli et al. 2017), abundance increased

when forests were more fragmented (Ethier and Fahrig 2011), and activity was not affected by anthropogenic noise (Bunkley et al. 2015). Unfortunately, their abundance in the Northeast and Southeast regions summarized here declined significantly post-WNS (Johnson et al. 2021, O'Keefe et al. 2019, Cheng et al. 2021), which may explain the lack of recent, identifiable habitat preferences. While this synurbic species was reported in many cities across North America, the only urban areas documented as supporting little brown bats in our regions were Boston, MA (Johnson and Gates 2019), Sandy Springs, GA (Loeb et al. 2009), and Washington, DC (Johnson et al. 2008, Lookingbill et al. 2010, Deeley et al. 2021*a*).

Little brown bat – Ecology. Little brown bats were reported roosting in bat boxes, buildings, mines, caves (Mering and Chambers 2014), and trees (Baer et al. 2016). In the Northeast region, tree species of *Acer* and *Ulmus* were used for roosting (Baer et al. 2016), but data on tree roosts in the Southeast were lacking, likely because of their propensity to roost in man-made structures (Drake et al. 2020). A recent metadata analysis characterized them as dead tree roost generalists occupying 82 % of the eastern temperate forest region and reported the average (mean ± SD) of overall tree roosts as follows: DBH of 40 ± 6.3 cm, tree height of 10.7 ± 3.3 m, roost height of 8.4 ± 2.9 m, canopy closure of 62.4 ± 19.4 %, 22.1 % deciduous tree species, 23.5 % dead trees, and 0 % roosts in tree foliage (Drake et al. 2020). Another North American metadata analysis reported that they selected smaller DBH roost trees in the eastern US region compared to their counterparts in other US regions (Lacki 2018). Their average wing loading, average aspect ratio, and short and rounded wing tips allow them to fly slowly, close to, and within clutter for long distances. Additionally, their feeding behavior is characterized as a narrow space hawking, capturing prey mid-air while flying, and trawling, foraging over water surfaces, strategy (Norberg and Rayner 1987). More recently, they were observed in a flight cage

gleaning their prey in addition to hawking; however, gleaning without sending a third harmonic in their calls indicated they are not as specialized at gleaning as Northern long-eared bats (Ratcliffe and Dawson 2003).

A long-term study in the Northeast reported little brown bat activity increased in wetlands, closer to roads and trails, and with more open canopy cover (Nocera et al. 2020). That study also reported plasticity in their habitat use, finding no change in occupancy across general habitat types. In the Southeast, one study reported their occupancy was highest when along edges, in interior corridor stands, and in habitat diversity corridor systems (Hein et al. 2009). Furthermore, Austin et al. (2018) identified their activity was highest in the edges between burned and unburned sites. In terms of general urban usage, their large colony sizes, breadth of habitats used and diets consumed, and affinity for anthropogenic structures make them a logical urban dweller. In the Northeast region, Hooton et al. (2016) reported they traveled each night between 0.32 ± 0.01 km and 2.81 ± 0.13 km. Another study in the Northeast calculated an average home range size of 143 ± 71.0 ha, and compared their findings to home ranges reported in the Midwest (515 ha), Quebec, Canada (18-30 ha), and New Brunswick, Canada (52 ha; Coleman et al. 2014). Unfortunately, we found no reported home range sizes or nightly travel distances for our Southeast region.

A recent diet study in the Northeast reported Lepidoptera, Coleoptera, Diptera, Trichoptera, Hymenoptera, Hemiptera, Neuroptera, Plecoptera, Psocoptera, Arachnida, Orthoptera, Ephemeroptera, and Blattodea in their diet (Thomas et al. 2012) while another study in the Northeast reported a slightly different composition of Lepidoptera, Diptera, Trichoptera, Coleoptera, Hymenoptera, Homoptera, and Hemiptera (Carter et al. 2003). We found no reported diet analysis for the Southeastern region.

Northern Long-eared bat – Abundance and Distribution. This species' range included the eastern and central United States, including all of Maryland, and Canada and all parts of Maryland (Paradiso 1969), though their distribution was patchy (Thompson 2006). Thalken et al. (2018) reported most Northern long-eared bats in their Southeastern study site remained in small summer colonies located less than 2,000 m from their winter hibernacula. Recently, there have been reports of significant population decreases in both Northeast and Southeast regions (Johnson et al. 2021, O'Keefe et al. 2019), attributed to WNS (Cheng et al. 2021). While they were reported in multiple cities in North America (Moretto et al. 2019, Robertson 2002, Ulrey et al. 2005), they were documented in only two cities in our region: Boston, MA (Johnson and Gates 2019) and Washington, DC (Johnson et al. 2008, Lookingbill et al. 2010, Deeley et al. 2021*a*).

Northern Long-eared bat – Ecology. Similar to other species, Northern long-eared bats roosted in artificial structures such as bat boxes, buildings, and mines, in addition to caves and trees (Mering and Chambers 2014). A recent metadata analysis characterized them as dead tree roost generalists occupying 67 % of the eastern temperate forest focused in more northern areas and calculated their average (mean ± SD) tree roost as 35.4 ± 5.9 cm in DBH, 16.8 ± 4.1 m in tree height, 7.9 ± 2.8 m in roost height, 67.7 ± 25.3 % canopy closure, 76.8 % deciduous tree composition, 41.9 % dead tree roosts, with 0 % in foliage (Drake et al. 2020). An additional metadata analysis reported they selected roost trees in the eastern US region with smaller DBH than roost trees in other US regions (Lacki 2018). Roost trees species used in the Southeast included a wide variety (Table 3) of live and dead species (Thalken and Lacki 2018, Grider et al. 2021, Jordan 2020), while Johnson et al. (2009*b*) found roosts in the Northeast included mostly *Robinia* spp. Thalken et al. (2018) reported roosts in the Southeast in mesic deciduous forests

were within 500 m of roads and 800 m of water. Interestingly, Cruz et al. (2018) found they selected artificial roost sites in the Northeast far from large contiguous forests (> 200ha) and were not affected by distance to forest gaps. Northern long-eared bats switched roosts an average (mean ± SD) 1.8 ± 1.3 days in the Southeast (Grider et al. 2021). Johnson et al. (2009*b*) reported they switched roosts more frequently (mean of 1.35 days) at their sites in West Virginia than the averages of 5.3 and 3.0 days previously reported in the same state.

Their low wing loading, average length wings, and rounded wing tips should help them maneuver well, but may create inefficient flight (Norberg and Raynor 1987). Their slow and highly maneuverable flight coupled with large ears should allow them to glean insects off vegetative surfaces while flying (Norberg and Raynor 1987). They have been observed both gleaning and hawking their prey (Ratcliffe and Dawson 2003) and are thus characterized as narrow-space hawking and gleaning foragers (Denzinger and Schnitzler 2013, Norberg and Rayner 1987). Given that their wing and ear morphologies are well suited for gleaning insects, it was suggested that they may be sensitive to urban noise (Moretto and Francis 2017). Species-specific behavior like frequent roost switching, preference for roost tree species with exfoliating bark, common use of snags, morphological restriction to heavily forested landscapes, and possible avoidance of urban noises, lead to a low likelihood of living in a typical heavily fragmented urban setting unless specific habitat requirements are provided.

Northern long-eared bats were reported foraging within small home ranges averaging (mean ± SE) 65 ± 5.2 ha inside a heavily managed forest with road corridors in the Northeast (Owen et al. 2003), and in small openings, occasionally over water (Thompson 2006). Relatively small home range sizes were also reported in the Southeast by Lacki et al. (2009). They reported average (mean ± SE) home ranges of 60.2 ± 14.1 ha before burns and 72.3 ± 6.2 ha following

prescribed burns, including heavily forested areas dominated more by pine than hardwoods, suggesting they selected for microhabitats with less clutter (Lacki et al. 2009). Northern long-eared bats in the Lacki et al (2009) study traveled a maximum of 1,300 m nightly. While nightly distance traveled was not reported in our literature review in the Northeast, a similar maximum nightly distance of 1,163 m was reported in Prince Edward Island, Canada along with a very small average (mean ± SD) home range (foraging area) of 6.09 ± 5.83 ha (Henderson and Broders 2008). We broadened our review following findings from Henderson and Broders (2008) and found conference proceedings that reported a nightly average (mean ± SE) travel distance of 602 ± 66.6 m and a maximum distance of 1,719 m in the Northeast (Sasse and Pekins 1995).

Their diet in the Northeast included Lepidoptera, Coleoptera, Trichoptera, Arachnida, Diptera, Hymenoptera, Hemiptera, Plecoptera, and Neuroptera (Thomas et al. 2012) while other studies at sites in Northeast and Southeast regions confirmed most of those orders except Plecoptera and they reported Homoptera (Carter et al. 2003, Dodd et al. 2014).

Indiana bat – Abundance and Distribution. This species was reported as distributed across much of eastern and central United States, though rare in Maryland and limited to the western portion of the state (Paradiso 1969), likely due to their populations concentrated mostly in four Midwestern states (O'Rourke et al. 2021). They are known to remain near (< 100 km) their winter hibernacula year-round in Northeast and Midwest regions (Britzke et al. 2006, Cable et al. 2021, Krauel et al. 2018, Pauli et al. 2017), though longer distance migrations > 100 km were recently documented in the Southeast (Roby et al. 2019, St. Germain et al. 2017). In Southeastern states where they were previously common, their populations have significantly declined (O'Keefe et al. 2019) likely from WNS (Cheng et al. 2021). Given their historic

restriction near their karst hibernacula year-round, it follows that the only cities known to support populations were in the Midwest near their hibernacula (Bergeson et al. 2020, Cable et al. 2021, Sparks et al. 2005, Timpone et al. 2010, Tuttle et al. 2006).

Indiana bat – Ecology. Indiana bats' low to average wing loading, broad wings with short and rounded wing tips should allow them to fly slowly and closely to or within clutter (Norberg and Rayner 1987) leading to their classification of using a narrow-space hawking strategy (Denzinger and Schnitzler 2013, Norberg and Rayner 1987). Being a clutter specialist, they roost and/or forage in forests. Female and male Indiana bats in the mountainous part of Virginia near their hibernacula had an average home range size of 250 ± 100 ha, roosted mostly in *Carya* and *Quercus* species in or near deciduous forests, changed roosts every 2 - 3 days, and spent more time in open deciduous forests than developed or closed deciduous forests or mixed forests (Brack 2006). Indiana bats were reported for the first time in the coastal part of Virginia with a roosting area of 61 ha and roosting in *Pinus* species inside a recently burned hardwood forest along a ravine next to shrub wetland (St. Germain et al. 2017). In the Northeast region, Indiana bats had an average (mean ± SE) home range size of 130.47 ± 28.75 ha (Jachowski et al. 2014) and an average (mean ± SD) size of 83 ± 82 ha (0.83 ± 0.82 km²; Watrous et al. 2006). Watrous et al. (2006) located Indiana bat home ranges an average (mean ± SD) 158 ± 88.48 m from water in mostly agriculture, forested, and wetland habitats. Jachowski et al.'s (2014) findings also suggested that forest habitat edges near water were important to Indiana bats in the northern portion of their range. Their resource use increased with the amount forested wetland and distance from development and decreased with increasing agriculture, development, shrub wetland, and distances to water and forest edge (Jachowski et al. 2014). Their tree roosts were found in northern mixed forests with larger DBH, high decay class, including species of *Ulmus*,

Robinia, *Acer*, *Carya*, *Pinus*, *Populus*, *Prunus*, *Fagus*, *Quercus*, and *Juglans* (Jachowski et al. 2016). The only nightly foraging distance reported for Indiana bats was outside of the Northeast and Southeast regions discussed here. In the Midwest, Sparks et al. (2005) calculated an average nightly distance as 3.02 km, ranging 0.80 – 8.37 km, mostly over woodlands with minimal urban areas. However, this distance is likely greater than Indiana bats in our region would travel as home ranges reported for the Midwest (Bergeson et al. 2013, Womack et al. 2013) are also much larger than those in Northeast and Southeast regions. A metadata analysis reported differences of Indiana bat roost trees between US regions; more specifically, roost trees used by Indiana bats in the eastern US region had smaller DBH than other populations (Lacki 2018). Another metadata analysis characterized them as dead tree generalists occupying 44 % of the eastern temperate forest, focused more towards the central region, and reported values including mean and standard deviation of tree roosts: 37.7 ± 6.1 cm in DBH, tree height of 18.3 ± 4.3 m, roost height 10.1 ± 3.2 m, 52.5 ± 34.9 % canopy cover, 64.9 % deciduous species, 60.9 % dead trees, in 0 % foliage (Drake et al. 2020). Summer roosts also included caves (Mering and Chambers 2014).

Indiana bat diet was not available in Northeast or Southeast regions but was available from studies in the Midwest. A detailed molecular diet analysis reported their diets were composed of Diptera, Lepidoptera, Arachnida, Hemiptera, Coleoptera, Psocodea, Trichoptera, and Ephemeroptera (O'Rourke et al. 2021).

Tri-colored bat – Abundance and Distribution. This species was reported as being distributed across eastern and central United States and Canada and most of Maryland, though records from the Coastal Plain (eastern shore) province were lacking (Paradiso 1969). Recently, their populations in Northeast and Southeast regions significantly declined (Johnson et al. 2021, O'Keefe et al. 2019) likely due to their high impact from WNS (Cheng et al. 2021). In terms of

urban areas, they have been documented in multiple eastern cities: Greensboro, NC (Parker et al. 2019), New York City, NY (Parkins et al. 2016), Washington, DC (Johnson et al. 2008, Lookingbill et al. 2010), and other cities in NC (Li et al. 2020, Li and Kalcounis-Rueppell 2018).

Tri-colored bat – Ecology. Tri-colored bats are readily distinguishable from others due to their small size, unique fur pattern, dental formula, and erratic flight resembling a moth (Paradiso 1969). In the Southeast, they were found hibernating alone or in small groups up to four and selected larger caves which had large temperature fluctuations within seasons (Briggler and Prather 2003). Interestingly, a literature review (Fujita and Kunz 1984) referenced their propensity to hibernate singly for long bouts in winter because of female's reproductive requirements for storing sperm. The review also reported fat levels as remaining constant throughout hibernation, blood levels containing the highest hemoglobin concentration of any small mammal, and strong fidelity to hibernacula each winter (Fujita and Kunz 1984).

Their high aspect ratio, low wing loading, short and rounded wing tips should indicate slow and agile movements around but not in clutter (Norberg and Rayner 1987), while their long wings allow them to travel long distances. The clutter-avoidance hypothesis was supported by Fujita and Kunz (1984) as they documented tri-colored bats foraging slowly in open fields and forest edges. The long distance travel hypothesis was supported by a study that documented a travel distance of 243 km within a two night migration in the Southeast region (Samoray et al. 2019). A 2003 literature review in one Southeastern state reported average nightly distance from roost to foraging sites of 1,137 m (Menzel et al. 2003). These two hypotheses were supported by one additional study outside our region. In the Midwest, one study reported nightly travel distances up to 4.3 km by females flying in canopy gaps, open areas, and edge habitats (Veilleux et al. 2003). We found no reported nightly travel distances for the Northeast.

Tri-colored bats were characterized as edge-space hawking foragers (Denzinger and Schnitzler 2013), and a past literature synthesis of bats in South Carolina by Menzel et al. (2003) reported a home range size of 389.2 ha. One study in the Southeast (O'Keefe et al. 2009) detailed summer roosts as live, tall, large DBH trees from *Carya, Acer, Betula, Populus, Quercus, Magnolia,* and *Pinus* species. Roost trees were located an average (mean ± SE) 35.59 ± 7.25 m from intermittent streams, 95.79 ± 21.58 m from perennial streams, 185.58 ± 35.78 m from nonlinear openings, and in mature stands over 72 years old. Roost canopy cover averaged 58 %, and the close proximity to nonlinear openings of roosts indicated that they did not select roosts under closed canopy (O'Keefe et al. 2009). Roosts were selected in a likely effort to minimize energetic costs associated with travel to foraging and drinking sites. In the Northeast, stream condition was a strong predictor for tri-colored bat presence in heavily forested sites (Ford et al. 2005). Roost tree species details and home range sizes were not available in the Northeast, though some details were available from studies further west. In the Midwest, Veilleux et al. (2003) reported they roosted in the foliage of mostly *Acer* and *Quercus* species, located within an average (mean ± SD) of 117 ± 154 m from water and 52 ± 47 m from forest edge. They changed roosts an average (mean ± SD) 3.9 ± 2.5 days. A metadata analysis across North America characterized them as foliage roost specialists, occupying 89 % of the eastern temperate forest, using roost trees with the following averages (mean ± SD): 27.5 ± 5.2 cm DBH, 20 ± 4.5 m height, 13.7 ± 307 m roost height, canopy closure of 49.1 ± 29.7 %, 89.5 % deciduous species, 33.1 % dead trees, in 100 % foliage (Drake et al. 2020). Unlike other species hibernating in our regions, tri-colored bats roosted completely in foliage while avoiding common tree roost types like tree hollows and bark (Drake et al. 2020), similar to their populations in the Midwest (Veilleux et al. 2003). They were also reported roosting in anthropogenic structures such as

barns, garages (Menzel et al. 2003), bridges (Ferrara and Leberg 2005), and mines (Agosta et al. 2005) as well as caves (Agosta et al. 2005) and demonstrated strong summer roost fidelity (Fujita and Kunz 1984). Perhaps their strong fidelity to hibernacula and summer roosts, avoiding common roost types, remaining in torpor for long bouts, and hibernating singly in caves, demonstrates interspecific competition avoidance. While some studies in the Southeast indicated they preferred more open habitats (Brooks et al. 2017, Moore and Best 2018), another study in the region indicated they may be broadening their niche post-WNS and becoming more summer habitat generalists (Shute et al. 2021). Their quite general habitat use was also supported by a recent study outside our region in Ontario, Canada (Mayberry et al. 2020). With an ability to travel far each night, roost in anthropogenic structures, and tolerate a wide variety of temperatures, it is logical that tri-colored bats would inhabit urban areas.

A recent study at sites occurring in Northeast and Southeast regions documented their diet consisted of Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Neuroptera, and Trichoptera (Dodd et al. 2014) making their diet the broadest of bat species covered during that study. Another study on Tri-colored bats in the Northeast reported those same insect orders plus Homoptera (Carter et al. 2003). Interestingly, their diet used to be the most selective of bat species as they consumed only Lepidoptera, Hymenoptera, Coleoptera, and Diptera during a previous study in the Southeast (Carter et al. 1998).

CONCLUSION

It has been over 50 years since the most comprehensive ecology of the six cave bat species of our case study state, Maryland, was published. While that publication provided general details about these species, characteristics such as home range sizes, insect orders consumed, foraging distances, foraging strategies, and tree roost species for many bat species were lacking. Over the last 50 years, extensive, individualistic research has been conducted filling in many of those holes; however, results varied widely between regions and within the same region. Regional variation could be due to attempting comparisons between different habitat types, seasons, and/or sexes. Synthesizing continuity in bat ecology is further complicated by research published across multiple journals and multiple time frames. We attempted to synthesize results into one comprehensive review, noting where bat ecology knowledge gaps still exist in our regions.

It appears WNS may have affected more than just bat population sizes. It may be leading to cascading changes in species composition, interspecific competition, and resource use and availability. Given the disproportionate impacts of WNS on bat species, massive changes in recent land cover use, and recent advancements in wildlife data collection and statistical analyses, it is imperative that we synthesize literature reviews such as this at least every decade. Each cave bat species responds to WNS uniquely, resource availability continues to change, and bats may alter their response to resources in unpredicted ways. It is also evident from our results that certain cave bat species likely occupy urban areas more readily than others, yet research into their relationships with urban habitat resources is sorely lacking in our region. Relationships between bat species and the specific use of habitat resources appears to operate at a fine-scale both spatially and temporally. Therefore, we urge researchers to design their studies based on the

newest ecology available at their local population level rather than the regional, national, or continental scale.

Table 1.—Literature review results from 1970-2021 involving Maryland cave bat research. Results include study's region, focus, method, urbanization level, bat species, timing, and source. Region codes are: A (Appalachian Plateau), C (Coastal), B (Blue Ridge), N (Not reported), P (Piedmont), V (Valley & Ridge). Method codes are: A (acoustics), C (capture), L (laboratory), R (literature review), T (telemetry), V (visual observation). Urban level codes: UB (urban), UR (urban-rural gradient), NR (not reported), RU (rural). Bat species codes are: EPFU (Big brown bat), MYLE (Eastern Small-footed bat), MYLU (Little brown bat), MYSE (Northern Long-eared bat), MYSO (Indiana bat), PESU (Tri-colored bat).

Region	Focus	Method	Urban level	Bat species	Timing	Source
C, P, B, V	habitat activity & distribution	A, C	UR	EPFU, MYLE, MYLU, MYSE, MYSO, PESU	pre- & post-WNS	Deeley et al. 2021*a*
C, P, B, V	sampling effort	A	UR	EPFU, MYLU, MYSE, MYSO, PESU	post-WNS	Deeley et al. 2021*b*
C	habitat activity & distribution	A	NR	EPFU, MYSPP, PESU	post-WNS	McGowan and Hogue 2016
V	Mercury levels	C, L	NR	EPFU, MYLU, MYSO, MYSE, PESU	pre-WNS	Yates et al. 2014
C	off-shore activity	A	NR	EPFU	pre- & post-WNS	Sjollema et al. 2014
C	seasonal activity	A	RU	EPFU, PESU	pre-WNS	Johnson et al. 2011*b*
N	WNS wing damage & BMI	C	NR	EPFU, MYLE, MYLU, MYSE, MYSO, PESU	pre-WNS	Francl et al. 2011
V	viral zoonosis	C, L	RU	EPFU, MYLU, PESU	post-WNS	Donaldson et al. 2010
P & B	habitat activity	A	UR	EPFU, MYLU, MYSE, PESU	pre-WNS	Lookingbill et al. 2010
V	foraging habitat	C, T	RU	MYLE	pre-WNS	Johnson et al. 2009*b*
V	roost preference	C, T	RU	MYLE	pre-WNS	Johnson and Gates 2008*a*
C	seashore activity	A, C	RU	EPFU, PESU	pre-WNS	Johnson and Gates 2008*b*
C, P, B, V	habitat activity & distribution	A, C	UR	EPFU, MYLU, MYSE, PESU	pre-WNS	Johnson et al. 2008

C	roost preference	C, T	RU	EPFU, MYSPP, PESU	pre-WNS	Limpert et al. 2007
A, V	cave/mine roost use	C	RU	EPFU, MYLE, MYLU, MYSE, MYSO, PESU	pre-WNS	Agosta et al. 2005
A	roost activity	C	RU	EPFU, MYLE, MYLU, PESU	pre-WNS	Agosta et al. 2003
A, V	diet	C, L	RU	EPFU	pre-WNS	Agosta and Morton 2003
N	diet & habitat use	R	RU	EPFU	pre-WNS	Agosta 2002
V, A	cave use	V	RU	EPFU, MYLE, MYLU, MYSO, PESU	pre-WNS	Raesly and Gates 1987
A, V	diet	C, L	RU	EPFU, MYLU, MYSE, PESU	pre-WNS	Griffith and Gates 1985
A, V, P	cave use	C	RU	EPFU, MYLE, MYLU, MYSE, MYSO, PESU	pre-WNS	Gates et al. 1984
P	toxicity levels	C, L	UB	MYLU	pre-WNS	Clark and Krynitsky 1978
V, P	toxicity levels	C, L	UB, RU	MYLU, EPFU, MYSE	pre-WNS	Clark and Prouty 1976

Table 2.—Results from broadened literature review of six cave bat species in North America using key words diet, distance, habitat, home range, roost, and urban. Bat species codes are: EPFU (Big brown bat), MYLE (Eastern Small-footed bat), MYLU (Little brown bat), MYSE (Northern Long-eared bat), MYSO (Indiana bat), PESU (Tri-colored bat).

Species	Diet	Distance	Habitat	Home range	Roost	Urban	Total
EPFU	31	12	87	1	98	43	272
MYLE	4	2	5	3	10	1	25
MYLU	29	19	102	5	72	10	237
MYSO	5	21	36	7	43	6	118
MYSE	9	13	61	3	53	3	142
PESU	6	10	54	1	56	7	134
All species	84	77	345	20	332	70	928

Table 3.—Updated ecology of six cave bat species in eastern North America. Results include known summer roost types, Genus of tree species used for summer roosting, home range size, distance traveled each night while foraging, insect orders consumed, foraging strategy utilized, and WNS threat level. Results are presented as mean ± SD or SE as indicated or with an asterisk * if neither were provided. Bat species codes are: EPFU (Big brown bat), MYLE (Eastern Small-footed bat), MYLU (Little brown bat), MYSE (Northern Long-eared bat), MYSO (Indiana bat), PESU (Tri-colored bat). Summer roost type codes: BA (barn), BB (bat box), BO (boulder), BR (bridge), BU (building), CA (cave), CL (cliff face), MI (mine), RC (rock crevice), RO (rocky outcrop), SS (storm sewers), TR (tree), TU (tunnel), WO (wood pile). Diet codes are: AR (Arachnida), BL (Blattodea), CO (Coleoptera), DI (Diptera), EP (Ephemeroptera), HE (Hemiptera), HO (Homoptera), HY (Hymenoptera), LE (Lepidoptera), ME (Megaloptera), NE (Neuroptera), OD (Odonata), OR (Orthoptera), PL (Plecoptera), PS (Psocodea), TR (Trichoptera). Foraging strategy codes are: ES (edge-space), G (gleaning), H (hawking), OS (open-space), NS (narrow-space), T (trawling). WNS threat codes are: H (high), M (medium), N (not reported), V (very high).

Species	Summer roosts	Tree species	Home range	Distance	Diet	Foraging strategy	WNS threat
EPFU	BB, BR, BU, CA, MI, RC, SS, TR, TU, WO	Fagus, Pinus, Populus, Pseudotsuga, Quercus, Sequoia, and Thuja	Southeast: 2,906 ha ± 1,129.8 (SE)	Southeast: 5 km* maximum	CO, HE, HY, DI, NE, LE, TR, OR, OD, AR	OS, H	M
MYLE	BA, BO, BU, BR, CA, CL, MI, RC, RO, TR, TU	Not reported	Northeast: 10.2 - 99.7 ha *	Northeast: 189.5 m ± 64 (SE) to 897.8 m ± 31.9 (SE)	LE, CO, DI, NE, HE, HY, TR, AR, EP, PL, OR	NS, H & G	NR
MYLU	BB, BU, CA, MI, TR	Acer, Ulmus	Northeast: 143 ha ± 71.0 (SE)	Northeast: 0.32 km ± 0.01 (SD) to 2.81 km ± 0.13km (SD)	LE, CO, DI, TR, HY, HE, NE, PL, PS, AR, OR, EP, BL, HO	NS, H, G, & T	H

MYSE	BB, BU, CA, MI, TR	Acer, Quercus, Liriodendron, Sassafras, Pinus, Juniperus, Oxydendrun, Nyssa, Fraxinus, Robinia, Carya, Ostrya, Cornus, Taxodium, Persea, Ulmus, Magnolia, Ilex, Gordonia	Northeast: 65 ha ± 5.2 (SE); Southeast: 60.2 ha ± 14.1 (SE) before burns, 72.3 ha ± 6.2 (SE) after burns	Northeast: 602 m ± 66.6 (SE) average, 1,719 m maximum; Southeast: 1,300 m maximum	LE, CO, TR, AR, DI, HY, HE, PL, NE, HO	NS, H & G	V
MYSO	CA, TR	Carya, Quercus, Pinus, Ulmus, Acer, Pinus, Populus, Prunus, Fagus, Juglans, Robinia	Northeast: 130.47 ha ± 28.75 (SE); Northeast: 83 ha ± 82 (SD); Southeast: 250 ha ± 100 (SD)	Midwest (only): 3.02 km *	DI, LE, AR, HE, CO, PS, TR, EP (Midwest only)	NS, H	M
PESU	BU, BR, CA, MI	Carya, Acer, Betula, Populus, Quercus, Magnolia, Pinus	Southeast: 389.2 ha *	Southeast: 1,137 m *	LE, DI, CO, HY, HE, NE, TR, HO	ES, H	H

Chapter 2: Activity and diversity of Maryland's cave bats across an urban-rural gradient

ABSTRACT

White-nose Syndrome (WNS) has caused significant population declines of cave bats living in many Northeastern states, such as Maryland. Most bat research in Maryland focused in the less developed, western region. Gaps in knowledge about bats in the urbanized, central, and coastal regions remain. Urbanized areas may provide important habitat to bats during the WNS crisis due to urbanization's heat-island effect and the additional anthropogenic resources they offer. However, published studies in Maryland's urban areas only occurred at National Parks rather than highly developed areas. Therefore, we investigated cave bat species' activity and diversity across an urban-rural gradient using a novel definition. We surveyed 18 sites along the gradient in four seasons from 2015 to 2017. Activity was highest for big brown (*Eptesicus fuscus)*, tri-colored (*Perimyotis subflavus*), and total bats at urban sites while not differing across the gradient for little brown (*Myotis lucifugus*) and Northern long-eared (*M. septentrionalis*) bats. Species richness was higher in urban and rural sites than suburban. Overall, our results indicated highly urban areas, additional to rural, may serve as important habitats to select bat species during WNS and deserve additional survey and management considerations. Furthermore, investigation into the specific available habitat resources along the urban-rural gradient is warranted to improve management recommendations for bat species of concern.

KEY WORDS

activity, bat, diversity, *Eptesicus fuscus*, *Perimyotis subflavus*, urban, White-nose Syndrome

INTRODUCTION

Bats throughout much of North America are in peril due to White-nose Syndrome (WNS). WNS is a fatal, cascading disease process initiated by the psychrophilic fungus *Pseudogymnoascus destructans* (Pd). Pd was first detected in North America in a cave in New York in 2006 and has decimated cave bat populations across much of North America (Hoyt et al. 2021). For example, one of North America's most common bat species has been so heavily impacted by WNS that modeling by Frick et al. (2010) predicted local extirpation in many regions.

Maryland is known to support six cave bat species that can contract WNS: big brown (*Eptesicus fuscus*), Eastern small-footed (*Myotis leibii*), little brown (*M. lucifugus*), Northern long-eared (*M. septentrionalis* formerly *M. keenii septentrionalis*), Indiana (*M. sodalis*), and tri-colored bat (*Perimyotis subflavus* formerly *Pipistrellus subflavus*; Paradiso 1969). Most of the studies on cave bats in Maryland were conducted before 2010, when WNS was first detected in the state (MDDNR 2011), and many of those studies occurred in rural, mountainous areas. Yet, Piedmont and Coastal Plain provinces support many of those same bat species and provide unique opportunities to study bats in various levels of urbanization, from low to extremely high. These differing levels of urbanization are of interest in terms of bat ecology because some urban areas have been reported as favoring certain bat species while generally harming others (Russo and Ancillotto 2014). Urban areas typically contain buildings, roads, manicured green spaces (Moretto and Francis 2017), and numerous water and light sources (Russo and Ancillotto 2014). While these resources are intended to directly provide services to humans, they can unintentionally affect wildlife species. The typically cited negative consequences of urbanization to bats include direct habitat loss, exotic predator introduction, vehicular collisions, anthropogenic light and sound pollution, chemical pollution, nutrient overloads, and vegetation

loss affecting insect prey availability (Hutson and Mickleburgh 2001; Baker and Harris 2007; Francis and Barber 2013; Russo and Ancillotto 2014; Santini et al. 2019; Frick et al. 2020). Conversely, some positive aspects of urbanization include providing roost sites such as trees, buildings, and bridges, natural predator protection, insects that are attracted to streetlights, and water sources such as stormwater retention ponds and swimming pools for drinking (Voigt et al. 2016). Additionally, the unique urban climate or the "heat-island effect" (Jung and Threlfall 2016) from their impervious surfaces may benefit certain bat species. More specifically, Russo and Ancillotto (2014) proposed that the warmer climate of cities may reduce energetic costs of pregnancy and increase the growth of pups.

In this study, we investigated urbanization's impact on bats in Mid-Atlantic North America. Specifically, we studied the relationships between cave bat species richness, species-specific activity, total bat activity, and level of urbanization from central to eastern Maryland, given that WNS has now been established in this region for over a decade to identify indications of bats benefitting from urbanization. We provide context for the presence of species and activity of bats across the gradient to identify patterns that would support or refute the idea of urbanization fostering bat populations or usage.

MATERIALS AND METHODS

Study area.—Our study sites were located in Maryland and overlapped with adjacent counties in Delaware, Virginia, and the District of Columbia. This region receives approximately 91-117 cm of precipitation a year, with greater amounts in summer. The average temperature of 8-14º C varies based on proximity to the Atlantic Ocean, Chesapeake Bay, and elevation. Maryland is composed of the following five physiographic provinces from west to east:

Appalachian Plateau, Ridge and Valley, Blue Ridge, Piedmont, and Coastal Plain with much of the state lying in Piedmont and Coastal Plain provinces (Paradiso 1969). Specific study sites were selected from Piedmont and Coastal Plain provinces due to their numerous opportunities to study the urban-rural gradient and documented bat ecology knowledge gaps. The Piedmont province was characterized by white oak (*Quercus alba*), black oak (*Q. velutina*), tulip poplar (*Liriodendron tulipifera*), smoothbarked hickories (*Carya* sp.), flowering dogwood (*Cornus florida*), chestnut oak (*Q. prinus*), scarlet oak (*Q. coccinea*), scrub pine (*P. virginiana*), pitch pine (*P. rigida*), beech (*Fagus grandifolia*), and hemlock (*Tsuga canadensis*). Elevations ranged from 91-244 m, with much land cleared for agriculture. The Coastal Plain province was characterized by marshes and estuaries with elevations ranging from 30 - 91 m, and the forested regions consisted mostly of scrub pine, pitch pine, white oak, and tulip poplar (Paradiso 1969).

Site Selection.—Overall, six sites were randomly selected within three urbanization levels (Urban, Suburban, and Rural) resulting in a total of 18 research sites (Fig. 1). Due to intense urban sprawl and development in Maryland, the 5th most densely populated state in the United States (Campbell 2018), properly defining the levels of urbanization was imperative. Yet, definitions of urbanization are highly variable throughout ecological literature (Saari et al. 2016; Padilla and Sutherland 2019). When investigating current literature, urban-rural gradients studied in bat research have typically defined urbanization based on distance from the research site to city/suburban center or limit (Geggie and Fenton 1985; Lesinski et al. 2000; Avila-Flores and Fenton 2005; Johnson et al. 2008; Coleman and Barclay 2012; Li et al. 2020). However, Maryland, Virginia, and the District of Columbia have numerous sprawling urban areas (Lichtenberg and Hardie 2007), which led to overlapping “urban” designations or edge effects from urban centers. So, not only did that urbanization definition not work for our region, but as

urban sprawl continues, it will become more ineffective in the future. Other studies have relied on political boundaries (Kurta and Teramino 1992; Loeb et al. 2009; Fabianek et al. 2011; Dixon 2012; Li and Wilkins 2014; Krauel and LeBuhn 2016; Moretto et al. 2019) or human population size (Gallo et al. 2018). However, sites selected using only these metrics may not have any correlation with the changing available resources to bats as areas become more or less urban. Therefore, we created a more appropriate, novel definition of completely discrete urbanization levels while accounting for the heterogeneity of development, forestation, and agricultural habitats using a combination of requirements including human population size and percentages of impervious surface, forest, and open land cover. To create the classification scheme, first, we reclassified land cover data with "forest" consisting of deciduous, evergreen, mixed forest, and woody wetlands. Then, "open" was reclassified as all barren, agriculture, grassland, shrub/scrub, and emergence herbaceous wetland, "water" as all water; and all remaining categories as "other". Next, we calculated percent forest, open, and impervious surface by applying block statistics in ArcGIS® using a square window of 150 m^2, which allowed for multiple representatives of each urbanization level plus movement of the bat detector if a random selection was deemed not appropriate. For any one site to be considered, there had to be > 10 % of non-impervious landscape represented by both open and forest to avoid any bias from a 100 % homogeneous forested or open landscape (Table 4). Forest and open land cover and impervious surface layers were overlaid with census data shapefiles and blocks for urbanization level were only retained if they met all the definition requirements (Table 4).

Human population size classification shapefiles were downloaded from United States Census Bureau's (USCB) 2010 census data (USCB 2010). USCB defined "urban" areas as cities with greater than 50,000 residents, "urban clusters" (aka suburban) as 2,500-50,000 residents,

and “rural” areas as non-urban areas with under 2,500 residents (USCB 2012). Impervious surfaces and land use data were downloaded from the National Land Cover Database (NLCD 2011). All ArcGIS® layers were projected into UTM Zone 18N.

Sites were visited in random order to ground-truth the urbanization classification and obtain permission to gain access. We ensured that sites were separated by ≥ 2 km to limit spatial autocorrelation in bat responses (McGowan and Hogue 2016; Moretto et al. 2019).

Surveys.—Unlike most other bat studies, we designed our surveys to span all parts of the evening hours across multiple seasons and years to increase our ability to identify any relationships between cave bat species and urbanization. This also enabled us to detect total species richness at all sites. Sites were surveyed within each season, in random order without replacement, four seasons per year in 2015, 2016, and 2017. Seasons were confined to four groupings: spring (March and April), early summer (May and June), late summer (July and August), and fall (September and October; Li and Wilkins 2014). This partitioning of surveys coincided with spring migration, pregnancy, parturition/pups volant, and fall migration/swarming. Sites were surveyed for ≥ four consecutive nights per season to increase the probability of capturing echolocation activity of all species present at each site (Hayes 2000; Niver et al. 2014), which can change nightly with differing climate conditions and distances of echolocation calls from microphone.

Detector programming.—Bat echolocation calls were recorded using Song Meter SM2BAT+ bat detectors (Wildlife Acoustics, Concord, MA) with SMX-U1 omnidirectional microphones placed 20’ above ground attached to a modified painter’s pole. That height avoided most echoes from impervious surfaces, and by aiming slightly under horizontal to the ground surface, we avoided collection of rain in the microphone. Detectors were programmed to trigger

from sunset to sunrise according to latitude, longitude, date, and time zone while recording ultrasonic acoustic sound waves in full spectrum wav files. Detectors were programmed with a sampling rate of 384,000Hz to record frequencies greater than the highest expected frequency along with the following settings: high pass filter (hpf) fraction sample fs/24, low pass filter (lpf) turned off, trigger level of 18 dB, trigger window of 2.0 s, and trigger maximum length of 5 s. Microphones were calibrated following the methods of Parkins and Clark (2015). Acoustical recordings do not require handling of mammals and are therefore exempt from University of Maryland's IACUC.

Species identification.—Bat echolocation calls were identified to species level using multiple characteristics of each analyzable sequence such as highest frequency, lowest frequency, characteristic frequency, call duration, and more (3 + search phase calls < 0.5 s; Parsons and Szewczak 2009). Non-bat calls were removed by running all files through Sonobat Batch Scrubber Utility using the default settings. Then, bat calls were run through Sonobat 4.2.2 (https://sonobat.com/) using default settings for NE automated classifier which uses a call quality of 0.80 and decision threshold of 0.90 to view echolocation calls, programs' attempted species identification, and associated identification likelihoods. Due to inconsistencies in automated identification programs (Lemen et al. 2015), we also ran calls through Kaleidoscope Pro 4.3.0 (https://www.wildlifeacoustics.com/) using the Bats of North America 4.3 classifier package with accuracy set to 0 Balanced (Neutral) for Maryland bats. Then, all calls identified by either program as *Myotis* spp. and ≥ 20 % of calls identified as non-*Myotis* spp. were manually vetted. Calls identified as migratory species or unknown species were removed prior to analysis.

Analysis.—Species richness for each night was calculated by first converting each species' nightly activity into absence (0) or presence (1), then summing presence for all possible

species each night at each site. Our analysis dependent response variables included nightly species richness, nightly activity of each cave bat species, and total bat activity. Then, we reviewed outliers, assumptions of normal distribution, and homogeneity of variance, using boxplots, Shapiro-Wilk tests, and Levene Tests in the *car* package (Fox and Weisberg 2019). Due to violations of assumptions in all response variables and the inability to transform variables to meet normalcy assumptions, we utilized nonparametric tests. We tested for equal variance in bat species richness, species-specific activity, and total bat activity using Kruskal-Wallace Rank Sum tests followed by Dunn's multiple comparison post hoc test with Bonferroni correction using *FSA* package version 0.8.30 (Ogle et al. 2020). Basic statistics (median and range) were calculated using psych package version 1.9.12 (Revelle 2020). All *P*-values were set *a priori* to 0.05. All analyses were conducted in R 3.6.3 (http://www.r-project.com).

RESULTS

We surveyed 1,202 nights and recorded 110,062 echolocation calls from big brown (n = 106, 283), little brown (n = 76), Northern long-eared (n = 16), and tri-colored (n = 3,687) bats. Eastern small-footed and Indiana bats were never detected. We recorded a median of 67.5 nights at each site, ranging from 55 – 84 nights. Species richness, species-specific activity, and total bat activity within each urbanization level were totaled (Table 5) and we calculated their nightly averages (median and range; Table 6). At urban sites, we recorded a total of 72,132 calls from big brown (69,959), tri-colored (2,155), and little brown bats (18) over 383 nights. While at suburban sites, we recorded a total of 11,566 calls from big brown (11,295), tri-colored (237), little brown (19), and Northern long-eared bats (15) during 413 nights. Finally, at rural sites, we recorded big brown (25,029), tri-colored (1,295), and little brown bat (39) calls totaling 26,364

across 406 nights. Maximum species richness was highest at suburban sites (n = 4) followed closely by both urban (n = 3) and rural sites (n = 3). Nightly species richness averages were equivalent in each urbanization level (median of one, ranging from zero to three each night; Table 6).

When acoustic data were analyzed each night at each site, there were significant differences for species richness, big brown, tri-colored, and total bat activity among urbanization levels (Table 7; Fig. 2.). Species richness was significantly higher (Table 8) in rural versus suburban sites ($Z = 6.22$, $P < 0.0001$). It was also higher in urban versus suburban sites ($Z = 4.54$, $P < 0.0001$). However, rural and urban species richness did not differ ($Z = 1.58$, $P = 0.34$). Big brown bat nightly activity closely mimicked species richness and was higher (Table 8) in urban compared to suburban sites ($Z = 5.68$, $P < 0.0001$) as well as rural versus suburban sites ($Z = 5.88$, $P < 0.0001$) while not differing between rural and urban sites ($Z = 0.10$, $P = 1.00$). Conversely, overall nightly activity from little brown and Northern long-eared bats did not differ (Table 8) across the three urbanization levels ($\chi^2 = 0.35$, $P = 0.86$; $\chi^2 = 3.30$, $P = 0.19$; respectively). Tri-colored bat activity was also significantly higher (Table 8) at either extreme of the urbanization categories with urban sites greater than suburban ($Z = 5.39$, $P < 0.0001$) and rural sites greater than suburban ($Z = 7.85$, $P < 0.0001$). Tri-colored bat activity did not differ between rural and urban sites ($Z = 2.34$, $P = 0.058$). When all cave bat species activities were analyzed together, total bat activity mimicked the results of the species richness, big brown, and tri-colored bats with significantly higher (Table 8) overall activity at urban versus suburban sites ($Z = 6.01$, $P < 0.0001$) and rural sites compared to suburban sites ($Z = 6.28$, $P < 0.0001$) with no difference between rural and urban sites ($Z = 0.18$, $P = 1.00$).

DISCUSSION

With each site selected randomly and surveyed among multiple seasons and years, we attempted to ensure significant findings were due to changes in some metric related to urbanization as opposed to temporal changes. Previously, studies investigating urbanization's effects on bats compared them across the gradient using different survey methods, time periods, or omitted test results (Kurta and Teramino 1992; Johnson et al. 2008; Krauel and LeBuhn 2016; Deeley et al. 2021a). Our relatively simple, yet intensive yearly data collection and analysis supports the premise that urban and rural areas seem to provide unique resources to multiple bat species in quantity or quality, while suburban areas seem somehow lacking.

First, across the three levels of urbanization, overall species richness was significantly greater at the theoretically opposing categories of urban and rural Maryland. This finding made us reconsider what might constitute quality habitat for bats without having analyzed any detailed microhabitat characteristics. If more urbanization generally equated to less quantity or quality habitat, we would have expected a more positive linear relationship between species richness and increasing rurality. The line of reasoning presented in past research was that urbanization typically increases the amount of impervious surface, while inherently decreasing natural land cover types, yet a positive linear relationship was never observed during our data analysis. Conversely, perhaps it was suburbia that functioned as an outlier. Bat species richness may have been much depressed in suburban sites due to a lack of specific habitats used by multiple bat species in our region such as specific tree heights, roost heights, tree composition, amount of canopy cover, amount of snags, and/or amount of foliage (Lacki 2018; Drake et al. 2020). In a past study, the concept of bats shifting to areas with unique levels of urbanization to support differing biological needs throughout the year was documented (Menzel et al. 2001).

Specifically, Menzel et al. (2001) found that big brown bats selected for areas with 26 - 50 % development during pregnancy, then shifted to higher levels of development (51 – 75 %) during early and late lactation. They also noticed female big brown bats occasionally used heavy development (76 - 100 %) throughout pregnancy while completely avoiding rural (0 – 25 %) areas (Menzel et al. 2001). That study, along with our study results, support the idea that different urbanization levels offer disparate, necessary resources. Investigation into roost conditions, available diet, and seasonal water quality and availability across the gradient could provide further clarification of what specific needs may or may not be met in each type of site for each species.

This research need is further supported by our data on tri-colored bats that were also not very active in our suburban category. In our region, tri-colored bats roosted in mostly deciduous species of trees common to the eastern temperate forest (Drake et al. 2020). Perhaps their activity in suburban sites was low due to the absence of specific preferred trees and tree conditions. Conversely, given tri-colored bats' avoidance of bark and tree cavity roosts, ability to roost in man-made structures, and consumption of a diverse diet (Menzel et al. 2003, Dodd et al. 2014, Veilleux et al. 2003), tri-colored bats may just be more synurbic in our highly developed region. Additionally, this species richness finding may have been enhanced by the decade-long impacts of WNS on the other cave species, especially the little brown bats, in our urban areas. Activity of the once prolific synurbic, urban dwelling, little brown bat was almost absent in our urban areas, which may have reduced competition for prey or roosts for tri-colored bats.

Standing in opposition to the reduced activity in suburbia, significant big brown and tri-colored bat activity was documented in urban and rural areas. Of all the bat species, big brown bats were the most active in our urban sites. This was not totally unexpected given their

morphological features which allow for efficient navigation throughout edges and ecotones (Norberg and Rayner 1987) and their broad foraging and roosting habitats (Kurta and Baker 1990). In fact, several past studies have documented big brown bats in such urban areas (Menzel et al. 2001, Parkins and Clark 2015, Johnson and Gates 2019, Li et al. 2020, Richardson et al. 2021, Deeley et al. 2021a). A telemetry study by Everette et al. (2001) reported big brown bats roosted in Denver's core urban area while foraging in a natural setting at a nearby national refuge. During another study, big brown bats were reported in all levels of that studies' urban-rural gradient while foraging activity levels were significantly higher in rural settings near water features and residential streetlights (Geggie and Fenton 1985). Both of those studies indicated that big brown bats were consistently using urban areas for roosting, with foraging locations varying based on available resources. That pattern may continue in our urban areas. Similarly, tri-colored bat activity was higher in urban areas, likely due to additional habitat availability in urban areas, such as water and roosting habitat. Other studies have also documented increasing tri-colored bat activity associated with more urban water sources, such as residential pools during hot summer periods as a nearby ephemeral urban park retention pond became less available (Nystrom and Bennett 2019), and tri-colored bats have specifically been reported roosting in anthropogenic structures (Fujita and Kunz 1984; Menzel et al. 2003).

Unlike our other species, little brown bats are highly susceptible to WNS (Moore et al. 2018) and some populations showed almost 100% mortality (Frank et al. 2014). In our WNS-positive state, we anticipated lowest activity towards the rural end of the gradient with a positive trend towards the urban end due to their general synurbic natural history (Sparks et al. 1998; Ulrey et al. 2005; Coleman and Barclay 2012; Thomas and Jung 2019). However, we documented no significant changes in activity across the three levels of urbanization. This could

be attributed to their massive population decline from WNS, changes in site-specific demographics, or interspecific competition. One study reported that after the establishment of WNS, little brown activity decreased concurrently with increasing big brown bat activity (Morningstar et al. 2019). So, the dramatic population declines of the synurbic little brown bat (Turner et al. 2011) may have led to big brown and tri-colored bats being able to take advantage of newly available roosts and prey, resulting in their higher activity in the urban areas. Like little brown bats, Northern long-eared bat activity was also low in our urban areas. This was somewhat surprising as they have been known to roost in multiple anthropogenic structures (Mering and Chambers 2014) and have been captured in mist nets in Washington, DC sites in recent years (Lindsay Rohrbaugh [Department of Energy and Environment, Washington, DC], personal communication, [August, 2020]; Deeley et al. 2021a). Unlike the other species, Northern long-eared bat activity was highest at our suburban sites but with such low frequency that it did not differ among urbanization levels. Perhaps their activity was lacking at our highly urban sites due to anthropogenic noise avoidance to improve foraging performance, supporting a previous prediction by Moretto and Francis (2017). Our urban sites were selected to be representative of truly urban settings rather than heavily forested habitat within urban jurisdictions. Very likely, our urban sites provided anthropogenic noises that could interrupt their highly specialized narrow-space hawking and gleaning foraging strategy (Ratcliffe and Dawson 2003).

With species richness and activity levels for big brown, tri-colored, and total bats combined appearing elevated at urban compared to suburban sites, we wonder if urban settings may serve as a source for cave bat species populations. Pregnant and lactating female big brown bats plus juveniles were captured inside Washington, DC (Lindsay Rohrbaugh [Department of

Energy and Environment, Washington, DC], personal communication, [August, 2020]) while pregnant and lactating female Northern long-eared bats were also captured nearby (Deeley et al. 2021a). These results support our notion but further investigation is needed to confirm.

We conclude that bat activity responses were species-specific and caution should be exercised when broadly claiming that urbanization has a negative effect on existing bat species diversity or activity now that WNS has long been established in our region. Our analysis indicated that investigation into many features of habitat availability should be further explored while surveying bats. Surveys regarding diet compared to food availability, roosts selected compared to their availability, roost exit surveys, water quality, sound pollution, home range size, nightly foraging distances, and activity timing would better help understand bat species' complex relationships with urbanization. Furthermore, from a wildlife management perspective, urban areas warrant reconsideration in terms of wildlife conservation agencies interested in providing habitat to species currently listed as threatened or under consideration for listing. As such, we specifically recommend future analyses investigate how changes in relationships between Northern long-eared, little brown, and tri-colored bats' presence, activity, and preferably abundance are related to urbanization and its associated specific characteristics.

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FIGURE LEGENDS

Fig. 1.—Map of our research sites in WNS-positive Maryland and adjoining counties in WNS-positive Delaware and Virginia. Our 18 research sites were distributed evenly among urbanization levels: six in highly urban (purple), six in suburban (yellow), and six in rural (red) areas. We defined urbanization levels according to human population size and percentages of impervious surface, forested, and open land categories.

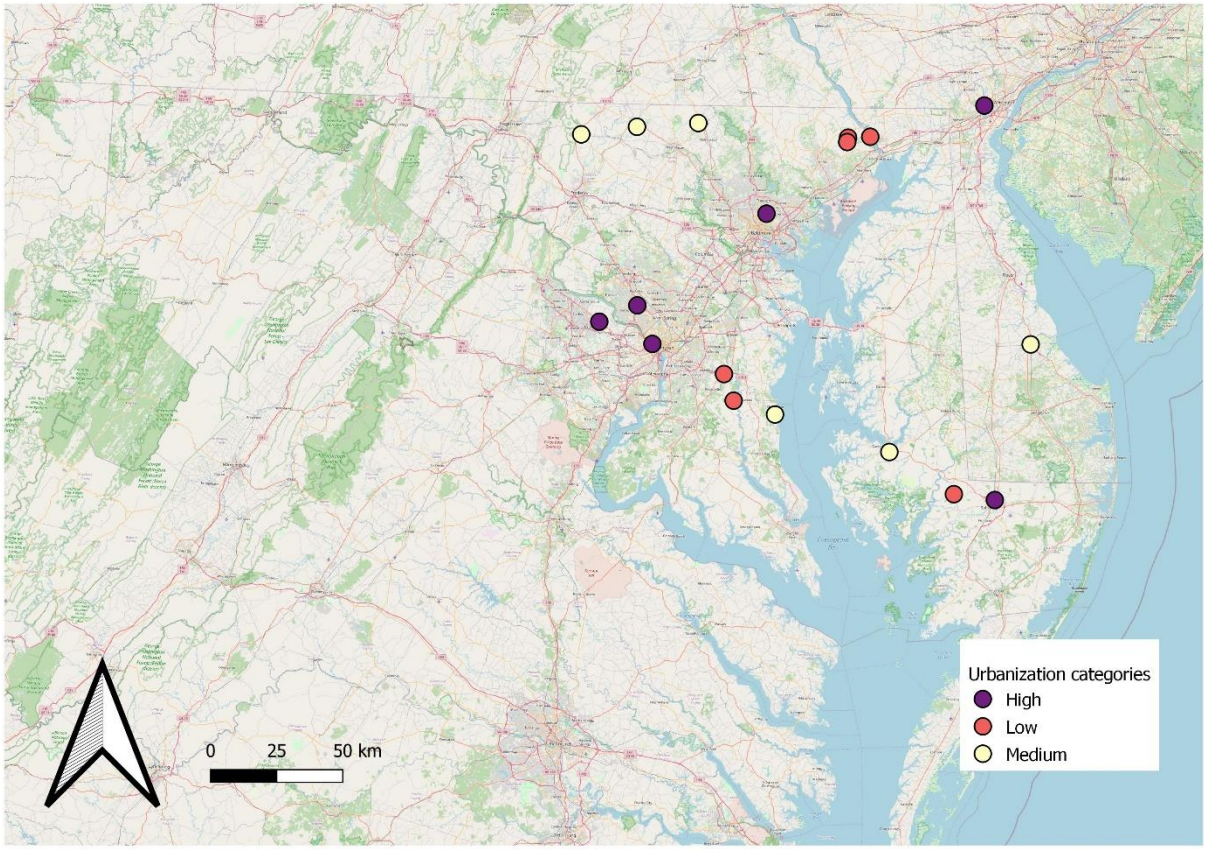
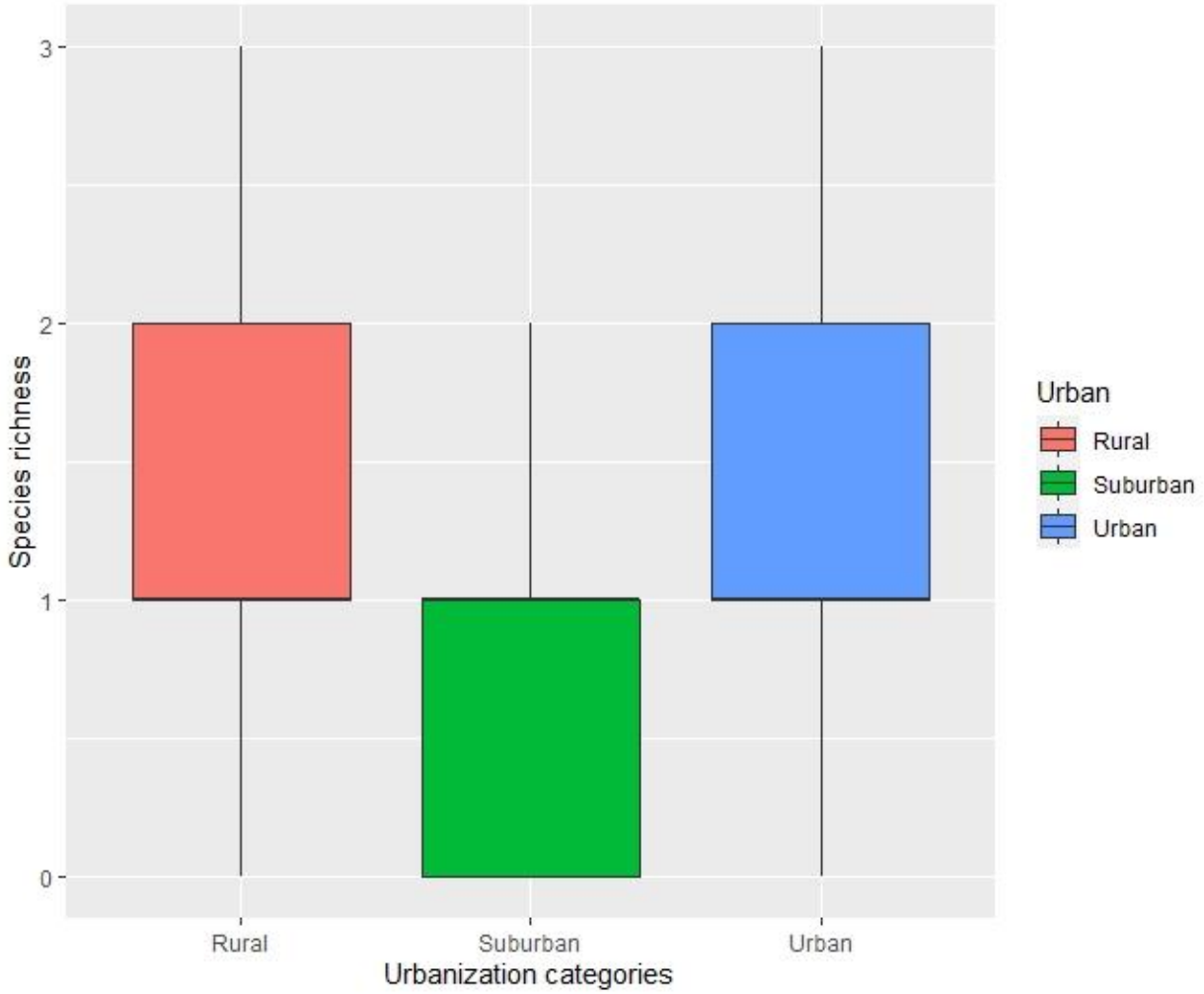


Fig. 2.—Species richness was calculated by adding the sum of all cave bat species present at each site each night then compared among urban, suburban, and rural sites. Nightly species richness was compared among three urbanization levels of Urban (blue), Suburban (green), and Rural (red) using Kruskal-Wallace Rank Sum tests followed by Dunn's multiple comparison post hoc test with Bonferroni correct. Richness was significantly lowest at suburban sites when compared to both urban (Z = -4.54, *P*. adjusted < 0.001) and suburban (Z = -6.22, *P*. adjusted < 0.001) sites.



TABLES

Table 4.—Three levels of urbanization were determined to accurately represent the urban-rural gradient in our study based on US Census Bureau human population size, percent imperviousness, percent forested, and percent open landscape. Percent forested and open landscapes were determined by taking 10% of any remaining non-impervious landscape to ensure heterogeneity at sites. Applying this novel definition allowed us identify possible sites accurately reflecting resources available along the gradient.

Variable	Urban	Suburban	Rural
Population size	50,000+	2,500 - 5,000	< 2,500
% I.S.	> 90.00% and < 100.00%	> 45.00 % and < 55.00 %	> 0.00 % and < 10.00 %
% Not I.S.	0.001-10%	45-55%	90-99.999%
% Forest (10% of Not I.S.)	> 0.1 %	> 4.50 %	> 9.00 %
% Open (10% of Not I.S.)	> 0.1 %	> 4.50 %	> 9.00 %

Table 5.—We recorded echolocation calls (aka activity) of cave bats in six urban, six suburban, and six rural sites from sunset to sunrise, over four nights per week, four seasons per year, from 2015-2017. Species-specific cave bat activity, total activity, and species diversity collected each night at each site were totaled within each urbanization level (Urban, Suburban, and Rural).

Variable	Urban (n=383)	Suburban (n=413)	Rural (n=406)	Total (n=1,202)
Maximum species richness	3	4	3	3
Big brown bat activity	69,959	11,295	25,030	106,284
Little brown bat activity	18	19	39	76
Northern long-eared bat activity	0	15	0	15
Tri-colored bat activity	2,155	237	1,295	3,687
Total bat activity	72,132	11,566	26,364	110,062

Table 6.— Cave bat species richness, species-specific activity, and total bat activity were recorded each night at each site. Results are presented within each urbanization level as median with ranges inside parentheses.

Variable	Urban (n=383)	Suburban (n=413)	Rural (n=406)
Species richness	1 (0 - 3)	1 (0 - 3)	1 (0 - 3)
Big brown bat activity	8 (0 - 2,841)	3 (0 - 1,139)	13 (0 - 1,009)
Little brown bat activity	0 (0 - 4)	0 (0 - 5)	0 (0 - 7)
Northern long-eared bat activity	0 (0 - 0)	0 (0 - 9)	0 (0 - 0)
Tri-colored bat activity	0 (0 - 426)	0 (0 - 19)	0 (0 - 95)
Total bat activity	9 (0 - 2,841)	3 (0 - 1,139)	18 (0 - 1,012)

Table 7.—Cave bat species richness, species-specific activity, and total bat activity (dependent variables) were recorded each night at each site and compared among three levels of urbanization (independent variable) using Kruskal-Wallace Rank Sum tests to determine if there were significant differences (indicated by asterisk *) among urbanization levels (Urban, Suburban, and Rural).

Dependent variable	Independent variable	χ^2	df	P - value
Species richness	Urbanization level	41.61	2	< 0.0001 *
Big brown bat activity	Urbanization level	44.99	2	< 0.0001 *
Little brown bat activity	Urbanization level	0.31	2	0.86
Northern long-eared bat activity	Urbanization level	64.8	2	0.057
Tri-colored bat activity	Urbanization level	64.8	2	< 0.0001 *
Total bat activity	Urbanization level	50.81	2	< 0.0001 *

Table 8.—Following Kruskal-Wallace Rank Sum tests, we ran Dunn's multiple comparison post hoc test with Bonferroni correction to determine where bat activity and richness differed between urbanization level comparisons. Asterisk * following comparisons indicates significant differences ($P < 0.05$).

Dependent variable	Independent variable	Comparison	Z	*P*. unadjusted	*P*. adjusted
Species richness	Urbanization level	Urban - Rural	-1.58	0.11	0.34
		Urban - Suburban	4.54	< 0.0001	< 0.0001 *
		Rural - Suburban	6.22	< 0.0001	< 0.0001 *
Big brown bat activity	Urbanization level	Urban - Rural	-0.11	0.91	1.00
		Urban - Suburban	5.68	< 0.0001	< 0.0001 *
		Rural - Suburban	5.88	< 0.0001	< 0.0001 *
Little brown bat activity	Urbanization level	Urban - Rural	-0.55	0.58	1.00
		Urban - Suburban	-0.26	0.79	1.00
		Rural - Suburban	0.29	0.77	1.00
Northern long-eared bat activity	Urbanization level	Urban - Rural	0.00	1.00	1.00
		Urban - Suburban	-2.05	0.04	0.12
		Rural - Suburban	-2.08	0.04	0.11
Tri-colored bat activity	Urbanization level	Urban - Rural	-2.34	0.02	0.06
		Urban - Suburban	5.39	< 0.0001	< 0.0001 *
		Rural - Suburban	7.85	< 0.0001	< 0.0001 *
Total bat activity	Urbanization level	Urban - Rural	-0.18	0.86	1.00
		Urban - Suburban	6.01	< 0.0001	< 0.0001 *
		Rural - Suburban	6.28	< 0.0001	< 0.0001 *

Chapter 3: Temporal relationships of cave bats and their prey along the urban-rural gradient

ABSTRACT

Cave bat populations in North America have significantly declined due to White-nose Syndrome (WNS), caused by a psychrophilic fungus occurring in cold and humid hibernacula. It has been posited that during the WNS crisis, warmer urban climates may offer valuable prey availability, but few studies have analyzed the relationships between bats and prey availability in urban areas. Our study is the first to analyze correlations of cave bat species activity and diversity in relation to prey availability across the urban-rural gradient. March through October of 2015 to 2017, we recorded bat activity and captured insects at 18 sites of varying levels of urbanization in Maryland. As we expected, there were significant changes between seasons across the gradient. Big brown bat activity was positively related to their preferred prey at urban sites while tri-colored bat activity was positively related to their preferred prey and negatively related to big brown bat preferred prey at rural sites. Big brown and tri-colored bats were also active at other parts of the gradient without correlation to insects, indicating they may be primarily roosting or seeking water resources there. Overall patterns of bats in this study indicated we may have been documenting temporal niche partitioning across the urban-rural gradient. The partitioning was likely in response to some level of competition, possibly enhanced from cascading long-term effects of WNS.

KEYWORDS

activity, bat, correlation, diversity, insect, urban, urban-rural gradient, WNS

INTRODUCTION

Cave bat populations have significantly decreased across North America due to the White-nose Syndrome (WNS) disease process. WNS is caused by the *Pseudogymnoascus destructans* fungus which awakens cave bats, causing behavioral changes (Brownlee-Bouboulis and Reeder 2013), expenditure of stored energy and water, malnutrition, dehydration, and eventual death. The psychrophilic fungus thrives in cold and humid environments, such as bat hibernacula, which are quite dissimilar to urban areas. Urban areas are typically warmer and drier than nearby natural areas (Russo and Ancillotto 2014; Jung and Threlfall 2016; Moretto and Francis 2017), and may potentially provide unique resources to bat species. For example, urban areas could provide potential drinking sources, including such sources as retention ponds and swimming pools. Anthropogenic structures, such as buildings and bridges, provide roosting sites and protection from predators while city streetlights, along with gardens and green roofs, attract insects (Gehrt and Chelsvig 2004; Russo and Ancillotto 2014; Parkins and Clark 2015; Voigt and Kingston 2016; Voigt et al. 2016). Yet, only a few studies in the literature reviewed the relationships among urbanization, cave bats, and insect availability. These studies provided inconsistent or conflicting results as to the nature of the relationships among urbanization and bats, as well as urbanization and insect availability (Avila-Flores and Fenton 2005; Kalcounis-Rueppell et al. 2007; Coleman and Barclay 2013). Furthermore, the few studies that have investigated such questions were primarily conducted prior to WNS (Avila-Flores and Fenton 2005; Kalcounis-Rueppell et al. 2007; Coleman and Barclay 2013) or inside one localized urban area, as opposed to crossing a gradient of urbanization levels (Partridge et al. 2020).

The state of Maryland represents a unique opportunity to study these relationships due to its high density of urban areas (Campbell 2018) in addition to long-term Pd surveying (MDDNR

2011). Our study is the first to evaluate potential relationships between bats and their prey across the urban-rural gradient in a state that has been facing WNS for more than a decade. Our primary objective was to investigate how cave bat activity and diversity related to insect counts and diversity across the urban-rural gradient.

MATERIALS AND METHODS

Study area.—Our study sites were located in Maryland and overlapped with adjacent counties in Delaware, Virginia, and the District of Columbia. This region receives approximately 91 - 117 cm of precipitation a year, and the average temperature ranges from 8 to 14º C (Paradiso 1969). Throughout the region, we created a unique definition of urbanization to appropriately represent the urban-rural gradient in this heavily urbanized forested and coastal region rather than relying on distance to urban center/edge like other studies (Geggie and Fenton 1985; Lesinski et al. 2000; Avila-Flores and Fenton 2005; Johnson et al. 2008; Coleman and Barclay 2012; Li et al. 2020). This was necessary because simple definitions of urbanization would likely lead to randomly oversampling urban areas due to the high concentration in our region. Our definition (Table 9) also maintained heterogeneity among sites by removing homogenous heavily forested or open agriculture land cover types, which could attract or deter certain bat species. We set "urban" as sites containing 90 - 99.99 % impervious surface, > 0.001 % forest, > 0.001 % open, and classified as urban by United States Census Bureau (USCB; 2012). To ensure no overlap of classifications, we set "suburban" as sites containing 45 – 55 % impervious surface, > 4.5 % forest, > 4.5 % open, and classified as urban clusters by USCB. We set "rural" as sites containing 0.01 – 10 % impervious surface, > 9 % forest, > 9 % open, and classified as rural by USCB. We set a moving window size of 150 m^2 to allow for movement around each site to select a detector location that avoided echoes from proximity to buildings or water and provide

numerous potential replicates within each urbanization level to randomly select from. Like others (McGowan and Hogue 2016; Moretto et al. 2019), we required sites to be at least 2 km apart to remove spatial autocorrelation. We downloaded human population size shapefiles from USCB (2010) and percent impervious surface raster data from National Land Cover Database (NLCD; 2011). Land cover raster data were also downloaded from NLCD (2011) and reclassified as "forest" (deciduous, evergreen, mixed forest, and woody wetlands), "open" (barren, agriculture, grassland, shrub/scrub, and emergence herbaceous wetland), "water" (all water), and "other" (all other categories).

We selected 18 sites in Maryland and adjacent counties equally along the urban-rural gradient (Fig. 7) from Piedmont and Coastal Plain provinces where predominately urban areas are concentrated. The Piedmont province was dominated by oak-pine forest with many areas converted to agriculture. The Coastal Plain province was dominated by pine, oak-hickory and mixed forests with many aquatic habitats (Paradiso 1969). Sites were surveyed four seasons per year from 2015 - 2017 in random order without replacement. Similar to Li and Wilkins (2014), we defined seasons as spring (March and April), early summer (May and June), late summer (July and August), and autumn (September and October) because these seasons are characterized by hibernacula emergence, pregnancy/parturition, nursing/pups volant, and swarming/hibernating preparation, respectively.

Bat detector settings.—Song Meter SM2BAT+ bat detectors (Wildlife Acoustics, Concord, MA) with SMX-U1 omnidirectional microphones were placed 20' above ground to record bat echolocation calls in full spectrum wav files from sunset to sunrise. Higher frequency sound waves can attenuate given changes in climate and distance; therefore, we attempted to survey greater than the minimum number of recommended consecutive nights at each site

(Hayes 2000; Niver et al. 2014) each season. Detectors were set to a sampling rate of 384,000 Hz, hpf fs/24, lpf off, trigger level of 18 dB, trigger window of 2.0 s, and recording a maximum file length of 5 s. We calibrated microphones each year according to Parkins and Clark (2015).

Bat species identification.—Bat echolocation calls were identified to species-level using two automated identification programs followed by manual vetting of 100 % *Myotis* classified calls and > 20 % of non-*Myotis* classified calls by reviewing spectrograms plus listening to audio files. We removed non-bat call files using Sonobat Batch Utility Scrubber, then ran call files through Sonobat 4.2.2 using default settings for Northeastern bats. We also ran call files through Kaleidoscope Pro 4.3.0 with accuracy set to 0 Balanced (neutral) for Maryland bats. Activity from migratory bat species or unidentified bat species were excluded to ensure our analysis focused only on cave bat species. Our study was observational in nature regarding bats, did not alter their behavior, and therefore was exempt from the University of Maryland's IACUC process.

Insects collected.—Aerial insects were collected during each survey by placing three 3" x 5" sticky traps (Olson Products, Medina, OH) around one cylinder (Kunz 2009) attached to a rigid pole 20 feet above ground (Coleman and Barclay 2013). This allowed us to capture flying insects in 360 degrees. Insect traps were placed at the beginning of each bat survey and collected one week later to capture maximum insect prey availability similar to Partridge and Clark (2018). Insects were later identified to order-level (Triplehorn et al. 2005) using a dissecting AmScope 20X microscope. Sticky traps were used to capture insects as this method revealed findings similar to other methods such as UV light or suction traps regarding number of insect orders, large (> 4 mm) or small (< 4 mm) insect sizes (Sleep and Brigham 2003), and avoided survey biases by not having attractants (Capinera 2008). To measure insect diversity, we

calculated insect order richness, the sum of insect orders captured during each survey, and insect Shannon H diversity index, which includes insect order richness and evenness (Coleman and Barclay 2013). Studies of invertebrates are exempt from the University of Maryland IACUC process.

Analysis.—Bat species richness was calculated by creating a binary nightly activity metric (0 = absent or 1 = present) for each species followed by summing presence for all species at each site each night. Bat species richness is the only metric we used for measuring bat diversity, as acoustics are based on activity not abundance. Site-specific dependent variables (bat species richness, species-specific activity, and total bat activity) were recorded nightly then averaged each week, allowing for comparable temporal comparisons with independent variables (insect counts, total insects, and insect diversity) collected each week. We reviewed outliers, reviewed assumptions of normality, and variance using boxplots, the Shapiro-Wilk test, and the Levene test, respectively, in the car package in program R (Fox and Weisberg 2019).

We tested variation in our response variables among three urbanization levels (urban, suburban, and rural), three years (2015, 2016, and 2017), and four seasons (spring, early summer, late summer, and autumn). Due to violations of assumptions in response variables and the inability to transform them to a normal distribution, we used the nonparametric Kruskal-Wallis Rank Sum tests to determine if there were any differences among urbanization levels, years, or seasons followed by Dunn's multiple comparison post hoc tests with Bonferroni correction to identify specifically where significant differences occurred using the FSA package in program R (v. 0.8.30, Ogle et al. 2020). We also tested variation in independent insect variables (insect order counts, total, and diversity) among urbanization levels, years, and seasons using the same approach. Then, we used the nonparametric Spearman correlation coefficient to

review auto-correlations between independent insect variables surrounding insect order counts, total, richness, and diversity. If one variable in each pair was highly correlated (rho > 0.50 ; Ford et al. 2006; Hale et al. 2012), we retained the variable that had the greatest biological justification. We used the same approach to identify possible auto-correlations between dependent variables surrounding bat species richness, species-specific activity, and total bat activity and retained the variable in the correlated pair that was most biologically relevant. Then, correlation was assessed between dependent bat variables and independent insect variables, similar to Partridge et al. (2020). Basic descriptive statistics were calculated in program R using the psych package version 1.9.12 (Revelle 2020). All *P*-value thresholds were set to 0.05. All analyses were conducted in R 3.6.3 (http://www.r-project.com).

RESULTS

Bat activity.—We collected 110,062 echolocation calls from cave bats and identified them as the following species: Big brown (96.6 %), tri-colored (3.3 %), little brown (<1 %), and Northern long-eared (<1 %). Total bat activity was highly correlated with bat species richness (rho = 0.77, $P < 0.0001$), big brown bat activity (rho = 0.99, $P < 0.0001$), and tri-colored bat activity (rho = 0.55, $P < 0.0001$) while bat species richness was highly correlated with both big brown bat activity (rho = 0.74, $P < 0.0001$) and tri-colored bat activity (rho = 0.84, $P < 0.0001$). We removed total bat activity and bat species richness to allow for analysis of species-specific activity. Little brown and Northern long-eared bat activity levels were too low during surveys to warrant further analysis and were therefore excluded.

Activity from big brown and tri-colored bats differed among urbanization levels and seasons but not years (Tables 10 through 14). Since big brown bat activity ($\chi^2 = 3.53$, $P = 0.17$)

and tri-colored bats ($\chi^2 = 0.85$, $P = 0.65$) did not change among years (Table 10), we combined data from all years for our analysis. Conversely, big brown and tri-colored bat activity changed significantly among urbanization levels ($\chi^2 = 12.31$, $P < 0.001$; $\chi^2 = 18.33$, $P < 0.001$, respectively, Tables 10 and 11). Activity also changed significantly among seasons for big brown and tri-colored bats ($\chi^2 = 35.28$, $P < 0.0001$; $\chi^2 = 50.60$, $P < 0.0001$, respectively; Tables 10 and 11). Because of the significant variation along the urban-rural gradient and among seasons, we reviewed all further big brown bat activity (Fig. 4) and tri-colored bat activity (Fig. 5) separately in each season and each urbanization level.

Seasonal bat activity changes across the gradient.—During spring, there was no difference in activity levels from both big brown and tri-colored bats ($P > 0.05$) along the gradient. During early summer, big brown bat ($Z = 2.77$; *P adj.* = 0.017) and tri-colored bat activity ($Z = 3.13$; *P adj.* = 0.0052) were significantly higher at rural sites when compared to suburban sites (Fig. 4 and 5). In late summer, big brown bats and tri-colored bats differed. Big brown bat ($Z = 2.79$; *P adj.* = 0.16) activity at urban sites was now higher than suburban (Fig. 4) while tri-colored bats continued their significantly higher activity at rural (Fig. 5) sites when compared to suburban ($Z = 3.89$; *P adj.* = 0.0003). Big brown bats continued their significantly higher activity at urban (Fig. 4) versus suburban sites ($Z = 2.66$; *P adj.* = 0.024) through autumn.

Insect count.—We collected a total of 10,439 insects (Table 15) and identified them to the following orders: Hemiptera (34.6%), Hymenoptera (25.3%), Diptera (22.2%), Coleoptera (10.5%), Blattodea (<1%), Ephemeroptera (<1%), Lepidoptera (<1%), Orthoptera (<1%), and Trichoptera (<1%). Total number of insects was highly correlated with Coleoptera (rho = 0.52, $P < 0.0001$), Diptera (rho = 0.64, $P < 0.0001$), Hemiptera (rho = 0.54, $P < 0.0001$), Hymnoptera

(rho = 0.61; $P < 0.0001$), and insect Shannon H diversity (rho = 0.58, $P < 0.0001$) while insect order richness was highly correlated with Coleoptera (rho = 0.62, $P < 0.0001$), insect Shannon H diversity (rho = 0.87, $P < 0.0001$). We removed the total number of insects and order richness, and analyzed the count of individual orders. We retained insect Shannon H diversity as it did not correlate strongly with any insect order and was a more complex metric involving insect diversity.

When comparing all insect data among years, most insect order counts did not differ ($P > 0.05$; Table 10). Only Coleoptera had a change between years ($\chi^2 = 14.38$, $P < 0.0001$), specifically a decrease between 2015 and 2017 ($Z = -3.77$, $P\ adj. < 0.001$). We did see significant differences for insect counts and diversity among years and urbanization levels (Tables 10 and 11). When comparing all insect data among urbanization levels, we saw significant differences in Coleoptera ($\chi^2 = 23.03$, $P < 0.0001$), Diptera ($\chi^2 = 6.52$, $P = 0.04$), and Trichoptera ($\chi^2 = 8.30$, $P = 0.02$) counts (Table 10). When comparing all insect data among seasons, significant differences were reported for Coleoptera ($\chi^2 = 20.16$, $P < 0.001$), Diptera ($\chi^2 = 12.55$, $P = 0.01$), Hemiptera ($\chi^2 = 33.98$, $P < 0.0001$), and insect Shannon H diversity ($\chi^2 = 25.11$, $P < 0.0001$; Table 10). As such, we further analyzed insect order counts and diversities in each season and each urbanization level separately.

Insect count seasonal changes across the gradient.—During spring, no insect values changed significantly across the gradient (all P values > 0.05). In early summer, Coleoptera (Fig. 6) were higher in rural sites when compared to urban sites ($Z = 3.115$, $P\ adj. = 0.0057$), Trichoptera were more abundant at rural versus suburban sites ($Z = 2.76$, $P\ adj. = 0.018$), and Hemiptera were higher in urban sites as opposed to rural ($Z = 2.60$, $P\ adj. = 0.028$). By late summer, Diptera and Hymenoptera were higher in rural versus urban sites ($Z = 2.50$, $P = 0.038$,

$Z = 2.54$, $P = 0.034$, respectively). In autumn, Coleoptera (Fig. 6) were higher in rural versus urban sites ($Z = 2.51$, $P = 0.037$).

Bat-insect correlations in urban areas.—Correlations between bat activity and insect count or diversity changed among seasons in our urban sites (Table 16). Urban sites during early summer supported big brown bat activity positively correlated with Coleoptera counts (rho = 0.632, df = 11, $P = 0.020$), Hemiptera counts (rho 0.754, df = 11, $P = 0.0029$), and Shannon H (rho = 0.692, df = 11, $P = 0.011$). However, later in the summer at urban sites, the previously identified relationships were no longer significant as insect counts and diversity were higher at other levels of the gradient, but by autumn big brown bat activity was again correlated positively with Coleoptera (rho = 0.628, df = 11, $P = 0.022$) and now with Diptera (rho = 0.608, df = 11, $P = 0.028$).

Bat-insect correlations in rural.—While there were no significant correlations between bat activity and insect counts for any season in suburban areas, there were in rural areas (Table 16). At rural sites in spring, tri-colored bat activity had a strong negative correlation with Hemiptera (rho = -0.610, df = 14, $P = 0.013$, Table 11), but by early summer at these same sites, their activity flipped to a significant positive relationship with Diptera (rho = 0.552, df = 13, $P = 0.033$) and Hemiptera (rho = 0.568, df = 13, $P = 0.027$). In late summer, their activity at rural sites were negatively related to Coleoptera (rho = -0.54, $P = 0.032$). By autumn, their relationship with insects flipped yet again as they were positively associated with the Shannon H metric (rho = 0.551, $P = 0.041$).

DISCUSSION

Reviewing patterns in bats and insects along the urban-rural gradient among seasons revealed unique relationships that may indicate possible niche partitioning or slight competitive exclusion. For example, during spring, tri-colored bat activity was reported all along the urban-rural gradient, but they had a strong negative relationship with Hemiptera in rural sites which changed to positive by early summer. Simultaneously in early summer at the urban side of the gradient, big brown bat activity was also positive with Hemiptera. Hemiptera were recently identified in a molecular DNA analysis study as an important part of the big brown bat diet (Whitby et al. 2020), and they were consumed more by big brown than tri-colored bats (Carter et al. 2003; Feldhamer et al. 2009; Dodd et al. 2014). We attribute tri-colored bat activity's negative relationship with Hemiptera at rural sites during spring as representing avoidance of areas where they anticipated big brown bat activity. Since both bat species consume Hemiptera and both were likely foraging for them during the energetically demanding pregnancy season, we interpret these findings as species partitioning the urban-rural gradient for competition avoidance.

During early summer, big brown bat activity at urban sites was highly correlated with Coleoptera, Hemiptera, and insect diversity, indicating they may be foraging in cities to meet increased energetic demands of pregnancy. Over half of big brown bat diet consisted of Coleoptera next followed by Hemiptera (Carter et al. 2003; Feldhamer et al. 2009; Dodd et al. 2014), plus their diet breadth increased following WNS impacts on little brown bats (Morningstar et al. 2019), so we feel this activity explanation is valid. However, big brown bat activity and Coleoptera counts were both greatest at rural sites during early summer, but without correlation between the two. Perhaps big brown bats were active in rural areas for reasons other than or in addition to foraging. Pregnant and lactating female big brown bats were recorded

traveling up to 5 km during this season (Menzel et al. 2001). Our urban and rural sites were separated further than their maximum foraging distance when considering populations across our study areas, so our interpretations of bat activity by urbanization category are general findings, assuming these are not reflective of differences in local populations or the same population traveling between sites. Meanwhile, tri-colored bat activity was highest during early summer at rural sites and displayed a positive relationship with Diptera and Hemiptera. Other studies have documented tri-colored bats preying on Diptera and Hemiptera (Carter et al. 2003; Dodd et al. 2014). However, Diptera counts did not differ among urbanization levels during this season while Hemiptera were most abundant on the urban side of the gradient. These findings may be evidence of spatial partitioning by tri-colored bats foraging in rural areas and therefore able to avoid big brown bats foraging in early summer in urban areas.

We documented multiple shifts in relationships during late summer. Big brown activity was still high in the urban areas; however, urban sites ceased to support large quantities of any insect orders or insect diversity. Other studies have documented big brown bat behavioral patterns of roosting in cities while foraging outside of cities in more natural, rural settings (Geggie and Fenton 1985; Thomas and Jung 2019). Our rural sites supported the greatest counts of Diptera, which big brown bats were reported consuming in large quantities (Whitby et al. 2020), while no urbanization level supported large counts of Coleoptera, their top preference, during late summer. Similar to early summer predictions, big brown bats may have continued using urban sites for roosting and hydration. Our observed big brown bat correlation shift at urban sites occurred while tri-colored bat activity was highest at rural sites. So, the partitioning between these two species seemed to occur even when big brown bat prey were not driving their activity pattern. Interestingly, rural areas supported higher tri-colored bat activity plus higher

quantities of their preferred prey, Diptera (Carter et al. 2003), yet there was no positive correlation identified. Tri-colored bat activity's negative relationship with Diptera indicates another correlation shift during this late summer season. The culmination of these overall late summer patterns further indicated that in addition to avoidance of competition, roosting and water availability may also drive where bats are active, especially as weather heats up and lactation needs increase. Nystrom and Bennett (2019) documented increased bat activity over residential swimming pools that were being used as a drinking water source for bats during hot summer months when nearby ephemeral retention pond water started to dry up. Further investigation into potential water sources is warranted as water has been reported by others to be important in explaining bat activity (Seibold et al. 2013; Nystrom and Bennett 2019).

In autumn, we saw a return of Coleoptera and Diptera in the identified relationships for big brown bats in our urban category. This highly positive correlation occurred in spite of the fact that Coleoptera were available in larger quantities at our rural sites. Concurrently in our urban sites, increases in tri-colored bat activity during autumn almost reached statistical significance ($P\ adj. = 0.059$), although no other relationships were significant for tri-colored bats in that space or time. Simultaneously, at the rural end of the gradient, tri-colored bat activity had a positive correlation with insect diversity and a moderately strong correlation with Lepidoptera ($P = 0.058$). In one study, tri-colored bats were reported as consuming the most diverse diet, with the largest portion from Lepidoptera (Dodd et al. 2014). Perhaps tri-colored bats were using rural areas during autumn to forage away from high levels of big brown bat activity but were willing to utilize cities for roosting and drinking water.

Insect counts and diversity in our study were lower in colder spring and fall months and increased in warmer summer months as expected (Mellanby and Gardiner 1939). The changes in

seasonal insect counts mirrored results from other urban studies (Parkins and Clark 2015; Partridge and Clark 2018; Partridge et al. 2020). Interestingly, Hemiptera were the most abundant across all urban sites as well as all our study sites combined. Other urban studies in North America reported dominance of Diptera (Kalcounis-Rueppell et al. 2007; Coleman and Barclay 2013; Partridge et al. 2020), Lepidoptera (Partridge et al. 2020), and Thysanoptera (Partridge and Clark 2018). However, those studies utilized different trap types and different site selection methods. Site selection for those studies ranged from what may be considered typical bat habitat to green roofs, and none included randomly selected, highly impervious sites.

In terms of our study design, we were limited to weekly composite collections of insects, so daily variation was not able to be accounted for, but we returned to the same sites in multiple seasons and years in random order. By pooling and averaging bat activity each week, we had comparable samples but inherently decreased our sample size. Additionally, our study design captured bat echolocation activity using stationary bat acoustic detectors which indicated activity of bat species within detection zones limited due to distances from bats, echolocation sound wave frequencies, and climate variables rather than counts of individuals within each bat species. Our acoustic data allowed us to evaluate relationships of bat species activity and richness with insect counts and diversity but could not imply causation. Therefore, we cautiously discuss our interpretation of plausible reasoning for the relationships observed rather than inferring cause and effect.

Management.—With big brown bats' generalist nature, lower impact from WNS compared to tri-colored bats (Turner et al. 2011; Cheng et al. 2021), tri-colored bats under consideration for listing by USFWS (2020), and tri-colored bats' apparent competition avoidance (Veilleux et al. 2003), it follows that management actions should focus on meeting tri-colored

bat habitat needs. Generally, tri-colored bats have been documented as roosting in older, moderate sized trees of species within *Carya, Acer, Betula, Populus, Quercus, Magnolia*, and *Pinus* near intermittent and perennial streams (O'Keefe et al. 2009). They used a home range of 389 ha (Menzel et al. 2003) and travelled up to 4.3 km nightly (Veilleux et al. 2003). In our study, tri-colored bats actively used urban and rural sites, with the strongest identifiable activity in urban areas during autumn. So, while it is a general recommendation, maintaining areas of mature mixed forest habitats with canopy gaps near streams, as well as other quality water features every 4 km in Mid-Atlantic urban settings would provide areas that mimic where we documented the most bat activity. The same can be said for suburban and rural sites as well, with the addition of supporting their prey base. Along with promoting roosting and drinking habitats for bats in urban areas, we recommend promoting high insect counts/diversity in both urban and rural areas which can be achieved through outreach and education promoting bat and insect ecosystem services and understanding financial conservation incentives. Landowners can request assistance from the US Department of Agriculture Natural Resources Conservation Service (NRCS) and Farm Service Agency (FSA) to develop habitat management plans and receive tax incentives through the Farm Bill (Conaway 2018). Habitat management plans in urban and rural areas could include planting and maintaining diverse native insect habitat and tree species used by bats for roosting, bat box placement/maintenance, identifying trees for certified arborists to safely prune and girdle to create canopy gaps/edge habitats, avoiding pesticides and insecticides, and providing safe drinking water.

For future bat research studies, we recommend collecting and analyzing habitat features such as prey availability, insect habitat, water, roosts, and climate in order to make specific recommendations along the urban-rural gradient in each local region. Relationships between bat

activity, insect availability, urbanization, and seasonality seem to indicate some cave bat species may readily use urban areas for foraging, roosting, and drinking while others may select more rural areas to avoid competition. Bat activity/diversity and insect count/diversity changed along the urban-rural gradient in each season, so surveying in just one season or in just one part of the gradient is not sufficient to understand the complex nature of bats and their prey, especially after the establishment of WNS.

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FIGURE LEGENDS

Fig. 3.—Map of our 18 research sites which were evenly distributed in each part of the urban-rural gradient. Urban, suburban, and rural sites were selected based on our novel definition using percent impervious surface, forest, and open land along with U.S. Census Bureau human population size data. Purple circles indicate highly urban, yellow indicates suburban, and red indicates rural sites.

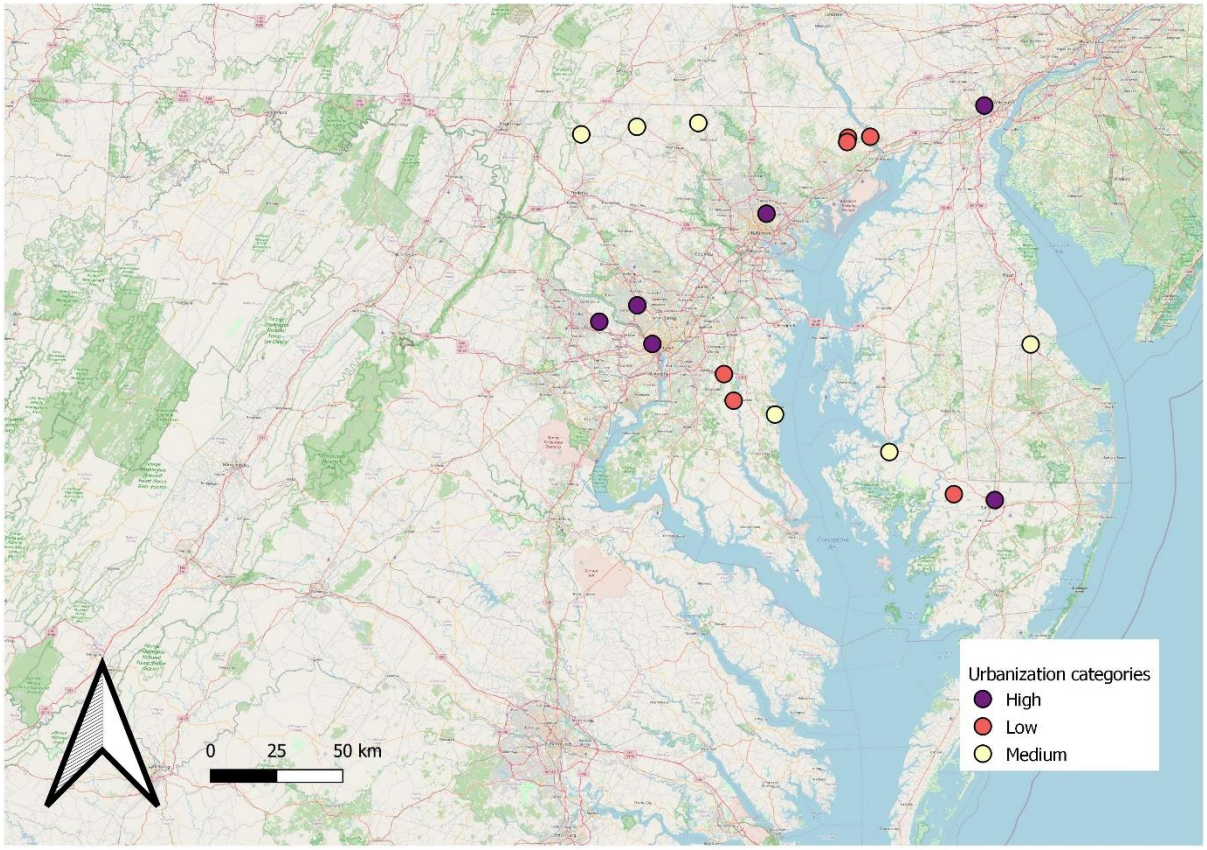


Fig. 4.—Big brown bat activity was collected each night then averaged each week of surveying. Their activity is displayed within each urbanization level (Urban in blue, Suburban in green, and rural in red) among four seasons. Season codes include Spring (1-Spr), Early summer (2-Sum), Late summer (3-Sum), and Autumn (4-Aut).

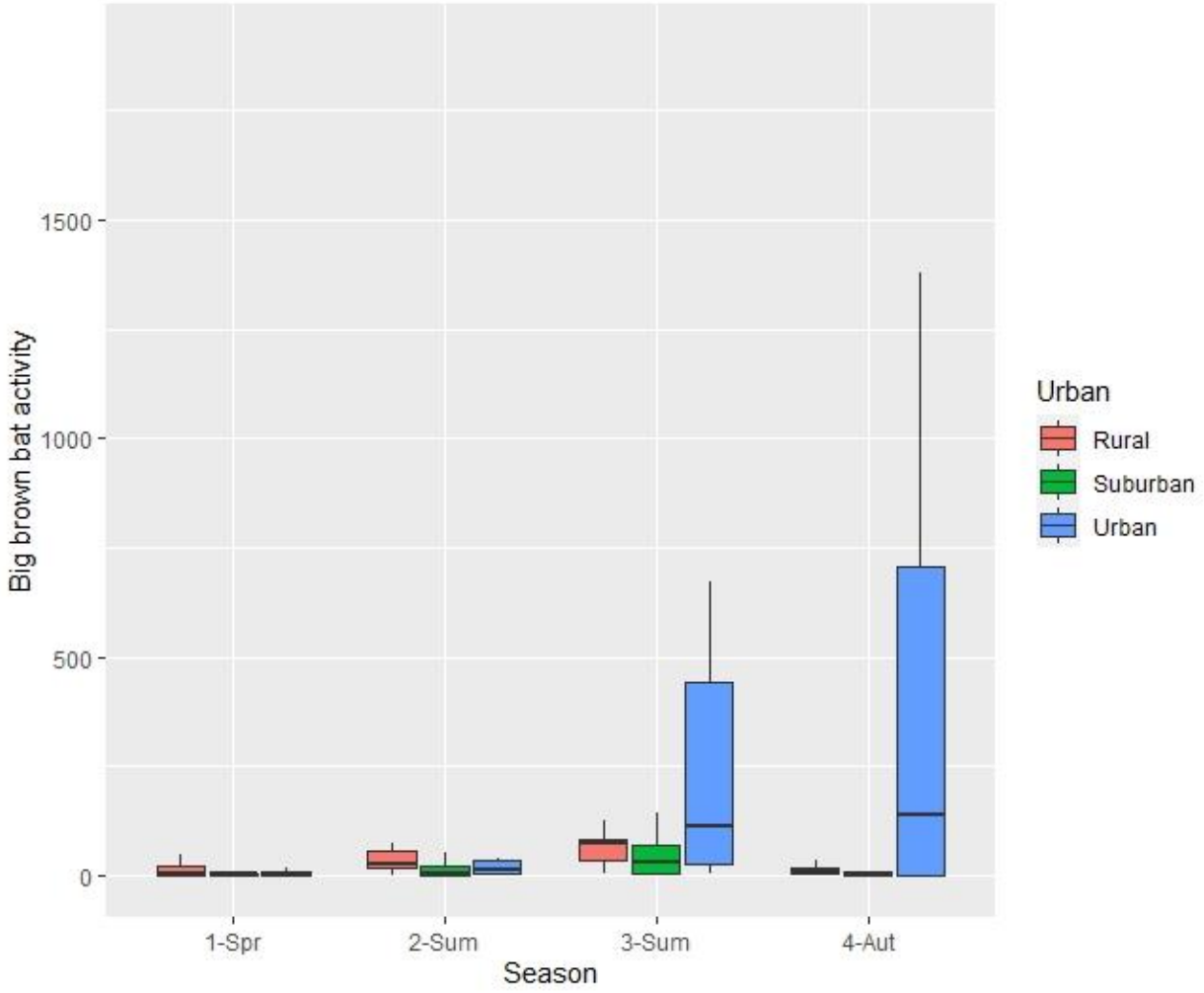


Fig. 5.—Tri-colored bat activity changed significantly among seasons along the urban-rural gradient, especially during late summer season when their activity was higher at rural sites (red) compared to suburban sites (green). Significance was set *a priori* to $P < 0.05$.

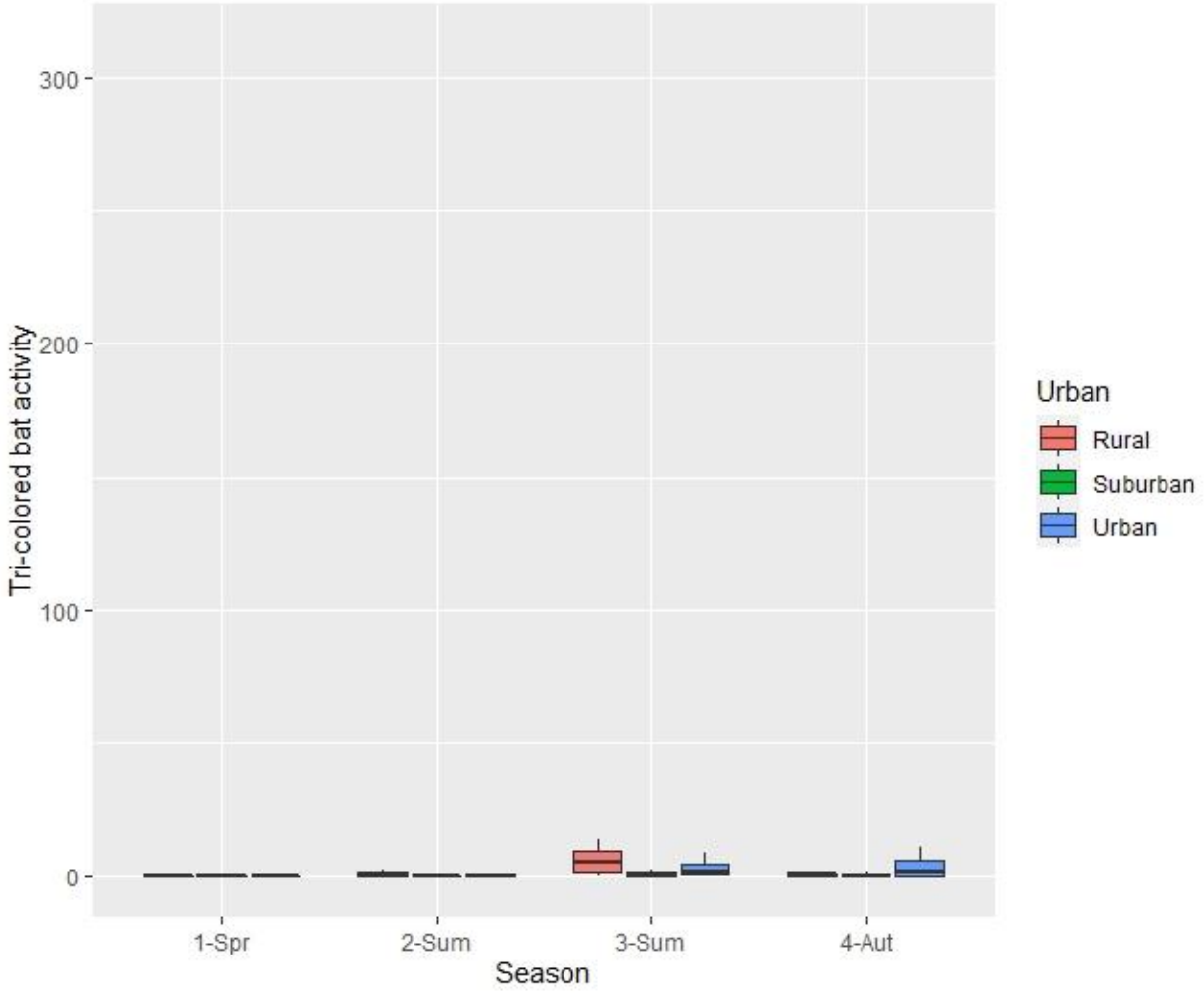
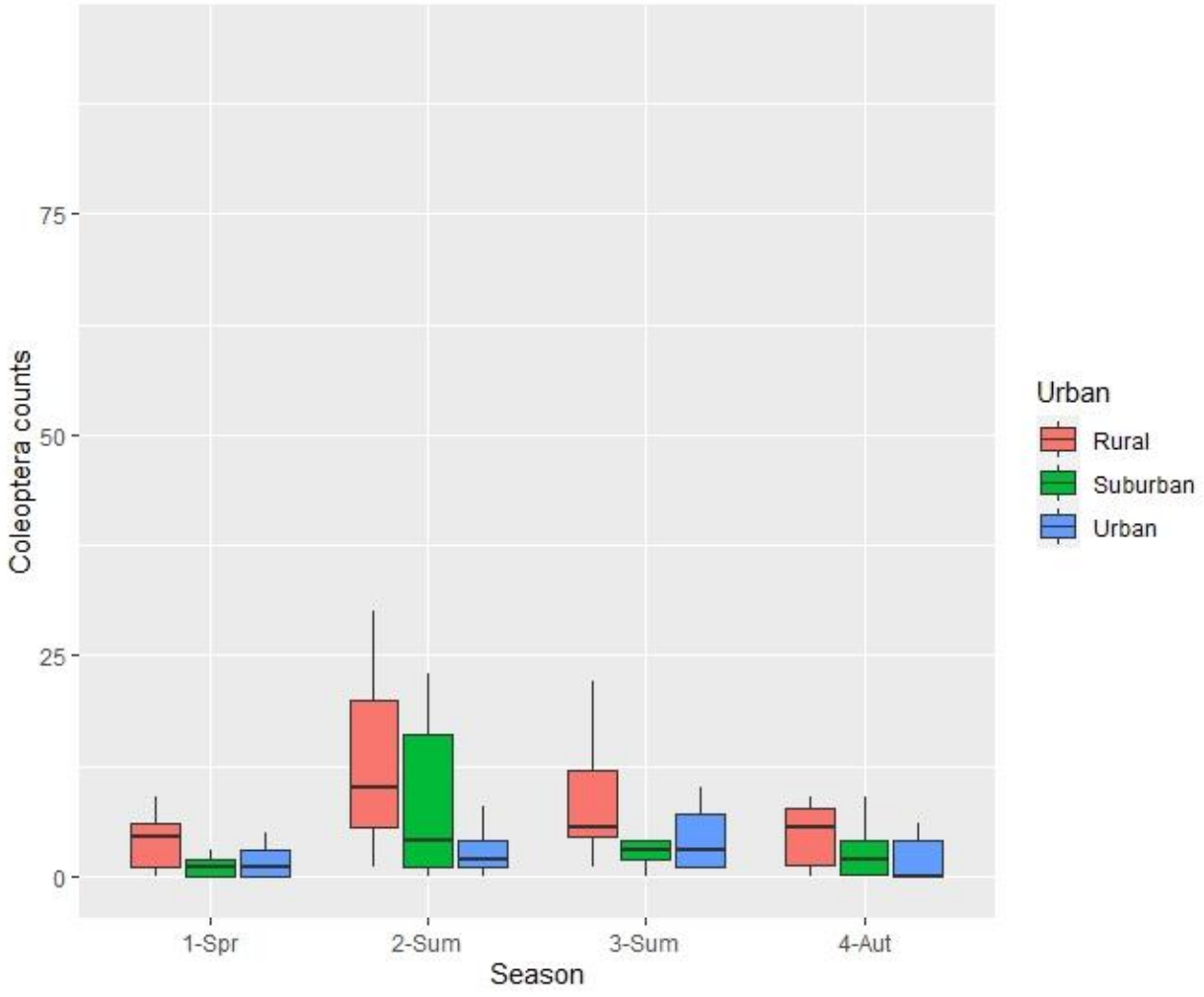


Fig. 6.—Coleoptera count changed across the urban-rural gradient each season. Coleoptera were significantly highest at rural (red) sites during early summer and autumn. Coleoptera were positively correlated with big brown bat activity at urban sites during early summer and autumn and negatively correlated with tri-colored bat activity at rural sites in late summer. Statistical significance levels were set at $P < 0.05$.



TABLES

Table 9.—We created a novel definition for three distinct levels of urbanization (Urban, Suburban, and Rural) due to historic definitions not applying in our Mid-Atlantic region. We required specific amounts of impervious surface (I.S.), forested, and open land categories in addition to human population size.

Variable	Urban	Suburban	Rural
Population size	50,000+	2,500 - 5,000	< 2,500
% I.S.	> 90.00% and < 100.00%	> 45.00 % and < 55.00 %	> 0.00 % and < 10.00 %
% Not I.S.	0.001-10%	45-55%	90-99.999%
% Forest (10% of Not I.S.)	> 0.1 %	> 4.50 %	> 9.00 %
% Open (10% of Not I.S.)	> 0.1 %	> 4.50 %	> 9.00 %

Table 10.—Bat activity and insects were compared among three urbanization levels (Urban, Suburban, and Rural), three years (2015, 2016, and 2017), and four seasons (Spring, Early summer, Late summer, and Autumn) using Kruskal-Wallace rank sum test to determine if there were any significant differences of $P < 0.05$ (indicated by * asterisk).

Dependent variable	Independent variable	χ^2	df	P - value
Big brown bat activity	Urbanization level	12.31	2	< 0.01 *
Tri-colored bat activity	Urbanization level	18.33	2	< 0.001 *
Blattodea	Urbanization level	1.04	2	0.59
Coleoptera	Urbanization level	23.03	2	< 0.001 *
Diptera	Urbanization level	6.52	2	0.04 *
Ephemeroptera	Urbanization level	1.04	2	0.60
Hemiptera	Urbanization level	2.48	2	0.29
Hymenoptera	Urbanization level	1.38	2	0.50

Lepidoptera	Urbanization level	0.65	2	0.72
Orthoptera	Urbanization level	5.00	2	0.08
Trichoptera	Urbanization level	8.30	2	0.02 *
Insect Shannon H diversity	Urbanization level	3.87	2	0.14
Big brown bat activity	Year	3.53	2	0.17
Tri-colored bat activity	Year	0.85	2	0.65
Blattodea	Year	0.87	2	0.65
Coleoptera	Year	14.38	2	< 0.001 *
Diptera	Year	4.55	2	0.10
Ephemeroptera	Year	0.87	2	0.65
Hemiptera	Year	2.77	2	0.25
Hymenoptera	Year	2.79	2	0.25
Lepidoptera	Year	3.54	2	0.17
Orthoptera	Year	2.20	2	0.33
Trichoptera	Year	3.52	2	0.17
Insect Shannon H diversity	Year	1.71	2	0.43
Big brown bat activity	Season	35.28	3	< 0.001 *
Tri-colored bat activity	Season	50.60	3	< 0.001 *
Blattodea	Season	6.12	3	0.11
Coleoptera	Season	20.16	3	< 0.001 *
Diptera	Season	12.55	3	0.01 *
Ephemeroptera	Season	1.85	3	0.60
Hemiptera	Season	33.98	3	< 0.001 *
Hymenoptera	Season	0.11	3	0.99
Lepidoptera	Season	4.29	3	0.23
Orthoptera	Season	5.42	3	0.14
Trichoptera	Season	2.19	3	0.53
Insect Shannon H diversity	Season	25.11	3	< 0.001 *

Table 11.—Differences in bat echolocation activity, insect order counts, and insect diversity in comparisons between each urbanization levels (Urban, Suburban, and Rural) and each season (Spring, Early summer, Late summer, and Autumn) were explored using Dunn's multiple comparison post hoc test with Bonferroni correction. Significant results ($P < 0.05$) are indicated by asterisk.

Dependent variable	Independent variable	Comparison	Z	P. unadjusted	P. adjusted
Big brown bat activity	Urbanization level	Rural - Suburban	2.92	0.00	0.01 *
		Rural - Urban	-0.24	0.81	1.00
		Suburban - Urban	-3.13	0.00	0.01 *
Tri-colored bat activity	Urbanization level	Rural - Suburban	3.95	< 0.001	< 0.001 *
		Rural - Urban	0.53	0.60	1.00
		Suburban - Urban	-3.38	< 0.001	< 0.01 *
Blattodea	Urbanization level	Rural - Suburban	0.86	0.39	1.00
		Rural - Urban	-0.04	0.97	1.00
		Suburban - Urban	-0.90	0.37	1.00
Coleoptera	Urbanization level	Rural - Suburban	3.47	< 0.001	< 0.01 *
		Rural - Urban	4.60	< 0.001	< 0.001 *
		Suburban - Urban	1.17	0.24	0.72
Diptera	Urbanization level	Rural - Suburban	1.82	0.07	0.21
		Rural - Urban	2.46	0.01	0.04 *
		Suburban - Urban	0.67	0.50	1.00
Ephemeroptera	Urbanization level	Rural - Suburban	0.87	0.38	1.00
		Rural - Urban	-0.03	0.98	1.00
		Suburban - Urban	-0.89	0.37	1.00
Hemiptera	Urbanization level	Rural - Suburban	-0.86	0.39	1.00
		Rural - Urban	-1.57	0.12	0.35
		Suburban - Urban	-0.73	0.47	1.00
Hymenoptera	Urbanization level	Rural - Suburban	0.83	0.41	1.00
		Rural - Urban	1.14	0.26	0.77
		Suburban - Urban	0.32	0.75	1.00

Lepidoptera	Urbanization level	Rural - Suburban	-0.73	0.47	1.00
		Rural - Urban	-0.06	0.95	1.00
		Suburban - Urban	0.66	0.51	1.00
Orthoptera	Urbanization level	Rural - Suburban	-2.21	0.03	0.08
		Rural - Urban	-0.83	0.41	1.00
		Suburban - Urban	1.37	0.17	0.52
Trichoptera	Urbanization level	Rural - Suburban	2.37	0.02	0.05
		Rural - Urban	2.60	0.01	0.03 *
		Suburban - Urban	0.27	0.79	1.00
Insect Shannon H diversity	Urbanization level	Rural - Suburban	0.37	0.71	1.00
		Rural - Urban	1.86	0.06	0.19
		Suburban - Urban	1.50	0.13	0.40
Big brown bat activity	Season	Spring - Early summer	-2.48	0.01	0.08
		Spring - Late summer	-5.88	< 0.001	< 0.001 *
		Early summer - Late summer	-3.31	< 0.001	0.01 *
		Spring - Autumn	-2.25	0.03	0.15
		Early summer - Autumn	0.17	0.86	1.00
		Late summer - Autumn	3.41	< 0.001	< 0.01 *
Tri-colored bat activity	Season	Spring - Early summer	-2.84	0.00	0.03 *
		Spring - Late summer	-6.96	< 0.001	< 0.001 *
		Early summer - Late summer	-4.02	< 0.001	< 0.001 *
		Spring - Autumn	-4.26	< 0.001	< 0.001 *
		Early summer - Autumn	-1.48	0.14	0.84
		Late summer - Autumn	2.40	0.02	0.10
Blattodea	Season	Spring - Early summer	-2.03	0.04	0.26
		Spring - Late summer	0.00	1.00	1.00
		Early summer - Late summer	2.07	0.04	0.23
		Spring - Autumn	0.00	1.00	1.00
		Early summer - Autumn	1.97	0.05	2.93

		Late summer - Autumn	0.00	1.00	1.00
Coleoptera	Season	Spring - Early summer	-3.62	< 0.001	< 0.01 *
		Spring - Late summer	-3.53	< 0.001	< 0.01 *
		Early summer - Late summer	0.19	0.85	1.00
		Spring - Autumn	0.40	0.40	1.00
		Early summer - Autumn	0.01	0.01	0.04 *
		Late summer - Autumn	0.01	0.01	0.06
Diptera	Season	Spring - Early summer	-2.66	0.01	0.05
		Spring - Late summer	-1.91	0.06	0.34
		Early summer - Late summer	0.82	0.41	1.00
		Spring - Autumn	0.38	0.70	1.00
		Early summer - Autumn	2.97	0.00	0.02 *
		Late summer - Autumn	2.24	0.03	0.15
Ephemeroptera	Season	Spring - Early summer	-1.01	0.31	1.00
		Spring - Late summer	-0.94	0.35	1.00
		Early summer - Late summer	0.10	0.92	1.00
		Spring - Autumn	0.00	1.00	1.00
		Early summer - Autumn	0.99	0.32	1.00
		Late summer - Autumn	0.91	0.36	1.00
Hemiptera	Season	Spring - Early summer	-4.26	< 0.001	< 0.001 *
		Spring - Late summer	-4.86	< 0.001	< 0.001 *
		Early summer - Late summer	-0.49	0.62	1.00
		Spring - Autumn	-4.99	< 0.001	< 0.001 *
		Early summer - Autumn	-0.83	0.41	1.00
		Late summer - Autumn	-0.37	0.71	1.00
Hymenoptera	Season	Spring - Early summer	-0.22	0.82	1.00
		Spring - Late summer	0.08	0.93	1.00
		Early summer - Late summer	0.31	0.76	1.00
		Spring - Autumn	-0.14	0.89	1.00

		Early summer - Autumn	0.08	0.93	1.00
		Late summer - Autumn	-0.22	0.83	1.00
Lepidoptera	Season	Spring - Early summer	-0.92	0.39	1.00
		Spring - Late summer	-0.37	0.71	1.00
		Early summer - Late summer	0.57	0.57	1.00
		Spring - Autumn	-1.94	0.05	0.31
		Early summer - Autumn	-1.04	0.30	1.00
		Late summer - Autumn	-1.62	0.11	0.63
Orthoptera	Season	Spring - Early summer	-0.91	0.36	1.00
		Spring - Late summer	-2.02	0.04	0.26
		Early summer - Late summer	-1.09	0.28	1.00
		Spring - Autumn	-1.91	0.06	0.34
		Early summer - Autumn	-1.02	0.31	1.00
		Late summer - Autumn	0.02	0.98	1.00
Trichoptera	Season	Spring - Early summer	-1.15	0.25	1.00
		Spring - Late summer	-0.30	0.77	1.00
		Early summer - Late summer	0.88	0.38	1.00
		Spring - Autumn	0.26	0.80	1.00
		Early summer - Autumn	1.37	0.17	1.00
		Late summer - Autumn	0.55	0.58	1.00
Insect Shannon H diversity	Season	Spring - Early summer	-3.52	< 0.001	< 0.01 *
		Spring - Late summer	-4.86	< 0.001	< 0.001 *
		Early summer - Late summer	-1.24	0.22	1.00
		Spring - Autumn	-2.75	0.01	0.04 *
		Early summer - Autumn	0.68	0.49	1.00
		Late summer - Autumn	1.91	0.06	0.34

Table 12.—Bat echolocation activity was recorded each night and averaged each week. Insects were collected during the entire week of bat surveys. Results are displayed within each year as median followed by range in parentheses.

Variable	2015 (n=55)	2016 (n=63)	2017 (n=64)
Bat species richness	1 (0 - 3)	1 (0 - 2.14)	0.94 (0 - 2)
Big brown bat activity	19.83 (0 - 1,900)	12.57 (0 - 1,367.2)	4.65 (0 - 707.5)
Little brown bat activity	0 (0 - 3.86)	0 (0 - 0.71)	0 (0 - 0.29)
Northern long-eared bat activity	0 (0 - 0.17)	0 (0 - 0)	0 (0 - 1.07)
Tri-colored bat activity	0.42 (0 - 9.14)	0.09 (0 - 312.4)	0.08 (0 - 13.57)
Total bat activity	20 (0 - 1,900.75)	14.14 (0 - 1,372.6)	5.17 (0 - 718.33)
Blattodea	0 (0 – 0)	0 (0 - 1)	0 (0 - 7)
Coleoptera	6 (0 - 94)	3 (0 - 51)	2 (0 - 23)
Diptera	9 (0 - 178)	9 (0 - 89)	5 (0 - 77)
Ephemeroptera	0 (0 - 0)	0 (0 - 1)	0 (0 - 1)
Hemiptera	7 (0 - 185)	10 (0 - 699)	7 (0 - 114)
Hymenoptera	5 (0 - 372)	6 (0 - 53)	4 (0 - 80)
Lepidoptera	0 (0 - 1)	0 (0 - 4)	0 (0 - 1)
Orthoptera	0 (0 - 2)	0 (0 - 1)	0 (0 - 6)
Trichoptera	0 (0 - 13)	0 (0 - 2)	0 (0 - 8)
Total insects	45 (2 - 379)	45 (6 - 725)	27.5 (1 - 166)
Insect order richness	4 (1 - 6)	4 (2 - 6)	4 (1 - 7)
Insect Shannon H diversity	6.73 (0.13 - 15.9)	7.12 (1.83 - 20.99)	6.71 (0 - 17.44)

Table 13. Species-specific echolocation activity, total bat activity, and species richness were collected each night at each site then averaged each week to match timing of insects which were collected each week. All seasons and all years were combined. Results are displayed within each urbanization level (Urban, Suburban, and Rural) as median with range inside parenthesis.

Variable	Urban (n=59)	Suburban (n=62)	Rural (n=61)
Bat species richness	1 (0 - 2.43)	0.82 (0 - 2.14)	1.2 (0 - 3)
Big brown bat activity	13.67 (0 - 1,900)	3.48 (0 - 343.57)	21.57 (0 - 503.43)
Little brown bat activity	0 (0 - 2)	0 (0 - 0.71)	0 (0 - 3.86)
Northern long-eared bat activity	0 (0 - 0)	0 (0 - 1.07)	0 (0 - 0)
Tri-colored bat activity	0.40 (0 - 312.4)	0 (0 - 6.79)	0.6 (0 - 28.57)
Total bat activity	14.5 (0 - 1,900.75)	4 (0 - 343.57)	23.86 (0 - 505.57)
Blattodea	0 (0 - 7)	0 (0 - 0)	0 (0 - 1)
Coleoptera	2 (0 - 24)	2.5 (0 - 94)	6 (0 - 51)
Diptera	6 (0 - 89)	6 (0 - 85)	10 (0 - 178)
Ephemeroptera	0 (0 - 1)	0 (0 - 0)	0 (0 - 1)
Hemiptera	10 (0 - 110)	8 (0 - 699)	7 (0 - 114)
Hymenoptera	5 (0 - 372)	5 (0 - 55)	6 (1 - 259)
Lepidoptera	0 (0 - 4)	0 (0 - 1)	0 (0 - 1)
Orthoptera	0 (0 - 6)	0 (0 - 1)	0 (0 - 1)
Trichoptera	0 (0 - 3)	0 (0 - 2)	0 (0 - 13)
Total insects	33 (1 - 379)	32 (3 - 725)	38 (7 - 308)
Insect order richness	4 (1 - 6)	4 (1 - 5_	4 (1 - 7)
Insect Shannon H diversity	6.61 (0 - 18.08)	7.28 (0.41 - 20.99)	7.29 (0.13 - 17.44)

Table 14.—Mean and ranges for bat echolocation activity, insect order counts and diversity were calculated within each season (Spring, Early summer, Late summer, and Autumn).

Variable	Spring	Early summer	Late summer	Autumn
Big brown bat activity	3.3 (0 - 119)	18 (0 - 503.43)	60.73 (0 - 1,900)	4 (0 - 1,376.9)
Tri-colored bat activity	0 (0 - 1.17)	0.08 (0 - 8.14)	1.34 (0 - 28.57)	0.5 (0 - 312.4)
Blattodea	0 (0 - 0)	0 (0 - 7)	0 (0 - 0)	0 (0 - 0)
Coleoptera	2 (0 - 22)	5 (0 - 94)	4 (0 - 37)	2 (0 - 34)
Diptera	6 (0 - 77)	10 (0 - 178)	9 (0 - 85)	4 (0 - 64)
Ephemeroptera	0 (0 - 0)	0 (0 - 1)	0 (0 - 1)	0 (0 - 0)
Hemiptera	2 (0 - 21)	8 (0 - 157)	9.5 (1 - 699)	11 (0 - 114)
Hymenoptera	5 (0 - 372)	6 (0 - 80)	5 (0 - 259)	5 (0 - 56)
Lepidoptera	0 (0 - 1)	0 (0 - 4)	0 (0 - 1)	0 (0 - 1)
Orthoptera	0 (0 - 0)	0 (0 - 6)	0 (0 - 1)	0 (0 - 2)
Trichoptera	0 (0 - 8)	0 (0 - 13)	0 (0 - 5)	0 (0 - 2)
Total insects	22 (1 - 379)	53 (3 - 233)	47 (4 - 725)	33 (6 - 169)
Insect Shannon H diversity	5.49 (0 - 11.1)	6.82 (0.41 - 18.08)	7.7 (1.57 - 20.99)	6.81 (1.75 - 13.67)

Table 15.—Three sticky traps were placed on the first day of each survey and collected seven days later. Individual insects were later identified to order-level and summed for each survey. Total insect orders, order richness, and insect Shannon H diversity are presented below within each urbanization level.

Variable	Urban (n = 59)	Suburban (n = 62)	Rural (n = 61)	Total (n = 182)
Blattodea	7	0	1	8
Coleoptera	181	368	543	1,092
Diptera	546	666	1,109	2,321
Ephemeroptera	1	0	1	2
Hemiptera	1,158	1,750	708	3,616
Hymenoptera	989	675	972	2,636
Lepidoptera	6	5	3	14
Orthoptera	9	7	1	17
Trichoptera	4	4	36	44
Total insects	3,195	3,671	3,573	10,439
Maximum insect order richness	9	6	9	9

Table 16.—Correlation results between big brown bat activity (EPFU) and tri-colored bat activity (PESU) for all insect counts, total insects, and insect Shannon H diversity. Results are presented within each discrete urbaniztation level (Urban, Suburabn, and Rural). Correlations were not adjusted and all comparisons are reported along with degrees of freedom (unless noted by NA indicating not applicable). Insect codes include: Blattodea (BLAT), Coleoptera (COLE), Diptera (DIPT), Hemiptera (HEMI), Hymenoptera (HYME), Lepidoptera (LEPI), Orthoptera (ORTH), Trichoptera (TRIC), total insects (TOTA), and insect Shannon H diversity (SHAN). Results presented below include Spearman rho, degrees of freedom, followed by *P* value inside parentheses. Significant differences ($P < 0.05$) are indicated by asterisk * and bolded.

Level	Spring	Early summer	Late summer	Autumn
Urban	EPFU & BLATT (NA, 14, NA)	EPFU & BLATT (-0.463, 11, 0.111)	EPFU & BLATT (NA, 15, NA)	EPFU & BLATT (NA, 11, NA)
	EPFU & COLE (0.305, 14, 0.250)	**EPFU & COLE (0.632, 11, 0.020) ***	EPFU & COLE (0.219, 15, 0.398)	**EPFU & COLE (0.628, 11, 0.022) ***
	EPFU & DIPT (-0.251, 14, 0.348)	EPFU & DIPT (-0.206, 11, 0.499)	EPFU & DIPT (0.229, 15, 0.378)	**EPFU & DIPT (0.608, 11, 0.028) ***
	EPFU & EPHE (NA, 14, NA)	EPFU & EPHE (0.231, 11, 0.447)	EPFU & EPHE (NA, 15, NA)	EPFU & EPHE (NA, 11, NA)
	EPFU & HEMI (0.411, 14, 0.114)	**EPFU & HEMI (0.754, 11, 0.003) ***	**EPFU & HEMI (-0.536, 15, 0.027) ***	EPFU & HEMI (-0.259, 11, 0.394)
	EPFU & HYME (-0.133, 14, 0.622)	EPFU & HYME (-0.233, 11, 0.444)	EPFU & HYME (0.031, 15, 0.906)	EPFU & HYME (0.249, 11, 0.412)
	EPFU & LEPI (-0.196, 14, 0.467)	EPFU & LEPI (0.096, 11, 0.755)	EPFU & LEPI (NA, 15, NA)	EPFU & LEPI (NA, 11, NA)
	EPFU & ORTH (NA, 14, NA)	EPFU & ORTH (-0.463, 11, 0.111)	EPFU & ORTH (NA, 15, NA)	EPFU & ORTH (-0.306, 11, 0.310)
	EPFU & TRIC (NA, 14, NA)	EPFU & TRIC (0.463, 11, 0.111)	EPFU & TRIC (0.408, 15, 0.104)	EPFU & TRIC (NA, 11, NA)
	EPFU & SHAN (0.250, 14, 0.435)	**EPFU & SHAN (0.692, 11, 0.009) ***	EPFU & SHAN (-0.318, 15, 0.214)	EPFU & SHAN (0.132, 11, 0.668)
	PESU & BLATT (NA, 14, NA)	PESU & BLATT (-0.353, 11, 0.237)	PESU & BLATT (NA, 15, NA)	PESU & BLATT (NA, 11, NA)
	PESU & COLE (0.035, 14, 0.898)	PESU & COLE (0.153, 11, 0.618)	PESU & COLE (-0.268, 15, 0.298)	PESU & COLE (-0.111, 11, 0.717)

	PESU & DIPT (-0.319, 14, 0.229)	PESU & DIPT (0.306, 11, 0.309)	PESU & DIPT (-0.088, 15, 0.738)	PESU & DIPT (0.368, 11, 0.216)
	PESU & EPHE (NA, 14, NA)	PESU & EPHE (-0.353, 11, 0.237)	PESU & EPHE (NA, 15, NA)	PESU & EPHE (NA, 11, NA)
	PESU & HEMI (-0.288, 14, 0.279)	PESU & HEMI (-0.095, 11, 0.758)	PESU & HEMI (-0.103, 15, 0.695)	PESU & HEMI (-0.136, 11, 0.659)
	PESU & HYME (-0.570, 14, 0.704)	PESU & HYME (0.384, 11, 0.195)	PESU & HYME (0.128, 15, 0.625)	PESU & HYME (0.203, 11, 0.506)
	PESU & LEPI (-0.123, 14, 0.649)	PESU & LEPI (-0.084, 11, 0.784)	PESU & LEPI (NA, 15, NA)	PESU & LEPI (NA, 11, NA)
	PESU & ORTH (NA, 14, NA)	PESU & ORTH (-0.353, 11, 0.237)	PESU & ORTH (NA, 15, NA)	PESU & ORTH (-0.097, 11, 0.754)
	PESU & TRIC (NA, 14, NA)	PESU & TRIC (-0.353, 11, 0.237)	PESU & TRIC (-0.281, 15, 0.275)	PESU & TRIC (NA, 11, NA)
	PESU & SHAN (-0.125, 14, 0.644)	PESU & SHAN (-0.357, 11, 0.231)	PESU & SHAN (-0.358, 15, 0.159)	PESU & SHAN (-0.044, 11, 0.886)
Suburban	EPFU & BLATT (NA, 12, NA)	EPFU & BLATT (NA, 15, NA)	EPFU & BLATT (NA, 15, NA)	EPFU & BLATT (NA, 12, NA)
	EPFU & COLE (0.053, 12, 0.857)	EPFU & COLE (0.245, 15, 0.344)	EPFU & COLE (0.075, 15, 0.775)	EPFU & COLE (0.162, 12, 0.581)
	EPFU & DIPT (-0.191, 12, 0.514)	EPFU & DIPT (0.133, 15, 0.611)	EPFU & DIPT (-0.178, 15, 0.495)	EPFU & DIPT (-0.236, 12, 0.416)
	EPFU & EPHE (NA, 12, NA)	EPFU & EPHE (NA, 15, NA)	EPFU & EPHE (NA, 15, NA)	EPFU & EPHE (NA, 12, NA)
	EPFU & HEMI (0.344, 12, 0.229)	EPFU & HEMI (0.069, 15, 0.792)	EPFU & HEMI (-0.020, 15, 0.940)	EPFU & HEMI (-0.396, 12, 0.162)
	EPFU & HYME (0.022, 12, 0.940)	EPFU & HYME (0.119, 15, 0.650)	EPFU & HYME (0.045, 15, 0.864)	EPFU & HYME (0.000, 12, 1.000)
	EPFU & LEPI (NA, 12, NA)	EPFU & LEPI (NA, 15, NA)	EPFU & LEPI (0.037, 15, 0.887)	EPFU & LEPI (-0.369, 12, 0.195)
	EPFU & ORTH (NA, 12, NA)	EPFU & ORTH (0.051, 15, 0.845)	EPFU & ORTH (0.085, 15, 0.746)	EPFU & ORTH (-0.153, 12, 0.603)

	EPFU & TRIC (0.380, 12, 0.180)	EPFU & TRIC (NA, 15, NA)	EPFU & TRIC (-0.408, 15, 0.104)	EPFU & TRIC (0.380, 12, 0.180)
	EPFU & SHAN (0.338, 12, 0.238)	EPFU & SHAN (-0.164, 15, 0.529)	EPFU & SHAN (-0.066, 15, 0.801)	EPFU & SHAN (-0.144, 12, 0.623)
	PESU & BLATT (NA, 12, NA)	PESU & BLATT (NA, 15, NA)	PESU & BLATT (NA, 15, NA)	PESU & BLATT (NA, 12, NA)
	PESU & COLE (-0.287, 12, 0.320)	PESU & COLE (0.063, 15, 0.810)	PESU & COLE (-0.165, 15, 0.528)	PESU & COLE (0.342, 12, 0.231)
	PESU & DIPT (-0.069, 12, 0.815)	PESU & DIPT (0.165, 15, 0.526)	PESU & DIPT (-0.201, 15, 0.440)	PESU & DIPT (0.311, 12, 0.279)
	PESU & EPHE (NA, 12, NA)	PESU & EPHE (NA, 15, NA)	PESU & EPHE (NA, 15, NA)	PESU & EPHE (NA, 12, NA)
	PESU & HEMI (0.141, 12, 0.631)	PESU & HEMI (-0.175, 15, 0.502)	PESU & HEMI (0.389, 15, 0.122)	PESU & HEMI (0.222, 12, 0.446)
	PESU & HYME (0.104, 12, 0.725)	PESU & HYME (0.053, 15, 0.840)	PESU & HYME (-0.399, 15, 0.113)	PESU & HYME (0.266, 12, 0.358)
	PESU & LEPI (NA, 12, NA)	PESU & LEPI (NA, 15, NA)	PESU & LEPI (-0.121, 15, 0.644)	PESU & LEPI (0.096, 12, 0.745)
	PESU & ORTH (NA, 12, NA)	PESU & ORTH (-0.137, 15, 0.600)	PESU & ORTH (-0.092, 15, 0.726)	PESU & ORTH (0.084, 12, 0.775)
	PESU & TRIC (-0.077, 12, 0.794)	PESU & TRIC (NA, 15, NA)	PESU & TRIC (-0.221, 15, 0.394)	PESU & TRIC (-0.229, 12, 0.432)
	PESU & SHAN (0.103, 12, 0.726)	PESU & SHAN (-0.270, 15, 0.295)	PESU & SHAN (0.102, 15, 0.696)	PESU & SHAN (0.174, 12, 0.553)
Rural	EPFU & BLATT (NA, 14, NA)	EPFU & BLATT (-0.310, 13, 0.262)	EPFU & BLATT (NA, 14, NA)	EPFU & BLATT (NA, 12, NA)
	EPFU & COLE (-0.098, 14, 0.717)	EPFU & COLE (0.085, 13, 0.763)	EPFU & COLE (0.208, 14, 0.440)	EPFU & COLE (0.357, 12, 0.210)
	EPFU & DIPT (-0.038, 14, 0.890)	EPFU & DIPT (0.412, 13, 0.127)	EPFU & DIPT (-0.070, 14, 0.798)	EPFU & DIPT (0.315, 12, 0.273)
	EPFU & EPHE (NA, 14, NA)	EPFU & EPHE (NA, 13, NA)	EPFU & EPHE (-0.364, 14, 0.166)	EPFU & EPHE (NA, 12, NA)

EPFU & HEMI (-0.146, 14, 0.590)	EPFU & HEMI (0.058, 13, 0.837)	EPFU & HEMI (0.093, 14, 0.732)	EPFU & HEMI (-0.068, 12, 0.817)
EPFU & HYME (0.077, 14, 0.778)	EPFU & HYME (0.308, 13, 0.263)	EPFU & HYME (0.219, 14, 0.416)	EPFU & HYME (-0.122, 12, 0.679)
EPFU & LEPI (NA, 14, NA)	EPFU & LEPI (0.400, 13, 0.262)	EPFU & LEPI (NA, 14, NA)	EPFU & LEPI (0.152, 12, 0.604)
EPFU & ORTH (NA, 14, NA)	EPFU & ORTH (NA, 13, NA)	EPFU & ORTH (-0.364, 14, 0.166)	EPFU & ORTH (NA, 12, NA)
EPFU & TRIC (-0.420, 14, 0.105)	EPFU & TRIC (0.297, 13, 0.282)	EPFU & TRIC (-0.263, 14, 0.324)	EPFU & TRIC (0.310, 12, 0.281)
EPFU & SHAN (-0.484, 14, 0.057)	EPFU & SHAN (-0.009, 13, 0.975)	EPFU & SHAN (-0.277, 14, 0.300)	EPFU & SHAN (0.213, 12, 0.464)
PESU & BLATT (NA, 14, NA)	PESU & BLATT (0.063, 13, 0.823)	PESU & BLATT (NA, 14, NA)	PESU & BLATT (NA, 12, NA)
PESU & COLE (-0.029, 14, 0.914)	PESU & COLE (0.453, 13, 0.090)	**PESU & COLE (-0.537, 14, 0.032) ***	PESU & COLE (-0.106, 12, 0.718)
PESU & DIPT (-0.211, 14, 0.433)	**PESU & DIPT (0.552, 13, 0.033) ***	PESU & DIPT (0.269, 14, 0.314)	PESU & DIPT (-0.248, 12, 0.393)
PESU & EPHE (NA, 14, NA)	PESU & EPHE (NA, 13, NA)	PESU & EPHE (-0.252, 14, 0.346)	PESU & EPHE (NA, 12, NA)
PESU & HEMI (-0.610, 14, 0.013) *	**PESU & HEMI (0.568, 13, 0.027) ***	PESU & HEMI (-0.212, 14, 0.430)	PESU & HEMI (0-0.063, 12, 0.830)
PESU & HYME (0.239, 14, 0.373)	PESU & HYME (0.502, 13, 0.057)	PESU & HYME (0.084, 14, 0.757)	PESU & HYME (-0.012, 12, 0.969)
PESU & LEPI (NA, 14, NA)	PESU & LEPI (0.158, 13, 0.575)	PESU & LEPI (NA, 14, NA)	PESU & LEPI (0.518, 12, 0.058)
PESU & ORTH (NA, 14, NA)	PESU & ORTH (NA, 13, NA)	PESU & ORTH (-0.252, 14, 0.346)	PESU & ORTH (NA, 12, NA)
PESU & TRIC (-0.215, 14, 0.424)	PESU & TRIC (0.438, 13, 0.103)	PESU & TRIC (-0.119, 14, 0.661)	PESU & TRIC (-0.317, 12, 0.270)
PESU & SHAN (-0.400, 14, 0.125)	PESU & SHAN (0.140, 13, 0.618)	PESU & SHAN (0.440, 14, 0.088)	**PESU & SHAN (0.551, 12, 0.041) ***

Chapter 4: Habitat features important to cave bats along the urban-rural gradient

ABSTRACT

Urban habitat resources are rarely investigated for bat species, especially cave bat populations across much of North America. Unfortunately, many of those species have been decimated by White-nose Syndrome (WNS), a condition caused by a psychrophilic fungus that occurs in cold and humid environments. Given the disparate nature of the preferred climate of the fungus that leads to WNS and the warmer, drier climate of most cities, urban areas, in some ways, may better serve vulnerable cave bat species. Therefore, we surveyed cave bat activity and available habitat features at 18 sites across the urban-rural gradient from March-October in 2015-2017. Using general linear mixed modelling, we identified the relationships between specific habitat features, seasons, urbanization, and bat activity. Habitat features included diet, climate, light pollution, roost, sound pollution, space, and water. These variables' conditions can change across time, so we incorporated seasonal and annual temporal changes. Individual species activity models were created for each part of the urban-rural gradient. In our urban models, the big brown bat (*Eptesicus fuscus*) model contained Coleoptera counts, season, and temperature while season, temperature, and year were used in the model for tri-colored bat (*Perimyotis subflavus*). Suburban models for big brown bat contained Coleoptera counts, season, temperature, and tree height; while for tri-colored bats, they contained just season. The big brown bat model at rural sites included season, temperature, and year while the tri-colored bat's rural model included Diptera counts, season, temperature, total insect counts, and year. Big brown bat activity increased at suburban sites with taller trees and higher Coleoptera counts while tri-colored bat activity was strongly related to higher Diptera counts at rural sites and sites generally

experiencing less big brown bat activity. Our results indicated interspecific competition avoidance may influence some of their activity. To further help species survive competition during WNS, we provided gradient-specific recommendations to support these species of greatest conservation need.

KEYWORDS

activity, bat, habitat, urban-rural gradient, *Perimyotis subflavus*, White-nose Syndrome

INTRODUCTION

Historically, urbanization has been known to negatively impact bats by removing roosting habitat, decreasing prey, introducing exotic predators, increasing vehicular collisions, and adding light, sound, and chemical pollution (Hutson and Mickleburgh 2001; Baker and Harris 2007; Russo and Ancillotto 2014; Santini et al. 2019; Frick et al. 2020). However, urban areas can provide additional habitat resources such as drinking water from ponds and pools, prey attracted to streetlights, roosting sites in buildings and bridges, and protection from natural predators (Gehrt and Chelsvig 2004; Russo and Ancillotto 2014; Parkins and Clark 2015; Voigt et al. 2016). Beyond these additional habitat resources, urban areas also provide stable, warmer, and drier climates due to the "heat island effect" where impervious surfaces absorb solar radiation during the day which slowly releases at night (Jung and Threlfall 2016). This warm and dry urban climate is a stark contrast to bat hibernacula which is characteristically cold and humid. Cold and humid environments allow fungi, such as *Pseudogymnoascus destructans* (Pd), to thrive. Pd is the fungus known to cause White-nose Syndrome (WNS), a cascading disease process in which Pd attaches to the skin membrane of cave bats, leading to excessive arousals and grooming (Brownlee-Bouboulis and Reeder 2013), causing significant depletion of

hydration, nutrition, electrolytes, and many times resulting in death. Unfortunately, much of the United States has lost much of their cave bat populations due to WNS (Hoyt et al. 2021).

While urban wildlife ecology is a blossoming field of science, urbanization as it relates to cave bat presence or activity during the WNS crisis has rarely been studied. Post-WNS' arrival, big brown bat (*Eptesicus fuscus*), tri-colored bat (*Perimyotis subflavus*), and total cave bat activity was reported as being higher at urban versus suburban sites (Chapter 2), which was supported by another study conducted prior to WNS' arrival (Gehrt and Chelsvig 2004). In Chapter 3, we reported significant correlation between cave bat species activity and insect counts/diversity along the urban-rural gradient, indicating that prey availability may be one of many key features to understanding the presence or lack of bat species activity across different levels of urbanization. These types of studies are crucial because cave bats known to inhabit urban areas during parts of the year (Timpone et al. 2010; Li and Kalcounis-Rueppell 2018; Bergeson et al. 2020; Deeley et al. 2021) are facing significant population declines due to WNS (O'Keefe et al. 2019; Nocera et al. 2020; Cheng et al. 2021; Hoyt et al. 2021). Their population sizes are so low that the United States Fish and Wildlife Service (USFWS) recently listed Northern long-eared bat (*M. septentrionalis*) as threatened and is considering adding little brown bat (*M. lucifugus*) and tri-colored bat to their threatened and endangered list (USFWS 2020).

Conservation agency efforts towards managing these populations would greatly benefit from learning these species' relationships with habitats where they are found across the urban-rural gradient. While a recent study in our region concluded one particular urban area appeared to serve as refugia to Northern long-eared bats (Deeley et al. 2021), most studies here were conducted prior to WNS' arrival in 2010 (MDDNR 2011) and did not investigate current relationships between individual species and available habitat. The objective of this study was to

identify specific habitat features that were related to cave bat activity across the urban-rural gradient. This the first study we are aware of to identify habitat features important to cave bats across the urban-rural gradient spanning multiple seasons and years during the WNS crisis.

MATERIALS AND METHODS

Study area.—To study the effects of urban habitats within a WNS region, we chose the state of Maryland as it is one of the most highly urbanized in the country (Campbell 2018) and has been managing for WNS since Pd was first discovered in the state in 2010 (MDDNR 2011). Maryland has an average annual precipitation of 91-117cm and temperature of 8-14º C. This small state contains five physiographic provinces, with urbanization highly concentrated in the Piedmont and Coastal Plain provinces. Much of the Piedmont province has been converted to agriculture with the remainder dominated by oak-pine forests. The Coastal Plain province supports many aquatic habitats with the remainder dominated by pine, oak-hickory and mixed forests (Paradiso 1969).

Site selection.—We selected 18 research sites in Maryland and adjacent counties equally among Piedmont and Coastal Plain provinces (Fig. 7). Traditional urbanization level definitions using distance from survey site to urban center/edge (Geggie and Fenton 1985; Lesinski et al. 2000; Avila-Flores and Fenton 2005; Johnson et al. 2008; Coleman and Barclay 2012; Li et al. 2020) were not appropriate for this region due to the high concentration of urban areas. Rather, we developed a novel definition for the urban-rural gradient using three unique levels of urban, suburban, and rural. Our new urbanization level definitions also allowed us to select sites that maintained heterogeneity and removed bias from dominance of any one habitat feature such as completely forested or completely agricultural landscapes. We set each urbanization level along

the gradient according to human population size and percentages of impervious surface, forest, and open land classifications. Specific amounts of each land class type were required to meet our definition (Table 17). Human population size data were downloaded from USCB (2010) while percent impervious surface data were downloaded from National Land Cover Database (NLCD; 2011). Land cover data were downloaded from NLCD (2011) and reclassified as "forest" (deciduous, evergreen, mixed forest, and woody wetlands), "open" (barren, agriculture, grassland, shrub/scrub, and emergence herbaceous wetland), "water" (all water), and "other" (all other land class categories). To maintain heterogeneity at sites, provide numerous replicates within each urbanization level to randomly select from, and allow for movement of the detector to avoid echoes or close proximity to water, we set our site selection scale to 150m^2. Each selected site was separated by > 2 km to avoid spatial autocorrelation (McGowan and Hogue 2016; Moretto et al. 2019). For our analysis, seasons were designated as spring (1) occurring from March through April, early summer (2) occurring from May through June, late summer (3) occurring from July through August, and autumn (4) was from September through October. These seasons are similar to Li and Wilkins (2014) and coincide with bats emerging from hibernacula, maintaining pregnancy, nursing/pup volancy, and swarming/mating/engorging prior to hibernation. To account for seasonal and annual variation, we selected to survey sites within each season without replacement using random selection of site ID, each year from 2015 to 2017. Bat activity can fluctuate drastically each individual night, therefore multiple consecutive nights of surveying are typically recommended (Hayes 2000; Niver et al. 2014). To account for such small-scale temporal variation, we surveyed ≥ four consecutive nights at each site within each season.

Bat detector settings.—We recorded bat echolocation calls at each site from sunset to sunrise (hereinafter referred to as nightly activity) for one week each season using Song Meter SM2BAT+ bat detectors (Wildlife Acoustics, Concord, MA). We placed SMX-U1 omnidirectional microphones 20 feet above ground to avoid echoes from solid surfaces, aimed them slightly below horizontal to avoid collection of rain, and calibrated them according to Parkins and Clark (2015). We set detectors to sample at a rate of 384,000 Hz, hpf fs/24, lpf off, trigger level of 18 dB, trigger window of 2.0 s, and maximum file length of 5 s.

Bat species identification.—We used two automated identification programs to identify bat echolocation calls to species-level. Manual vetting of calls was performed by primary author for all calls identified by either program as *Myotis* species and > 20 % of non-*Myotis* species using parameters such as highest frequency, lowest frequency, call duration, and more. Non-bat call files were removed using Sonobat Batch Utility Scrubber. Bat call files were run through Sonobat 4.2.2 (https://sonobat.com/) using the default setting for NNE bats. Bat call files were also run through Kaleidoscope Pro 4.3.0 (https://www.wildlifeacoustics.com/products/kaleidoscope-pro) with accuracy set to 0 Balanced (neutral) for Maryland bats. We excluded calls from migratory or unidentified bat species to allow our analysis to focus only on cave bat species activity.

Diet.—At the start of every survey, we placed three (Coleman and Barclay 2013) 3” x 5” sticky traps (Olson Products, Medina, OH) 20 feet above ground around a cylinder (Kunz 2009) to capture maximum number of insects available during our surveys. We selected sticky traps as they are affordable, less biased, and perform comparably to other traps in regards to insect order composition, numbers, and sizes (Sleep and Brigham 2003, Capinera 2008). We identified insects to order-level (Triplehorn et al. 2005) using a dissecting AmScope 20X microscope. We

calculated total insects and Shannon H diversity index based on the order counts collected during each survey (Moore and Best 2018).

Climate variables.—We placed a Kestrel Drop D3 wireless data logger (Kestrel Instruments, Boothwyn, PA) at each acoustic microphone to record temperature, relative humidity, and barometric pressure once per hour of surveying at each site. Data were downloaded and then averaged for each night from 1800 to 0600 hours. Hours of darkness, precipitation, and wind were obtained from nearest weather stations using WeatherUnderground.com and averaged for each night from 1800 to 0600 hours. Moon phase percent was obtained from Farmer's Almanac (www.almanac.com) for each night surveyed.

Anthropogenic variables.—A Hobo UA-002-64 pendant data logger (Onset, Cape Code, MA) was attached using zip ties to the top of the pole with each bat acoustic microphone to record ambient light levels once per hour at each site. A CEM DT-173 data logger (Ruby Electronics, Saratoga, CA) was attached half-way up the pole using zip ties to record ambient sound levels once per hour at each site. Light and sound levels were downloaded and then averaged for each night 1800 to 0600 hours.

Habitat variables.—Distances from bat detector to the closest tree, building, and light were measured using a Legend 1200 ARC rangefinder (Bushnell, Overland Park, KS). Distances to closest water, road, and edge were calculated using ArcGIS®. Diameter at breast height (DBH) and height of closest tree to bat detector in each cardinal direction were calculated using a Metric Fabric Tape (Forestry Suppliers, Jackson, MS) and a Tangent Height Gauge (Forestry Suppliers, Jackson, MS). Then the measurements were averaged per site. Four measures of percent canopy cover were calculated at the location of the microphone facing each cardinal direction using a spherical crown densitometer (Forestry Suppliers, Jackson, MS). Then, the four

measures were averaged. The total count of the number of snags and trees (> 16 cm DBH; Lacki et al. 2009) within a 30 meter radius of the detector, which was our microphone's average range of detection (Wildlife Acoustics, Concord, MA), was recorded.

Analysis.—To test for variables that affected bat activity, we modeled the number of calls per night with generalized linear mixed models (GLMM) using the package glmmTMB (Brooks et al. 2017). Models were fit with a zero-inflated negative binomial distribution and log link. Zero-inflated models are appropriate to use in ecology when experiencing excessive zeroes and overdispersion, causing variance to greatly exceed the mean, and assumes zeroes are due to two separate processes: sampling variance or process variance (Zuur et al. 2009). In our case, bat activity could have been truly absent because of process variations such as inclement weather, poor habitat quality, etc. or absent because of sampling variation such as incorrect species identification, distance of bat echolocation beyond our microphone's range, or attenuation of highest echolocation frequencies. If model residuals did not pass inspection, we evaluated if the cause was related to zero inflation by adding variables to the zero inflation component of the model (Long 1997). Our initial large number of possible explanatory variables identified from the literature compared to the limited number of sites we sampled, needed to be reduced to prevent overfitting and avoid issues with multicollinearity. We attempted PCA to reduce the number of variables but it did not result in any axis well describing multiple variables. We also tried forward selection of all variables. However, urbanization levels greatly interacted with most variables which needed to be addressed and resulted in models having convergence issues. Therefore, we used a novel three-step approach to decrease the number of variables used in big brown and tri-colored bat activity global models.

First, we subsetted the data frame into datasets for each urbanization level and reviewed continuous variables for high autocorrelation (rho = > 0.50) using Spearman's rank correlation, retaining the variable in the correlated pair that was the most biologically relevant based on the work of Ford et al. (2006) and Hale et al. (2012). Second, we created a global model for each species by identifying and removing variables that showed no correlative biological importance for big brown or tri-colored bat activity (rho < 0.3) following Hale et al. 2012. Global models also contained categorical fixed effects of season and year to address potential temporal variation. To control for the changing night length across the year, we included an offset of the log of the number of hours of darkness each night in all models. Third and finally, we created a simplified version of each global model through forward selection using the buildmer package (Voeten 2021) which retained only variables meeting significance thresholds. After running the GLMMs, our model selection was conducted in the package MuMIn (Bartoń 2020) by comparing AICc (Burnham and Anderson 2002). Then, we checked top model residuals for interactions using the interactions package (Long 2021) and included variable interactions when significant. We further tested residuals of the best performing models using QQ plot and Dotchart, and we checked for linear responses, overdispersion, zero inflation, and uniformity using DHARMa (Hartig and Lohse 2021). Final model fit was assessed with pseudo R^2 (conditional and marginal R^2) using MuMIn (Bartoń 2020) and performance (Lüdecke et al. 2021) packages. All statistical analyses were conducted in program R (R Core Team 2021). Significance threshold levels were set at $P < 0.05$.

RESULTS

Bat activity.—Combining all sites in all urbanization levels, we collected 110,062 echolocation calls during 1,202 nights of surveying from the following cave bat species: big

brown, little brown, Northern long-eared, and tri-colored, all of which are currently listed in this state as Species of Greatest Conservation Need in Maryland (SGCN; MDDNR 2016). Nightly activity for each species and total bats across the gradient are presented (Table 18). Activities from little brown and Northern long eared bats were too low in all urban, suburban, and rural datasets to include in any modeling.

Highly correlated variables.—Total bat activity was highly correlated with big brown activity at urban, suburban, and rural sites (rho = 0.71, $P < 0.0001$; rho = 0.99, $P < 0.0001$; rho = 0.99, $P < 0.0001$; respectively). Total bat activity was also correlated with tri-colored bat activity at urban and rural sites (rho = 0.50, $P < 0.0001$; rho = 0.50, $P < 0.0001$; respectively). As such, we removed total bat activity and investigated how big brown and tri-colored bat activity changed across urbanization levels to provide the best specific management recommendations.

When evaluating site-level independent variables, distances to light and building were highly correlated at suburban and rural sites (rho = .94, $P < 0.0001$; rho = 1, $P < 0.0001$; respectively). We retained distance to building as buildings can serve as important roosts for our bat species (Mering and Chambers 2014). Tree height and DBH were also highly correlated at our rural sites (rho = 0.90, $P < 0.0001$), however, we retained both variables given their reported significance to bat species in our region (Drake et al. 2020). While total insect count was highly correlated with multiple insect order counts, as well as insect Shannon H diversity index, we retained total insect counts at all sites to allow us to better allocate gradient-specific habitat recommendations. We finalized a set of possible models in each urbanization level for both the tri-colored and big brown bats, including a null model, a global model, a simplified model, and a model that included interactions. In certain instances, final model performance improved after

interactions were directly added or variables were added to the zero inflation component of the model (Table 19).

Urban bat activity model.—At highly urban sites, the best model for explaining big brown bat activity included Coleoptera, season, and temperature (Table 20). Additionally, season significantly interacted with both Coleoptera counts and temperature, and temperature also interacted with Coleoptera counts. Therefore, we re-fitted the final model with interactions between all these variables. Effects of Coleoptera, season, and temperature were also included in the zero inflated component of the model, which did improve the AIC and model residuals, resulting in an adequate model goodness-of-fit (Conditional R^2 of 0.46 and Marginal R^2 of 0.31).

Tri-colored bat activity was best predicted at urban sites by season, temperature, and year (Table 20). Year significantly interacted with season and temperature; therefore, we re-fitted the final model with its interactions. Additionally, the top tri-colored bat urban model improved significantly when adding season and year to the zero inflated component. The resulting final model had a high Conditional R^2 (0.86) and Marginal R^2 (0.80).

Big brown bat activity was greatest at urban sites during early summer ($P < 0.0001$) followed by late summer ($P = 0.02$). Nights with zero activity were explained by cold temperatures ($P < 0.0001$) and occurred more often in early summer ($P < 0.0001$). Our urban sites experienced highly variable temperatures during this early summer season from 7.16 º C up to 32.73 º C. Early summer nights with extremely low temperatures likely explained their activeless nights. Across the year, their documented activity was higher during warmer nights overall ($P < 0.0001$), but specifically, they were most active during the slightly cooler nights of early summer ($P < 0.0001$) followed by warmer nights of autumn ($P = 0.01$).

In urban areas, tri-colored bats activity was best predicted when nights were warmer during the second year of our study (2016). However, little average (mean ± SE) temperature difference was seen in the urban sites across years: 17.79 (± 0.63 º C), 17.66 (± 0.63 º C), and 17.95 (± 0.43 º C) in 2015, 2016, and 2017, respectively. Nights with no tri-colored bat calls at urban sites were not able to be explained by any variables added to the model.

Suburban bat activity.—At suburban sites, the best model explaining big brown bat activity included Coleoptera, season, temperature, and tree height (Table 20). Seasons interacted with Coleoptera, temperature, and tree height requiring us to re-fit the final model with those interaction terms. We added a polynomial term for temperature and fitted the zero inflation component with Coleoptera, season, and temperature resulting in a well fit model (Conditional R^2 of 0.62 and Marginal R^2 of 0.51).

Big brown bats were significantly more active with taller trees ($P < 0.001$), during warmer nights in both late summer ($P < 0.01$) and autumn ($P < 0.01$) at suburban sites. Their activity also responded positively with Coleoptera during early summer ($P < 0.01$) and late summer ($P = 0.02$). No variable was able to significantly explain nights with no big brown activity at suburban sites. Tri-colored bat activity at suburban sites varied by season; they were most active at our suburban sites during late summer ($P < 0.0001$), followed closely by autumn ($P < 0.001$; Table 20). The final tri-colored bat activity model met all assumptions, but had a poor fit (Conditional R^2 of 0.51 and Marginal R^2 of 0.15).

Rural bat activity.—In rural sites, big brown bat activity was best modeled by season, temperature, and year (Table 20). Season, temperature, and year all had significant interactions, requiring us to refit the model. The adjusted model was significantly improved when season and temperature were added to the zero inflated component and a polynomial term was added for

temperature (Conditional R^2 of 0.81 and Marginal R^2 of 0.78). Tri-colored bat activity in rural sites was best predicted by Diptera, season, temperature, total insects, and year (Table 20). Many of these variables interacted (season with Diptera, total insects, and year; year with Diptera and temperature).

At rural sites, big brown activity was lowest during autumn ($P < 0.001$) and lowest overall in 2017 ($P < 0.0001$), but their activity increased during early ($P = 0.02$) and late ($P = 0.03$) summer of 2017. Their rural activity significantly increased on warmer nights of autumn ($P = 0.03$). Suburban temperatures averaged (mean ± SE) 16.88 ± 0.63 º C, 17.37 ± 0.55 º C, and 17.42 ± 0.46 º C in 2015, 2016, and 2017, respectively. Cooler nights ($P = 0.02$), early summer ($P = 0.002$), late summer ($P = 0.02$), and autumn ($P = 0.02$) all contributed significantly to nights lacking big brown bat activity. Meanwhile, tri-colored bats were most active at rural sites during late summer ($P = 0.01$), followed closely by autumn ($P = 0.03$), and when total insects ($P = 0.02$) were more abundant. Their relationship with total insect counts flipped negatively during late summer ($P = 0.01$) and autumn ($P < 0.001$). Their activity also increased on warmer nights ($P = 0.001$). This highly complex model resulted in excellent fit (Conditional R^2 of 0.96 and Marginal R^2 of 0.95)

DISCUSSION

We documented seasonal differences and shifts in big brown and tri-colored bat activity in different levels of urbanization and identified differing habitat characteristics that likely related to those changes in activity. Given the uniqueness of the provinces in the state of Maryland, in many areas, within one home range, bats would have more than one choice of discrete urbanization levels during any time of the year. So, gaining this understanding of how bats may use different areas throughout the year or how it may influence their home range

placement will allow us to make habitat management recommendations for each species as detailed as possible.

Urbanization. —Big brown bat activity stood out in our very urban areas throughout most seasons, but especially from spring into late summer. Cooler nights of early summer, the season including pregnancy and parturition, had significant big brown activity. A radio-telemetry study reported female big brown bats remained near their roosts during this season, most likely to stay close to care for their young (Menzel et al. 2001). Additionally, female big brown bats may have been using cooler evenings in the urban sites to prevent evaporative heat loss involved in flying on warmer nights, thus conserving energy for pregnancy, parturition, and pup-rearing needs. As the year progressed, big brown bat activity increased slightly on warmer nights in these urban areas, indicating thermoregulation was playing a role in their activity. Issues of temperature impacting big brown activity was supported by the significant variables in our model including season and temperature. In contrast to the high levels of activity by big brown bats in our urban areas (Fig. 8A), tri-colored bats had low activity there (Fig. 8B), especially during late summer, but the model reflected the same impact of temperature, along with a yearly effect.

In suburban areas, big brown bats were active across all seasons, but increasingly active in late summer and autumn. Along with temperature, big brown bat activity seemed to be more influenced by Coleoptera counts in early and late summer as well as tree heights in suburbia. Specifically, Coleoptera counts, big brown bats' preferred prey (Agosta 2002), were more abundant at suburban than urban sites (Fig. 8C). As such, we were not surprised by this finding. Foraging by big brown bat adults and their volant juveniles would be expected at sites supporting higher quantities of their preferred prey. In fact, Menzel et al (2001) reported that female big brown bats foraged during the early and late lactation season of summer in areas with 51 – 75 %

development, which is comparable to our suburban site definition. Big brown activity was also positively related to tree height in our suburban areas, and our suburban roosting tree species and average tree heights (11.88 ± 0.12 m) were similar to previously reported averages used (13.9 ± 3.7 m; Drake et al. 2020; Agosta 2002). We presume that female big brown bats roosted and foraged during lactation time periods near sites with high prey amounts, like those in suburbia, thereby preventing additional energy expenditure traveling. Tri-colored bats were also active in our suburban sites, but not highly, and no relationships with any habitat variables were identified. Perhaps searching for potential mates during the hibernation preparation season allowed for our documented activity in these spaces.

In comparison to urban sites, big brown bats in the rural sites had lower activity, but we did document a slight increase in early summer, late summer, and autumn, especially in 2017. However, as summer progressed, tri-colored bat activity grew at these rural sites. Interestingly, Diptera counts were highest at the rural sites, which are a preferred food of tri-colored bats (Carter et al. 2003; Dodd et al. 2014). In fact, counts of Diptera were greater than any single order in rural sites and almost twice as abundant as Coleoptera, which is the preferred prey of big brown bats. Tri-colored bat females and their volant juvenile pups during late summer were likely the individuals most active in our rural sites, as their relationship with Diptera counts stayed relatively high ($P = 0.06$), even though it decreased with total insect counts ($P < 0.001$), indicating females and juveniles were possibly being selective toward their preferred prey. Hibernation preparation at rural sites would explain tri-colored bat activity as its positive relationship with Diptera during autumn almost met significance ($P = 0.09$). So, overall there seemed to be prey selection happening because of high levels of tricolored bats' preferred prey

across multiple seasons. This would also allow them to forage successfully, gain mass prior to entering hibernation, and avoid any heavy competition with big brown bats.

Perhaps the primary differences we have documented during autumn in bat activity along the gradient can be explained by issues surrounding thermoregulation and some level of interspecific competition or avoidance. None of these gradients exhibited big brown bat activity related specifically to diet during this pre-hibernation period, a period characterized by high foraging activity to gain mass prior to entering hibernation. Therefore, we presume they were using all levels of urbanization to feed and search for mates prior to hibernation.

Our data provide evidence that big brown and tri-colored bats might partition the urban-rural gradient for avoiding interspecific competition. These species diets significantly overlap (Carter et al. 2003; Dodd et al. 2014) though their wing morphologies and foraging strategies are more distinguishable. Big brown bats unique wing size, shape, and heavy body mass create slow flight which would be most tailored to open and uncluttered habitats (Norberg and Rayner 1987) leading to their open-space hawking strategy (Denzinger and Schnitzler 2013). Meanwhile, the smaller tri-colored bats with lighter body mass, shorter, and rounder wing tips (Norberg and Rayner 1987) allow them more agility in slightly more cluttered airspace leading to their classification as edge-space hawkers (Denzinger and Schnitzler 2013). Perhaps tri-colored bats select better quality foraging sites because their agility makes them better competitors at capturing more insects compared to less agile big brown bats. Conversely, big brown bats can travel long distances to capture prey at lower quality sites, thus could potentially avoid competition with tri-colored bats. A recent study documented evidence of “eavesdropping” by bats with similar characteristics as big brown bats while avoiding competition for space with agile bats of similar morphological traits as tri-colored bats (Roeleke et al. 2018). More

specifically, Roeleke et al. (2018) noticed strong interspecific competition avoidance between two bat species in Germany. They found the larger, less agile species abandoned sites when calls were played by a competing, smaller, more agile species as prey became less abundant during the cooler, later season. They concluded that slow, less agile bats that can travel far distances may find refuge from interspecific competition in suboptimal habitats. Perhaps our tri-colored bats thrive during interspecific competition utilizing their agility at high quality rural sites while our big brown bats survive competition by leaving for more urbanized settings. Further research into heterospecific echolocation call-backs and telemetry could support our speculation.

Analysis.—In order to best capture relationships between habitat features and bats of SGCN as accurately as possible, we returned to the same sites on consecutive nights for multiple seasons and years rather than moving within each night to additional sites which inherently decreased our potential power. We addressed potential pseudoreplication involved in repeating surveys at the same sites by running GLMM models with site set as random effect. Our study capitalized on the potential benefits of utilizing stationary bat acoustic detectors thus allowing us to learn relationships between cave bats and the highest of urbanized locations, rather than limiting our data collection to less urbanized sites supporting features capable of capturing bats; therefore, we were cautious in any attempts to explain relationships between population sizes and habitat features. Variations in species population sizes might explain the activity dominance by big brown bats, but it is challenging to evaluate based on stationary bat acoustic surveys. Activity patterns seen here may also have been influenced by migratory bat activity, warranting further analysis for all bat activity rather than restricting analysis to only cave species. Due to resource limitations, we allowed bat detectors to run every night of the week rather than excluding nights with inclement weather; therefore, we anticipated many nights with no activity.

We addressed activeless (or inactive) nights by fitting GLMM models with zero inflation and adding to the zero inflation component when necessary.

Management.—Tri-colored bats appeared to limit foraging to rural areas. This could indicate their selection for high quality or avoidance of big brown bat activity. They are known to spatially partition winter hibernacula and this avoidance of others may be helping them decrease exposure to the Pd fungus causing WNS (Hoyt et al. 2018). Perhaps the drive to survive WNS at habitats year-round may partly explain the activity we witnessed and supports their greater survivability than other species such as the gregarious, synurbic, little brown bat (Turner et al. 2011; Cheng et al. 2021). With tri-colored bat's possible avoidance of interspecific competition plus their current listing by MDDNR (2016*a*) and consideration by USFWS (2020), it seems prudent to provide additional assistance to tri-colored bats. One way to help tri-colored bats could be to diversify multiple parts of the gradient with habitat known to be used by both tri-colored and big brown bats to decrease perceived competition for habitat resources. A recent metadata analysis of trees used by these bats in the eastern temperate forest ecosystem (Drake et al. 2020) provided some specific details. Big brown bat tree roosts averaged (mean ± SD) a height of 13.9 ± 3.7 m, diameter breast height (DBH) of 26.4 ± 5.1 cm, and 61 ± 4.9 % canopy closure. They roosted in 22.7 % deciduous species in mostly (86.4 %) dead trees and used non-foliage. Quite uniquely, tri-colored bat roosts were completely (100%) under foliage of mostly (66.9 %) live trees that averaged 20 ± 4.5 m tall, 27.5 ± 5.2 cm DBH, in 49.1 ± 29.7 % canopy cover. Tri-colored bats were reported roosting in *Carya, Acer, Betula, Populus, Quercus, Magnolia,* and *Pinus* (O'Keefe 2009) while big brown bats were reported roosting in *Fagus, Pinus, Populus, Pseudotsuga, Quercus, Sequoia,* and *Thuja* species (Agosta 2002). For big brown bat roosts, we recommend maintaining *Fagus, Pinus, Populus, Pseudotsuga, Quercus,*

Sequoia, and *Thuja* 10-17 m tall with 21-31 cm DBH under moderately closed (55-65 % canopy) conditions in all parts of the gradient to decrease their congregation in any one area. For tri-colored bats, we recommend maintaining taller *Carya, Acer, Betula, Populus, Quercus, Magnolia,* and *Pinus* species around 15-25 m in height with 22-32 cm DBH with moderate gaps to allow more open (~50%) canopy cover in each part of the gradient to provide possible roosting conditions where they may find other valuable resources nearby.

Allowing trees to become this tall and wide in residential and commercial settings could potentially cause hazards during major storm events resulting in damaged property, power, and utility lines. Therefore, we recommend consulting with certified urban arborists to manage tree health of these valuable tree species and provide bracing when appropriate. Additional roosting opportunities from artificial bat boxes could benefit big brown bats and other bat species that use them (Mering and Chambers 2014) thus releasing additional natural tree roosts across the gradient for tri-colored bat usage.

Foraging opportunities could be provided by additional native vegetation plantings focused on butterfly gardens, wildflower garden road corridors and medians, rainwater gardens, green walls, green roofs, replacing artificial residential yards with native landscaping, and more. Native plant species should be selected from those which provide habitat to support high insect diversity, especially for the insect orders consumed by tri-colored bats in each local region. Our local region's tri-colored bats consumed insects in the orders of Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Homoptera, Neuroptera, and Trichoptera (Carter et al. 2003; Dodd et al. 2014). Native wildlife populations that forage on insects, such as tri-colored bats, do not benefit from application of broad-spectrum pesticides. Therefore, we highly discourage their misuse while reinvesting finances into incentivizing native vegetation plantings

which could also be done through local grassroots efforts and creating financial streams through new tax revenues (i.e., plastic bottle tax, soda tax). Additional incentivizing could involve lowering property taxes of properties that maintain habitat certification from examples such as National Wildlife Federation's (NWF's) Certified Wildlife Habitat ®, Audubon Bird-Friendly Habitat, Audubon At Home Wildlife Sanctuary, University of Maryland Extension Bay-Wise program, Habitat Partners© Corporate Habitat Program, Habitat at Home ©, and others.

Drinking opportunities could be provided through additional open (rather than enclosed) storm water runoff retention areas, wetland creation, and restoring hypoxic dead zones. Over fertilizing residential yards, gardens, and agricultural fields should be discouraged as well as applying salt over impervious surfaces during icy weather. Planting native grasses, replacing impervious surfaces with pervious options or Xeriscape landscaping, utilizing non-salt methods for clearing frozen paths/roads, and planting cover crops should be more financially incentivized during the WNS crisis. Many of the recommendations presented here require significant investments of financial and human resources, so we recommend landowners work with their local USDA NRCS office for financial and technical support when considering any of our recommendations.

Overall, we detected significant activity changes for both big brown and tri-colored bats across the urban-rural gradient and among seasons. Big brown bats appeared to use suburban areas for foraging and roosting and possibly urban areas for foraging while tri-colored bats appeared to forage in rural areas. Both species appeared to partition the urban-rural gradient during certain seasons possibly to avoid interspecific foraging competition. These significant gradient-level temporal results might have been missed if we surveyed short periods of the night, only one season, or restricted surveys to capturing bats in more natural settings within cities and

suburbs. Therefore, we highly recommend conservation agencies adapt our urban-rural gradient definition to their local region as appropriate and survey bats by incorporating the entire evening, returning consecutive nights each season, and spanning the entire urban-rural gradient.

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FIGURE LEGENDS

Fig. 7.—This map indicates locations of our 18 research sites which were evenly distributed along the urban-rural gradient in the Piedmont and Coastal Plain region of Maryland, neighboring counties, and Washington, DC. Urban, suburban, and rural sites were selected using a novel definition based on percent impervious surface, forest, and open land along with U.S. Census Bureau human population size data. Purple indicates urban, yellow indicates suburban, and red indicates rural sites.

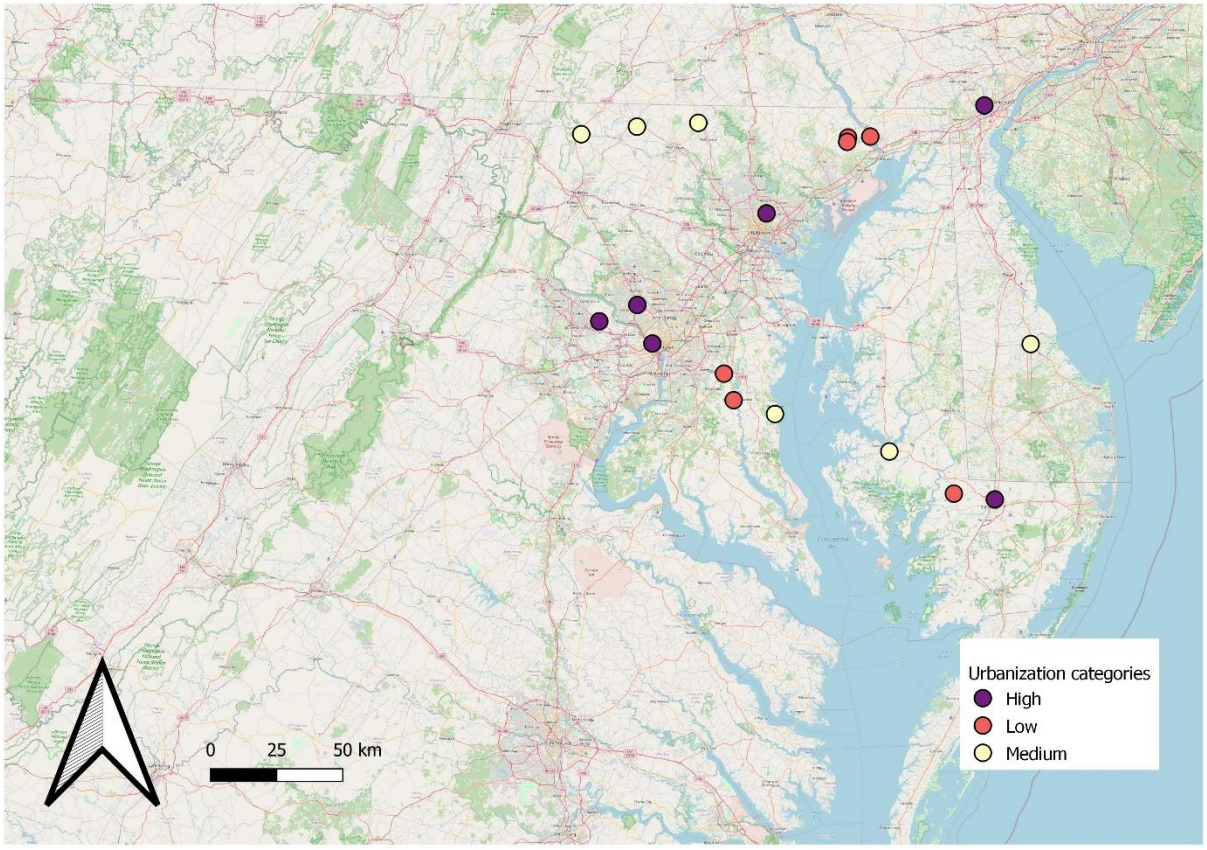

Fig. 8.—Acoustic activity for Big brown and Tri-colored bats were recorded each night at each site and compared among four seasons (1 = Spring, 2 = Early summer, 3 = Late summer, 4 = Autumn) across the urban-rural gradient. Insects were collected each week at each site. Coleoptera counts and total insect counts are displayed below among our four seasons across the urban-rural gradient. Big brown bat activity (A) appears high in urban sites (blue) during late summer and fall while their preferred prey (C) appear higher during these seasons at rural sites (red). Tri-colored bat activity (B) appears high during late summer at rural sites (red) while total insect counts (D) were also highest during that season at rural sites.

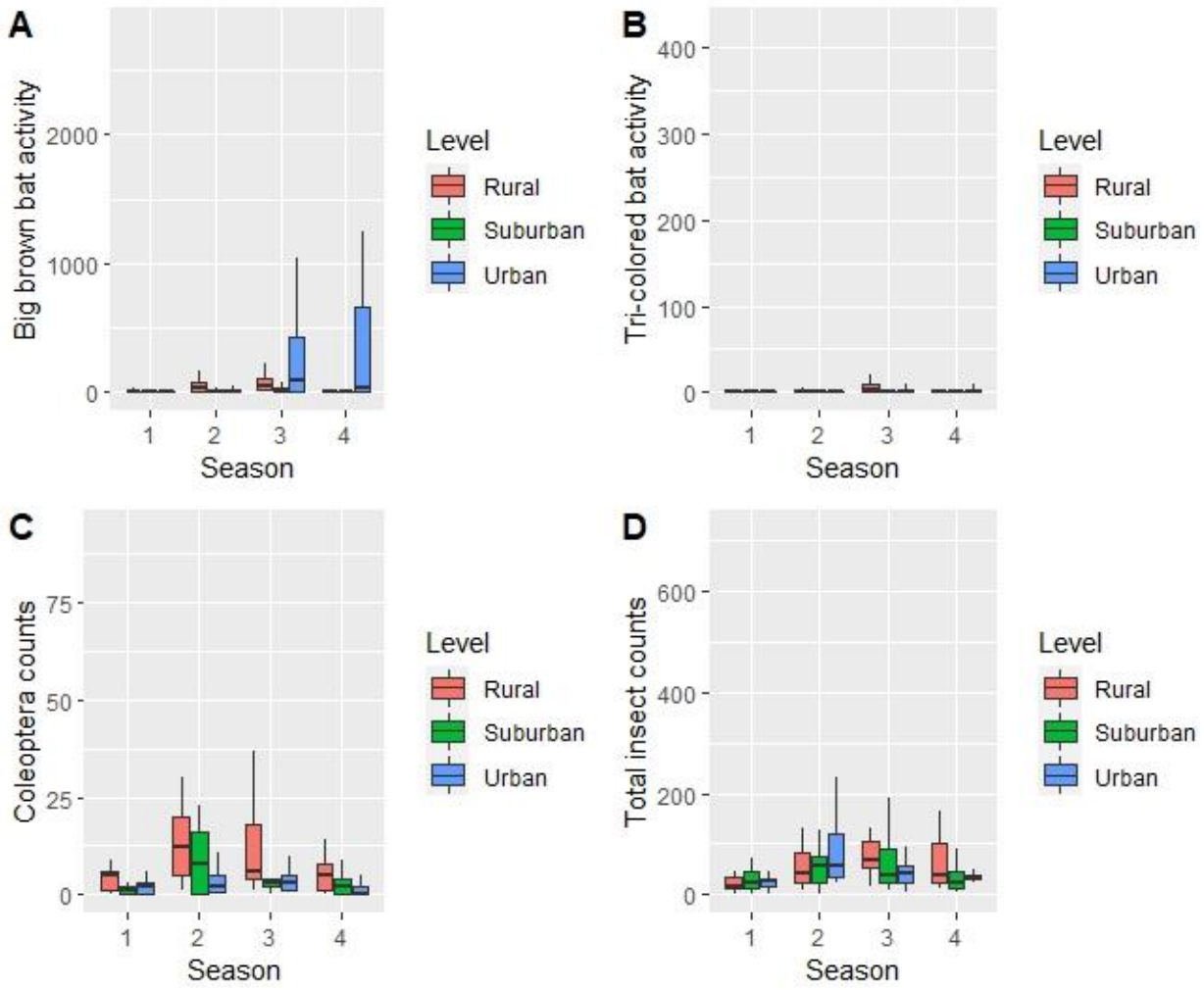

Table 17.—We defined three distinct levels of urbanization (Urban, Suburban, and Rural) within our urban-rural gradient in the Mid-Atlantic region using U.S. Census Bureau human population size data and percentages of imperviousness, forested, and open landscape. We first set a range for percent impervious surface. Then, we required a minimum amount of land devoid of impervious surfaces and required specific amounts of forest and open land classes ensuring potential sites maintain heterogeneity and avoid biasing our results.

Variable	Urban	Suburban	Rural
Population	50,000 +	2,500-50,000	< 2,500
% I.S.	> 90.00 % and < 100.00 %	> 45.00 % and < 55.00 %	> 0.00 % and < 10.00 %
% Forest	> 0.01 %	> 4.50 %	> 9.00 %
% Open	> 0.01 %	> 4.50 %	> 9.00 %

Table 18.—Dependent and independent variables and their measurement units are presented in each part of the urban-rural gradient. Variable averages are presented as mean ± (SE).

Variable	Urban (n=383)	Suburban (n=413)	Rural (n=406)
Big brown (calls/night)	182.66 (21.98)	27.35 (4.36)	61.61 (6.14)
Little brown (calls/night)	0.05 (0.02)	0.05 (0.02)	0.1 (0.03)
Northern long-eared (calls/night)	0 (0)	0.04 (0.02)	0 (0)
Tri-colored (calls/night)	5.63 (1.88)	0.57 (0.09)	3.19 (0.45)
Total bat (calls/night)	188.33 (22.09)	28.0 (4.36)	64.94 (6.17)
Light (lumens/ft^2)	96.28 (10.98)	142.79 (15.19)	128.1 (12.54)
Sound (dBA)	46.99 (0.44)	42.71 (0.41)	41.16 (0.38)
Temperature (Celsius)	17.79 (0.33)	17.79 (0.31)	17.23 (0.30)
Humidity (%)	77.69 (0.78)	80.68 (0.7)	79.84 (0.81)
Barometric pressure (Hg)	29.87 (0.01)	29.71 (0.02)	29.91 (0.01)
Wind (mph)	5.63 (0.18)	5.55 (0.17)	5.51 (0.18)
Precipitation (inches)	0.15 (0.03)	0.07 (0.01)	0.08 (0.02)
Total insects (count/week)	54.22 (2.94)	61.71 (5.03)	59.22 (2.76)
Insect Shannon H (calculation)	6.83 (0.14)	7.54 (0.17)	7.58 (0.16)
Blattodea (count/week)	0.09 (0.04)	0.01 (0.01)	0.02 (0.01)
Coleoptera (count/week)	3.05 (0.19)	5.25 (0.47)	9.26 (0.51)
Diptera (count/week)	11.07 (0.88)	10.82 (0.67)	18.19 (1.28)
Hemiptera (count/week)	19.03 (1.13)	32.45 (4.94)	11.75 (0.91)
Hymenoptera (count/week)	15.25 (2.41)	9.99 (0.65)	15.93 (1.77)
Lepidoptera (count/week)	0.1 (0.03)	0.04 (0.01)	0.04 (0.01)
Orthoptera (count/week)	0.13 (0.04)	0.12 (0.02)	0.02 (0.01)
Trichoptera (count/week)	0.07 (0.04)	0.1 (0.02)	0.51 (0.09)
Lunar (%)	0.53 (0.02)	0.49 (0.02)	0.44 (0.02)
Distance to water (m)	212.72 (13.47)	641.98 (28.02)	266.5 (4.74)

Distance to light (m)	38.99 (0.78)	32.44 (0.97)	80.11 (2.57)
Distance to building (m)	45.49 (1.55)	31.35 (1.02)	80.11 (2.57)
Distance to edge (m)	30.42 (1.66)	38.85 (3.11)	36.65 (1.61)
Distance to road (m)	63.54 (1.86)	65.71 (1.03)	167.58 (8.39)
Distance to tree (m)	5.3 (0.21)	4.08 (0.11)	7.15 (0.33)
Tree height (m)	20.6 (0.28)	11.88 (0.12)	15.39 (0.33)
Tree density (count)	61.99 (1.75)	38.44 (1.35)	25.86 (0.58)
DBH (cm)	58.01 (2.12)	44.06 (1.84)	25.95 (0.72)
Canopy cover (%)	57.54 (1.26)	71.67 (1.45)	42.01 (1.59)
Tree species (list)	red maple, sweetgum, Norway maple, black locust, red oak, tree of heaven	black cherry, tulip tree, sweetgum, black walnut, black locust, red oak	white pine, red oak, sweetgum, sycamore

Table 19.—Competing GLMM models for cave bat activity were compared for each urbanization level. Models were ranked based on AICc. Each global model consisted of habitat variables correlated (rho > 0.3) to individual species in each urbanization level and were fitted with season and year as fixed effects. Forward section was used to create simplified global models for comparison. Best performing models were compared to null models. All models were set with site as a random effect. Final models were reviewed for effects from interactions and zero inflation and adjusted when necessary. Pseudo R^2 results (CR^2 and MR^2) were provided for best performing models. Species include big brown (EPFU) and tri-colored bat (PESU). Model codes include: Global (global models contained all correlated independent variables), Inter (models fitted for interactions), Null (models containing only random effect of site), Simple (global models simplified using forward selection to retain only significant variables), ZIF (the zero-inflated component of the model was fitted with variables when needed). Final variables included in best performing models were Coleoptera counts (COLE), Diptera counts (DIPT), season (SEAS), temperature (TEMP), tree height (TREE), and year (YEAR).

Level	Species	Model	Variables	K	AICc	ΔAICc	ω	CR^2	MR^2
Urban	EPFU	Simple Int ZIF	COLE, SEAS, TEMP	21	3230.9	0.00	1	0.463	0.308
		Simple Int	COLE, SEAS, TEMP	16	3298.2	67.32	0		
		Simple	COLE, SEAS, TEMP	9	3340.0	109.05	0		
		Global	COLE, SEAS, TEMP, YEAR	11	3343.7	112.79	0		
		Null	NONE	4	3534.1	303.19	0		
	PESU	Simple Int ZIF	SEAS, TEMP	23	1044.4	0.00	1	0.857	0.796
		Simple Int	SEAS, TEMP	11	1069.6	25.13	0		
		Global	SEAS, TEMP, YEAR	10	1089.1	44.67	0		
		Simple	SEAS, TEMP	8	1091.3	46.84	0		
		Null	NONE	4	1221.2	176.80	0		

Suburban	EPFU	Simple Int ZIF	COLE, TEMP, TREE	29	2563.0	0.00	1	0.624	0.5129
		Simple Int	COLE, TEMP, TREE	20	2608.1	45.13	0		
		Global	COLE, SEAS, TEMP, TREE, YEAR	12	2629.9	66.96	0		
		Simple	COLE, TEMP, TREE	9	2630.1	67.14	0		
		Null	NONE	4	2735.9	172.88	0		
	PESU	Simple	SEAS	7	603.9	0.00	0.649	0.506	0.149
		Global	SEAS, YEAR	9	605.2	1.23	0.351		
		Null	NONE	4	645.5	41.55	0		
Rural	EPFU	Global Int ZIF	SEAS, TEMP, YEAR	31	3304.6	0.00	1	0.811	0.782
		Global Int	SEAS, TEMP, YEAR	21	3371.0	66.31	0		
		Simple Int	TEMP, YEAR	9	3466.1	161.48	0		
		Global	SEAS, TEMP, YEAR	10	3471.6	166.95	0		
		Simple	TEMP, YEAR	7	3475.1	170.50	0		
		Null	NONE	4	3578.4	273.75	0		
	PESU	Global Int	DIPT, SEAS, TEMP, TOTA, YEAR	28	1304.4	0.00	1	0.958	0.947
		Global	DIPT, SEAS, TEMP, TOTA, YEAR	12	1333.3	28.87	0		

	Simple	DIPT, SEAS, TEMP, TOTA, YEAR	9	1336.6	32.21	0
	Null	NONE	4	1496.5	192.05	0

Table 20. Coefficient estimates for each species' best performing generalized linear mixed models (GLMM) along the urban-rural gradient. All models contained site as a random effect. Asterisk * denotes variable holding statistical significant ($P < 0.05$) within the model. Species codes include EPFU (big brown bat) and PESU (tri-colored bat). Variables adjacent to and immediate following ZIF indicate results for the zero inflated component of the GLMM model.

Urbanization level	Species	Variable	Estimate	SE	z value	*P* value
Urban	EPFU	Intercept	-3.16	0.86	-3.64	<0.001 *
		Temperature	0.19	0.04	4.25	<0.0001 *
		Early summer	5.52	1.12	4.94	<0.0001 *
		Late summer	2.59	1.11	2.32	0.02 *
		Fall	-1.01	1.31	-0.77	0.44
		Coleoptera	0.06	0.13	0.44	0.66
		Temperature X Early summer	-0.25	0.06	-4.5	<0.0001 *
		Temperature X Late summer	-0.07	0.06	-1.28	0.20
		Temperature X Fall	0.19	0.08	2.51	0.01 *
		Temperature X Coleoptera	-0.01	0.01	-0.65	0.51
		Coleoptera X Early summer	0.12	0.11	1.06	0.29
		Coleoptera X Late summer	0.11	0.11	1.04	0.30
		Coleoptera X Fall	-0.07	0.12	-0.55	0.59
		ZIF Intercept	2.90	0.68	4.26	<0.0001 *
		Temperature	-0.28	0.06	-4.99	<0.0001 *
		Early summer	1.36	0.58	2.35	0.02 *
		Late summer	-16.56	2325.87	-0.01	0.99
		Fall	0.65	0.57	1.14	0.26

		Coleoptera	-0.13	0.09	-1.39	0.17
	PESU	Intercept	-23.74	7317.75	0	0.99
		Early summer	20.97	7317.75	0	0.99
		Late summer	22.24	7317.75	0	0.99
		Fall	21.11	7317.75	0	0.99
		2016	16.31	7317.75	0	0.99
		2017	20.37	7317.75	0	0.99
		Temperature	0.01	0.06	0.23	0.82
		Early summer X 2016	-21.13	7317.75	0	0.99
		Late summer X 2016	-20.41	7317.75	0	0.99
		Fall X 2016	-15.69	7317.75	0	0.99
		Early summer X 2017	-19.5	7317.75	0	0.99
		Late summer X 2017	-18.51	7317.75	0	0.99
		Fall X 2017	-16.94	7317.75	0	0.99
		Temperature X 2016	0.18	0.08	2.04	0.04 *
		Temperature X 2017	-0.12	0.09	-1.28	0.20
		ZIF Intercept	-2.96	14.51	-0.2	0.84
		Early summer	-20.57	60668.28	0	1.00
		Late summer	-17.32	4267.79	-0.004	0.99
		Fall	0.88	14.04	0.06	0.95
		2016	0.88	3.13	0.28	0.78
		2017	-0.32	3.87	-0.08	0.94
Suburban	EPFU	Intercept	-8.27	2.46	-3.36	< 0.001 *
		Temperature	18.88	8.87	2.13	0.03 *
		Temperature^2	-12.19	7.46	-1.63	0.10
		Early summer	2.76	2.62	1.05	0.29

	Late summer	2.26	2.23	1.01	0.31
	Fall	2.00	3.03	0.66	0.51
	Tree height	0.68	0.19	3.53	<0.001 *
	Coleoptera	-0.24	0.31	-0.78	0.44
	Temperature X Early summer	9.78	11.36	0.86	0.39
	Temperature² X Early summer	-14.18	12.25	-1.16	0.25
	Temperature X Late summer	70.38	21.81	3.23	<0.01 *
	Temperature² X Late summer	-33.54	13.09	-2.56	0.01 *
	Temperature X Fall	88.50	31.00	2.85	<0.01 *
	Temperature² X Fall	-24.01	25.11	-0.96	0.34
	Tree height X Early summer	-0.29	0.20	-1.43	0.15
	Tree height X Late summer	-0.34	0.16	-2.08	0.04 *
	Tree height X Fall	-0.31	0.22	-1.42	0.16
	Coleoptera X Early summer	0.18	0.06	2.98	<0.01 *
	Coleoptera X Late summer	0.21	0.09	2.37	0.02 *
	Coleoptera X Fall	0.02	0.11	0.15	0.88
	Coleoptera X Tree height	0.004	0.02	0.19	0.85
	ZIF Intercept	-1.52	1.68	-0.91	0.36
	Temperature	-0.004	0.10	-0.05	0.96
	Early summer	1.62	0.94	1.73	0.08
	Late summer	-1.64	2.06	-0.8	0.43
	Fall	1.77	0.97	1.82	0.07
	Coleoptera	-0.53	0.32	-1.65	0.10
PESU	Intercept	-5.96	0.86	-6.91	<0.0001 *
	Early summer	0.90	0.59	1.54	0.12
	Late summer	2.76	0.48	5.8	<0.0001 *

		Fall	2.06	0.56	3.69	<0.001
Rural	EPFU	Intercept	1.64	0.58	2.84	0.005 *
		Temperature	48.43	9.75	4.97	<0.0001
		Temperature²	-32.65	13.40	-2.44	0.015 *
		Early summer	-0.10	0.68	-0.15	0.88
		Late summer	-1.54	1.05	-1.47	0.14
		Fall	-2.09	0.58	-3.62	<0.001 *
		2016	-0.47	0.52	-0.9	0.37
		2017	-2.25	0.53	-4.28	<0.0001 *
		Temperature X Early summer	-10.79	11.08	-0.97	0.33
		Temperature² X Early summer	13.20	14.18	0.93	0.35
		Temperature X Late summer	28.76	31.81	0.9	0.37
		Temperature² X Late summer	-4.42	18.80	-0.24	0.81
		Temperature X Fall	20.73	9.32	2.24	0.03 *
		Temperature² X Fall	-10.89	11.85	-0.92	0.36
		Temperature X 2016	-35.46	10.37	-3.42	<0.001 *
		Temperature² X 2016	19.49	9.75	2	0.046 *
		Temperature X 2017	-7.87	12.64	-0.62	0.53
		Temperature² X 2017	2.01	11.07	0.18	0.86
		Early summer X 2016	0.77	0.67	1.14	0.26
		Late summer X 2016	0.92	0.70	1.38	0.17
		Fall X 2016	-0.46	0.60	-0.77	0.44
		Early summer X 2017	1.48	0.63	2.35	0.02 *
		Late summer X 2017	1.55	0.72	2.17	0.03 *
		Fall X 2017	4.34	0.62	6.97	<0.0001 *
		ZIF Intercept	1.63	0.79	2.06	0.04 *

	Temperature	-0.13	0.06	-2.42	0.02 *
	Early summer	-2.06	0.66	-3.1	0.002 *
	Late summer	-2.16	0.96	-2.27	0.02 *
	Fall	-1.37	0.57	-2.42	0.02 *
PESU	Intercept	-6.57	1.23	-5.33	<0.0001 *
	Temperature	0.19	0.06	3.28	0.001 *
	2016	1.55	1.35	1.14	0.25
	2017	-17.33	3284.70	-0.01	0.99
	Early summer	-1.26	1.17	-1.07	0.28
	Late summer	2.95	1.03	2.88	< 0.01 *
	Fall	2.20	1.01	2.19	0.03 *
	Diptera	-0.61	0.32	-1.87	0.06
	Total insects	0.12	0.05	2.42	0.02 *
	Temperature X 2016	-0.12	0.07	-1.72	0.09
	Temperature X 2017	-0.14	0.08	-1.79	0.07
	Early summer X 2016	0.88	1.21	0.72	0.47
	Early summer X 2017	21.43	3284.70	0.01	0.99
	Late summer X 2016	0.13	1.10	0.12	0.91
	Late summer X 2017	18.33	3284.70	0.01	0.99
	Fall X 2016	0.55	1.02	0.54	0.59
	Fall X 2017	18.77	3284.70	0.01	0.99
	Diptera X 2016	0.02	0.03	0.72	0.47
	Diptera X 2017	0.03	0.02	1.34	0.18
	Diptera X Early summer	0.60	0.32	1.85	0.06
	Diptera X Late summer	0.61	0.32	1.9	0.06
	Diptera X Fall	0.55	0.32	1.7	0.09

	Total insects X Early summer	-0.09	0.05	-1.8	0.06
	Total insects X Late summer	-0.12	0.05	-2.56	0.01 *
	Total insects X Fall	-0.13	0.05	-2.58	< 0.001 *

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