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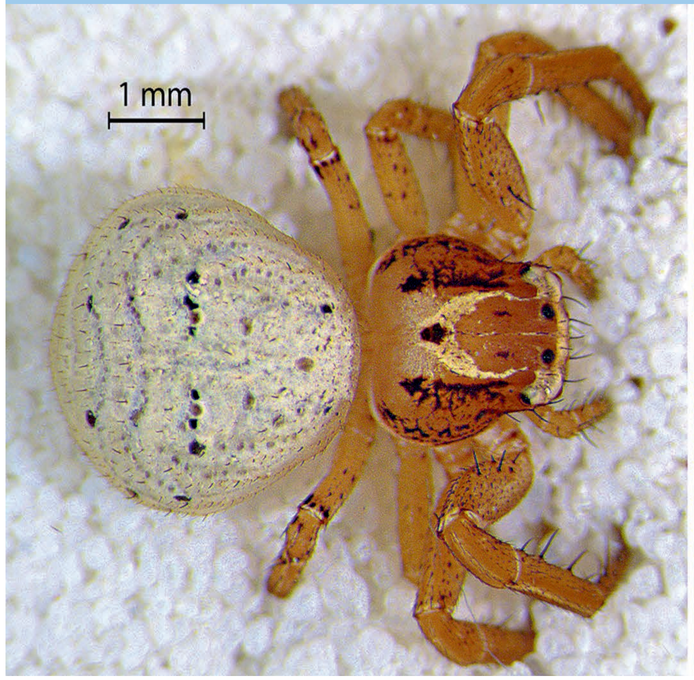

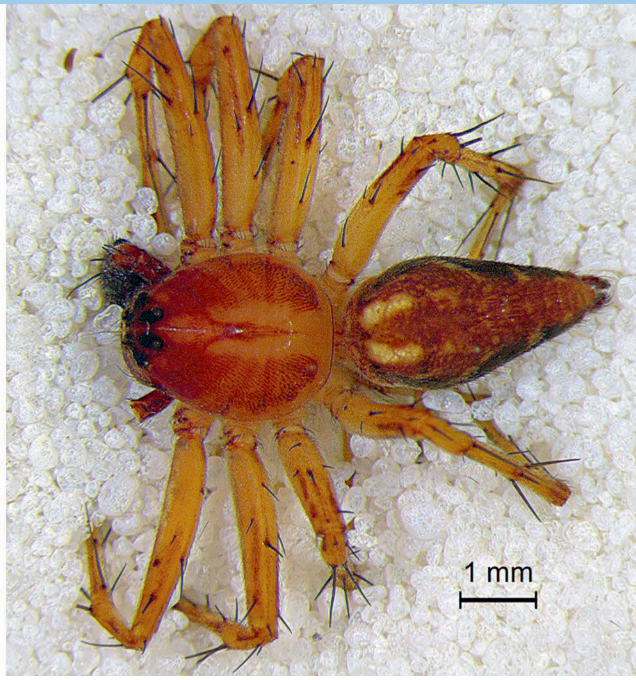

VOLUME 127 **2018** **NUMBER 1**

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Cover: Two new spider distribution records for Indiana collected in June 2017 at the Red-Tail Biodiversity Survey held at McVey Memorial Forest in Randolph County and White River Woods in Delaware County. Left: *Xysticus fervidus*, Ground crab spider, this female was found among tall weeds and low-lying vegetation near a pond at McVey Memorial Forest and commonly lives in this habitat, searching for small arthropods upon which to prey using its large, spiny anterior legs. Right: *Oxyopes scalaris*, Western lynx spider, this male is found on low vegetation, often preying on insects or other spiders that also live in herbaceous habitats. For more information on the spider fauna, as well as other flora and fauna observed during the bioblitz, see the article entitled "Results of the 2017 Red-Tail Land Conservancy Biodiversity Survey, Delaware and Randolph Counties, Indiana" in this issue. (Photographs by Marc Milne)

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A BIOGEOGRAPHIC COMPARISON OF SPIDERS WITHIN ILLINOIS AND INDIANA

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ABSTRACT. In March 2017, the Indiana Academy of Science held a biodiversity symposium prior to their annual meeting. Spiders were among the taxa discussed. Although published data on spider distribution records in both states (Indiana and Illinois) are sparse – especially in Indiana – an online collection network (SCAN) exists that supplements these data. I examined each recorded species from the online collection network that contained the most spider records and attempted to determine each record's validity by comparing the distance from its previously known range to either Indiana or Illinois. In addition, I calculated the numbers of species present in each state and within both states using published records. I also determined the general geographic range of each species based on known distribution data (northern, eastern, mid/eastern, southern, western, and widespread) and used a chi-square analysis with an adjusted residual post-hoc analysis to reveal significant differences from expected values. There were a significantly higher than expected number of spiders found in Indiana only that had eastern distributions and there were a significantly higher than expected number of spiders found in Illinois only that had western and northern distributions. Finally, there were a higher than expected number of spiders found in both states that possessed mid/eastern and widespread distributions. Records from the online database were not used because it became apparent that 10%–21% of the records may be misidentifications. These results emphasize that although the two states are adjacent to each other, the spider composition between the states have significant differences.

Keywords: Illinois, Indiana, biogeography, spider species richness, biodiversity, distribution

INTRODUCTION

The spiders of Illinois (IL) and Indiana (IN) have been documented since the late 1800s. The first published record of a checklist for either of these states was in a talk given by Fox in 1891 to the Washington Entomological Society, which noted 77 spider species known to Indiana (Fox 1891). This list was more than doubled by Banks (1906) to 148. Indiana's fauna list was later updated by Elliot (1932) to 218, Elliot (1953) to 303, Parker (1969) to 378, Beatty (2002) (with a reduction) to 367, Sierwald et al. (2005) to 383, and finally Milne et al. (2016) to 454. Meanwhile, the first published checklist of spiders in Illinois was written by Kaston (1955) in which he documented 350 species. This number was increased by Moulder (1966) to 363, by Beatty & Nelson (1979) to 500, Beatty (2002) to 550, and Sierwald et al. (2005) to 646.

A significant comparison and biogeographic examination of the spider fauna between the two states was not conducted until Beatty (2002) and then Sierwald et al. (2005). Unlike plants and other well-studied organisms, spider distributions are very rarely known at the county level (commonly, they are even poorly known on a state level; "P" in Table 8 in Sierwald et al. (2005)). The exception is medically-important species, such as the brown-widow spider (Brown et al. 2008) and brown recluse spider (Cramer & Maywright 2008). Therefore, it is difficult to answer habitat-specific biogeographic questions using spider distribution data. Alternatively, researchers – such as Beatty (2002) – classified each known species into ten pre-determined range categories and then compared the presence or absence of certain species of different ranges in the combined states (IL and IN together). This large-scale biogeographic analysis, while difficult to relate to specific habitats or environmental features (other than, perhaps, temperature and humidity), can inform us about both states' resemblance to other geographic areas based on their combined spider fauna.

Both IL and IN have physiographic similarities, such as being part of the Central Hardwood Forest, containing largely oak-hickory forest communities that are slowly being succeeded by beech-maple forest communities (Ebinger & McClain 1991; Shotola et al. 1992; Ebinger 1997; Fralish 2004), and having similar above-

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ground biomass density (Brown et al., 1999). However, within both states almost all of these forests have been cleared at least once and much of it is now farmland (Ebinger 1997). Both states also were split north-south by the most recent Wisconsinan glaciation, which retreated approximately 11,000 years before present. However, Illinois was historically primarily prairie (~61% according to early European surveyors) while Indiana only had small sections of prairie (Ebinger 1997).

The main network used for spider distribution records is Symbiota Collections of Arthropod Network (SCAN), which – as of this data analysis – possesses over 10 million records (SCAN 2017). SCAN is a subnetwork of Symbiota, a collection database system built on the internet, and aggregates data from museums, universities, and publicly-fed data aggregators such as the Global Biodiversity Information Facility (GBIF). Therefore, some specimens may have been identified by experts (e.g., museum-employed staff) while others may have been identified by amateurs (e.g., most GBIF submissions are from the public).

In 2017 the Indiana Academy of Science held a two-state biodiversity symposium that examined the similarities and differences in multiple groups of taxa between the two states. Within this symposium, I presented known spider species richness in each state, the shared species between states, and the role of online databases in supplementing traditional formally-published data from journals. Herein, these findings are expanded upon to better understand the similarities and differences in spider fauna between the two states. Moreover, the findings from the symposium are explored, analyzed, and used to build upon Beatty's (2002) biogeographic analysis of spider distributions between the states.

METHODS

The most recent spider distribution records for Illinois were obtained from Sierwald et al. (2005) while the most recent records for Indiana were obtained from the same source but updated with Milne et al. (2016). Distribution records of species were obtained from a combination of the American Arachnological Society's North American species list (Bradley et al. 2017), various manuscripts detailing the original description of the species (or a genus revision), and Sierwald et al. (2005).

Six different geographic distributions were established: northern, eastern, central/eastern, western, southern, and widespread (see Beatty (2002) for example maps of eastern and widespread). These geographic distributions more closely reflect those used by the main identification manual for spiders in North America (Ubick et al. 2017) than Beatty's (2002) geographic distributions. Northern distributions contained Canadian provinces but did not include southern, southwestern, or Gulf coast states. Eastern distributions included eastern Canadian provinces and US states. Eastern distributions also may have included Texas, Midwestern states, and Canadian provinces north of the Midwest. The central/eastern distribution may have included all locations within the eastern distribution in addition to states west of the Mississippi River or Canadian provinces west of Manitoba. However, a central/eastern distribution did not include states or provinces on the west coast or west of the Rocky Mountains. A western distribution mostly includes states west of the Mississippi River and the Canadian provinces north of those states. A southern distribution was constrained to states along the Gulf coast, east coast up to Maryland, and southwestern US but not Canadian provinces (Fig. 1). Finally, a widespread distribution was defined as having records from the east and west coast of the US and Canada and several states in between. Species were placed into one of these six categories based on their known distribution records. As explained in Beatty (2002), the placement of species into these categories can be subjective, but most species fit one of these six categories well. Once placed into a category, species were determined, based on Sierwald et al. (2005) and Milne et al. (2016), to be present in IN only, IL only, or present in both states.

A chi-square test was performed to determine if differences existed between observed and expected values within each category (e.g., the observed number of spiders in Illinois with an Eastern distribution versus the expected number, etc.) using Excel 2016. Expected values were calculated by multiplying the sum of a row by the sum of the column and dividing by the total of all cells (684). A significant p-value of 0.05 was established. Due to the high number of tests (18), a Bonferroni correction was conducted, creating a critical p-value of 0.0028. A two-tailed z-criterion was then created by taking the inverse of this corrected p-value. Using this z-statistic, a post-hoc test was then performed by calculating the adjusted

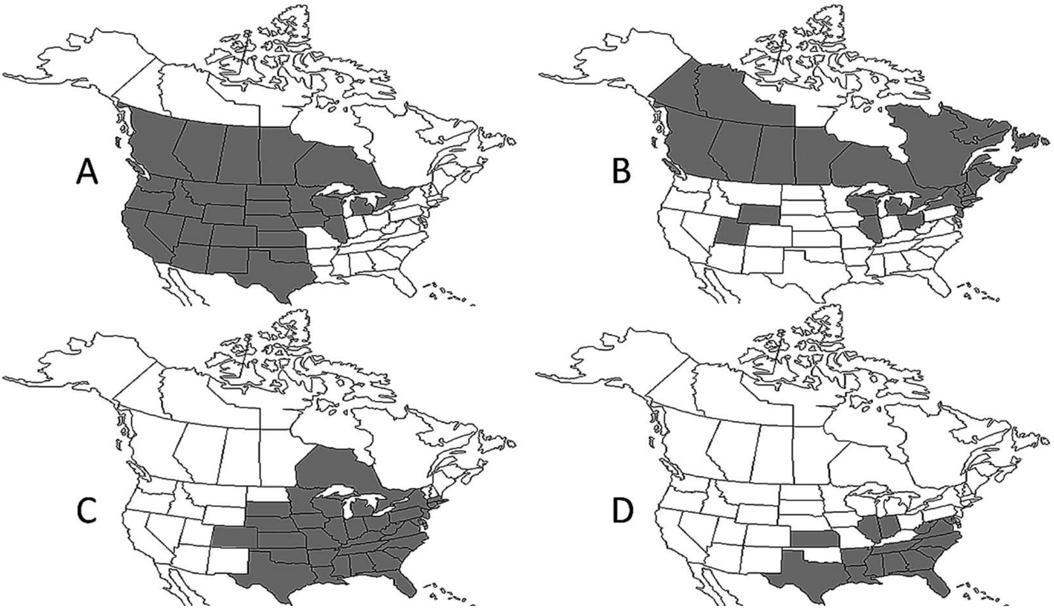

Figure 1.—Known range of four species. A. Western distribution exemplified by *Schizocosa mccooki* (based on Dondale & Redner 1978; Sierwald et al. 2005). B. Northern distribution exemplified by *Walckenaeria castanea* (based on Millidge 1983; Sierwald et al. 2005). C. Central/eastern distribution exemplified by *Schizocosa ocreata* (based on Dondale & Redner 1978; Sierwald et al. 2005; Milne et al. 2016; Bradley et al. 2017). D. Southern distribution exemplified by *Tigrosa georgicola* (based on Brady 2012; Bradley et al. 2017).

residuals for each category using the formula: {[observed value - expected value]/√[expected value × (1-row total/sum) × (1-column total/sum)]}.

Finally, all records of spiders (5,842 records) were examined within Indiana and Illinois on SCAN and cross-referenced against the most recent peer-reviewed publications. Since most of these records were not accompanied by any photographs nor were most of them available for examination, I attempted to determine the validity of all new state records by determining either the distance from the closest known distribution range or the country of the species' known range. Records that represented 1000+ mile range extensions were rejected as unlikely to be correct.

RESULTS

Of the 684 species documented, 405 were found in both states (59%). Illinois was found to possess far more unique species (236) than Indiana (43; Table 1). As presented in Table 1, most species documented had a central/eastern distribution (170; 24.9%). In descending order, the next most common were widespread species (166; 24.3%), eastern species (147; 21.5%), northern species (114; 16.7%), southern species (64; 9.4%), and finally western species (23; 3.4%).

The chi-square analysis of these data was highly significant ($p < 0.0001$). The z-criterion was 2.99 or -2.99, so all values greater than 2.99 and less than -2.99 were determined to be significant (bolded values in Table 1). The adjusted residuals indicated that the occurrence of species with an eastern distribution was significantly higher in Indiana than expected and significantly lower in both states than expected (Table 1). The adjusted residuals also indicated that the occurrence of spiders in Illinois was significantly higher than expected for northern and western species. Moreover, the occurrence of species in both states was significantly lower than expected for northern, southern, and western species. The occurrence of spiders with a central/eastern distribution within both states was significantly higher than expected, but significantly lower than expected in each state alone. This pattern also was present for spiders with a widespread distribution, except that it was non-significant for Indiana only (Table 1).

Table 1.—A comparison of the number of species found in each state by geographic range. Numbers in parenthesis represent adjusted residuals from chi-square post-hoc analysis. A residual of > 2.99 and < -2.99 indicates a value significantly different than expected. Significant adjusted residuals are bolded.

	IL only	IN only	Both	Total
North	64 (**5.32**)	7 (-0.07)	43 (**-5.11**)	114
East	58 (1.43)	20 (**4.13**)	69 (**-3.42**)	147
Central/East	32 (**-4.96**)	2 (**-3.17**)	136 (**6.36**)	170
West	16 (**3.60**)	3 (1.36)	4 (**-4.15**)	23
South	31 (2.46)	8 (2.15)	25 (**-3.44**)	64
Widespread	35 (**-4.18**)	3 (-2.73)	128 (**5.39**)	166
Total	236	43	405	684

After cross-referencing the SCAN records against both Sierwald et al. (2005) and Milne et al. (2016), there were 84 new state records for Indiana and 131 new state records for Illinois. However, the new Indiana records included six species that were rejected due to unlikely ranges, i.e., two Palearctic species, and one European species (10.7% of the total number of new distribution records). Similarly, the Illinois records included twenty-five 1000+ mile range extensions, i.e., one Chinese species, one Japanese species, and one Palearctic species (21.4% of the total number of new distribution records).

DISCUSSION

In his analysis, Beatty (2002) found that most spider species in IL and IN possessed an eastern distribution (37%), while the remaining species possessed (in descending order of occurrence) a northeastern (18.4%), northern (15.9%), southeastern (13.6%), widespread (10.6%), western (1.7%), central (1.5%), and southern distributions (1.3%). Because this study used different geographic regions than Beatty (2002), it is difficult to directly compare the two analyses. However, when the eastern and central/eastern categories of this analysis were compared to Beatty's (2002) eastern and northeastern categories – a similar categorization – they make up approximately half of the species found in both analyses (this analysis is ~46% while Beatty's is ~55%). Moreover, both analyses found that species with northern distributions make up approximately 16% of the species and western species are quite rare in IL and IN (Table 1). The most notable difference was that Beatty (2002) found that only 11% of the species were considered widespread while the current analysis put that value at 24.3%. This may be due to how each author categorized distributions as "widespread." The higher value in this analysis may be attributed, in part, to an increased number of distribution records throughout the US and Canada added within the last 15 years, thereby giving a more recent analysis of any particular species a higher likelihood of being considered "widespread."

As expected, species with eastern distributions had a significantly higher occurrence than expected in the more eastern state (IN) while species with western distributions had a significantly higher occurrence than expected in the more western state (IL). Spiders that possessed a widespread or central/eastern distribution had significantly higher occurrences in both states than was expected. This may be because spiders that have a widespread occurrence will likely occur in both IL and IN as well as many other Midwestern states. What was interesting to note was that species with a northern occurrence were significantly more prominent in Illinois than would be expected. This may be due to the presence of the northern part of Illinois in higher latitudes, to the west of Lake Michigan – latitudes not present in Indiana. This is undoubtedly also due to the makeup of the taxa. Many spiders with northern distributions are in the family Linyphiidae, sheet-web weaving spiders. Many of these spiders are small, rare, and have not been found in Indiana, likely due to a lack of searching (Sierwald et al. 2005).

These conclusions are dependent on reliable distribution record data, but the data used to come to these conclusions are incomplete. Sierwald et al. (2005) predicted the presence of hundreds of species in Indiana that have not yet been found. Moreover, the greatest predictor of knowing the distribution of species within a state was found not to be geographic area or time since state founding, but human population size. This suggests that as human population increases, sampling effort increases due to the higher likelihood of the presence of arachnologists within that state actually looking for spiders (Sierwald et al. 2005). Indeed, very recent studies (e.g., Milne et al. 2016) represent the ongoing faunistic work that is occurring within Indiana.

Reliability of these data is also an important aspect when considering faunistics, the study of species lists and distributions. The peer-review of species lists is critical in ensuring correct distribution maps for species. Spider identification is notoriously difficult and is therefore a slow process, so much so that computer algorithms and programs have been developed in attempts to bolster identification speed and accuracy (Do et al. 1999). This difficulty hampers identification by amateurs and specialists alike. While it is likely that most of the species I found in SCAN were legitimate new records that have yet to be recorded in the published literature, the fact that they were interspersed with 10%–21% likely incorrect species identifications "muddies the water." With this hindrance to accuracy, the only way to determine if these specimens were legitimate would be to examine them in person or to view detailed pictures of the specimens, neither of which were readily available. Due to this unreliability, I would recommend not using SCAN data without examining specimens first. To improve the quality of SCAN data, I recommend that these specimens' identity be comfirmed by an arachnologist prior to being added to the database.

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A COMPARATIVE BIOGEOGRAPHY OF THE VASCULAR FLORAS OF ILLINOIS AND INDIANA

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ABSTRACT. A symposium on γ biodiversity and the natural history collections of Illinois and Indiana was held at the 2017 annual meeting of the Indiana Academy of Science and included an analysis of the biogeography of the vascular plant floras of these two states. The analysis documented a rich temperate zone flora: the species shared by the two states numbered 2540 and a total count of 3450 species (native and non-native). Although the two states have much in common physiographically, on a per $\log_{10}$ km^2 basis Illinois possesses the richer flora. Illinois has at least 360 native species in its flora that do not occur in Indiana while only 165 species are limited to Indiana. The richer Illinois flora was due to a larger influence by the Great Plains flora, larger numbers of species reaching their northern limit in the Mississippi Embayment, more species reaching their southern limits especially within the Driftless Area of Illinois, and elements of the Ozark flora reaching into southern and western Illinois. Furthermore, at least 58 species introduced into Illinois (but not into Indiana) have a nativity from western US. Although Indiana has a notable Appalachian component in its flora, these deciduous forest species tend to also be found in the southern Illinois hill country. However, unique to Indiana was a suite of coastal plain disjunct species with populations in northwestern Indiana and also southwestern Michigan.

Keywords: Illinois, Indiana, biogeography, plant biodiversity

INTRODUCTION

For the past 125 years botanists have actively sampled the floras of Illinois and Indiana, amassing in excess of a half million herbarium specimens. This work has resulted in the compilation and publication of state floras by Mohlenbrock (1st edition 1975; 4th and most recent edition 2014) for Illinois and Charles Deam (1940) for Indiana. In addition, a major floristic series, started by Floyd Swink, documented the floristically diverse Chicago region (Swink 1969; Swink & Wilhlem 1994; Wilhelm & Rericha 2017).

These floras not only recorded the species of plants growing spontaneously within these two states, they also provided insight into the phytogeography of this region of the Midwest and a defining of natural regions (Schwegman et al. 1973; Homoya et al. 1985). The natural boundaries often have a physiographic basis including glacial history, bedrock type, and proximity to Lake Michigan. In addition there is a moisture gradient that, along with fire, drove historic vegetation to include expanses of tallgrass prairie and oak savanna in addition to eastern deciduous forest (Omernik & Griffith 2014, EPA 2017).

The Indiana Academy of Science at its 2017 annual meeting sponsored a two-state biodiversity and natural history collections symposium. For the first time, a broad range of organism groups, including mammals, invertebrates, plants, and fungi, was analyzed for their γ diversity (Whittaker 1972) across the two states. The two states have much in common physiographically. Both have glaciated as well as unglaciated regions and both have a long north–south axis resulting in a diverse mix of species from cooler and warmer climes. Likewise both have large river systems (Illinois, Mississippi, Ohio, and Wabash) with extensive bottomlands as well as frontage on Lake Michigan. At the same time, the two-state analysis, especially for plants, revealed some unexpected differences in their floras and faunas. In this paper, the Venn diagram approach is used to ask which vascular plant species are in common between the two states and which are unique to each and to explore potential causation behind these patterns.

MATERIALS AND METHODS

Species lists for Illinois and Indiana were compiled from Biota of North America Project (BONAP, Kartesz 2017), a database that treats the two states and nearby regions in a similar manner (but see Franz & Sterner (2017) for cautions related to aggregated biodiversity databases overall). In Microsoft's Access® database,

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species in common across the Illinois and Indiana were sorted and enumerated. Each remaining species was scored for state occurrence, nativity, and a series of phytogeographic range limits. The latter were determined through the study of species distribution maps found in Kartesz (2017). In particular, these maps provided information on which species reached their northern or southern range limit in each state, which reached their eastern limit in Illinois or western limit in Indiana, which had disjunct distributions (i.e., outlier populations in Illinois or Indiana widely separated from the species' center of distribution), and which could be defined as regional endemics. Two forms of regional endemism were defined: those of limited geographical range from the Ozark Mountain region into Illinois and the second centering on the Interior Low Plateau that lies between the Appalachian range and Mississippi River floodplain and extends from southern Indiana to northern Alabama.

RESULTS AND DISCUSSION

Whittaker (1972) envisioned three levels of species diversity: α diversity is the richness of species at the community level, β diversity is species turnover along habitat gradients, and γ diversity is the total diversity of a landscape or geographical area. The latter may be thought of as a product of α and β diversity. Thus, the following interstate floristic comparisons largely fall into the category of γ diversity. An estimated 3450 species (2594 native species) are found in the two-state region (Table 1), with a larger number of plant species known for Illinois (3283) than for Indiana (2865). Illinois, in fact, had more native species (2429) than any of the states or province examined including Indiana. Indiana was more species-rich than several more northern states (Wisconsin and especially Minnesota) and comparable in richness with Michigan and Kentucky (Table 1). The greater species richness of Illinois compared to Indiana could be attributed to the larger size of the former and, on an unscaled species/km^2 basis, Indiana does indeed seem more species rich. However, when scaled on either a semilog (Table 1) or log-log basis (not shown) basis, as is typical for species-area relationships (Connor & McCoy 1979), several interesting trends emerge. The historically prairie states of Kansas and Iowa had the fewest native species per unit area. Likewise more northern states (Minnesota, Wisconsin) and the province of Ontario had low numbers of species. Species richness often correlates with factors such as ecosystem productivity, precipitation, and temperatures (e.g., Bai et al. 2007; Wang et al. 2009). Based upon the $\log_{10}$ km^2 calculation, Illinois has 471 native species per unit area compared to only 439 for Indiana, i.e., Illinois enjoys a 7% advantage over Indiana.

Illinois and Indiana share 2540 species in common. Indiana has 230 species not known from Illinois, about 8% of its flora (Table 2). In contrast, the Illinois flora has an estimated 680 species unknown in Indiana or more than 20% of its species list. Since these values are for all vascular plant species, whether native or non-native, it could be that these differences reflect a more thorough monitoring of recent introductions. In part this is true. Nonetheless the pattern

Table 1.—Number of vascular plant species known for Illinois, Indiana, and neighboring regions. Data based upon BONAP (Kartesz 2017). SR = species richness; SR_total = species richness of native + non-native species.

State or Province	SR_total	SR_native	Native_SR/km^2	Native_SR/$\log_{10}$ km^2
Illinois	3283	2429	0.017	471
Indiana	2865	2180	0.023	439
Illinois + Indiana	3450	2594	0.011	483
Iowa	2274	1733	0.012	336
Kansas	2302	1808	0.008	339
Kentucky	2899	2191	0.021	437
Michigan	3099	2207	0.015	422
Minnesota	2398	1863	0.009	351
Missouri	3026	2262	0.012	429
Ohio	3146	2213	0.019	437
Ontario	3336	2382	0.002	395
Pennsylvania	3567	2408	0.020	476
Wisconsin	2698	2005	0.014	390

Table 2.—Number of vascular plant species unique to Illinois versus Indiana.

	Illinois	Indiana
Unique native + non-native species	680	230
Unique native species	360	165
Unique native species per $\log_{10}$ km^2	69.8	33.2

persists when comparisons are made with native species only (Table 2), which are well known for both states. Most telling was that on a per $\log_{10}$ km^2 basis, Illinois had 70 unique native species per unit area compared to only 33 for Indiana.

The data suggest that Illinois has a greater endowment of plant species. An exploration of biogeographical patterns might provide clues as to why this is the case. First, to-date Illinois does have a larger number of introduced species that are not recorded for Indiana. Interestingly, 58 of these Illinois introductions are from the western US and 11 from southern US. Indiana has only nine introduced species from other portions of the US that are not also recorded for Illinois. Unpublished work by Kay Yatskievych (pers. comm.), however, is rapidly enlarging the list of introduced species in Indiana. These have not been analyzed for their co-occurrence in Illinois.

Each state possesses a unique suite of species that have a relatively narrow geographic range overall. Southern and western Illinois has about 12 regional endemics that collectively display an Ozark influence. Example species include *Ruellia pedunculata* Torr. ex A. Gray and *Symphyotrichum anomalum* (Engelm.) G. L. Nesom. Indiana on the other hand has approximately three regional endemics whose distribution is limited to the Interior Low Plateau: *Carex picta* Steud., *Hypericum dolabriforme* Vent., and *Viola egglestonii* Brainerd. *Solidago shortii* Torr. ex A. Gray, a species whose narrow distribution consists of one site on the Indiana side of the Ohio River and three Kentucky counties, could be included on this list. Two additional species were not included as Indiana regional endemics. *Eleocharis bifida* S. G. Sm. has unresolved taxonomic questions and may be an undescribed species. *Physaria globosa* (Desv.) O'Kane & Al-Shehbaz has its sole and ecologically puzzling Indiana station in the Wabash River Valley rather than on the Interior Low Plateau.

Table 3.—Number of species reaching their range limit in either Illinois or in Indiana.

	Illinois	Indiana
Species reaching their northern limit	97	29
Species reaching their southern limit	51	23
Species reaching their eastern limit (in Illinois) or western limit (in Indiana)	56	27

The greater geographical reach of Illinois, compared to Indiana, both to the south as well as the north, accounts in part for that state having more species that attain their north–south limit (Table 3). Illinois has 97 species that reach their northern limit compared to Indiana's 29. Species such as *Nyssa aquatica* L. extend northward up the Mississippi Embayment from the coastal regions into southern Illinois and often do not make it into Posey County in the extreme southwestern corner of Indiana. At the opposite end of these two states, 51 northern species have occurrences in northwest Illinois. This is the Driftless Area, unglaciated during the most recent glacial maximum, and home to species such as *Gymnocarpium dryopteris* (L.) Newman (Pusateri et al. 1993). The Driftless Area also harbors *Dodecatheon amethystinum* (Fassett) Fassett and *Solidago sciaphila* Steele, species with narrow geographic range globally that are limited to dolomite and sandstone cliffs and talus slopes (Pusateri et al. 1993).

An analysis of species reaching their eastern and western range limits again reveals greater numbers of species for Illinois. *Astragalus crassicarpus* Nutt. and 55 other species of the Great Plains flora reach into Illinois but have not been observed in the limited area of tallgrass prairies of presettlement western Indiana. Conversely only 27 species, such as *Lilium canadense* L., have their western limit in Indiana. By and large it appears that those elements of the Appalachian flora whose range extends as far west as Indiana also have found suitable habitat in the southern Illinois hill country.

One suite of species sharply favors Indiana over Illinois, the so-called coastal plain disjunct species. Indiana has at least 12 species with a bimodal distribution pattern in which the center of distribution is along the Atlantic and Gulf of Mexico coastal plain and a distinct secondary center near the end of Lake Michigan. Example species include *Eleocharis melanocarpa* Torr. and *Xyris difformis* Chap. Due to the quirks of postglacial geology, sand dune formation, and placement of the state line, Illinois has no coastal plain species that do not also occur in Indiana.

In summary, both Illinois and Indiana have richer floras than more northern states, Ontario, and the prairie-dominated states such as Kansas and Iowa. The flora of Indiana, while rich and interesting, is on a par with neighboring states of Kentucky, Michigan, and Ohio, and, aside from a suite of coastal plain disjunct species, supports fewer biogeographically limited species than its neighbor state to the west. On the other hand, Illinois has a remarkably rich vascular flora, i.e., high γ diversity. This stems in part from a confluence of privileged geography and geological history, such as observed by Ricklefs & He (2016). Illinois' high γ diversity may be attributable to its long north–south axis, its pattern of glaciation, and the influence of the Ozark flora. In addition, the state straddles two biomes, so that prairie species are abundant and yet Appalachian and floral elements of the deciduous forest biome also are abundant. Thus, one might envision that the state has high species turnover or high β diversity across the biome transition zone (Kark & vanRensburg 2006). As more historic and current herbarium records become available in digital databases (e.g., see midwestherbaria.org), it should be possible to address interesting species distributional questions across transition zones and how these zones are changing in response to climatic shifts and the influx of non-native species.

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INFRARED SPECTROSCOPIC STUDIES OF C_{60} AND C_{70} NANOPARTICLE INTERACTIONS WITH δ–VALEROLACTAM

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ABSTRACT. Fourier transform infrared spectroscopy was used to investigate the interactions between C_{60} and C_{70} nanoparticles and *δ*-valerolactam (*δ*-lactam or D-lactam, the six-member heterocyclic lactam) in a toluene solvent environment. Changes in the carbonyl absorption in the lactam ring (both shape and position) were used to identify interactions between the *δ*-valerolactam and these nanoparticles. In a previous study, shifts in the carbonyl absorption were observed for 2-pyrrolidone (*γ*-lactam, the five-member heterocyclic lactam) treated with C_{60}, but only very small broadenings of the carbonyl absorption were observed when 2-pyrrolidone was treated with C_{70}. In this study, significant changes in the carbonyl absorption were observed when *δ*-valerolactam was treated with both C_{60} and C_{70} in a toluene solvent environment.

Keywords: Infrared spectroscopy, nanoparticles, C_{60} and C_{70}, fullerenes, δ-valerolactam (δ-lactam or D-lactam)

INTRODUCTION

The interactions of C_{60} and C_{70} nanoparticles with a number of molecular systems have been studied using a variety of analysis methods such as UV-visible, infrared, and Raman spectroscopy along with X-ray, neutron scattering, and theoretical analysis methods (Holleman et al. 1999; Kyzyma et al. 2008; Jurow et al. 2012; King et al. 2012; Kyrey et al. 2012; Tropin et al. 2013; Bowles et al. 2014; Kirsch et al. 2015, 2017; Behera & Ram 2015; Li et al. 2016). This paper describes the results of an infrared spectroscopic investigation of the interactions between both C_{60} and C_{70} nanoparticles and δ-valerolactam using toluene as a solvent environment (Aksenova et al. 2013).

The compound δ-valerolactam is a six-member, heterocyclic amide sometimes referred to as delta-lactam (δ-lactam) or D-lactam. The cyclic lactam structures have important pharmaceutical and biological applications (Midgley et al. 1992; Harreus et al. 2011). C_{60} and C_{70} nanoparticles dissolve in toluene forming a blue solution with C_{60} and a red solution with C_{70} (Ruoff et al. 1993). In this study infrared spectroscopy was used to investigate changes (both shape and position) in the carbonyl absorption of the δ-valerolactam as an indication of its interactions with the C_{60} and C_{70} nanoparticles in a toluene solvent environment. In a previous study (Kirsch et al. 2017), infrared spectra showed significant changes in the carbonyl absorption of 2-pyrrolidone (γ-lactam, the five-member heterocyclic lactam) treated with C_{60} indicating interactions between the 2-pyrrolidone and C_{60} nanoparticles; however, only minimal changes in the carbonyl absorption were observed when 2-pyrrolidone was treated with C_{70} nanoparticles. In this study, significant changes in the carbonyl absorption of δ-valerolactam were observed on treatment with both C_{60} and C_{70} nanoparticles indicating molecular interactions of δ-valerolactam with both C_{60} and C_{70} nanoparticles.

EXPERIMENTAL METHODS

A Digilab FTS 7000 infrared spectrometer and a circle cell fitted with a ZnSe ATR rod were used to collect spectra for the investigation by averaging 500 scans at a spectral resolution of 2 cm^{-1}. The empty circle cell was used as the single-beam spectral background for the study. The spectrometer was purged with dry air for at least an hour prior to spectral collection to minimize potentially interfering atmospheric water vapor absorptions. Solution spectra were collected over a concentration range of ~ 4 to ~ 20 mg of δ-valerolactam per mL toluene or toluene saturated with the nanoparticles (C_{60} and C_{70}) to identify a suitable concentration range to observe spectral changes.

INVESTIGATION RESULTS

Figure 1 contains the spectrum of δ-valerolactam (D-lactam) in toluene, the spectrum of δ-

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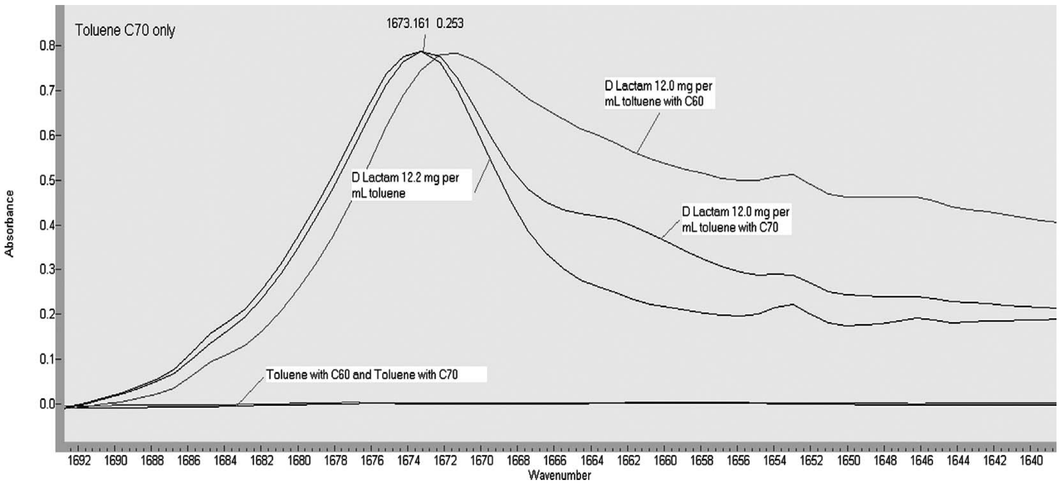

Figure 1.—The spectra of 12.2 mg of δ–valerolactam (D-lactam) per mL toluene, the spectra of 12.0 mg of δ–valerolactam per mL toluene saturated with C_{60}, and the spectra of 12.0 mg of δ–valerolactam per mL toluene saturated with C_{70} are shown. The spectra of toluene saturated with C_{60} and C_{70} also are included.

valerolactam in toluene saturated with C_{60}, and the spectrum of δ-valerolactam in toluene saturated with C_{70} at concentrations of 12 mg of δ-valerolactam per mL toluene or toluene saturated with the nanoparticles. The spectra of toluene saturated with the nanoparticles also are included in Fig. 1, and they do not show any spectral absorption in the region of the δ-valerolactam carbonyl absorption, between 1640–1690 cm^{-1}.

Examination of the spectra in Fig. 1 shows both a small shift (~ 2 cm^{-1}) to lower wavenumbers and significant broadening on the low wavenumber side of the carbonyl absorption of the δ-valerolactam (D-lactam) resulting from its treatment with C_{60}. The spectra in Fig. 1 also show the appearance of a new spectral absorption (at ~1662 cm^{-1}) when δ-valerolactam was treated with C_{70} at this concentration level. These changes in the shapes and positions of the carbonyl absorptions indicate interactions between δ-valerolactam and both C_{60} and C_{70} nanoparticles.

The interactions between the nanoparticles (C_{60} and C_{70}) and δ-valerolactam are very likely governed by an equilibrium process. Of course, shifting the equilibrium toward a complex formed between δ-valerolactam and the nanoparticles could result from increasing the concentration of either the nanoparticles or the δ-valerolactam. Changing the concentrations of the nanoparticles is limited by their low solubility in the toluene solvent and the use of saturated solutions of the nanoparticles in this study. The solubility of C_{60} is 2.8 mg of C_{60} per mL toluene (Ruoff et al. 1993). Increasing the concentration of δ-valerolactam would certainly shift the equilibrium toward any complex formed between the δ-valerolactam and the nanoparticles; however, it would also increase the amount of the δ-valerolactam not interacting with the nanoparticles that contains the infrared probe, the lactam carbonyl bond. The carbonyl absorption of the non-interacting δ-valerolactam at higher concentrations could cover up the carbonyl absorption of any complex formed between the δ-valerolactam and nanoparticles and interfere with its observation. So, the experimental challenge is to add enough δ-valerolactam to form a complex, but not enough δ-valerolactam to cover up the absorption of the complex formed with the absorption of non-interacting δ-valerolactam.

Concentration studies were carried out to determine the optimum concentration level of δ-valerolactam to generate a δ-valerolactam - nanoparticle complex and still allow the observation of its carbonyl absorption. Figure 2 shows spectra collected at approximately 19, 12, and 7 mg of δ-valerolactam per mL toluene and toluene saturated with C_{60}. The spectra in Fig. 2 show a small shift (~2 cm^{-1}) to lower wavenumbers and significant broadening of the carbonyl absorption by δ-valerolactam treated with C_{60} at 19 and 12 mg of δ-valerolactam per mL toluene saturated with C_{60}. At a concentration near 7 mg only a minimal change of the carbonyl absorption is

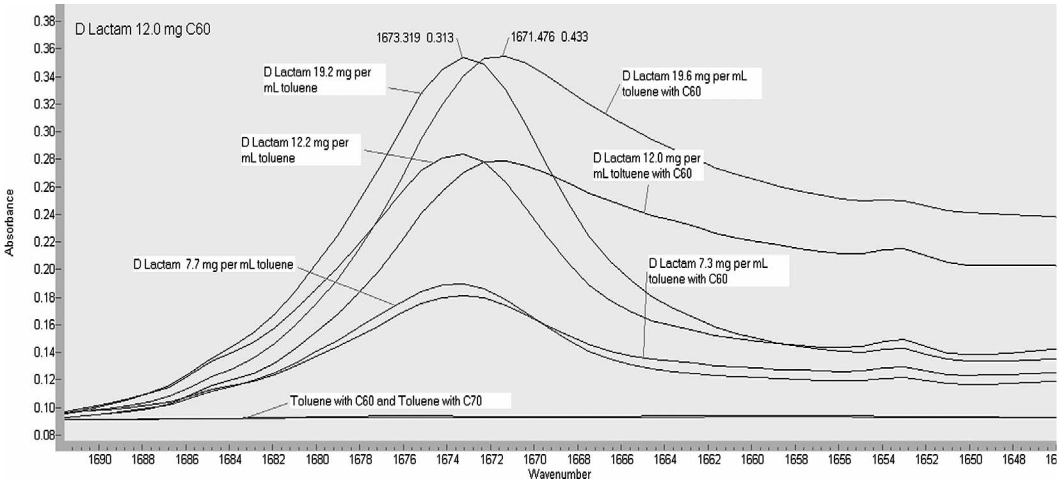

Figure 2.—The spectra of 19.2, 12.2, and 7.7 mg of δ–valerolactam (D-lactam) per mL toluene, and the spectra of 19.6, 12.0, and 7.3 mg of δ–valerolactam per mL toluene saturated with C_{60} are shown. In addition, the spectrum of toluene saturated with C_{60} is included. The spectral peak heights have been adjusted to help clarify spectral changes along the wavenumber axis; therefore, peak intensities do not quantitatively represent concentrations of δ–valerolactam.

observed on treatment of the δ-valerolactam with C_{60}.

As suggested earlier, the broadening of the carbonyl absorption of the δ-valerolactam on treatment with C_{60} results from an equilibrium process generating an overlap of the carbonyl absorptions from both non-interacting and interacting δ-valerolactam. Spectral subtraction has been shown to be a valuable tool in separating overlapping spectral absorptions resulting from a mixture of absorbing species (Gillette & Koenig 1984; Honigs et al. 1985; Yang 1994; Siyuan et al. 2010). Figure 3 shows the result of the subtraction of the spectrum of δ-valerolactam times a 0.73

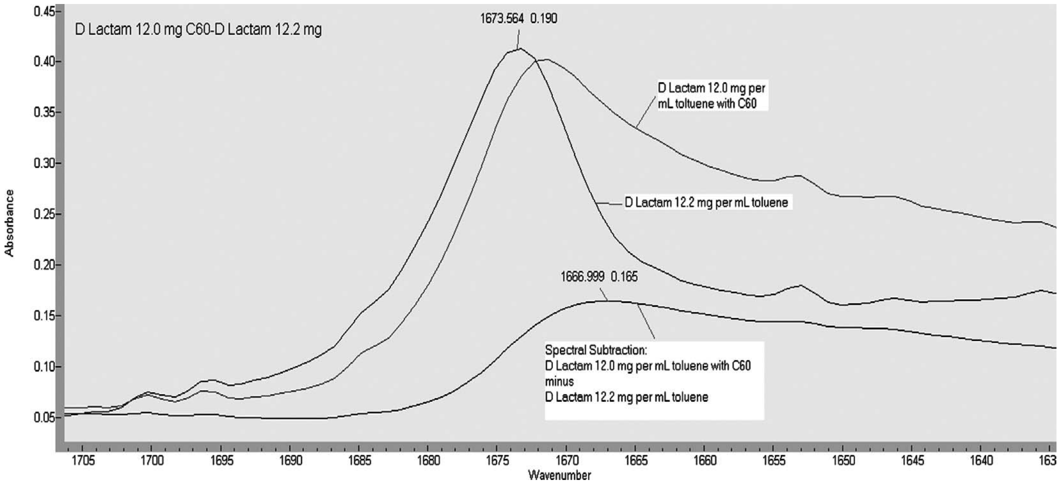

Figure 3.—The spectra of δ–valerolactam (D–lactam) in toluene and toluene saturated with C_{60} are shown at a 12 mg per mL concentration level. In addition, the spectra resulting from the subtraction of the spectra of δ–valerolactam in toluene from the spectra of δ–valerolactam treated with C_{60} are shown. The spectral subtraction factor was 0.73. Subtracted Spectra = (12.0 mg of δ–valerolactam (D-lactam) per mL toluene saturated with C_{60}) minus (12.0 mg of δ–valerolactam (D-lactam) per mL toluene) × 0.73. Spectral peak heights have been adjusted to clarify positioning along the wavelength axis.

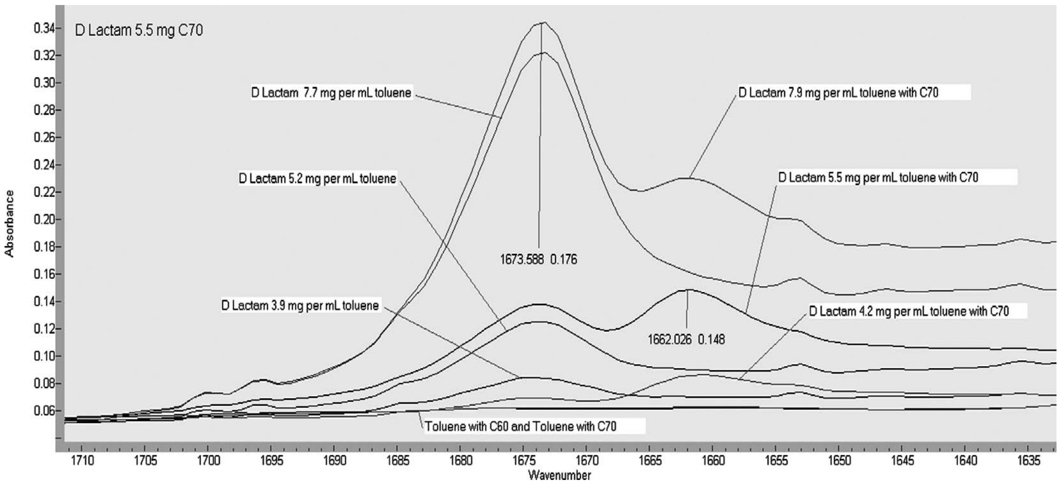

Figure 4.—The spectra of 7.7, 5.2, and 3.9 mg of δ–valerolactam (D-lactam) per mL toluene, and the spectra of 7.9, 5.5, and 4.2 mg of δ–valerolactam per mL toluene saturated with C_{70} are shown. In addition, the spectrum of toluene saturated with C_{70} is included. Spectral peak heights have been adjusted to clarify positioning along the wavelength axis and do not indicate absolute concentrations of δ–valerolactam.

subtraction factor from the spectrum of δ-valerolactam treated with C_{60}. The subtraction factor adjusts for differences in concentrations, baselines, and molar absorptivity of the absorbing species.

The idea of the subtraction process is to remove the absorption of the non-interacting δ-valerolactam from the spectrum of overlapping absorptions yielding the spectral absorption of the δ-valerolactam interacting with the nanoparticles. The subtraction process yields a spectrum with absorption at 1667 cm^{-1} that corresponds to the carbonyl absorption of δ-valerolactam interacting with C_{60}.

Figure 4 shows spectra collected at approximately 8, 5, and 4 mg of δ-valerolactam per mL toluene and toluene saturated with C_{70}. The spectra in Fig. 4 show the appearance of a new carbonyl absorption near 1662 cm^{-1} resulting from the treatment of δ-valerolactam with C_{70}. As the concentration changes from about 8 to 4 mg of δ-valerolactam per mL toluene saturated with C_{70}, the relative intensity of the absorption at 1662 cm^{-1} increases significantly compared to the parent absorption at 1674 cm^{-1}. The spectra in Fig. 4 indicate the complex formed between δ-valerolactam and C_{70} is characterized by carbonyl absorption near 1662 cm^{-1}.

DISCUSSION AND CONCLUSIONS

The observed shifts of the δ-valerolactam carbonyl absorptions to lower wavenumbers on treatment with C_{60} and C_{70} suggest that its interaction with the nanoparticles occurs through the lone-pair electrons of the oxygen part of the carbonyl bond in its amide structure (Kirsch et al. 2015, 2017). A number of studies suggest that nanoparticles are good electron acceptors for molecular systems (Charvet et al. 2012; Schubert et al. 2013; Stranius et al. 2014). A recent study of the interactions of 2-pyrrolidone (γ-lactam, the five-member heterocyclic lactam) with C_{60} also showed a shift of the carbonyl absorption to lower wavenumbers suggesting an interaction through the oxygen lone-pair electrons of the carbonyl bond in the lactam structure (Kirsch et al. 2017). The amide structure has been traditionally described by two resonance structures resulting from the lone-pair electrons on the nitrogen being delocalized into the amide carbon–nitrogen chemical bond generating a minor resonance structure with a carbon–oxygen single bond (Avram & Mateescu 1970). If the lone pair electrons of the oxygen on the δ-valerolactam are donated into the antibonding molecular orbitals of the C_{60} or C_{70} (Feng et al. 2008), the resonance structure containing the single bonded CO is stabilized a bit more yielding a lower wavenumber infrared absorption of the carbonyl group in the amide structure. Figure 5 shows this oxygen lone-pair donation model, and describes its impact on the carbonyl absorption (Behera & Ram 2015).

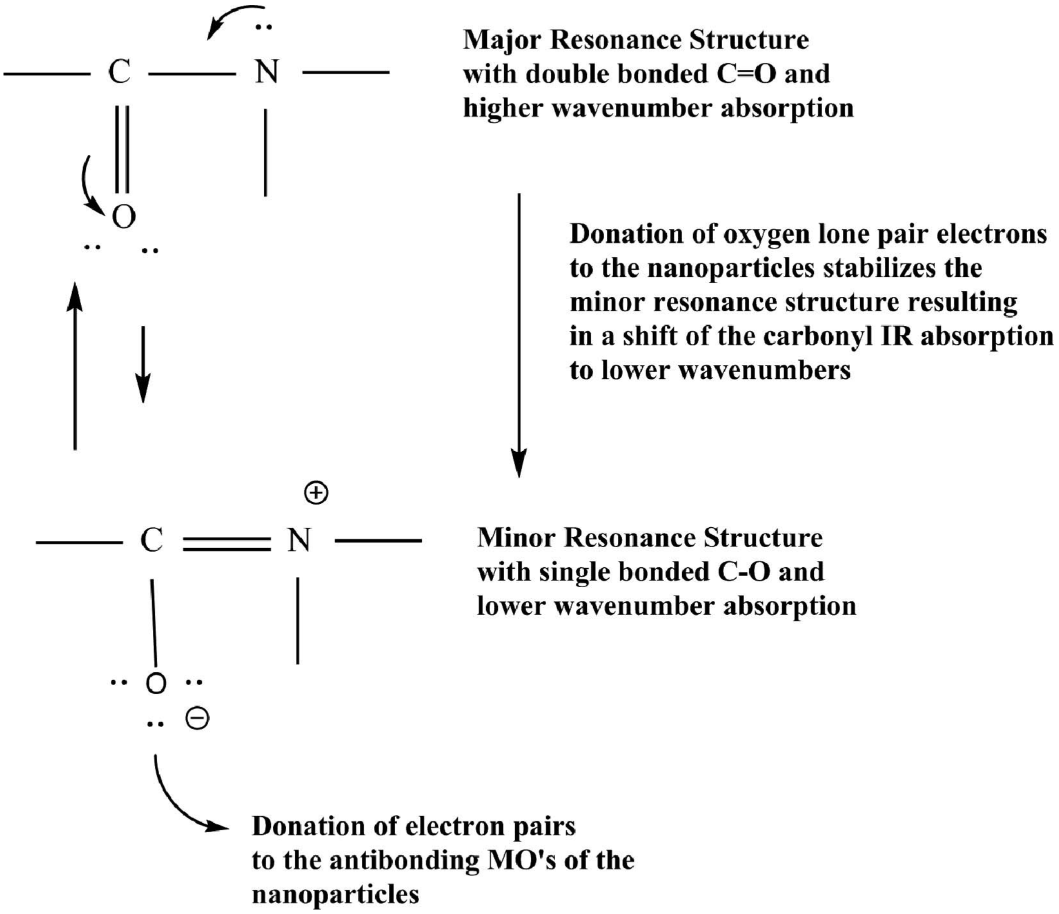

Figure 5.—Resonance structure model with oxygen, lone pair donation to the nanoparticles resulting in an increase in the stability of the resonance structure with the single bonded carbonyl supports a shift of the carbonyl absorption to lower wavenumbers.

In summary, the observed changes in the carbonyl absorption of δ-valerolactam indicate that interactions occur (a complex is formed) between δ-valerolactam and both C_{60} and C_{70}. The shifts of the carbonyl absorption to lower wavenumbers suggest an interaction resulting from donation of the electron pairs from the oxygen part of its amide functionality into the antibonding MO's of the nanoparticles (C_{60} and C_{70}). Spectral subtraction of δ-valerolactam in toluene from δ-valerolactam treated with C_{60} in toluene generates a spectrum with an absorption at 1667 cm^{-1} that is in reasonable agreement with the new carbonyl absorption observed at 1662 cm^{-1} when δ-valerolactam (D-lactam) is treated with C_{70}.

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USING GENERAL LAND OFFICE SURVEY NOTES TO DEFINE REFERENCE ECOSYSTEMS FOR BALL STATE UNIVERSITY'S GINN WOODS, DELAWARE COUNTY, INDIANA

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ABSTRACT. Nineteenth century land surveyors listed the species and size of witness trees. From this information we have first-person accounts for the timber comprising Indiana's presettlement forests. The goal of this investigation was to use historic General Land Office (GLO) survey notes to establish reference ecosystems for Ginn Woods, a Ball State University Field Station property. For the region surrounding Ginn Woods, witness tree species and sizes were charted on a mile section grid and a presettlement map of plant associations was created. Results show that Ginn Woods was historically part of a larger Beech-Maple community geographically isolated between the Mississinewa River and Pipe Creek floodplain ecosystems. Species associated with the Oak-Hickory community were located near Ginn Woods, but these species were not historically recorded in what became the Ginn Woods site. GLO data also identified the presence of prairies, swamps, and springs in or around the Ginn Woods property. GLO results were compared to more recent examinations of the composition of Ginn Woods, and Geographic Information Systems (GIS) land use data were incorporated to illustrate the dramatic loss of historic forest and wetland complexes to modern agriculture.

Keywords: Reference ecosystems, presettlement landscapes, Ginn Woods, ecological restoration, landscape management

INTRODUCTION

Reference ecosystems are historic ecosystems that existed prior to European settlement, and have since been altered by human activities such as urbanization and agriculture (Egan & Howell 2001). Indiana landscapes have multiple reference ecosystems, for instance glacial, inter-glacial, pre, and post settlement. Typically, however, the community type and composition of presettlement landscapes is used as a benchmark or goal for restoration projects (Egan & Howell 2001; Barr et al. 2002). GLO survey notes produced for east-central Indiana in the early decades of the nineteenth century offer a glimpse of the Hoosier presettlement landscape. An awareness of historic reference ecosystems, along with observed changes over time (clearing of vegetation, species loss, invasive species, woodland pasturing of livestock, hydrological alterations, etc.), inform restoration and management decisions in the 21st Century.

General Land Office survey.—The General Land Office (GLO) was an agency created by the US Government in 1812 to quickly and efficiently divide and sell publically owned land. The government sought to extend its influence, and secure its control, over recently acquired public lands from European and Native American competition (i.e., lands acquired in the 1783 Treaty of Paris and the 1803 Louisiana Purchase)(White 1991).

The GLO adopted the Rectangular Land System where surveyors divide the landscape into townships made up of thirty-six, square-mile (1.6 km), 640 acre (259 ha) sections demarcated by mile-long north-south and east-west lines. To delineate these lines in forested areas surveyors placed a wood post at the corner of each section, and the half-mile point between each section corner. The surveyor used an ax to "blaze" (chop out a flat surface in the tree's trunk) two nearby trees as more permanent markers of the wood post. Since blazed trees identified legally surveyed locations, they became known as "witness trees" and later "bearing trees" (BLM 1980).

For each mile-long section line the surveyor was directed to record specific information about the landscape: the species of the witness trees and undergrowth typically found along the section line, and the potential of the land for agriculture. For each witness tree the surveyors were instructed to record its "kind and diameter," and to use

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compass bearings to locate it in the landscape. Witness trees at township and section corners were "not less than five inches in diameter" in size (12.7 cm). This size was later changed to "not less than two and a half inches in diameter" (6.35 cm). The beech tree (*Fagus grandifolia*) is the only species specifically mentioned in the guidance to surveyors, who were permitted to scar the tree's smooth bark rather than cutting through the thick bark typically required of other timber species. Vegetation for a section line was to be summarized by accounting for the "several kinds of timber and undergrowth in the order in which they predominate." Finally, topography, or "Land Surface" was described either as "level, broken, or hilly—1st, 2d, or 3d rate on each mile—1st rate to indicate extra quality, 2d rate good average, and 3d rate inferior quality" (Hawes 1868; GLO 1871). Surveyor's notes were used by prospective buyers to judge a property's value for farming or industry. Since the surveyors were required to list the tree's species, we have first-person accounts for the timber comprising Indiana's historic presettlement forests.

GLO data are used in a variety of research in both the sciences and humanities. Several articles and texts are effective in describing the agency's history (Bourdo 1956; Rohrbough 1968), and nineteenth century guidelines for GLO surveyors are available online from the Bureau of Land Management's web site (Hawes 1868; GLO 1871; BLM 2017).

Using GLO data for reconstructing nineteenth century forest composition is limited by potential surveyor bias of species, size, and witness tree location (Bourdo 1956; Manies & Mladenoff 2000; Schulte & Mladenoff 2001; Fralish & McArdle 2009; Hanberry et al. 2012). Bourdo explains how some surveys used fraudulent data or contained errors that were never corrected. More importantly, he suggests that the guidelines directing surveyors to choose witness trees of "the soundest and most thrifty in appearance" led to a bias in species selection in that tree species were not randomly selected and not a purely objective sample of the presettlement forest (Bourdo 1956:760). However, given that many of the witness tree sizes in the area surrounding what is now Ginn Woods are small (Table 3), Bourdo's claim appears to be overstated. Schulte & Mladenoff (2001) stated that surveyors were paid by the mile, and likely selected witness trees that were easiest to locate. Therefore, the patterns of species in the landscape "tempered surveyor choice through reduced availability of tree species and sizes in the environment surrounding the [section] corner (Schulte & Mladenoff 2001:7)." Manies & Mladenoff (2000) concluded that GLO data somewhat underestimates species diversity and size, but GLO reconstructions are still representative of the landscape for large-scale studies.

Since GLO data is, in essence, a "snapshot in time", it is most often used to define reference ecosystems for modern day ecological restorations and management decisions. While there is a multitude of published research describing the use of GLO data to inform restoration and management decisions, those that directly influence this project are highlighted here. Indiana presettlement landscape maps built on GLO data, and illustrating major vegetative communities, have been assembled by Gordon (1936), Potzger et al. (1956), Crankshaw & Lindsey (1965), and Jackson (1997). Barr et al. (2002) used Geographic Information Systems (GIS) software and GLO data to map presettlement vegetative communities to guide ecological restoration decisions in Marion County, Indiana. Barr et al., with modification (see Methods), could act as a model for GLO studies in Indiana.

Ginn Woods and the GLO study site.—Ginn Woods is a 65.2 ha (161 ac), Ball State University owned and managed property containing one of the largest stands of old-growth forest remaining in Indiana (Badger et al. 1998; Ginn Woods 2017). In *Natural Areas of Indiana and their Preservation* Alton Lindsey et al. (1969) described the woods as "largely flat, and in some places the soil is imperfectly drained so that water is ponded at least in early spring." The survey valued the property for its large and intact size, and the potential use as an educational facility (Lindsey et al. 1969:312). Studies of Ginn Woods have looked at flora and vegetation (Schoultz 1997; Ruch et al. 1998), its old-growth structure and composition (Badger et al. 1998), the influence of soils on vegetation (McClain 1985), and the distribution of specific species (Crankshaw & Cartwright 1978). While this on-going body of research examines the property from within, this study's use of GLO survey notes attempts to recreate the historic vegetative context of Ginn Woods within the presettlement landscape.

The GLO study site encompasses the two square mile sections in which Ginn Woods is

Table 1.—Location of Ginn Woods study site. The Ginn Woods study site consists of thirty township sections located in Blackford, Delaware, and Grant counties.

Township/range	County	Township name	Township sections	Number of sections
T22N, R9E, 2nd Meridian	Grant	Jefferson	1–2	2
	Delaware	Washington	11–14, 23–26, 35–36	10
T22N, R10E, 2nd Meridian	Blackford	Licking	4–6	3
	Delaware	Union	7–9, 16–21, 28–33	15

located (Section 18 and 19 of Union Township, Delaware County), and the two tiers of townships encircling the site (Fig. 1). Therefore, the study area contains 30 mile-square (640 acre, 258.9 ha) sections totaling 19,200 acres (7,769.9 ha). Two sections are in Blackford County, three in Grant County, and twenty-five in Delaware County (Table 1).

METHODS

From the GLO notes, the quantity of each tree species, percent of total trees, and average diameter for each tree species were recorded. Tree species were assigned a forest association using Whitaker & Amlaner (2012:327–336) (Table 2 & 3), a reference specific to Indiana In a similar study from Marion County, Barr et al. (2002) used a less precise, geographically broad guide (Kricher & Morrison 1988) for assigning witness tree species to specific communities. Several witness trees recorded in the GLO notes were listed with only their genus (e.g., elm, hickory, ash), but not their species. Since they could not be assigned a community association, they were listed as non-indicators. For instance, if the surveyor simply listed “ash,” he could have meant a green ash associated with a Floodplain Forest, or a white ash associated with a Beech-Maple forest. Another set of non-indicators were species with multiple associations (i.e., black cherry).

Tree species from GLO survey notes were mapped on a one-mile section grid using GIS sofware (ESRI 2017). Species associated with the Beech-Maple community are represented on the map with a solid circle (•), species associated with the Oak-Hickory community are represented with a plus (+), generalist species are represented with solid square (■), and non-indicator species are represented with a circle (○). To simplify the illustration, the two Floodplain Forest species (one willow and one sycamore) were not mapped. Landforms identified in the survey (swamp, spring, prairie) were mapped (Fig. 2.).

A pressttlement map was created using the GLO data (Fig. 3.). Trees with an Oak-Hickory association were rare in the study area, and the community was mapped around the cluster of

Table 2.—Assumed Forest Association for Ginn Woods Species. Species were recorded in the 1820 General Land Office surveyor notes. Assumed forest association from Table P-5 Whitaker & Amlaner (2012:327–336). Hickory, ash, and elm are considered non-indicators because only their genus was listed.

Assumed Forest Association for GinnWoods Species	
Beech-Maple	
Fagus grandifolia	Beech
Ulmus rubra	Red elm
Oak-Hickory	
Celtis occidentalis	Hackberry
Cornus florida	Dogwood
Liquidambar styraciflua	Sweetgum*
Ostrya virginiana	Ironwood
Quercus macrocarpa	Bur oak
Quercus velutina	Black oak
Beech-Maple and Oak-Hickory (Generalist Species)	
Acer negundo	Box elder
Acer saccharum	Sugar maple
Aesculus glabra	Ohio buckeye
Carpinus caroliniana	Hornbeam
Fraxinus americana	White ash
Fraxinus quadrangulata	Blue ash
Juglans nigra	Black walnut
Morus rubra	Red mulberry
Populus grandidentata	Aspen
Quercus alba	White oak
Quercus rubra	Red oak
Floodplain	
Platanus occidentalis	Sycamore
Salix spp.	Willow
Non-indicator	
Carya spp.	Hickory
Fraxinus spp.	Ash
Prunus serotina	Cherry
Ulmus spp.	Elm

* Sweetgum is not common to Delaware County (Deam 1953), and is likely Black gum (*Nyssa sylvatica*) which is an Oak-Hickory association.

Table 3.—Results of General Land Office survey notes for the Ginn Woods study area: scientific name, witness tree common name, number surveyed, species percentage of total trees surveyed, average DBH, and assigned forest association (Beech-Maple, Oak-Hickory, Floodplain, or Non-indicator). Witness trees recorded with only their genus (e.g., elm, hickory, ash), but not their species, are listed as non-indicators.

Inferred scientific name	Witness tree recorded name	Number surveyed	Percent of total trees	Average DBH (in)	Assumed forest association
Acer negundo	Box elder	1	0.3	6	Beech-Maple, Oak-Hickory
Acer saccharum	Sugar maple	24	7.6	16	Beech-Maple, Oak-Hickory
Aesculus glabra	Ohio buckeye	13	4.1	10	Beech-Maple, Oak-Hickory
Carpinus caroliniana	Hornbeam	1	0.3	5	Beech-Maple, Oak-Hickory
Carya	Hickory	19	6	14	Non-indicator
Celtis occidentalis	Hackberry	1	0.3	26	Oak-Hickory
Cornus florida	Dogwood	4	1.2	6	Oak-Hickory
Fagus grandifolia	Beech	138	44	16	Beech-Maple
Fraxinus americana	White ash	11	3.5	15	Beech-Maple, Oak-Hickory
Fraxinus	Ash	15	4.7	12	Non-indicator
Fraxinus quadrangulata	Blue ash	16	5	9	Beech-Maple, Oak-Hickory
Juglans nigra	Black walnut	2	0.6	13	Beech-Maple, Oak-Hickory
Liquidambar styraciflua	Sweetgum*	4	1.2	24	Oak-Hickory
Morus rubra	Red mulberry	2	0.6	10	Beech-Maple, Oak-Hickory
Ostrya virginiana	Ironwood	15	4.7	6	Oak-Hickory
Platanus occidentalis	Sycamore	1	0.3	24	Floodplain
Populus grandidentata	Aspen	1	0.3	12	Beech-Maple, Oak-Hickory
Prunus serotina	Cherry	2	0.6	18	Non-indicator
Quercus alba	White oak	15	4.7	24	Beech-Maple, Oak-Hickory
Quercus macrocarpa	Bur oak	4	1.3	20	Oak-Hickory
Quercus rubra	Red oak	5	1.6	18	Beech-Maple, Oak-Hickory
Quercus velutina	Black oak	5	1.6	33	Oak-Hickory
Ulmus rubra	Red elm	1	0.3	22	Beech-Maple
Ulmus	Elm	15	4.7	13	Non-indicator
Salix	Willow	1	0.3	4	Floodplain
	Total	316			

* Sweetgum is not common to Delaware County (Deam 1953), and is likely Black gum (*Nyssa sylvatica*) which is an Oak-Hickory association.

associated witness trees in the central portions, and on the western edge, of the project site. Section lines with more than one species from the Oak-Hickory association were identified, then GIS was used to create a half-mile buffer around each witness tree to estimate an area of potential environmental conditions supporting Oak-Hickory association species (Tulowiecki & Larsen 2015). Since few Floodplain Forest species were identified (2% of species), this community was defined using the floodplain soils identified in county soil surveys (USDA1985, 2004). Wetlands were mapped using the Historic Wetlands GIS shapefile from the Indiana Map website (2017).

To estimate change in the study area over time, the GLO presettlement map was compared to current land uses using Gap Analysis Project data (GAP), and gains or losses in vegetative communities were reported (Tables 4 & 5). GAP data is land cover information mapped by the United States Geological Survey and used for conservation planning. It is used in the Ginn Woods study to estimate land cover change over time.

RESULTS

Representing 25 different species, 316 witness trees were identified in the study area (Table 2 & 3). Of the trees 44% (139) are representative of a Beech-Maple community, 10% (33) represent an Oak-Hickory community, and 1% (2) represent a Floodplain Forest community. Generalist species common to multiple associations accounted for 29% (91) of the total. Sixteen percent (51) of the trees were non-indicators for a specific community, or the GLO notes recorded the genus but not the specific species.

The presettlement map created using GLO witness trees, in conjunction with soil and wetland

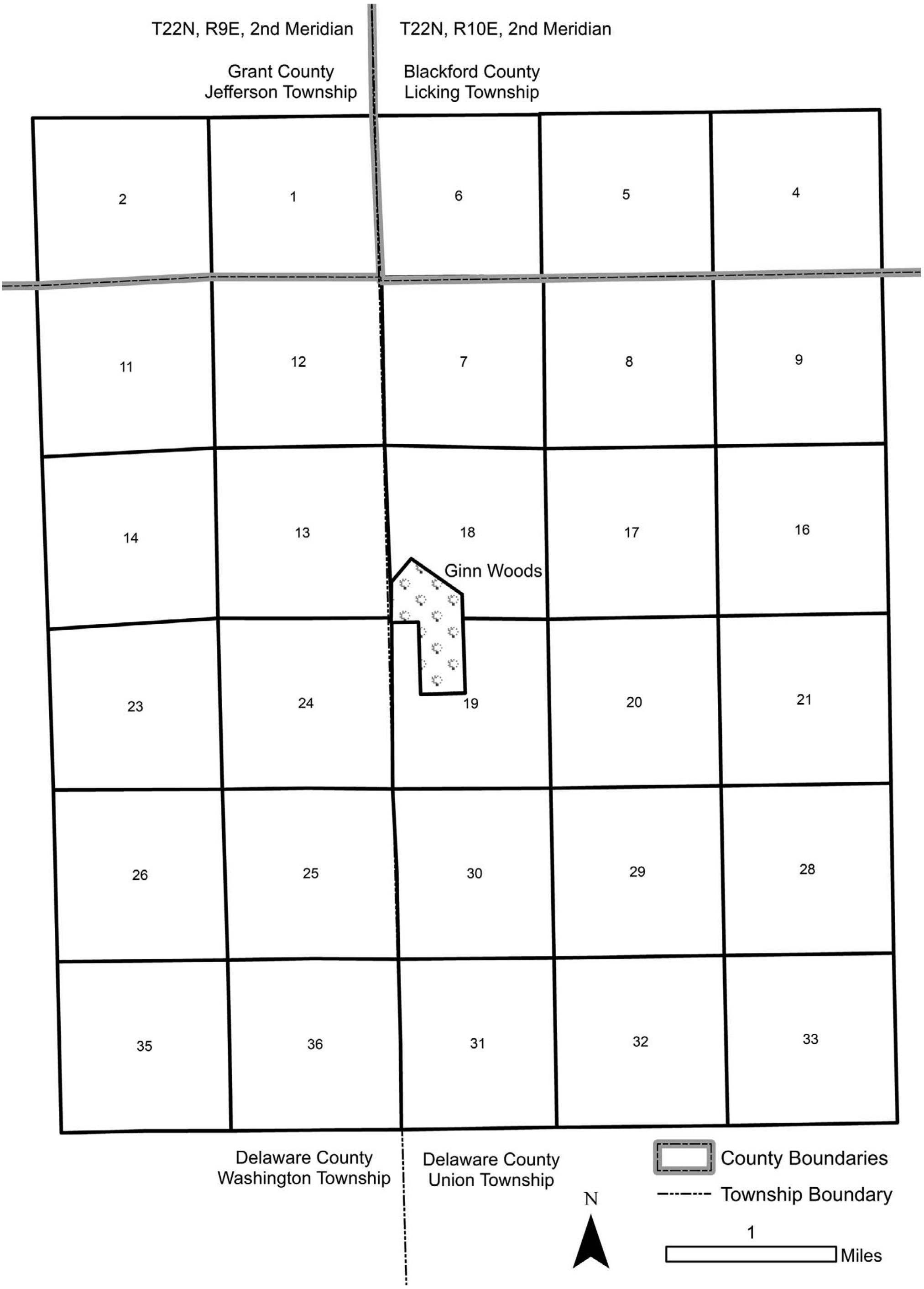

Figure 1.—Ginn Woods and accompanying GLO study area. The study area encompasses thirty square miles of Delaware, Grant, and Blackford Counties. Ginn Woods straddles the line between Sections 18 and 19 in Union Township, Delaware County.

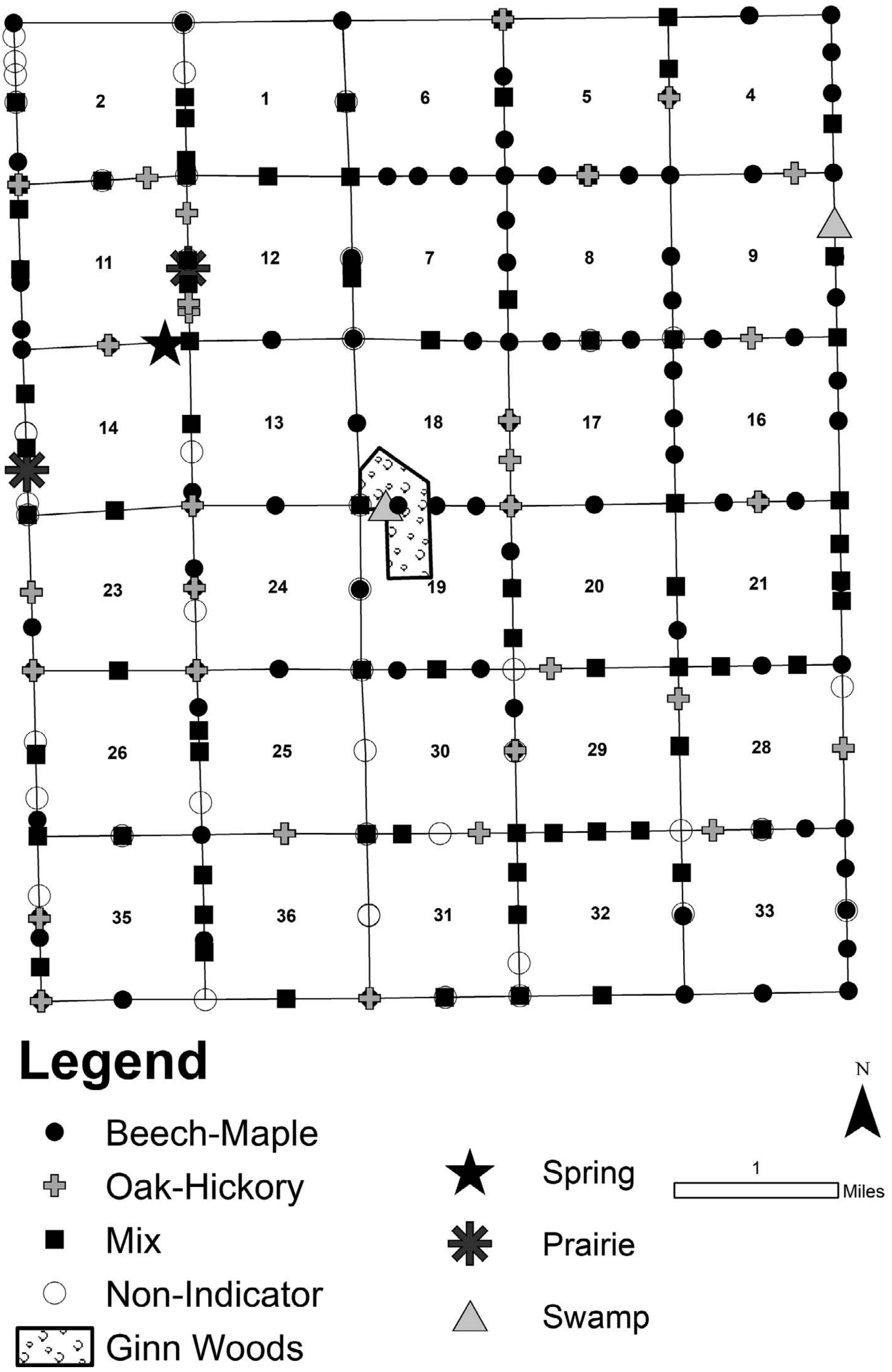

Figure 2.—Study Area Witness Tree Locations. Witness tree locations and species associations charted onto the square-mile section grid. The illustration includes the surveyor's locations for prairies, swamps, and springs.

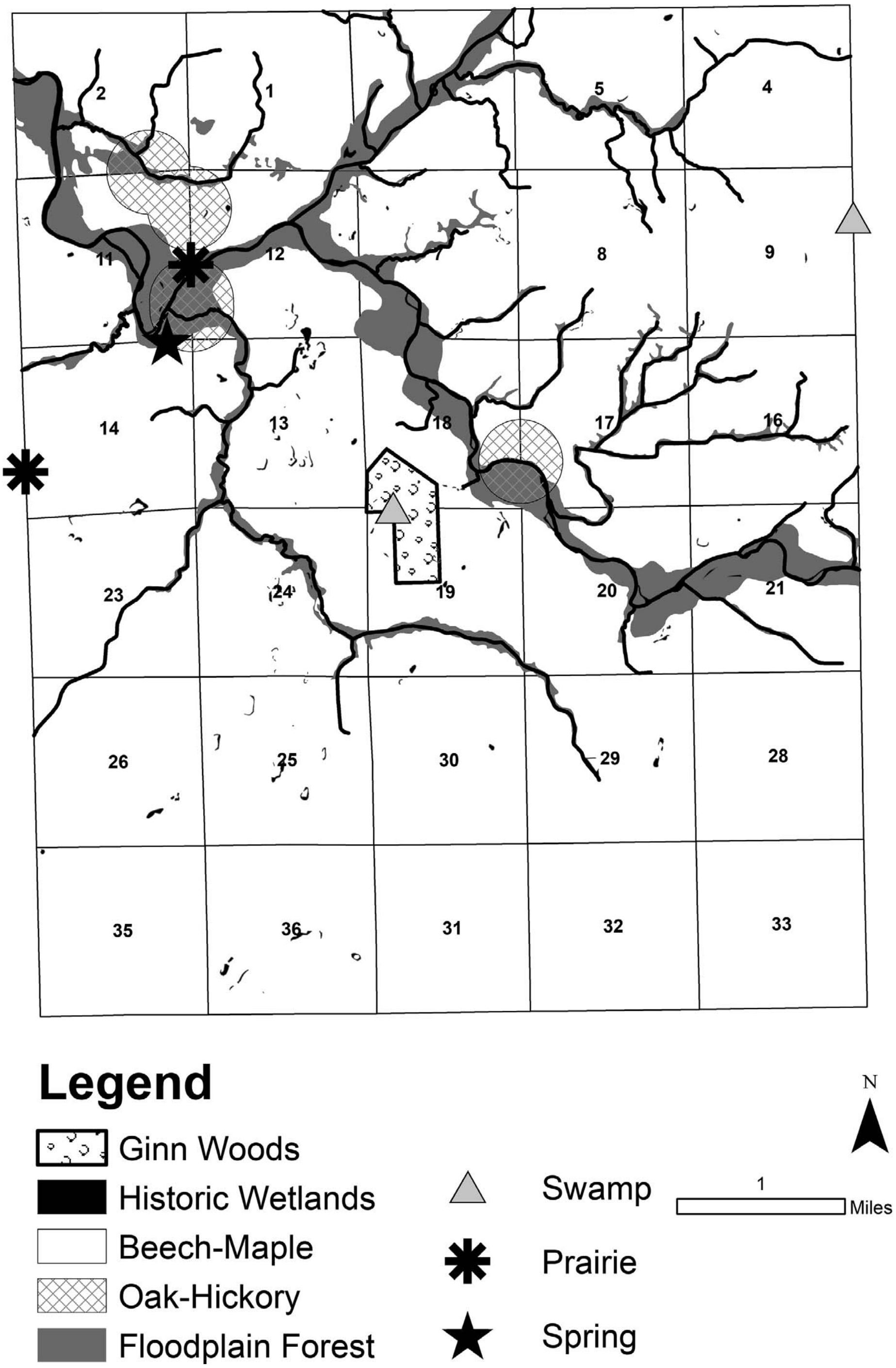

Figure 3.—Presettlement Map for Township Sections surrounding Ginn Woods. The map was assembled using methods from Barr et al. (2002) and represents the study site's historic 1820 forest associations and landscape features.

Table 4.—Cumulative totals of Historic Land Associations for the Ginn Woods study area for acreage and percent of total acres (See Fig. 3).

Historic Land Associations	Historic acreage	Percent of total acres
Beech-Maple	13,392	69
Oak-Hickory	4,223	22
Floodplain	1,597	8.5
Wetlands	88	0.5
Total	19,200	100

data, illustrates that the Beech-Maple community accounts for 69% of the study area, followed by Oak-Hickory at 22%. The hydrological system-related associations of Floodplain (8.5%) and Wetland (0.5%) account for about 9% of the study area (Table 4).

A comparison of presettlement GLO and GAP data illustrates changes to the study site over time (Tables 4 & 5). The historic forest/wetland/floodplain landscape composition documented by GLO surveyors is now dominated by agriculture (78%). The presettlement forest, identified as "deciduous" in the GAP data, has shrunk to only 13% of the study site. Wetland and floodplain communities that represented about 9% of the presettlement landscape have contracted to only 2%.

DISCUSSION

The Ginn Woods study site is located at the southern end of the Bluffton Till Plain, and is characterized by poorly drained clayey till. A common characteristic of the soils are small depressions that facilitate ponding. Red maple, bur oak, swamp white oak, American elm, and green ash are timber species characteristic of poorly drained areas. Beech, sugar maple, tulip poplar, and white ash are timber species typical of the plain's better drained but moist soils (Homoya et al. 1995). Braun (1950) reported that the Beech-Maple region of the US is "so uniform throughout its extent" that vegetative changes are unrecognizable (1950:309). Till plain forests are characterized by "low relief where minor differences in elevation (a foot or two), resulting in differences in soil moisture and aeration, determine the soil type" (1950:316). Oak-Hickory association species are typically found in gravel or sandy areas too dry for Beech-Maple species. Therefore, vegetative changes are based on local edaphic and topographic changes.

The level plain of the study site is divided by the Mississinewa River valley that runs southeast to northwest approximately thirty feet (10 m) below the adjacent uplands. The river channel is characterized by a level flood plain containing mounds of soil deposits from seasonal flooding. Additional topographic changes in the study area are created by small stream tributaries to the river.

The sections were surveyed by John McDonald in 1819 and 1820, and digital copies of the notes were viewed at the Ball State University Applied Anthropology Laboratories. Along with the identification of witness trees, McDonald identified several distinct landscape features. In two locations he recorded "prairie" in the notes; the first as part of the Mississinewa River floodplain, and a second on the uplands of Section 14. From notes describing a wet "prairie" in the township just west of the study site, and his inability to set a post or blaze a tree, we know that the surveyor used the term to describe a treeless landscape. Conversley, the term "swamp" was used to describe a forested wetland in the uplands of Section 9, and in what would become Ginn Woods in Sections 18 and 19. A "spring" was also identified in the Mississinewa River floodplain between Sections 11 and 14, although there is no

Table 5.—Cumulative totals of current land associations for the Ginn Woods study area for acreage and percent of total acres.

Current GAP land use categories	Current acreage (from GAP data)	Percent of total	Percent loss from historic woodlands and wetlands
Agriculture	14, 974	78.0	
Deciduous Forest	2,489	13.0	(85.7)
Evergreen Forest	34	0.2	
Other Vegetation	262	1.3	
Development	1,070	5.6	
Wetlands	371	1.9	(78.4)
Total	19,200		

indication in the notes that it supported a significant vegetative community.

Ginn Woods is located on a flat upland site between the Mississinewa River and Pipe Creek. It straddles the east-west survey line between Sections 18 and 19. Four of the six witness trees in the property are beech, and the other two are white ash and white oak. The notes also identify a swamp fifteen chains (990 ft, 302 m) from the west boundary of the Ginn Woods property, and demonstrates the presence of ponding within the woods. Spicebush (*Lindera benzoin*; denoted by the surveyor as spicewood*)* and prickly ash (*Zanthoxylum americanum*) are listed as understory species.

Mapped witness trees from GLO survey notes illustrate that, historically, Ginn Woods and its surrounding landscape represented an upland, mostly level Beech-Maple forest association dotted with small ponded wetlands. More than 50% of the trees in the GLO sample were either beech or sugar maple. American beech (*Fagus grandifolia*) accounted for 138 trees and 44% of the total trees in the study area. Sugar maple (*Acer saccharum*) was the next most populous tree at 24 and counted for 7.6% of witiness trees. The section of landscape containing Ginn Woods was mostly isolated between the floodplain communities associated with the Mississenawa River and Pipe Creek.

The GLO notes identified treeless prairies in both floodplain and upland settings. The surveyor used the term "swamp" twice, indicating locations of forested wetlands in contrast to open wet meadows. One of the swamp areas is part of Ginn Woods. A review of aerial photography shows how ditches drain the farm fields surrounding the current forest, a practice common in northern Delaware County.

GLO notes indicate the presence of trees representating the Oak-Hickory association in the sections west of Ginn Woods (Sections 11–14, 23–26, and 35), and east (Sections 17–20). Till plain soil characteristics, hydrology, and topography are consistent throughout the upland area on the south side of the Mississenawa, and no patterns of soil types driving a change in species composition, as described by Crankshaw & Lindsey (1965) and Braun (1950), were discernable. Absent of these patterns, a designation of Beech-Maple-Oak mix might better represent these woodlands. Kricher & Morrison (1988) describe how Oak-Hickory forests "intermingle with virtually all other forest types" on moist sites, and are typically characterised by "both" oak and hickory species. The GLO data tend to support this characterization. Twenty-nine oaks, and nineteen hickories were recorded as witness trees (15% of all trees). Twenty-eight share a point with a witness tree having a Beech-Maple association, or are within a quarter mile of a witness tree with a Beech-Maple association.

GLO notes provide a sample of trees species and sizes that can be compared to the current species composition of Ginn Woods. Badger et al. (1998) defined the structure and composition for the woods, and described it as a Maple-Beech-Basswood old-growth forest. The study identified twenty-eight species within Ginn Woods. Nine are not listed as witness trees in the GLO study (i.e., red maple, silver maple, black ash, green ash, Kentucky coffeetree, tulip poplar, cottonwood, chinquapin oak, and basswood). The GLO study identified twenty-five species, five of which are not found in Ginn Woods (i.e., dogwood, black gum, ironwood, aspen, and willow). The composition of the study area is also significantly different than that of the property. Badger et al. found a dominance of sugar maple (36%) over beech (8%) when the three areas of Ginn Woods were averaged. Conversely, the GLO study identified a composition of beech (44%) over sugar maple (8%). Therefore, the current composition of Ginn Woods is significantly different from the presumed composition recorded in the GLO data. There are several potential explanations for this difference, such as change in species composition over time, the local environmental conditions creating Ginn Woods is distinct from the study area as a whole, surveyor bias, or the GLO data is too coarse to define composition at the Ginn Woods scale.

While wet prairies were not identified in the area that became Ginn Woods, the GLO notes do indicate that it was common to find these wetland openings in the study area's forest canopy. The notes identify Ginn Woods as "swamp." Unfortunately, they do not identify the wetland species for comparison to present conditions. Regardless, GLO notes identify this body of water as historic, and management and future land purchases should focus on preserving the offsite hydrologic systems supporting the woods.

A comparison of the historic GLO and contemporary GAP landscapes illustrates the dramatic change from presettlement forest to agriculture (Table 5). The presettlement forest, identified as "deciduous" in the GAP data, has shrunk to only 13% of the study site—a loss of more than 85% of the historic woodland landcover. The GAP data does not provide a measurement of the ecological quality of the current forest cover, but these woodlands are mainly restricted to fencerows, ravines, floodplain, and the occasional 2^{nd} growth, degraded woodlot. Equally dramatic is the loss of approximately 78% of the site's wetlands. While not yet studied, agricultural practices have likely caused changes to the landscape affecting Ginn Woods, including hydrological changes, the introduction of exotic species, and the use of pesticides, herbicides, and fertilizers that drift or flow into the woods.

Finally, GLO and GAP data indicate, to a degree, how species makeup has changed in the study area. For instance, GAP data indicates the presence of 34 acres of non-native "evergreen forests" at four Christmas tree farms. Species have surely disappeared as well. Ash of various species (possibly black, blue, white, and green) account for 13% of the GLO witness trees, and elm another 5% (with American elm likely represented). However, due to the emerald ash borer and Dutch elm disease infestations, these species have declined, and it is unclear what species will fill the void. Fortunately, in the light of all these changes, Ginn Woods remains a rare and protected example of Indiana's presettlement forests.

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CONSTRUCTION OF A SUBURBAN-RURAL RECREATIONAL TRAIL PRODUCES DEVELOPING EDGE EFFECTS

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ABSTRACT. The development of edge effects due to increased light penetration attendant to the construction of a bicycle–pedestrian recreational trail through a forest in southwest Indiana was studied. Though the trail roughly followed the route of an existing path through the forest, light penetration was dramatically increased by the construction of the trail and transiently increased in the forested edge within 10 m of the trail. Decreased light penetration in the forested edge compared to the forest interior had also developed five years after trail construction, indicating the development of a side canopy. Over the study period of five years post-trail construction, there was no evidence of decreasing light penetration over the trail itself. The findings will be relevant for land managers and others involved in recreational trail construction through Indiana forests.

Keywords: Forest edges, forest understory, light penetration, magnitude of edge influence, side canopy, PAR, photosynthetically active radiation

INTRODUCTION

Urban recreational trails can confer diverse benefits to human communities. They allow individuals using the trails to experience greater connection with the natural world and opportunities for outdoor exercise and play; they may also provide for a nexus of community social connection. Locally greater biodiversity is observed at edges created along corridors between habitat types as these corridors support a mixture of introduced species, edge specialists, and core habitat species (Harris 1988; Hall & Kuss 1989; Parendes & Jones 2000; Roovers et al. 2004; Honu & Gibson 2006; Avon et al. 2010). Green spaces and forest fragments at the urban – rural interface provide habitat for wildlife. However, roads, trails, lawns, and maintained park landscapes also cause fragmentation of wild and semi-natural habitats. Such fragmentation reduces the effective habitat size for species of core habitats and can result in increased vulnerability to disease, predation, parasitism, and competition from invasive species as well as generating direct effects from reduced habitat area (Ries et al 2004). Habitat size is reduced further because the edges of natural habitat fragments adjacent to constructed environments are affected for some distance into the fragment. While such edge-effects have been long studied, efforts to develop comprehensive and mechanistic models have met with success only relatively recently (Ries et al. 2004). Reviews and careful descriptive work allow recognition of different classes of fragments and edges, and all of these efforts have pointed to the importance of light penetration at edges in driving edge effects at the interface between forests and other habitat types (Matlack 1993; Murcia 1995).

Edge effects in northern hemisphere temperate deciduous forests are characterized by gradual changes in both abiotic and biotic features as one moves from one habitat type to another or from an anthropogenic matrix into natural habitat fragments. Among the abiotic changes observed are changes in light penetration into the forest, temperature, soil moisture, relative humidity, and vapor pressure deficit (Matlack 1993). Where forests adjoin more open habitats, increased light penetration in the forest edge — a positive edge effect — is seen (Matlack 1993; Murcia 1995). Biotic changes often include changes in plant species richness, occurrence of introduced species, and increased understory foliage growth along edges as these edges age, but many changes in plant and animal communities have been inconsistent among different studies (Murcia 1995). Increased growth of understory foliage at an edge is observed as the increased light availability drives proliferation of lateral branches (Matlack 1993). As a newly created edge ages, the development of this "side canopy" then decreases light penetration into the edge. Thus, side canopy

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growth may produce a zone just inside the edge where light penetration is decreased relative to the habitat interior (Murcia 1995; Ries et al. 2004).

Because the forest canopy may close or nearly close over them, trails and roads present a type of edge that can be expected to differ from edges between forest fragments and agricultural or other anthropogenically influenced habitats. Paved and graded recreational trails with engineered improvements for drainage and slope retention can be expected to affect the forest habitat through which they pass as if they were narrow roads.

This study had two goals. The first was to document the increased light penetration over a recreational trail and at the edge of the trail due to its construction and the attendant opening of the canopy. This was accomplished by measuring PAR (photosynthetically active radiation) in 2010, one year before trail construction, and again in 2012, 2014, and 2016. The second was to examine the development of any complex edge with side canopy growth that may have occurred. This work, with higher spatial resolution, was conducted in 2016. A strong positive edge effect resulting from the construction of the trail, followed by a diminution of this effect as the side canopy developed, was predicted.

METHODS

Study site.—The USI–Burdette Park Trail, a locally developed trail that is part of the larger American Discovery Trail, extends from the campus of the University of Southern Indiana (USI) to Burdette Park, a municipally owned and operated park in southern Vanderburgh County in southwest Indiana. From the southern portion of the developed part of the USI campus, the trail continues south-southeast through an agricultural area and into a mesic, upland, mixed hardwood forest before it descends to a creek bottom and re-ascends into Burdette Park. Most of the forested portion of the trail south of the USI campus-proper follows a ridge above a tributary of Bayou Creek, which flows into the Ohio River. Along this section of the trail to the southwest, is a hillside that descends into a large forest tract owned by USI (Forest side) while the northeast side of the trail drops sharply by 27 to 29 m over a distance of about 300 m to the creek (Creek side) and an abandoned agricultural field on its opposing side. The route taken by the trail roughly follows the route of a former log skidding trail that had been infrequently used for recreational purposes as a footpath and ATV trail by local residents. Built to Indiana Department of Transportation standards with efforts taken to minimize overall width and cutting of large trees, the trail is asphalt with gravel and grass borders and occasional small retaining walls and concrete curbs where it either cuts into or runs over the edge of the ridge. Engineering drawings for the trail specified that the width of the cleared corridor would be 20 feet (6.1 m); the resulting trail was then to be 10 feet (3.05 m) wide. This asphalt trail was to be flanked with a gravel verge and a mowed border on each side that would add another 10 feet (3.05 m). The actual width of the cleared corridor was measured shortly after trail paving and found to average 10.5 m (± 1.6, SD) and vary between 7.5 and 12.9 m. Along the forested portion of the trail, the width of the asphalt portion is typically 3.8 m (± 0.4, SD) and the gravel and grass border on either side is normally 2.6 m (± 0.6, SD), varying between 1.6 m and 3.7 m at the transects where we worked. Presently, periodic mowing and herbicide applications are used to maintain the grassy edges next to the trail. The width of the mowed border and herbicide applications varies somewhat from year to year as does mowing frequency.

Along the ridge top and down the slopes on either side of the trail, the composition of the shrub layer in the understory typically included *Asimina triloba* (pawpaw), *Lindera benzoin* (spicebush), and *Sassafras albidum* (sassafras). The canopy layer included *Acer saccharum* (sugar maple), *Carya* species including *C. glabra* and *C. laciniosa,* and *Liriodendron tulipifera* (tulip poplar). The herb layer, especially near the trail, included *Impatiens capensis* (jewelweed), *Parthenocissus quinquefolia* (Virginia creeper), and *Podophyllum peltatum* (mayapple). Introduced invasive species, such as *Alliaria petiolata,* (garlic mustard) and *Lonicera japonica* (Japanese honeysuckle), were present but coverage was not extensive.

Four 150 m transects were installed roughly perpendicular to the footpath and ridgeline in 2010 prior to the construction of the trail. These transects extended across the ridge, 75 m to the north-northeast and south-southwest on either side of the ridge. Stations were marked along each transect with painted wooden grade stakes that were later replaced with metal fence posts at 10 m intervals. The transects were parallel to each other and spaced roughly 80 m apart along the trail such that the last transect crossed the trail 235 m from the first. Posts marking transect stations were numbered with number 1 at the southwest end and number 16 at the northeast end; the trail crossed transects between stations 8 and 9.

PAR measurements.—Available light was measured as the fraction of PAR (photosynthetically active radiation) in the forest relative to open sky PAR. To do this, light was measured along the transects with a PAR sensor (LiCOR 190R, LiCOR Biosciences, Lincoln, NE) mounted on a telescoping fiberglass pole for heights over 1 m or a horizontal aluminum rod affixed to a vertical aluminum pole for heights ≤ 1 m. The sensor was leveled with two orthogonal bubble levels for each measurement and read with a LiCOR 1400 datalogger. At points where PAR measurements were made, the time of measurement to the nearest minute was noted. Measurements were made at 0, 1, 2, and 4 m above the ground at each measurement location; on each transect, measurements were made at transect stations 1, 3, 5, 7, 9, 10, 12, 14, and 16. To allow for increased sampling in the region where transects crossed the trail, additional measurements were made halfway between transect stations 7 and 8, 8 and 9, and 9 and 10. Concurrent with measurements along transects, a separate set of time-marked PAR measurements were recorded simultaneously on a gravel road in a nearby (less than 1 km distant) agricultural field. The PAR sensor here (Apogee MQ200, Apogee Instruments, Logan UT) was mounted ~2 m above ground on a fiberglass pole. All PAR measurements were made in the early morning or late afternoon with the sun low in the sky such that little or no direct sunlight struck either sensor. On rare occasions when direct sunlight did strike either sensor, notes were made and any PAR readings at these times were excluded from analysis. Forest PAR measurements were divided by open sky PAR measurements taken at the same time.

Measurements from each sensor were found to be slightly different but highly correlated ($r^2 > 0.97$). Accordingly, measurements made with the open sky sensor were adjusted to match the sensor used in the forest using a calibration equation previously obtained by linear regression (Corrected field measurement $= 1.0103 \times$ field measurement $+4.6465$ µmol $m^{-2}s^{-1}$). Measurements were made along each transect in 2010, 2012, 2014, and 2016. Trail construction began with preliminary surveys and flagging of the proposed route in 2010 after we completed our measurements. In spring 2011, brush and trees were cleared and the trail was completed before summer.

Magnitude of edge influence.—In 2016 a separate set of measurements were made that were designed to allow direct evaluation of edge development and the distance that any edge effect penetrated into the surrounding forest. For these, three measurements were made 0.5 m apart along 1 m bands perpendicular to the transects at heights of 0, 1, 2, and 4 m above the forest floor. Measurements were made along each transect at the center of the paved trail, at either edge on the border between the mowed grass and the forest, and at distances along transects of 1, 2, 5, 10, and 15 m in both directions into the forest. In this way, the distance of edge effect influence could be evaluated on both sides of the trail.

Data analysis.—Because the paved trail was wider than the previous footpath/ATV path, the strip of land under it and within the boundaries of the mowed grass edges were exposed to a greater fraction of open sky by trail construction. To reveal this and to test for an edge effect in the forest adjacent to the cleared edge, the measurement locations were divided into three groups along each transect. Measurements termed "Trail" measurements included those measurements that were taken over the paved trail or inside the edge of the mowed border. Measurements termed "Edge" measurements were measurements from locations at the border or within 10 m (shortest straight line distance) from the trail's mowed border, and measurements termed "Forest" were taken at locations greater than 10 m from the trail border. The shortest straight line distance to the mowed border of the trail edge was measured because, while the transects cut straight lines across the trail, the trail itself and the mowed border adjacent to it had varying widths and meandered markedly such that distances measured along transects often varied from the shortest straight line distance to the forest opening. With measurement locations grouped in this way, PAR fractions were evaluated with repeated measures analysis of variance using SPSSx v. 23 so that we could examine the change in light penetration over time, at different heights in the understory, and at different distances from the mowed edge – forest border. Measurement location groups (Trail, Edge, Forest) were the treatment levels and measurement years (2010, 2012, 2014, 2016) were the levels of the within-subjects factor. F statistics and degrees of freedom reported reflect the Huynh-Feldt correction for any violations of sphericity (Huynh & Feldt 1976).

Analysis of the distance of edge influence with the separate measurements made in 2016 followed the randomization method of Harper & MacDonald (2011). In this method, the magnitude of edge influence (MEI) is estimated as a difference between the average of edge measurements at a given distance from the border and a reference set of interior measurements. In our case, replicate reference measurements were made at a distance of 15 m deep into the forest on both sides of the trail. MEI is calculated as:

$$MEI = \frac{E - R}{E + R}$$

where E is the mean of edge measurements at a given distance and R is the mean of reference measurements. This quantity will vary between -1 and 1. Here, positive values indicate more light penetration than the reference while negative values indicate less. The significance of the difference between the estimated MEI and zero is then calculated with a randomization test (RTEI) at each distance from the edge where measurements are made such that the distance of edge influence can be assessed. Because reference measurements included all the measurements made at all the plots that were 15 m deep, MEI calculations account for natural variation in the extent of undisturbed canopy cover.

RESULTS

Construction of the trail strongly increased light penetration over the region within the edge as well as over the location of trail itself ($F = 22.60$, $p < 0.0009$ for years–within subjects effect; Fig. 1). However, the significant main effects of years and distance ($F = 41.48$, $p < 0.0009$ for distance–between subjects effect) showed a significant interaction ($F = 19.27$, $p < 0.0009$ for year by distance interaction) because the effect of distance arose after construction of the trail. At sites on the paved trail or within the cleared region that included the mowed edges as well as the asphalt, light levels were more than three fold higher one year after trail construction and remained similarly high for the length of the study (Figs. 1 & 2). Light levels for measurements within 10 m of the trail were approximately doubled in the year after trail construction, but had fallen by the third year after trail construction and were not significantly different from light levels deeper in the forest for the remainder of the study (Fig. 1). No significant changes in light levels were found for forest measurements. There was a strong effect of height; light level was greater as heights increased above the forest floor ($F = 48.00$, $p < 0.0009$ for height–within subjects effect; Fig. 2) and increased from 2.9 (± 0.2, SE) percent of open sky to 5.4 (± 0.4, SE) between the ground and 5 m. Despite the fact that measurements made on the paved trail contrasted markedly with measurements made under forest canopy, there was no significant height by distance interaction following adjustment of degrees of freedom with the Huynh-Feldt correction ($F = 1.521$, $p = 0.213$). However, the effect of height varied with measurement years (significant height by year interaction, $F = 2.077$, $p = 0.042$).

MEI estimates and depth of edge estimates were different on each side of the trail. On the northeast side of the trail, there was a significant positive edge influence at the border of the mowed edge and forest (0 m distance; Table 1) at all heights except 4 m. This positive effect extended 1 m into the forest at ground level and at 1 m above ground. At greater distances into the forest, there were no significant edge effects. On the southwest side both positive and negative edge effects were observed. The edge of the mowed border and forest was significantly brighter at all heights and, at 1 m into the forest, light levels were higher at the 1 m height (Table 2). Strong negative edge effects were present at 2 and 5 m distances past the mowed border at the 4 m height; there was also a negative edge effect on the ground at 5 m (Table 2).

DISCUSSION

While a strong edge effect for light penetration two years after the construction of the paved trail was observed, this was transient and light levels at measurement points between the edge of the mowed border and within 10 m of the edge were indistinguishable from forest light levels after three years had passed. Conceptual analyses predict that, following creation of an edge between forest and more open terrain, increased

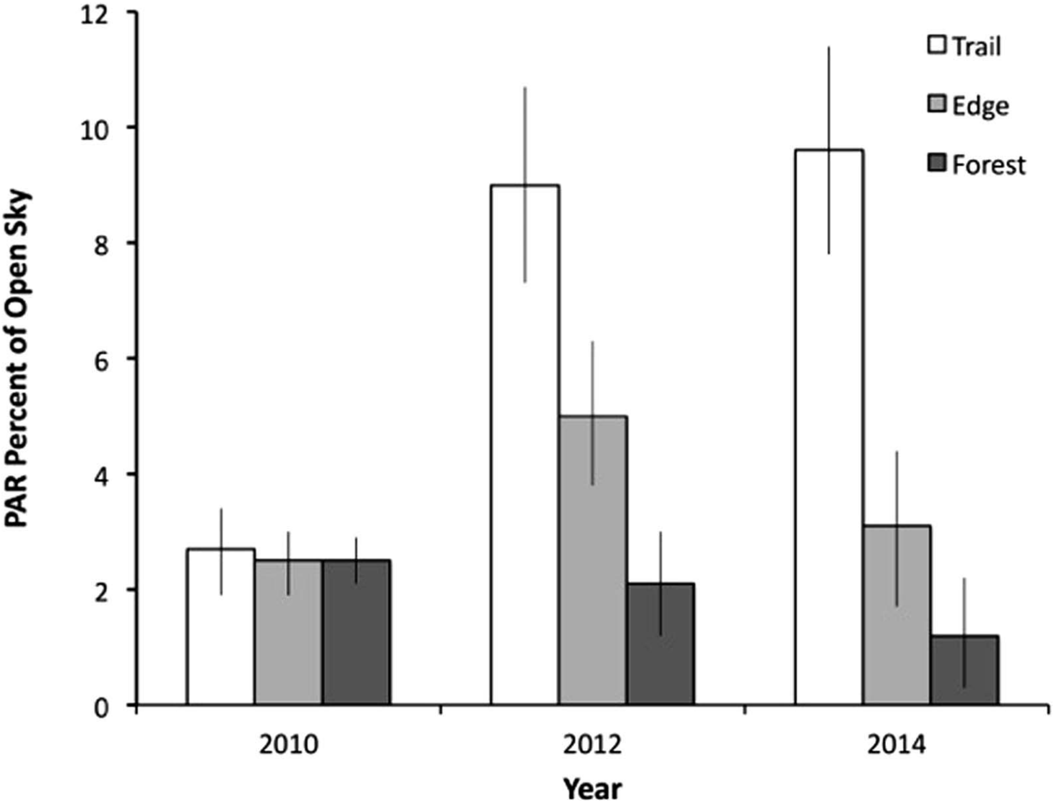

Figure 1.—Light penetration around the USI–Burdette Park bicycle and pedestrian trail. Clear bars (trail) show mean percent photosynthetically active radiation (PAR) above the paved trail and mowed borders, light grey bars (edge) show mean radiation above the forested zone within 10 m of the mowed edges, and dark grey bars (forest) show mean radiation above the forested zone greater than 10 m and up to 80 m beyond the mowed edges. Whiskers represent 95% confidence intervals.

light availability at the edge should stimulate shoot proliferation and foliage density should increase just beyond the edge. This has been termed "side canopy" and is thought to seal edges and create a shallow zone of decreased light availability just beyond the edge (Matlack 1993; Murcia 1995). While a clear decrease in light penetration in this region was observed, increased shadiness close to the trail edge was not noted in either the first or third year after trail construction with our transect measurements. It is possible that the side canopy may not have fully developed by the point of our 2016 measurements. Alternatively, the spatial resolution needed to observe this predicted dip in light availability may have been absent. Previous work has indicated that development of side canopy and closing of forest edges can take at least 5 years (Matlack 1993).

Over the paved and mowed trail itself, light penetration was greatly increased. Interestingly, this increase showed little sign of diminution over the study period although the ground level measurements may be an exception (Fig. 2a). This decrease may have been the result of proliferation of lateral shoots and development of side canopy near the ground, particularly on the southwest side of the trail.

The findings for individual transect by height combinations show little evidence of increased shadiness (Fig. 2b) but, by 2016, five years post construction, side canopy effects, if present, should have been detectable. MEI measurements on the edge of the mowed trail border and the forest had higher light levels than the reference plots as did measurements at lower heights 1 m deep into the forest. Mowing and herbicide treatments varied somewhat in the years following trail construction; higher light levels at lower heights just inside the mowed border likely reflected this practice. The sign of edge influence

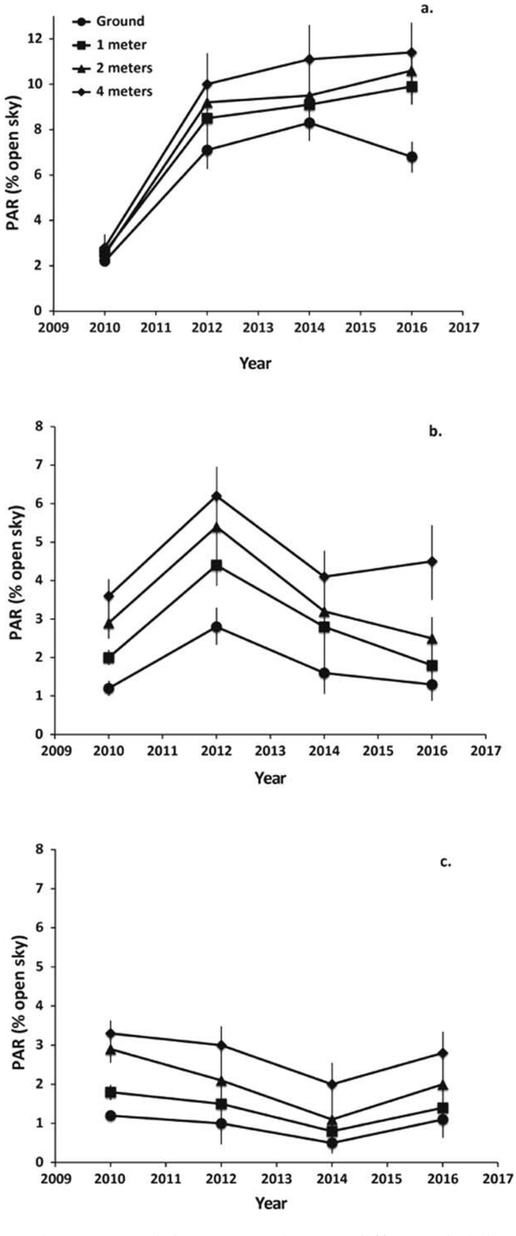

Figure 2.—Light penetration at different heights above ground around the USI–Burdette Park bicycle and pedestrian trail. Circles, squares, triangles, and diamonds represent different heights above ground (0, 1, 2, and 4 m, respectively). Lettered panels show bands around the trail: (a) paved trail and mowed border, (b) forested zone within 10 m of the mowed edges, and (c) forested zone greater than 10 m and up to 80 m beyond the mowed edges. Whiskers represent standard errors. Note that in some cases, symbols obscure error bars and ordinate for panel (a); trail data, in particular, have a different axis scale to improve clarity.

changed from positive to negative at 2 m deep into the forest. At a distance of 2 m or greater, five of 12 MEIs on the northeast side and eleven of 12 MEIs on the southwest side were negative (Tables 1 & 2). Distinct and significant decreases in light levels were observed at distances of 2 and 5 m into the forest on the southwest side of the trail, while negative edge effects on the northeast side were of marginal significance ($p = 0.055$ at 10 m deep into the forest; Table 1). These findings suggest that a side canopy had developed and that it resulted in lowered light levels in belts on either side of the trail, ending somewhere before 10 m deep into the forest and beginning somewhere past 1 m deep. Further, development of the side canopy was more distinct on the southwest side of the trail. Greater development of side canopy on the southwest side is expected given the position of the trail on a ridge where its southwest exposure should yield greater sun exposure (Matlack 1993; Ries et al. 2004).

In other work, edge effects of narrow roads and trails on light availability and other responses have been rather shallow compared to edge effects where two extensive habitat types adjoin. For example, changes in plant diversity and the influence of exotic species were found to extend 3 m past the edge of gravel walking trails (LaPaix et al. 2012) in Nova Scotia, while Avon et al. (2010) found typical road influences, including light levels, not to extend 5 m past the road embankment–forest edge in French managed oak forests. In contrast, edges between forests and adjacent open habitats tend to extend more deeply into the forest. In a study of edges in the Shawnee National Forest (Illinois, USA), Honu & Gibson (2006) found that canopy openness was significantly greater 40 and 50 m from the edge for crop–forest and hayfield–forest interfaces, while the influence for access road–forest interfaces was 30 m. Additionally, in their study of forest edges in Nova Scotia, LaPaix et al. (2012) found edge influences to extend 50 m into the forest along boundaries between forest and open habitat.

Thus, edge effects created by roads and trails are smaller than effects generated by boundaries with extensive open habitat and may scale with the size of the road or trail (Parendes & Jones 2000). Nevertheless, direct and indirect effects on animals may extend further into the forest than expected as roads and paved trails represent a type of qualitatively different "habitat", especially to mobile animals (Ries et al. 2004). For example, edge effects on salamander abundance and

Table 1.—Changes in light penetration on the northeast side of trail. Magnitude of edge influence (MEI – top number in each cell), average PAR (PAR percent of open sky – middle number in each cell), and *p* values (two – tailed tests; bottom number in each cell) at distances from the mowed edge-forest border into the forest. Reference values were taken at 15 m deep into forest. Significant *p* values are in bold print.

		Distance into forest from mowed edge (m)					
Height (m)		0	1	2	5	10	Reference
Ground	MEI	0.271	0.311	0.150	-0.006	-0.089	-
	PAR	1.75	1.91	1.36	0.99	0.84	1.00
	p	**0.008**	**0.023**	0.094	0.940	0.25	-
1 meter	MEI	0.534	0.440	0.417	-0.043	-0.133	-
	PAR	5.06	3.96	3.74	1.41	1.18	1.54
	p	**0.023**	**0.047**	0.125	0.656	0.055	-
2 meters	MEI	0.484	0.333	0.304	0.236	-0.096	-
	PAR	5.95	4.13	3.87	3.34	1.70	2.07
	p	**0.016**	0.328	0.391	0.484	0.055	-
4 meters	MEI	0.497	0.315	0.256	0.208	-0.092	-
	PAR	6.80	4.39	3.86	3.49	1.90	2.28
	p	0.102	0.469	0.469	0.469	0.133	-

diversity caused by narrow and lightly used logging roads in the Nantahala National Forest extended between 35 m and 60 m into the surrounding forest (Smelitsch et al. 2007). Abundance of interior bird species was affected up to 75–100 m from recreational trails running through forests and grasslands around the city of Boulder, Colorado and rates of nest predation were higher near trails (Miller et al. 1998). In contrast, Smith-Castro & Rodenwald (2010) found no effect of distance from recreational trails on nest survival in Northern Cardinals, which are highly tolerant of humans.

In their conceptual model of edge effects, Ries et al. (2004) argued that altered ecological flows of energy, materials, and individuals, as well as increased access to spatially separated resources, change edge habitat quality while the ecological responses of resource mapping and altered species interactions at edges combine to influence species abundance and distribution. Thus, while altered light environment (an energy flow) may be the primary driver of edge effects for neotropical forest birds, resulting indirect effects may play a role as well (Patten & Smith-Patten 2012). In this work, the authors noted that landscape charac-

Table 2.—Changes in light penetration on the southwest side of trail. Magnitude of edge influence (MEI – top number in each cell), average PAR (PAR percent of open sky – middle number in each cell), and *p* values (two – tailed tests; bottom number in each cell) at distances from the mowed edge-forest border into the forest. Reference values were taken at 15 m deep into forest. Significant *p* values are in bold print.

		Distance into forest from mowed edge (m)					
Height (m)		0	1	2	5	10	Reference
Ground	MEI	0.295	0.115	-0.043	-0.105	-0.084	-
	PAR	2.14	1.47	1.07	0.94	0.98	1.16
	p	**0.000**	0.383	0.328	**0.023**	0.063	-
1 meter	MEI	0.481	0.178	0.086	-0.018	-0.094	-
	PAR	5.06	3.96	3.74	1.41	1.18	1.36
	p	**0.023**	**0.047**	0.125	0.656	0.055	-
2 meters	MEI	0.353	0.090	-0.062	-0.124	-0.005	-
	PAR	4.90	2.81	2.07	1.83	2.32	2.34
	p	**0.000**	0.328	0.336	0.305	0.946	-
4 meters	MEI	0.253	0.063	-0.248	-0.293	-0.038	-
	PAR	6.45	4.36	2.31	2.10	3.56	3.84
	p	**0.000**	0.664	**0.047**	**0.008**	0.578	-

teristics can be expected to affect resource mapping and the degree of spatial separation of resources; when roads and trails run through more developed residential areas, edge influences on bird nest predation were greater than forested areas.

Our work focused on a forest fragment at the edge of an interdigitated rural–suburban landscape. A strong but transient positive edge effect was found for light penetration. Forest regrowth at and near the edges possibly accounts for the transience of this positive effect. Further regrowth and the likely development of a side canopy allowed the development of negative edge effects by the fifth year following trail construction. A side canopy is expected to develop further as these edges age. Nevertheless, variation in mowing and herbicide treatments inherent in the current haphazard management scheme will likely maintain a strong positive edge effect near ground level. Oddly, there was no evidence of decreased light penetration directly over the trail that would be driven by canopy overlap and merging of driplines on opposite sides of the trail. Additional time may be required for this to happen.

Although landscape characteristics may modulate edge effects and light penetration may be the primary driving factor for edge effects in some species, indirect effects related to species movements, resource mapping, and interactions are expected to add complexity to edge effects, even for narrow edges with overlapping canopy. Wildlife managers and planners can minimize edge effects due to light penetration by minimizing the width of recreational trails and keeping mowed edges at a constant width to allow side canopies to develop and seal the forest beyond. Our findings on light penetration into edges and over trails should help to provide guidance for recreational trail construction through forests in Indiana, especially those using INDOT trail specifications.

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RESULTS OF THE 2017 RED-TAIL LAND CONSERVANCY BIODIVERSITY SURVEY, DELAWARE AND RANDOLPH COUNTIES, INDIANA

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ABSTRACT. The Red-Tail Land Conservancy (RLC), the Indiana Academy of Science (IAS), the Robert Cooper Audubon Society, and the Oakwood Retreat Center hosted a biodiversity survey or bioblitz in east-central Indiana on the 10th and 11th June 2017. The event was held on two properties owned or maintained by RLC; i.e., White River Woods, a 47.4 ha preserved located 9 km southeast of Muncie, Indiana in Delaware County, and McVey Memorial Forest, a 100.4 ha forest located 11 km north of Farmland, Indiana in Randolph County. Over 75 scientists, naturalists, students, and other volunteers on 19 different taxonomic

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teams observed and reported 1086 taxa during the event. The nineteen taxonomic teams included ants, aquatic macroinvertebrates, bats, bees, beetles, birds, butterflies, odonates, fish, freshwater mussels, herpetofauna, small mammals, moths, mushrooms/fungi and slime molds, non-vascular plants (mosses), singing and non-singing insects, snail-killing flies, spiders, and vascular plants. State endangered species included the northern long-eared bat (*Myotis septentrionalis*; also federally threatened), the Indiana bat (*Myotis sodalist*; also federally endangered), the cerulean warbler (*Setophaga cerulea*), the osprey (*Pandion haliaetus*), and warty spurge (*Euphorbia obtusata*). In addition shell material of the federal and state endangered Northern Riffleshell (*Epioblasma torulosa rangiana*), Clubshell (*Pleurobema clava*), and Rayed Bean (*Villosa fabalis*) was found but none are still known live on either property. The spider team recorded six state records: *Emblyna hentzi* (meshweaver), *Gladicosa bellamyi* (wolf spider), *Pirata triens* (pirate wolf spider), *Schizocosa mccooki* (wolf spider), *Oxyopes scalaris* (western lynx spider), and *Xysticus fervidus* (ground crab spider). In addition many Delaware County and Randolph County records were reported. This manuscript presents both a brief history of the bioblitz sites and a summary overview of the results. Detailed results are available on the IAS website.

Keywords: Bioblitz, biodiversity survey, White River Woods, McVey Memorial Forest, Red-Tail Land Conservancy, Indiana state records, Delaware County records, Randolph County records

INTRODUCTION

The Indiana Academy of Science's 2017 biodiversity survey, or bioblitz, was held on two properties owned or maintained by the Red-Tail Land Conservancy (RLC). The two sites were White River Woods (WRW) in Delaware County and McVey Memorial Forest (MMF) in Randolph County; the sites being approximately 24 km (15 mi) apart (Fig. 1). Since east-central Indiana, the home of the RLC, is located in the Central Till Plain Natural Region (Homoya et al. 1985), most of the non-urban land is agricultural. As a result, natural areas in the region are small, scattered, and usually isolated islands. Therefore, in order to have a site large enough to conduct a bioblitz, two natural areas were included.

The two sites have a combined area of 148 ha (365 ac). White River Woods, a 47.4 ha (117 ac) site Fig. 2), lies 9 km (5.6 miles) southeast of downtown Muncie, Indiana and 480 m (0.3 miles) north of Prairie Creek Reservoir. McVey Memorial Forest, a 100.4 ha (248 ac) forest (Fig. 3), lies on State Road 1 approximately 11 km (7 mi) north of Farmland, Indiana. Edna McVey established this nature park in her will so that generations to come could enjoy it.

The biodiversity survey, the first held on RLC property, was conducted on 10–11 June 2017. The bioblitz attracted more than 75 scientists, naturalists, students, and others volunteering their time and expertise to make the event an enormous success. Food and lodging for the participants were provided through the generous support of the Red-tail Land Conservancy, the Indiana Academy of Science, the Robert Cooper Audubon Society, and the Oakwood Retreat Center.

This manuscript provides a brief history of the bioblitz sites and a summary of the biodiversity results. For additional details see the Red-Tail Biodiversity Survey Final Report (2018) on the Indiana Academy of Science website.

BRIEF HISTORIES OF MCVEY MEMORIAL FOREST AND WHITE RIVER WOODS

Red-tail Land Conservancy (RLC) is a 501(c)(3) land trust whose mission is to preserve, protect, and restore natural areas and farmland in east-central Indiana while increasing awareness of our natural heritage. RLC accomplishes its mission by offering conservation options to landowners and providing nature education programs and events to the general public. RLC was launched in March 1999. RLC's successes and accomplishments are well chronicled on its website at **www.fortheland.org**.

McVey Memorial Forest is a 100.4 ha (248 ac) wildlife sanctuary along Indiana Highway 1 South and adjacent to the Mississinewa River.

In 1958, Edna McVey set up a perpetual trust under the authority of the Randolph Circuit Court with a number of Successor Trustees appointed by the court over the years. In September 2012, the Randolph Circuit Court appointed RLC the Successor Trustee to McVey Memorial Forest.

The park shares a common property line with an IDNR Fish and Wildlife Preserve of 141.6 ha (350 ac) north of the Mississinewa River, making this the largest protected natural area in east-central Indiana. It is open to the public and has a trail system, shelter, and off-road parking. In 2017, RLC designed and installed a new trail

Figure 1.—Map illustrating the counties of Indiana. D = Delaware County; R = Randolph County. The dot in Delaware County indicates the location of White River Woods and the dot in Randolph County illustrates the location of McVey Memorial Forest. The two site are approximately 24 km (15 mi) apart.

system on the northern section, north of CR 750N, which includes a third parking area and canoe launch on the river. The land types are quite diverse, with a riparian area along the Mississinewa River and Bush Creek, a mature upland wooded area dominated by shellbark hickory, and a 30 year-old planting of native hardwoods along the western side. Moreover, there are numerous wetland areas along the river and in the creek bottom.

The other 2017 bioblitz site is along the White River in Delaware County, just north of Prairie Creek Reservoir. This site permanently protects the east bank/riparian zone of 0.8 km (0.5 mi) stretch of the river along with a splendid 12.1 ha (30 acre) mature upland flatwoods that is open to the public with a trail system and parking lot. This site was brought to the attention of Barry Banks, founder and Executive Director of RLC, by

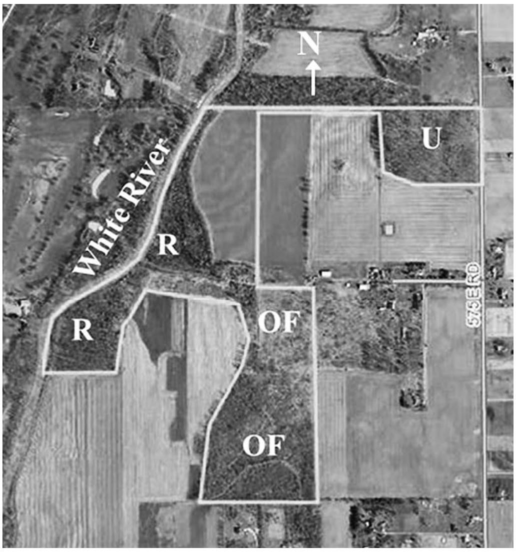

Figure 2.—White River Woods (outlined) is a 47.4 ha (117 ac) preserve located in Delaware County, Indiana. The White River runs along the west border. R = riparian woodlands; U = mesic upland forest; OF = old-fields in various stages of development. Figure modified from RLC image.

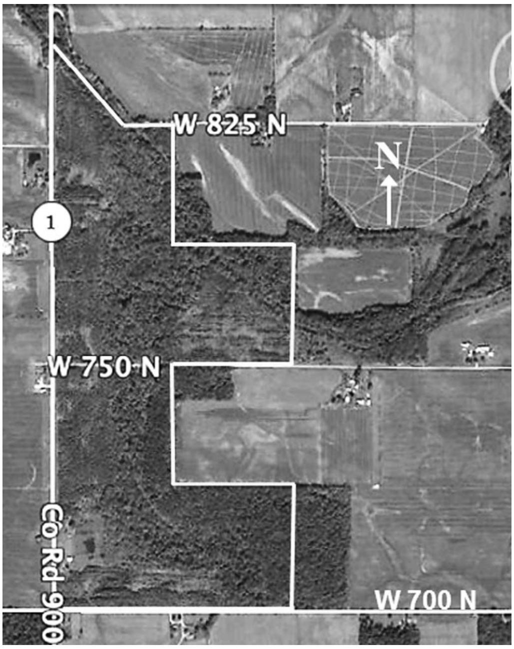

Figure 3.—McVey Memorial Forest (outlined) is a 100.4 ha (248 ac) primarily forested preserve located in Randolph County, Indiana. State Road 1 defines the western border, County Road W 700 N is the southern border, and the Mississinewa River runs along most of the northern border.

Rainbow Farm director Donna Blodget in 2004. She invited Barry to walk the woods at a time when the riparian area was sporting acres of Virginia bluebells in full bloom. Barry was "thrilled" by the natural beauty of that flood plain. The entire farm was owned at that time by Emissaries of Divine Light. Following years of negotiation with that organization, in 2009 they decided to sell a number of their real estate holdings around the world. RLC is most fortunate to have had funding available via the Bicentennial Nature Trust and the Land Conservation Fund with which it purchased 47.4 ha (117 acres) in December 2014.

The Oakwood Retreat Center, who bought their campus from the Emissaries, co-hosted the participants of the 2017 bioblitz in their equipment storage barn. It was the perfect setting for the morning gatherings and evening wrap-ups of the dozens of natural scientists and volunteers who participated in this wonderful event.

SUMMARY GEOMORPHOLOGICAL ASSESSMENT OF WRW AND MMF

Matthew Purtill, Applied Anthropology Laboratories, Ball State University, graciously prepared the following geomorphological assessment of the sites. Both the McVey Memorial Forest and White River Woods are located upon, geologically speaking, a young landscape still adjusting to its glacial past. As little as 16,000 years ago, both areas would have been directly beneath the Late Wisconsin glacial ice sheet associated with the East White sublobe of the Huron-Erie Lobe. Both the White and Mississinewa Rivers that border the nature preserves have gravel-to-cobble dominated bedload, moderate sinuosity, low gradient, and moderate entrenchment ratios. These streams likely would be classified as a Type E or F stream following Rosgen methodology. LiDAR data reveal abundant relict braid bars and abandoned channels that reflect a time when both rivers were still transporting coarse bedloads associated with glacial outwash from retreating glaciers. MMF is situated on the edge of the Mississinewa Moraine that provides noticeable relief and well-drained soils. A prominent geomorphological feature at McVey is the remains of a large, now abandoned, meander bend of the ancient Mississinewa River. This infilled channel scar extends through the central portion of McVey and is characterized by gleyed soils indicative of high water-holding soil capacity. This meander undoubtedly provides a localized micro-habitat for modern plants and animals. Modern-day Bush Creek flows through a portion of this abandoned meander before it enters the Mississinewa River. Based on meander scars readily visible, and review of 1960s USGS topographic maps, it is apparent that portions of Bush Creek that run through McVey have been artificially straightened sometime over the last 50 years. Possible alteration in hydrology and erosion due to stream straightening were not studied.

CULTURAL RESOURCES ASSESSMENT OF THE MCVEY MEMORIAL FOREST

James Martin and J. Ryan Duddleson, Orbis Environmental Consulting, kindly provided the following cultural resources assessment of MMF. Volunteers from Orbis Environmental Consulting conducted an above ground survey for potential archaeological and historical resources in the MMF. The team identified known cultural resources such a pioneer cemetery but also located the original location of County Road West 750 North as it once followed the Mississinewa River. This original layout of the county road also crossed Bush Creek and the team found the remains of a bridge there. Historic records show an old pioneer town, known as Steubenville, in the McVey Forest. Our survey did not observe any remnants of historic structures in this area, but this location contains recently planted trees which might obscure historic foundations and/or artifacts. Additional survey may locate remnants of this former town. There also are known prehistoric archaeological sites within the forest property and the team was able to identify numerous areas in the forest that are likely to contain additional unidentified prehistoric sites.

SUMMARY OF RESULTS AND METHODS

The Red-Tail Land Conservancy bioblitz attracted over 75 scientists, naturalists, students, and other volunteers. Nineteen taxonomic teams reported 1086 taxa (Table 1). This two-day survey provided a floral and faunal "snapshot in time" of the natural resources at these sites and exposed the remarkable species richness present. Of course to obtain a complete picture of the biodiversity present at these sites, long-term seasonal surveys are necessary. An overview of the results from the nineteen taxonomic teams follows; see Table 1 for a summary. To view the complete results, visit the Indiana Academy of Science website at **https://**

www.indianaacademyofscience.org/, lay the cursor over Resource Center at the top of the page, click BioBlitz Events and Data, then select Red-Tail Conservancy BioBlitz complete report.

Ants.—There were no surprises in the ant species found at the sites, with all of them being either found across the country or in the eastern United States specifically. The taxa found at both sites tend to be either generalist species or ones that prefer forested environments, but there were some exceptions. *Lasius alienus* prefers open fields and the presence of agricultural fields surrounding both survey sites likely explains its presence. Similarly, *Tapinoma sessile* thrives in disturbed habitats, so its presence at the White River site is unsurprising. The rest of the species occupying the White River site all tend to be more tolerant of the shrub and woodland ecosystems present there, while the species present solely at the McVey site all favor older, more pristine wooded habitats.

Aquatic macroinvertebrates.—A 500 μm mesh D-frame aquatic dipnet was used to collect samples from riffles, then all additional in-stream habitats in a 50 m section of the stream at each site were sampled. Samples were combined, elutriated through a 500 μm mesh sieve and then picked in the field for 20 minutes. Collected specimens were identified to lowest practical taxon by use of standard texts (Merritt et al. 2008; Thorp & Covich 2001).

Aquatic macroinvertebrates were collected from three locations, two in MMF and one in WRW. The first site, located on the White River at WRW, was approximately 500 m west of the Oakwood Retreat Center barn. At this location the White River was ~ 15 m wide in the riffle, widening to 25 m in the slower run areas. Substrate within the sampled area was composed primarily of gravel, small cobble, and sand with several submerged logs, emergent vegetation, and root wads located within 1 m deep pools along the east bank. The sample collected at this location on the White River was the most diverse with 67 taxa, including 17 taxa of the Ephemeroptera, Plecoptera, and Trichoptera (EPT; mayflies, stoneflies, and caddisflies). In terms of the macroinvertebrate diversity, this sample contained a greater number of taxa than 98% of the macroinvertebrate samples collected by the Indiana Department of Environmental Management (IDEM) using this method from 2004–2013 (Todd Davis, IDEM Office of Water Quality, Pers. Comm., 11 August 2017); this site also ranked in the top 5% of IDEM samples in number of EPT taxa collected.

The second site, located on the Mississinewa River, was ~ 150 m north of the parking area located on County Road West 750 North in MMF. At this location the Mississinewa River was ~ 15 m wide in the riffle, widening to 30 m upstream and downstream of the riffle. Substrates within the sampled area were composed of cobble, gravel, and a greater amount of silt than was seen at the White River site. Pools were not present but there was a larger accumulation of woody debris on the shoreline. The sample at this location was relatively diverse with 50 taxa including 11 EPT taxa.

The third site, located on Bush Creek at MMF, was ~ 50 m north of the County Road West 700 North bridge crossing. Brush Creek was much smaller than the other sites, narrowing from eight to two m wide over the length of the sampled zone. Substrate in this stream was composed entirely of sand with some gravel and silt in depositional areas and almost no additional habitat types. The macroinvertebrate community was reduced at this site with only 27 taxa with three EPT taxa.

In summary, a total of 573 individuals were collected and identified, representing 91 macroinvertebrate taxa. None of the taxa are known to be species of special concern in Indiana.

Bats (order Chiroptera, family Vespertilionidae).—Using mist nets to capture bats, Tim Carter sampled the White River at WRW and Jeremy Sheets sampled the Mississinewa River at MMF. Tim Carter reported three species, i.e., Indiana bat (*Myotis sodalis*), little brown bat (*M. lucifugus*), and big brown bat (*Eptesicus fuscus*). Jeremy Sheets reported three species, i.e., big brown bat (*E. fuscus*), hoary bat (*Lasiurus cinereus*), and eastern red bat (*L. borealis*). Additionally, the historical records of bat collections (2009 and 2015) from MMF were examined. Including the three species reported by Jeremy Sheets above, a total of eight species have been reported. The additional five species were the Indiana bat (*M. sodalis*), little brown bat (*M. lucifugus*), northern long-eared bat (*M. septentrionalis*), evening bat (*Nycticeius humeralis*), and Seminole bat (*Lasiurus seminolus*).

Both of these sites support a wide array of bat species. Indiana bats are a federally endangered species and have been documented at both

Table 1.—Summary of the 1086 taxa reported at the 2018 Red-Tail Land Conservancy Biodiversity Survey, Delaware and Randolph Counties, Indiana. WRW = White River Woods; MMF = McVey Memorial Forest.

Team	Leader	Taxa found
Ants	Mathew Dittmann	15 species, all common
Aquatic macroinvertebrates	Paul McMurray	91 taxa, none of special concern in Indiana
Bats	Tim Carter	WRW: 3 species (1 federally endangered species, 1 federally threatened species); MMF: 3 species during the bioblitz and 8 species (including the 3 noted during the bioblitz) at MMF from historical records
Bees	Robert P. Jean	36 species (32 from WRW and 20 from MMF); 18 Delaware County and 19 Randolph County records
Beetles (Coleoptera)	Jeffrey D. Holland	92 taxa, none unusual or unexpected
Birds	Kamal Islam	78 species (47 common to both sites); highlights included cerulean warbler and osprey (both state endangered species), bald eagle (state species of 'Special Concern'), bobolink and dickcissel (species with declining populations rangewide), and 34 species of long-distance migrants that winter in Central and South America and breed in Indiana
Butterflies	Kirk Roth	22 species; 4 Delaware and 5 Randolph County records; range extension south for the eyed brown (*Lethe eurydice*)
Odonates (dragonflies and damselflies)	Kirk Roth	28 species of odonates (18 dragonflies and 10 damselflies); 14 Delaware County and 15 Randolph County records
Fish	Brant E. Fisher	47 species (42 species from MMF and 37 from WRW); 2 non-native species; no state listed fish species were collected
Freshwater mussels	Brant E. Fisher	25 species; evidence of 3 federal/state endangered species and 3 species of state special concern were reported; although a relatively diverse freshwater mussel community still persists, both sites have lost around a third of their historic diversity.
Herpetofauna	Robert Brodman	12 species (5 reptile and 7 amphibian species); 2 Delaware County and 4 Randolph County records; 2 species of special concern.
Small Mammals	John Whitaker, Jr. & Angie Chamberlain	7 species, all common
Moths (Lepidoptera)	Megan McCarty	51 taxa (including 38 species, 3 to genus, and 10 unidentified); none unusual or unexpected
Mushrooms	Stephen Russell	56 species in total from both sites representing 46 genera, mostly wood rot fungi; interesting finds included the mushrooms *Rhodotus palmatus*, *Pluteus americanus*, and the slime mold *Reticularia* (*Enteridium*) *lycoperdon*, only the second report of this species from Indiana
Non-vascular plants	Linda Cole	30 species (29 mosses and 1 liverwort); 16 species occurred at both sites; the mosses are characteristic of shaded, moist, calcium-enriched sites
Singing and non-singing insects	Carl Strang	11 species (4 species of singing insects and 7 species of non-singing insects), all common
Snail-killing flies (Diptera: Sciomyzidae)	William L. Murphy	11 species: 2 species from the tribe Sciomyzini, 9 species from the tribe Tetanocerini; 6 Randolph County records; total number of Sciomyzidae species now known from Randolph County, 19.
Spiders	Marc Milne	81 taxa; 6 state records

Table 1.—Continued.

Team	Leader	Taxa found
Vascular plants	Donald Ruch	476 taxa (406 at MMF and 289 at WRW; 218 taxa occurring at both sites, 71 only at WRW, and 187 only at MMF); 22 potential Delaware County records and 24 potential Randolph County records; 1 endangered species, *Euphorbia obtusata*, at WRW.

locations. Northern long-eared bats are a federally Threatened species and have been documented at McVey and are, or were likely present at White River. The capture of a Seminole bat at McVey in 2015 is a very unusual occurrence. This species is typical of the southeastern US and its range is usually the Gulf Coast States and SE Atlantic States. This is only the second record from Indiana of which we are aware.

The bat community in this region has been severely impacted by the disease White-Nose Syndrome (WNS). Historic netting efforts in McVey in 2009 prior to WNS resulted in 70 bats in two nights. Those same sites were trapped post WNS in 2015 and only 13 bats were captured. Netting at WRW in 2017 resulted in only four bat captures in two nights. Both McVey and White River represent excellent bat habitat, including excellent roosting and foraging habitat for all bats. The rivers are in good condition with strong insect communities. Both sites will likely continue to support remnant populations of bat species that are affected by WNS and strong populations of bats not affected.

Bees.—Bee sampling was conducted on 27 June 2017. Bee surveys were performed at a later date than other taxonomic groups due to scheduling conflicts and weather conditions. Bees were collected using passive (bowl trapping) and active (netting at flowers) sampling techniques. WRW was passively sampled using 30–12 oz bowls (ten white, ten fluorescent blue, and ten fluorescent yellow) separated by 5 m each in random color order (for a total of 60 bowls) along two-75 m transects. One transect was setup in an old field area with several flowering species and the other was positioned along a forest edge with flowers present. MMF was sampled with a single 75 m transect comprised of 30 bowls along the open grassy area near the pond. Bowls were placed out in the morning and then collected in the late afternoon. Each site was net collected for approximately 3 hours for a total 6 hours across both sites. Voucher specimens are housed in the Environmental Solutions & Innovations, Inc. (ESI) entomology collection in Indianapolis.

Thirty-six species, ~ 8% of the Indiana bee fauna, representing all five common bee families in Indiana were collected. Overall floral diversity was moderate and bee activity was low. Flowering was mainly concentrated in openings, forest edges, old fields, and a prairie restoration (MMF) and these were the focus of the net collections. Thirty-two bee species were collected at WRW of which 18 were Delaware County records. Twenty bee species were collected at MMF of which 19 were Randolph County records (as only one bee species had been vouchered from Randolph County in the past).

In total, 36 bee species were collected from both sites with 16 species collected only in Delaware County and 4 species collected only in Randolph County. Overall bees in the families Apidae and Halictidae represented a large portion of the species richness (13 spp. and 16 spp. respectively) and much of the bee abundance (44% and 49% respectively; 93% collectively). Interesting species include *Andrena persimulata* Viereck, 1917, *Andrena wilmattae* Cockerell, 1906, and *Melissodes illatus* Lovell and Cockerell, 1906 all of which have been rarely recorded in Indiana. Overall, even with the relatively low diversity, a combined 37 new county records were noted demonstrating these areas had been little collected in the past. There were two introduced bee species, including the honey bee (Apis *mellifera* Linnaeus, 1758) and the giant resin bee, *Megachile sculpturalis* Smith, 1853. For the latter species these are among the first records of this species using natural areas in the state as it is often found in urban settings. In addition, this is one of the first published records of the *M. sculpturalis* for the state although it has been collected in a few other counties and has been suspected of occurring throughout the state.

These collections demonstrate the importance of WRW and MMF for bee conservation and

Figure 4.—Beetle team at work after sunset. (*Photo by John Taylor*)

habitat and they help fill in some areas of the state that have been very poorly collected in the past. Further management to increase native wildflower diversity, expand prairie plantings, maintain some openings, and reduce invasive plant species will enhance bee populations even further. It should be noted that the forests in these areas likely provide valuable nesting and overwintering resources for native bees and likely provide floral resources for bees in the spring when flowering is lower in other habitats. Collections in these areas in spring and fall will likely add many more species and should be considered to establish a baseline for these important pollinators.

Beetle (Coleoptera).—The Coleoptera were surveyed using a variety of methods. Two flight intercept type traps were left for a week to collect beetles at both sites. The traps at each site consisted of a clear acrylic plastic window trap and a black panel trap (Advanced Pheromone Technologies, Inc., Marylhurst, USA) at each site. An ethanol lure was used in all traps. Beetles also were collected at a 175 W mercury vapor light at MMF during 9–12 PM Saturday evening (Fig. 4). During the day on Saturday and Sunday morning, the beetle team hand collected, aspirated small beetles from plants, and used sweep nets to sample beetles from vegetation.

Ninety-two taxa (species or genera) were detected during the beetle surveys. These came from 29 different beetle families. None of the species were unusual or unexpected. By far, the most abundant species seen was a soldier beetle, *Chauliognathus pensylvanicus*. This species was very abundant on the flowering vegetation at both sites. One very attractive specimen was the six-spotted tiger beetle (*Cicindela sexguttata*; Fig. 5) observed at WRW. Voucher specimens have been deposited in the Purdue Entomological Research Collection.

The number of species detected is slightly lower than expected for this amount of collecting effort. Mid-June is a very good time of year for beetle collecting with many species active in Indiana. The low number of species is likely due to the location of the conservation areas that were surveyed. The surrounding landscape is dominat-

Figures 5–10.—Images of various organisms observed during the Red-Tail Land Conservancy biodiversity survey. 5. Six-spotted tiger beetle (*Cicindela sexguttata* Fabricius; family Carabidae) observed at White River Woods. (*Photo by John Taylor*) 6. Eyed Brown (*Lethe eurydice*) at White River Woods which represents a range extension for the species. (*Photo by Kirk Roth*) 7. Handsome Clubtail (*Gomphus crassus*) photographed at McVey Memorial Forest. (*Photo by Paul McMurray*) 8. Northern watersnake (*Nerodia sipedon*) in the White River. (*Photo by Paul McMurray*) 9. Green-striped grasshopper (*Chortophaga viridifasciata*) at McVey Memorial Forest. (*Photo by Carl Strang*) 10. A species of marsh fly, *Limnia boscii* (Robineau-Desvoidy), was the most abundant species of Sciomyzidae collected from the margin of the pond at McVey Memorial Forest. (*Photo by Steve Marshall*, used with permission)

ed by intensive row crop agriculture. This makes colonization of the habitat less likely, leading to a reduction in the number of species. As well, much of the forested area is recent second-growth forest. The actual forest area was much smaller 40 years ago, thus the beetle fauna likely represents what would be found in a small, isolated forest and may increase over time.

Birds.—Seventy-eight species of birds were recorded during the 2-day event with 47 species common to both properties. Reports of birds from individuals participating on other taxonomic teams were included in the final tally. All birds observed or heard appeared to be on territory and were considered potential breeders. Highlights of the count included Cerulean Warbler and Osprey (both state endangered species), Bald Eagle (state species of 'Special Concern'), Bobolink, and Dickcissel (species with declining populations rangewide), and 34 species of long-distance migrants that winter in Central and South America and breed in Indiana.

On 10th June, the bird team birded MMF. A total of 66 bird species were detected. Highlights of this count included all 6 resident species of woodpeckers, two long-distance grassland/fallow field specialists (Bobolink and Dickcissel), six species of migratory flycatchers, and eight species of long-distance migratory warblers. The most surprising find was a male singing Cerulean Warbler on territory; this species has declined by over 70% throughout its rangewide distribution during the last five decades and its stronghold in Indiana is restricted to the southern forested parts of the state. On 11th June, the team surveyed WRW along mowed pathways and trails. A total of 59 species of birds were recorded. Notable species included Osprey and Bald Eagle. A Rose-breasted Grosbeak was observed singing on territory; normally, this species breeds further north in the state.

More species (66) and more individuals (465) were observed at MMF than at WRW (59 species, 276 individuals). This discrepancy in number of species recorded and individuals counted between the two properties is likely a result of a difference in acreage rather than effort. In addition, MMF has a much larger contiguous block of mature forest with Bush Creek meandering its way into the Mississinewa River. Along Bush Creek and other areas of the property, several mature forest dependent species were detected and these species were largely absent at WRW, such as Yellow-billed Cuckoo (3 vs. 0), Yellow-throated Vireo (5 vs. 0), Red-eyed Vireo (18 vs. 3), Ovenbird (1 vs. 0), Kentucky Warbler (1 vs. 0), American Redstart (1 vs. 0), Cerulean Warbler (1 vs. 0), and Scarlet Tanager (2 vs. 0).

Forty-seven species were found at both properties consisting of many edge species or generalists such as Mourning Dove, Warbling Vireo, House Wren, American Robin, Common Yellowthroat, Yellow-breasted Chat, Field Sparrow, and Indigo Bunting among others. Five species with the highest count were Red-winged Blackbird (62), Indigo Bunting (37), European Starling (37), Canada Goose (28), and Brown-headed Cowbird (25). European Starlings are an introduced commensal that have successful colonized North America. The Brown-headed Cowbird, a known brood parasite, has also benefitted from human modification of the landscape, especially with the removal of forests for agriculture.

Butterflies.—Species diversity of butterflies was similar at both sites with 15 species at MMF and 19 species at WRW (22 total species from both sites). However, WRW had more individual butterflies detected (182) compared to MMF (101), likely due to greater sampling effort. Abundant species at both sites included Silver-spotted Skipper (*Epargyreus clarus*), Cabbage White (*Pieris rapae*), Summer Azure (*Celastrina neglecta*), and Red Admiral (*Vanessa atalanta*). It is notable that the date of the bioblitz was between or before the main flights of several skipper species (Belth 2013), which may explain why only three species of skipper were detected. A surprising absence was that of Pearl Crescents (*Phyciodes tharos*), which are often abundant and easily found during butterfly surveys.

The most unexpected find was an Eyed Brown (*Lethe eurydice eurydice*) at WWR (Fig. 6); it was several counties south of its expected range in Indiana (Belth 2013). One individual was found in an open seep dominated by *Carex stricta*, its larval host plant. The Eyed Brown is typically a northern species, so it is possible that this represents a remnant population, or simply that a small number have dispersed to Delaware County. This record indicates that other areas of *Carex stricta* in Indiana should be checked for this species in June and July.

Other notable species found during the butterfly survey were the White-M Hairstreak (*Parrhasius m-album*) at WRW and four Banded Hairstreaks (*Satyrium calanus*) at each location. The White-M Hairstreak is a canopy dwelling species, which is rarely observed closer to convenient observation level. Hairstreaks in general are often localized in distribution, so these sites may represent important local habitat for them. The Eyed Brown and both hairstreaks represent county records for each county in which they occurred. Other county records include Zabulon Skipper (*Poanes zabulon*) in Randolph, Delaware Skipper (*Anatrytone logan*) and Hackberry Emperor (*Asterocampus celtis*) in Randolph, and Northern Pearly-eye (*Lethe anthedon*) in Delaware and Randolph counties. These likely represent lack of sampling rather than rarity, as these four species are not uncommon.

Odonates (dragonflies and damselflies).—A total of 28 species of odonates, comprised of 18 dragonflies and 10 damselflies, were detected during the bioblitz, with 20 species at each property. Numbers of individuals were similar at both locations, with 122 at MMF and 108 at WRW. Abundant species at both locations included Widow Skimmer (*Libellula luctuosa*), Twelve-spotted Skimmer (*Libellula pulchella*), Ebony Jewelwing (*Calopteryx maculata*), and Blue-tipped Dancer (*Argia tibialis*).

The diversity of aquatic habitats at both sites is a likely driver of the odonate diversity observed, as species composition was typical of standing and running waterways. The pond at the southern end of MMF was an excellent location for skimmer species (Libellulidae), as may be expected from a large, isolated, and shallow pond. Both sites have extensive running waterways which provide excellent habitat for the many damselflies identified during the bioblitz. However, many larger and interesting species were found in upland habitats, including Swamp Darner (*Epiaeschna heros*), Handsome Clubtail (*Gomphus crassus*; Fig. 7), Illinois River Cruiser (*Macromia illinoiensis*) on both sites; Arrowhead Spiketail (*Cordulegaster obliqua*) at MMF; and Ruby Meadowhawk (*Sympetrum rubicundulum*) and Spot-winged Glider (*Pantala hymenaea*) at WRW. Delaware and Randolph Counties are not well represented for odonate collection (see Curry 2001) so most species encountered represented county records, i.e., 14 Delaware County and 15 Randolph County records. Most of these were photographed, and records will be documented at the Odonata Central website (Abbott 2006–2017).

Fish.—A total of 47 species of fish were collected from the four sites sampled in WRW (one site on the West Fork White River) and MMF (one site on the Mississinewa River and two sites on Bush Creek). Fish diversity was slightly higher within MMF, with 42 species collected compared to 36 from WRW. Eleven species were unique to MMF, including Steelcolor Shiner (*Cyprinella whipplei*), Western Creek Chubsucker (*Erimyzon claviformis*), Spotted Sucker (*Minytrema melanops*), Black Bullhead (*Ameiurus melas*), Tadpole Madtom (*Noturus gyrinus*), Brindled Madtom (*N. miurus*), Rainbow Trout (*Oncorhynchus mykiss*), Orangespotted Sunfish (*Lepomis humilis*), Least Darter (*Etheostoma microperca*), Slenderhead Darter (*Percina phoxocephala*), and Freshwater Drum (*Aplodinotus grunniens*). Western Creek Chubsucker, Spotted Sucker, Black Bullhead, and Tadpole Madtom do inhabit areas of the upper West Fork White River drainage, but with the limited aquatic habitat to sample in the WRW property, they were not encountered. Of the five species unique to WRW, i.e., River Chub (*Nocomis micropogon*), Silver Shiner (*Notropis photogenis*), Rosyface Shiner (*N. rubellus*), Mimic Shiner (*N. volucellus*), and Channel Catfish (*Ictalurus punctatus*), only the Channel Catfish (Fig. 11) would be possible from the MMF property; the other four minnow species are not known from the upper Mississinewa River drainage. Of the remaining 31 species found on both properties, 26 were found at least three of the four sites sampled and would be considered common inhabitants of central Indiana streams. No state listed fish species were collected from either property.

Figure 11.—Channel Catfish (*Ictalurus punctatus*) collected from the West Fork White River, White River Woods. Drew Holloway with the Muncie Bureau of Water Quality is holding the fish. (*Photo by Brant Fisher*)

Freshwater mussels.—Evidence of 24 native species of freshwater mussels and one non-native mollusk (Asian Clam – *Corbicula fluminea*) was found from the five sites sampled in WRW (one site on the West Fork White River) and MMF (two sites on the Mississinewa River and two sites on Bush Creek). While fourteen native species of freshwater mussels were found live/fresh dead on both properties, Mucket (*Actinonaias ligamentina*), Spike (*Elliptio dilatata*), Wavyrayed Lampmussel (*Lampsilis fasciola*), and Rainbow (*Villosa iris*) were only found live in WRW. None of these four species are still found live anywhere in the upper Mississinewa River drainage. Live/fresh dead White Heelsplitter (*Lasmigona complanata*), Giant Floater (*Pyganodon grandis*), Mapleleaf (*Quadrula quadrula*) and Paper Pondshell (*Utterbackia imbecillis*) were only collected from the MMF property. Giant Floater and Paper Pondshell are known to be live in the upper West Fork White River drainage near the WRW property and could be found there in future surveys. Shell material of the federal and state endangered Northern Riffleshell (*Epioblasma torulosa rangiana*), Clubshell (*Pleurobema clava*), and Rayed Bean (*Villosa fabalis*) was found but none are still known live on either property. Two live individuals and additional fresh dead shell material of the Wavyrayed Lampmussel, a state species of special concern, were collected from the WRW property; only weathered shell material was found on the MMF property and it is likely not live there. Weathered shell material of two additional state species of special concern, Kidneyshell (*Ptychobranchus fasciolaris*) and Purple Lilliput (*Toxolasma lividum*), was found on both properties, but neither is likely still live. Overall, a relatively diverse freshwater mussel community, compared to other central Indiana streams, still persists on both the WRW and MMF properties, even though both have lost around a third of their historic diversity.

Herpetofauna.—Amphibians and reptiles were surveyed by a combination of methods. Terrestrial and wetland habitats were sampled by visual searches and sampling cover objects. Calling frogs were identified and wetlands were sampled by dip-nets for larvae. Turtles and amphibian larvae were also sampled by turtle traps and minnow traps in wetlands, ponds, and the river. Effort was evenly split between the two sites.

The herp team found a total of 53 herpes from 12 species including 25 reptiles representing 5 species and 28 amphibians representing 7 species. Two species (*Lithobates catesbeianus* and *Lithobates clamitans*) are listed by Minton (2001) as present but had never been vouchered in Delaware County. Two species (*Acris blanchardi* and *Anaxyrus americanus*) are listed by Minton (2001) as present but had never been vouchered in Randolph County. Two species (*Graptemys geographica* and *Hyla versicolor*) represent new Randolph County records. *Acris blanchardi* is a species of special concern in Indiana and has declined greatly throughout the northern half of its geographic range. They were common at each wetland and pond surveyed at MMF. *Rana pipiens* is also a species of special concern. Although common, a northern watersnake (*Nerodia sipedon*; Fig. 8) was seen in the White River.

Voucher photos of *Graptemys geographica, Acris blanchardi, Hyla versicolor, Anaxyrus americanus, Lithobates catesbeianus*, and *Lithobates clamitans* are kept by Dr. Robert Brodman and Herp Mapper. A specimen of *Acris blanchardi* is deposited in the Indiana State Museum.

Small mammals.—Sixteen lines consisting of 50 snap-traps were set in WRW and maintained during the time period June 12–17, 2017. This work comprised 3400 trap nights and approximately 192 person-hours. Only three species of small mammals were taken in traps. Several mole burrows, chipmunks, raccoons, and a woodchuck were observed. In all, seven species of small mammal were reported. These numbers were lower than expected; mostly because of human interference. Four lines were run over, one line was flooded, one line was mowed, and most of the traps of one line were pulled out of the woods and deposited into the adjacent lane. We classed the habitats at WRW into four types, i.e., nine lines in grassy weedy fields, five lines along the edge of woods, one line in the woods, and one line in a marshy field.

The diversity with regard to species caught was low with only three species taken, i.e., white-footed mouse (*Peromyscus leucopus*), northern short-tailed shrew (*Blarina brevicauda*), and the meadow jumping mouse (*Zapus hudsonicus*). Species expected but not captured included the prairie deer mouse (*Peromyscus maniculatus bairdii*), meadow vole (*Microtus pennsylvanicus*), prairie vole (*Microtus ochrogaster*), and perhaps the masked shrew (*Sorex cinereus*). Species less likely to occur but possible are the least shrew (*Cryptotis parva*) and the bog lemming (*Synaptomys cooperi*).

Moths (Lepidoptera).—Moths were surveyed using lights (ultraviolet and mercury vapor lights) and white sheets from the beetle team. Surveying took place on June 10th from 8:30 pm to midnight in a forested area at MMF. Voucher specimens were collected and will be housed in the Purdue Entomological Research Collection.

A total of 51 taxa of moths (50 identified to at least family level) were collected. Nine different families of moths were recorded, but Geometridae (geometer moths or inchworm moths) made up the bulk of the specimens present at the light sheets. Geometridae was the most diverse family recorded, having 15 of the 51 taxa observed. The second most abundant family present (in terms of individuals) was Erebidae, with the majority of the moths belonging to the subfamily Herminiinae (litter moths). A total of seven erebid species was recorded. The abundance of these two groups was expected, given that they are commonly found in forested areas. However, erebid moths in the genus *Catocala* were surprisingly absent, since they are a very diverse group with a preference for forested habitat. Other families found include Tortricidae (8 taxa), Limacodidae (3 species), Crambidae (6 taxa), Pyralidae (2 species), Saturniidae (1 species), Sphingidae (1 species), Notodontidae (1 species), and Noctuidae (5 species). There were two "micro" moth specimens that could not be identified to family level.

Mushrooms (fungi and associates).—Team Fungi went out Saturday, June 10th, and encountered 68 observations of 54 different species at two different sites, i.e., the woodlands to the mesic upland woods in the northwest corner of WRW and the woodland at MMF. These species ranged across 45 genera. There were few mushrooms growing from the ground at the time of year this event was held, but a fair number of lignicolous species were encountered. Of particular interest was *Rhodotus palmatus*, known as the netted rhodotus or wrinkled peach. It is one of the most stunningly beautiful mushrooms in North America, featuring a pinkish cap with a veined/ridged surface. It was observed at five locations across the two survey sites. In addition, an interesting *Pluteus* (genus of wood-loving, pink-spored mushrooms), named *P. americanus*, was found. It is one of the few *Pluteus* species known to contain psilocybin, a hallucinogenic chemical.

Another interesting specimen found during this survey was *Reticularia* (*Enteridium*) *lycoperdon*, a slime mold known as a "false puffball." There is only one other record of this species from Indiana, with no current collections in fungaria. Slime molds are no longer classified as true fungi, but we report them as they are generally only studied by mycologists. Another species of note is *Tyromyces galactinus*; there is only one other record of this species in fungaria, dating back to 1917 in Sullivan County. Many collections of *Tyromyces* are documented under the name *Tyromyces chioneus*, a common species in field guides. However, the DNA results indicate that *Tyromyces galactinus* is the most common member of the genus found in Indiana and that many, if not most collections of *T. chioneus*, are likely misidentified specimens of *T. galactinus* (the present specimens included). We will be publishing updated information about this species group in future years. A final interesting find was *Hohenbuehelia angustata*, a genus that is often misidentified as belonging to *Crepidotus* or *Pleurotus* (oyster mushrooms). Despite being somewhat common across the state (pers. observ.), there is only one other record of this species in fungaria dating back to 1925 from Turkey Run State Park.

Physical specimens were collected and dried for 39 of the observations at this event. These specimens are housed at Purdue University's Kriebel Herbarium (PUL). All of the specimen records have been uploaded to MyCoPortal (**www.mycoportal.org**), a consortium containing the records of North American fungaria. These records contain additional specimen information including PUL accession numbers. Color images for the species encountered at the bioblitz can be found on MycoMap (**www.mycomap.com**). Lastly, 25 of the specimens from this event underwent DNA sequencing of the ITS region, and these DNA sequences and their GenBank accession numbers have been made publicly available through GenBank (**www.ncbi.nlm.nih.gov/genbank**). Several of these sequences represent the first time a DNA sequence for the species has been made publicly available. These include *Agrocybe acericola* (MG748577), *Gymnopus spongiosus* (MG748574), and *Mycena niveipes* (MG748570). A species name has not been finalized for the *Inocybe* (MG748575), but this record also represents the first time a sequence for the species has been made publicly available. Most of the other sequences that were generated represent the first publicly available DNA records for the species from Indiana.

Non-vascular plants (bryophyte).—The sampling of the bryophyte community on the bioblitz properties demonstrates a suite of bryophytes suited to moist, enriched soils of temperate deciduous flatwoods. This Central Till Plains terrain has been scoured down, valleys filled, and the whole surface much smoothed over in a random mix of till. Along with wind-blown dust from pulverized stone and 10,000 years of humus from the decay of grasses, trees, and herbaceous vegetation, and with the help of bryophytes, nature has created this swath of nearly flat, fertile landscape which contains the WRW and MMF preserves. The specimens were collected in approximately three hours of field work followed by seven hours of microscopic study. The collection process was limited by thick cover by vascular plants, conditions that restricted access and visibility of ground-level bryophytes. Such a survey would ideally be conducted in early spring before tall foliage obscures the bryophytes. Nevertheless, a substantial number of species were obtained from trees, stumps, rotting logs and humus, many of which are indicative of moist, calcium enriched mesic flatwoods.

It is interesting to note that mosses collected from humus growing under the massive growth of vascular vegetation appeared healthy and robust even though only about 3% of available sunlight reaches the forest floor in some areas; and the moist, shaded substrates provide cooler habitats that bryophytes prefer. Although a few mosses access water and nutrients from the ground via primitive vascular systems (i.e., Polytrichaceae), only one of those acrocarps, *Atrichum undulatum*, was present, occupying more open areas of the moist forest floor.

Both preserves were comparable in species diversity and composed generally of mosses characteristic of shaded, moist, calcium enriched sites. A total of 30 species were identified, including 29 species of mosses and one species of leafy liverwort. Twenty-four species were identified from MMF, while 22 species were identified from WRW. Sixteen of the 30 total species occurred at both sites. No rare species or any species considered indicators of acid, nutrient deficient soils were encountered. Most notably in this particular survey were the beautiful *Plagiothecium* mosses appearing to grow in abundance. These glossy, yellow-green, calciphilous pleurocarps, also known as "silk mosses", were found frequently hugging the dark substrates of decaying logs. Also worth noting was a healthy specimen of *Anomodon attenuatus* growing on an oxidized metal stake sunk into the ground, which begs the question, 'What is a calciphile doing here?' Perhaps it only goes to show the remarkable ability of mosses to tolerate and adapt to substrates that would be impossible for vascular plants, maintaining their unique purpose evolved biochemically over millions of years. Certainly each species of moss is a variation on a theme, a unique creation designed for success in tiny niches in virtually every ecosystem. Therefore, knowing mosses adds depth and intimacy to our knowing the world.

Singing and non-singing insect.—The timing of the 2017 bioblitz was early in the singing insects' season, as most species do not mature until mid- to late-summer. The four species identified all are common and expected, i.e., spring trigs (*Anaxipha vernalis*) abundant at both sites, the non-native Roesel's katydid (*Roeseliana roeselii*) at WRW, and the green-striped grasshoppers (*Chortophaga viridifasciata*; Fig. 9) and protean shieldbacks (*Atlanticus testaceus*) at MMF. There was an additional species of katydid singing at McVey that I was unable to see for identification. It had the pattern of a meadow katydid, with one or two quick ticks attached to the beginning of a buzz, but did not exactly match any species of my acquaintance. They may have been newly matured common meadow katydids, which had not fully developed their songs and were singing at a higher frequency than they will have when fully mature. The lack of spring field crickets, not only at the sites but in the area generally, was a surprising absence. Seven species of non-singing insects were observed.

Snail-killing flies (Diptera: Sciomyzidae).—All specimens were collected over the course of two days by use of a sweep net from the margin of a pond at the southern end of MMF, in full sunlight. As expected, no sciomyzids were found in vegetation adjacent to either the Mississinewa or White rivers, where the muddy banks had been scoured by spring floods. The mature woodlands in both areas undoubtedly contain *Euthycera flavescens* (Loew) and *Trypetoptera canadensis* (Macquart), the larvae of which prey on land snails. Both species are found throughout Indiana in deciduous forests. In North America, *E. flavescens* has been found feeding within the land snails *Mesodon inflectus* (Say), *Stenotrema hirsutum* (Say), and *Ventridens ligera* (Say), while *T. canadensis* is known to feed on small pulmonate land snails. Both species of sciomyzids rarely are collected by use of a sweep net. They are most often captured in Malaise traps, which were not used in this study.

Sixty-eight snail-killing flies (Diptera: Sciomyzidae) of 11 species were recorded. Two species (*Ditaeniella parallela* and *Pherbellia nana nana*) are members of the sciomyzid tribe Sciomyzini, larvae of which live chiefly as parasitoids in exposed aquatic, hygrophilous, and terrestrial snails. The other nine species are members of the tribe Tetanocerini, the aquatic larvae of which are overt predators of aquatic and semi-aquatic snails in fens, marshes, pond margins, and even roadside ditches. New for Randolph County are *Dictya expansa*, *D. sabroskyi*, *D. stricta*, *Ditaeniella parallela*, *Limnia boscii* (Fig. 10), and *P. nana nana*, bringing to 19 the number of sciomyzid species known from Randolph County. All species are native. Surprisingly, when one considers the extensive expanses of tilled soil in Randolph County that is unsuitable habitat for sciomyzids, the county now ranks third in the state (after Tippecanoe and Marshall) for the greatest diversity of sciomyzid species.

In Indiana, nine of the species recorded are widespread, with *D. stricta* approaching its northern limit, whereas two species (*D. parallela* and *P. nana nana*) are far less common, being found mainly where falling water levels have stranded their snail hosts. All 11 species would be expected to occur in suitable habitat anywhere in Indiana. The two specimens of *S. fuscipennis* were of the southern form (*S. f .fuscipennis* Loew), which in Indiana generally is found from approximately the latitude of Indianapolis south; no individuals were of the northern form (S. *f. nobilis* Orth). These findings indicate a southern influence on the sciomyzid fauna in east-central Indiana. All specimens will be deposited in the U.S. National Museum of Natural History, Washington, DC.

Spiders.—During this bioblitz, the spider team employed a variety of methods to find and collect spiders. The most common collection method was sweep netting. This technique involved the use of a sweep net to collect spiders from low vegetation. A second technique employed was litter sifting. Litter sifting used a long canvas tube separated on the inside by metal screens (called a litter sifter). Leaf litter was put into the top of the litter sifter and the tube was held over a white sheet and shaken so that spiders that leave the leaf litter could then be collected on the sheet below. Finally, hand collecting was used to capture spiders, especially at night when headlamps were used to find spiders by eye shine and then scooped up into vials. All specimens are housed at the University of Indianapolis except for new state records, which are held at Indiana State University.

The bioblitz was considered a success by the spider team. We expected to find ~ 72 species through one day and night of searching. However, after spending two weeks identifying spiders back in the lab post-bioblitz, we accumulated a tally of 81 species. Among the species found were many rare and infrequently collected species. Moreover, our collecting uncovered six spider species never recorded from the state. These notable species were *Emblyna hentzi* (meshweaver, Dictynidae), *Gladicosa bellamyi* (wolf spider, Lycosidae), *Pirata triens* (pirate wolf spider, Lycosidae), *Schizocosa mccooki* (wolf spider, Lycosidae), *Oxyopes scalaris* (western lynx spider, Oxyopidae), and *Xysticus fervidus* (ground crab spider, Thomisidae). For details, see the final report at **https://www.indianaacademyofscience.org/IAS/media/Documents/BioBlitzInfoandData/RTC-2017-Bioblitz-Final-Report.pdf**.

The spider species richness at both sites is higher than reported here. It's estimated that it takes over 3,000 spider specimens to accurately gauge the species richness of a habitat but only ~500 specimens were captured over our sampling period. However, as evidenced by the large number of new spider distribution records found through only 21 man-hours of collecting during this bioblitz, these areas may represent refuges for biodiversity in Eastern Indiana – an area where most of the land has been cleared for agriculture. WWR possesses habitats such as riparian woods, marshland, and temperate forest that are becoming rarer in this area and are therefore critically important for the conservation of a variety of animals. Although relatively young, MMF possesses high spider species richness. Our prediction is that, through time, spider species richness will increase as the leaf layer increases, the canopy closes, and the area recovers from its recent disturbances. As leaf layer increases, smaller arthropods such as collembola will increase in prevalence, providing ideal living conditions for litter-dwelling spiders such as small linyphiids, dictynids, lycosids, and gnaphosids. Moreover, a closed canopy combined with a thick leaf litter will help retain moisture close to the soil, preventing the desiccation of small arthropods. The conservation of these two sites would be important in preserving arthropod biodiversity in Eastern Indiana.

Vascular plants.—It is clear from the results of the bioblitz that east-central Indiana supports a rich and diverse vascular plant flora. In summary 477 taxa (406 at MMF and 289 at WRW) were reported. Of the total, 218 taxa occurred at both sites, 71 taxa only occurred at WRW, and 187 taxa only occurred at MMF. The details of each site are presented separately here.

McVey Memorial Forest: The woodland at MMF was divided into two sections, i.e., the northern woods (all land north of CR 750 N) and the southern woods (all land between CR 700N and 750 N). A total of 406 species were observed at MMF. Of these 15 occurred only in the northern woodland, 142 occurred in both woodlands, and 249 species occurred only in the southern woodland. Of the 406 taxa, 312 (76.8%) were native and 94 (23.2%) were non-native. From the northern woodland, 157 taxa were reported of which 111 (70.7%) were native species and from the southern woodland, 388 taxa were reported of which 302 (77.8%) native species.

Among the 406 species were 24 potential Randolph County records. Most notable among these were *Callitriche terrestris*, *Elodea canadensis*, *Erythronium americanum*, *Monotropa uniflora*, *Najas flexilis*, *Potamogeton nodosus*, *Quercus palustris*, *Scutellaria nervosa*, *Silphium laciniatum*, and *Solidago rigida*. Lastly, two species, *Hydrastis canadensis* and *Viola pubescens*, are on the state watch list. No endangered, rare, or threatened species were encounter.

The native FQI and mean C for MMF were 62.9 and 3.6, respectively, while the total (native + non-native species) FQI and mean C were 55.1 and 2.7, respectively. The vascular plant taxa documented and the native FQI at MMF were typical of other floristic inventories of vegetation in east-central Indiana (see Ruch et al. 2014; Hubini et al. 2017). The native matrices suggest that MMF is of remnant natural quality and contains some noteworthy remnants of the natural heritage of the region (Swink & Wilhelm 1994; Rothrock & Homoya 2005). Although low for sites outside the Central Till Plain region, the native mean C for MMF is typical for sites within this region. See Hubini et al. (2017) for an explanation of the lower native mean C values in the Central Till Plain region, especially east-central Indiana. Of the 406 species reported from MMF, 33 (8.1%) had C-values equal to or greater than seven ($C \geq 7$), include five $C = 10$ species, i.e., *Carex bromoides*, *Ranunculus hispidus* var. *caricetorum*, *Silphium laciniata, Taxodium distichum*, and *Thuja occidentalis*. However, the later three species were likely planted.

For all species (native + non-native), the FQI = 55.1 or 7.8 units lower than the FQI for native species alone. Likewise, for all species the mean C was 2.7 or 0.9 units lower than the mean C for native species alone. Rothrock & Homoya (2005) have suggested that natural quality of an area is compromised when non-native diversity lowers mean $C \geq 0.7$ units. Based on these numbers, it would appear that the non-native flora is having a negative impact on the native flora. However, based on visual observations and species distribution, the negative impact is not equal across all habitats. Non-native flora is negatively impacting the native flora along the roadside, in old fields, along drainage ditches, in tree plantations along SR 1, and in and around the man-made pond. However, within the older woodland, the impact is negligible. Problematic non-natives include *Alliaria petiolata, Bromus* spp., *Cirsium arvense, Conium maculatum, Festuca arundinacea* (=*Schedonorus arundinaceus*), *Hemerocallis fulva* (in patches), *Leucanthemum vulgare, Lonicera maackii*, *Melilotus officinalis, Phalaris arundinacea* (along the river), *Poa pratensis*, *Rosa multiflora, Setaria* spp., *Trifolium* spp., and *Vicia cracca*.

White River Woods: Figure 12 shows the plant team working along the White River. Of the 289 species observed at WRW, 220 (76.1%) were native and 69 (23.9%) were non-native. From the mature woods, 161 taxa were reported of which 129 (80.1) were native species, including several large tree species (Fig. 13); from the old fields, 172 taxa were reported of which 121 (70.3%) were native species; and from the floodplain woods, 173 taxa were reported of which 123 (71.1%) were native.

Among the 289 species reported, 22 were potential Delaware County records. Most notable among these were *Carex oligocarpa, Desmodium illinoense, Echinocystis lobata, Euphorbia obtusata, Heracleum maximum, Iris pseudacorus* (non-native), *Prenanthes crepidi-*

Figure 12.—Members of the plant team working the banks along the river at White River Woods. (*Photo by John Taylor*)

nea, Robinia pseudoacacia (surprisingly!), *Schoenoplectus acutus*, and *Vicia villosa*. In addition, *Euphorbia obtusata*, which was collected in an old field, is listed as state endangered, and *Prenanthes crepidinea*, which was collected in the floodplain woods, is listed on the state watch list.

Figure 13.—Stephanie Schuck from the plant team measuring the dbh of a large bur oak (*Quercus macrocarpa*) at White River Woods. The dbh was over 90 cm! (*Photo by John Taylor*)

The native FQI and mean C for WRW were 46.3 and 3.1, respectively, while the total (native + non-native species) FQI and mean C were 40.4 and 2.6, respectively. The flora observed and the native FQI and mean C at WRW were typical of other floristic inventories in east-central Indiana (see Ruch et al. 2014; Hubini et al. 2017). Although these matrices represent observations and data collection for only one weekend, the numbers indicate that WRW is of nature preserve quality (Swink & Wilhelm 1994; Rothrock & Homoya 2005).

Of the 289 species reported from WRW, only 14 (4.8%) had $C \geq 7$. Although there were no species with C-values of 9 or 10, there were five $C = 8$ species, i.e., *Carex amphibola, C. oligocarpa, Carya laciniosa, Elymus trachycaulus*, and *Symplocarpus foetidus*.

For all species (native + non-native) at WRW, the $FQI = 40.4$ was ~ 6 units lower than the FQI for native species alone. Likewise, for all species the mean C was 2.4 or 0.7 units lower than the mean C for native species alone. As stated earlier, Rothrock & Homoya (2005) have suggested that natural quality of an area is compromised when non-native diversity lowers mean C $\geq$ 0.7 units. As described above, the negative impact is not equal across all habitats. Clearly, the non-native flora is negatively impacting the native flora along the roadside and in all the old fields. The negative impact of exotics is negligible in the interior of the woodlands, especially the older woodland in the northeast corner of the property. The non-native species presenting the greatest problem are *Bromus inermis, Cirsium arvense, Conium maculatum, Dipsacus fullonum, Elaeagnus umbellata, Festuca arundinacea, Galium mullugo, Iris pseudacorus, Lonicera maackii, Melilotus* spp., and *Vicia villosa*.

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ATYPICAL AMERICAN BEECH TREE USED BY INDIANA BAT MATERNITY COLONY

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ABSTRACT. Encountered in Clermont County in southwest Ohio during the summer of 2013, an American beech (*Fagus grandifolia* Ehrh.) snag was being used as a roost by two juvenile male Indiana bats (*Myotis sodalis*) for six days with at least 25 other bats (presumably Indiana bats). More bats were seen emerging from this tree than any of five roost trees at our study site and suggests that it may have been a primary maternity roost tree. Beech trees are rarely observed in use as Indiana bat maternity roosts.

Keywords: Indiana bat, *Myotis*, roost, American beech

INTRODUCTION

The Indiana Bat Draft Recovery Plan (USFWS 2007) states that one American beech tree (*Fagus grandifolia* Ehrh.) was used as a roost by one Indiana bat prior to 2004; it also mentions that beech are rarely used as primary roosts and unlikely maternity roosts. Britzke et al. (2006) describes a female Indiana bat that used a dead American beech for eight days during spring migration. Indiana bats typically use tree species as roosts that provide certain characteristics, such as slabs of exfoliating bark among other criteria (Kurta 2005), which American beech trees do not typically exhibit (USFWS 2007). Trees most commonly used by Indiana bat as roosts include ashes (*Fraxinus* spp.), elms (*Ulmus* spp.), cottonwoods and poplars (*Populus* spp.), hickories (*Carya* spp.), maples (*Acer* spp.), and oaks (*Quercus* spp.) (Callahan et al. 1997; Kurta 2005; Sparks et al. 2005; USFWS 2007; Whitaker & Sparks 2008).

METHODS

Two juvenile male Indiana bats were captured in 2013 during a mist net survey, following federal guidelines (USFWS 2013), in Clermont County, Ohio, southeast of the town of Bethel. A 0.31 g radio transmitter (model no. LB-2N Holohil Systems Ltd., Ontario, Canada) was placed on each bat between their scapula using nontoxic medical glue (Torbot Liquid Bonding Cement, Torbot Ostomy and Medical Supplies, RI). The bats were tracked to day roosts with TRX 2000 radio receivers (Wildlife Materials, Inc.) with either a three-element directional Yagi antenna or an omnidirectional whip antenna. Emergence counts were conducted at each roost for two nights.

RESULTS AND DISCUSSION

Bats were tracked for seven days each to five roost trees; a dead American beech, two dead pin oaks (*Quercus palustris* Münchh.), and two live shagbark hickories (*Carya ovata* (Mill.) K. Koch). Both bats were found in the beech-roost more frequently than other roosts. Other roosts were used only once for a single night when occupied by either bat. Indiana bats are known to switch roosts for a variety of reasons throughout the maternity season (Barclay & Kurta 2007).

Overall the beech-roost appeared to have suitable Indiana bat maternity roost characteristics such as solar exposure and exfoliating bark, even though American beech trees typically do not usually retain slabs of exfoliating bark. The beech-roost had a DBH of 58 cm with approximately 80% exfoliating bark present. The beech-roost was completely broken approximately 4 m from the ground, located in an opening without canopy, and adjacent to many toppled or broken trees, suggesting a weather event may have damaged these trees in the past. The bats were roosting 3 m from the ground, exiting from under a slab of bark on the western portion of the roost.

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A total of 110 bats were seen to emerge from the five roost trees occupied by Indiana bats: 45 from the beech and between 3 and 30 from the other four roosts. The beech-roost had the highest bats emerge over the two consecutive nights of sampling. It also had maternity roost characteristics, thus suggesting that the beech is a primary maternity roost.

Biologists that conduct bat surveys may ignore beech trees and many other tight/smooth barked tree species because they usually lack the characteristics of potential roost trees. This use of a beech could be a rare event, as exfoliating bark is very rare for beech trees. However, this roost tree should demonstrate that biologists should not ignore tree species because they are not considered to be typical.

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PREDICTING POTENTIAL CONFLICT AREAS BETWEEN WIND ENERGY DEVELOPMENT AND EASTERN RED BATS (*LASIURUS BOREALIS*) IN INDIANA

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ABSTRACT. Wind turbines pose threats to bats due to the risk of collisions, barotrauma, habitat loss, and environmental changes. To assess potential conflicts between wind energy development and the summer habitat of the eastern red bat (*Lasiurus borealis*) in Indiana, we used a species distribution modeling approach (MaxEnt) to generate two predictive models. We created a model representing areas with the potential for future wind energy development based on six environmental characteristics along with the locations of wind turbines. To create models of habitat suitability for summer resident eastern red bats, we used detections of eastern red bats collected via mobile acoustic surveys. We modeled these with 20 environmental variables that characterize potentially suitable eastern red bat summer habitat. Wind power at a height of 50 m, wind speed at a height of 100 m, and land cover type were the most influential predictors of wind energy development. Proportion of forest within 500 m and 1 km and forest edge within 5 km were the most important variables for predicting suitable summer habitat for red bats. Overlaid maps revealed that approximately three-quarters of the state was unsuitable for both wind development and red bats. Less than 1% of the state showed areas suitable for both wind development and red bats, which made up an area of about 4 km^2. Primarily, these were rural areas where cropland was adjacent to forest patches. Predicting areas with potential conflicts can be an invaluable source for reducing impacts of wind energy development on resident red bats.

Keywords: Bats, EchoClass, *Lasiurus borealis*, MaxEnt, wind energy

INTRODUCTION

Understanding habitat preferences of a species can be important for assessing potential ecological impacts of large-scale developments, such as the establishment of wind energy facilities (Roscioni et al. 2013; Santos et al. 2013). The installation of wind turbines can result in environmental costs, including habitat fragmentation, habitat loss (Larsen & Madsen 2000), and the direct threat to wildlife through collisions with turbine blades (Arnett & Baerwald 2013) and barotrauma (Baerwald et al. 2008). In particular, concerns for birds and bats have increased due to high mortality rates reported at wind energy facilities (Orloff & Flannery 1992; Barrios & Rodríguez 2004; Kunz et al. 2007). Both the placement of wind turbines and the habitat selected by wildlife depend on environmental and geographic variables (Limpert et al. 2007; Brower et al. 2010; Roscioni et al. 2013). As such, understanding the conditions needed for both imperiled species and high quality wind energy may allow us to identify areas where development poses a risk to a species and its habitat. Such understanding can aid in setting conservation priorities and managing wind energy development.

The wind energy sector is an emerging threat to eastern red bats (*Lasiurus borealis*), henceforth referred to as red bats (Johnson et al. 2004; Kunz et al. 2007; Arnett & Baerwald 2013). This is a migratory, relatively common, and widely distributed foliage-roosting bat in North America (Shump & Shump 1982; Cryan 2003). However, the red bat is one of the few species most

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frequently killed by wind turbines (Johnson et al. 2003; Kunz et al. 2007; Arnett et al. 2008) and is a state-listed species of special concern in Indiana (Whitaker & Mumford 2009; IDFW 2015).

Red bats are considered to be declining in many parts of their range (Winhold et al. 2008). Although fatality rates are highest during fall migration, they also occur throughout the entire summer (Arnett & Baerwald 2013; Foo et al. 2017). Currently, Indiana has 1,203 wind turbines in operation with wind energy development expected to increase (AWEA 2018). To reduce the detrimental effects wind turbines have on this species, an accurate understanding of the potential for conflict between red bats and present and future wind energy development is critical.

The red bat is often associated with hardwood, and occasionally, coniferous, forests and use water sources, such as streams, for foraging, drinking, and traveling (Hutchinson & Lacki 1999; Jung et al. 1999; Limpert et al. 2007). Their roosts are found in forests with varying degrees of tree density but most often occur in low to moderately dense forests (Hutchinson & Lacki 2000; Elmore et al. 2005; Limpert et al. 2007). Furthermore, red bats often forage along forest edges (Krusic et al. 1996; Mager & Nelson 2001; Morris et al. 2010). Although developed areas are not a preferred habitat, they will utilize such areas for foraging (Furlonger et al. 1986; Mager & Nelson 2001; Walters et al. 2007). Red bats have been known to forage over 5 km from a roost site in a single night (Hutchinson & Lacki 1999). With the ability to fly long distances, bats likely select habitat in a hierarchical manner (Johnson 1980; Limpert et al. 2007). Thus, it is important to consider habitat preferences of red bats at several scales, such as the scale at which they consider optimal foraging habitat (Limpert et al. 2007).

Species distribution models (SDMs) are useful tools for quantifying suitable habitat for wildlife. MaxEnt is an SDM tool that combines presence-only data with a set of environmental features within a geographic spatial grid and uses machine-learning to predict the potential distribution and/or habitat of a species (Phillips et al. 2006; Merow et al. 2013; Elith et al. 2011). This method has been used for several organisms, including bats (Rebelo & Jones 2010; Razgour et al. 2011). Because of their nocturnal behavior bats can be difficult to survey, so absence data may not be reliable or accurate (Hirzel et al. 2006; Rebelo & Jones 2010). Thus MaxEnt, with its use of presence-only data, offers an efficient and valuable solution for creating SDMs for bats.

The goal for this study was to determine the potential for habitat conflict between summer resident red bats and wind turbines in Indiana. Our objectives were to examine the presence locations of resident red bats and known locations of wind turbines, along with environmental variables that influence red bat habitat and wind energy development selection, to (1) identify those variables that most influence suitability for both red bats and wind energy; (2) identify habitats with a high probability of suitability for this species and for wind energy development; and (3) use bat and wind energy suitability maps to quantify areas of potential conflict by generating a map of low conflict and high conflict areas.

METHODS

Study area.—Our field sites consisted of 17 areas in Indiana, the majority occurring in and around state forests. Thirteen of these publicly managed regions occurred within 8 km of an Indiana state forest, one occurred within the Indiana Dunes National Lakeshore, and three were rural areas in east-central Indiana being sampled for another project. The dominant forest types of these areas include oak-hickory, beech-maple, mixed hardwood, and pine (Shao et al. 2014). Forests of white oak (*Quercus alba*), red oak (*Q. rubra*), chestnut oak (*Q. montana*), and hickories (*Carya* spp.) were predominate (Shao et al. 2014). Study areas were chosen in order to incorporate much of the area that red bats in Indiana were expected to use, which included agricultural, forested, and developed areas (Cryan 2003; Limpert et al. 2007; Walters et al. 2007). These sites were used to obtain red bat presence data, but our modeling study area consisted of the entire state of Indiana.

MaxEnt Species Distribution Modeling Software.—Several SDMs require information on the presence and absence of a species. However, absence data can be difficult to obtain for some species and false absences may bias model results (Hirzel et al. 2006). So, rather than comparing presence data to absence data, MaxEnt contrasts presence data and background data (Phillips et al. 2009). Background data is the set of conditions where the focal species could have been found based upon the survey technique (Phillips et al. 2009). MaxEnt randomly samples the area containing the background data (creating pseudo-absences) and contrasts these against the presence data (Merow et al. 2013).

MaxEnt models utilize Area Under the Curve (AUC) of the receiver operating characteristic to evaluate model performance (Elith et al. 2011). AUC measures the models ability to discriminate between a random presence point and a random absence point (or a random background point) on a scale from 0 to 1; a value of 1 represents perfect discrimination, while a value of 0.5 represents random discrimination (Fielding & Bell 1997; Rebelo & Jones 2010).

MaxEnt has grown in popularity for its use in predicting species distributions since it is accurate in its predictions and user-friendly (Merow et al. 2013). However, the predictive ability of MaxEnt is dependent upon the quality of input data and the satisfaction of model assumptions such as data independence and random sampling.

Red bat presence data.—A total of 28 mobile acoustic surveys was conducted in and in proximity to Indiana state forests from 30 May to 7 August 2012 (Tonos et al. 2014). An additional 19 surveys were carried out in northwestern Indiana between 7 July and 8 August 2013 (D'Acunto et al. 2018). Each route was surveyed once. Although fall is generally when bat mortalities peak (Arnett & Baerwald 2013), we chose to focus on red bats in the summer (Britzke & Herzog 2009) because it may be particularly informative to identify habitat that could potentially put resident summer bats at risk. Additionally, summer bat surveys are easier to conduct since summer is a broader window of time and does not require timing of surveys to perfectly coincide with migration. Surveys traversed all major cover types in the region (agriculture, forests, developed areas, open water) and occurred throughout Indiana, including in some of the same general areas as the wind turbines.

Ultrasonic echolocation calls of bats were recorded with a microphone mounted to the roof of a vehicle connected to an Anabat SD2 (Titley Scientific, Inc., Ballina, NSW, Australia) and an iPAQ Personal Digital Assistant (PDA; Hewlett-Packard Company, Palo Alto, CA; Britzke & Herzog 2009). The length of the routes ranged between 16.1 and 51.2 km (mean = 39.4 km, SD = 10.6 km). Each route was driven at a consistent speed between 24–32 kph. The locations of recordings were registered using a CompactFlash SiRF STAR III Global Positioning System (GlobalSat, New Taipei City, Taiwan). To maximize likelihood of red bat identification, surveys began 20 min after sunset when the temperature was at least 12.8° C, there was little to no chance of rain, and wind speeds were forecasted to be less than 24 kph.

The automated acoustic bat identification software, EchoClass (v2), was used to identify red bats from echolocation call files, and thus obtain presence locations. Echolocation call files were identified using "Species Set 2" in EchoClass, which includes a suite of nine species to which the calls can be identified, i.e., big brown bats (*Eptesicus fuscus*), silver-haired bats (*Lasionycteris noctivagans*), red bats, hoary bats (*Lasiurus cinereus*), eastern small-footed bats (*Myotis leibii*), little brown bats (*M. lucifugus*), northern long-eared bats (*M. septentrionalis*), Indiana bats (*M. sodalis*), and tricolored bats (*Perimyotis subflavus*). These are the most commonly encountered species in our study area throughout the summer. Classification accuracy of these species often exceeds 90% from call libraries, though field recordings are expected to introduce more potential for misidentification (Britzke et al. 2002, 2011). Given a particular call, the species identified by the program is referred to as the "prominent species". If another bat is present, that species is the second prominent species. Files that identified red bats as the prominent species or those that classified the red bat as the second most prominent species when the first prominent species was unknown were included in our presence data.

Red bat environmental variables.—For the habitat suitability model of red bats, five major feature types were selected following Weber & Sparks (2013), i.e., proportion of forest, proportion of area with forest edge, proportion of area with streams in forest, length of streams, and proportion of developed area. These variables are relatively consistent throughout the study area and represent habitat over a long time period. All maps were created in ArcMap 10.2.2 (Environmental Systems Research Institute, Inc., Redlands, CA). Focal statistics was used to calculate the proportion of each cover type within circular plots at four spatial scales: 500 m, 1 km, 3 km, and 5 km. Red bats have been observed foraging 5 km away from their roost sites (Hutchinson & Lacki 1999) and was considered the maximum area they could explore when selecting habitat. Variables relating to forest, forest edge, and developed area were calculated using the U.S. Geological Survey's 2006 National Land Cover Database (Xian et al. 2011). Forest edge was identified as forested raster cells adjacent to non-forested areas (e.g., hay/pasture, cultivated crops, water, developed areas with open space). In order to calculate stream lengths, the number of stream raster cells within circular plots at each scale was determined and we assumed that any cell designated as a stream constituted a stream length of 30 m (due to raster cells being 30 × 30 m). Stream variables were based on maps created from the U.S. Geological Survey National Hydrography Dataset (intermittent code 46003; perennial code 46003 and 55800; nhd.usgs.gov). Because our sampling effort was concentrated along country roads, we only included high–intensity and moderate–intensity areas of development for our proportion of developed area variable so as not to impose bias based upon our sampling along low–intensity developed roads. Cells of all raster maps were 30 × 30 m.

Wind presence data.—For locations of current and developing wind turbines, we obtained archives generated by the Federal Aviation Administration (FAA). Turbines and meteorological towers that were determined to be "no hazards" to air navigation by the FAA between 2008 and 2013 were selected because these serve as the best representation of where turbines are located in Indiana (GEC 2005). Meteorological towers are used to gather on-site environmental data, including wind parameters, near a potential wind energy facility and assess the wind resource availability for wind energy sites (Brower et al. 2010). These sites were included as presence data in the model. Any turbine categorized as a "work in progress" also was included since the environmental characteristics of these proposed wind turbine locations were considered to be informative of future development. A number of large-scale wind energy projects are situated in northwestern Indiana and compose the majority of the wind turbines in the state (GEC 2005).

Wind environmental variables.—For wind energy development potential, variables that are considered to influence wind resource potential or wind turbine construction were chosen (Bailey et al. 1997; Brower et al. 2010; Copeland et al. 2013; Pocewicz et al. 2013; Petrov & Wessling 2015). Variables included wind power in watts (W/m^2) at a height of 50 m and 100 m, wind speed (m/s) at a height of 50 m and 100 m, percent slope, and land cover. The wind resource maps were produced by the Mesoscale Atmospheric Simulation System and WindMap (TrueWind Solutions). The NLCD served as the basis for our land cover data. Percent slope was calculated using elevation data from the U.S. Geological Survey's National Map Viewer and the slope tool on ArcMap 10.2.2.

Spatial autocorrelation.—MaxEnt assumes that the presence data input into the software is independent and free from spatial autocorrelation (Merow et al. 2013). Therefore, a random distribution of occurrence data should be utilized within MaxEnt. Failure to account for spatial autocorrelation would introduce error into the model that may affect the model performance and result in overfitting and errors in prediction (Elith et al. 2011; Merow et al. 2013). As stated below, to alleviate some of the spatial autocorrelation in the wind turbine data, we randomly selected data for model training, while the rest were used for model evaluation (Pocewicz et al. 2013). However, the wind turbine data in this study represents a census, rather than a sample, of all wind turbines within the state of Indiana. Thus, any bias revealed is intrinsic to the entire wind turbine "population" and should be included in the model without modification to produce accurate predictions.

Sampling bias.—MaxEnt models assume that every point within a landscape has an equal chance of being sampled (Merow et al. 2013). However, sampling along roads violates this assumption, thus, giving rise to sampling bias (Reddy & Dávalos 2003; Merow et al. 2013). If such bias is not accounted for, the model's output may only represent the survey effort and/or intensity rather than the species' actual distribution (Phillips et al. 2009; Merow et al. 2013). To account for this bias, it is necessary that the background data be drawn from the area actually sampled (Phillips et al. 2009). Because our acoustic surveys took place along roads, our sampling area was considered to be all locations located within a 30 m buffer area along all routes surveyed, representing the sampling limits of our acoustic detectors. Similarly, the placement of wind turbines is not random. Therefore, we considered only the counties in Indiana in which turbines were located based on the FAA archived data to be the sampling area for background data, including counties with "work in progress".

Model selection.—MaxEnt utilizes a user-adjustable regularization parameter to constrain model complexity (Phillips et al. 2006; Warren & Siefert 2011; Merow et al. 2013). Comparison of models with various regularization values provides a method to determine the model that best balances model fit and complexity (Warren & Seifert 2011). For this study, ten models were created with varying regularization values (1, 3, 5, 7, 9, 11, 13, 15, 17, and 19) in MaxEnt 3.3.3k (Phillips et al. 2006) using red bat and wind development datasets and their respective environmental variables following the methods outlined by Warren & Siefert (2011). Results from each set of models were compared using ENMTools 1.4.4 (Warren et al. 2008). The model with the lowest AICc value was chosen for both red bats and wind turbines with the corresponding regularization value for each "best" model used for the empirical models.

Empirical models.—Empirical models for both red bats and wind energy development were run in MaxEnt using the appropriate regularization value obtained from the model selection method outlined above. Duplicate presence records in the same grid cell were removed within MaxEnt in order to prevent further autocorrelation (Diniz-Filho et al. 2003). Five red bat presence records were removed within MaxEnt for occupying the same grid cell, thus 450 red bat presence records were used for MaxEnt modeling – 315 for model training, 135 for model evaluation. All 1678 wind turbine records were used for MaxEnt modeling – 1,175 for model training and 503 for model evaluation. For each model, 70% of total presence records were used for model training and the remaining 30% were withheld for model evaluation. Background data for both red bats and wind turbines consisted of 10,000 points randomly distributed throughout the respective sampling areas. From our MaxEnt models we obtained raw output representations depicting relative occurrence probabilities for red bat habitats and wind energy development potential.

Both models had greater than 455 presence records, and this sample size allows MaxEnt to create complex response curves, or features, for the environmental variables (i.e., linear, quadratic, product, hinge, and threshold). In our case, MaxEnt utilized all features (linear, quadratic, hinge, product, and threshold) because of our large number of presence records (Elith et al. 2011; Merow et al. 2013).

MaxEnt null models.—AUC is the most popular predictor used in the literature to assess model accuracy of presence-only data in MaxEnt (Merow et al. 2013; Raes & ter Steege 2007). However, the use of background data (acting as pseudo-absences) decreases the maximum achievable AUC value to less than 1.0 and it is not always possible to determine based upon this value alone if a model contributes significantly to predicting suitable habitat (Raes & ter Steege 2007; Phillips et al. 2006). Therefore, it is necessary to assess whether the AUC value of a model significantly differs from that expected by chance through comparison to null models with AUC values from models created using randomly distributed presence locations (Raes & ter Steege 2007). Null models for both red bats and wind turbines were created by generating 500 sets of random locations (each set representing the same number of presence locations from original models) within each sampling area (Raes & ter Steege 2007). Each set of presence data for the null models was processed in MaxEnt utilizing the exact same parameters used for each empirical model. The AUC value for each empirical model was then compared to the distribution of AUC values of the corresponding null-model to determine whether the discrimination power of the empirical model was significantly greater than random.

Conflict potential.—Based on the best model, MaxEnt provides a map for both the red bat and wind energy models. Each cell within the maps is given a value that represents the relative probability of suitability for either red bats or wind energy. Each map was classified into distinct suitability categories using the maximum sum of sensitivity and specificity (max SSS) for each model as a threshold (Liu et al. 2005). Values above this threshold in each model were considered 'suitable' while values below this threshold were deemed 'unsuitable.' To quantify the potential for conflict between suitable red bat habitat and wind energy development potential, these maps were overlaid and the amount of area for each possible combination of suitability levels from both maps was determined. For each of these groups, the area and the percentage of each

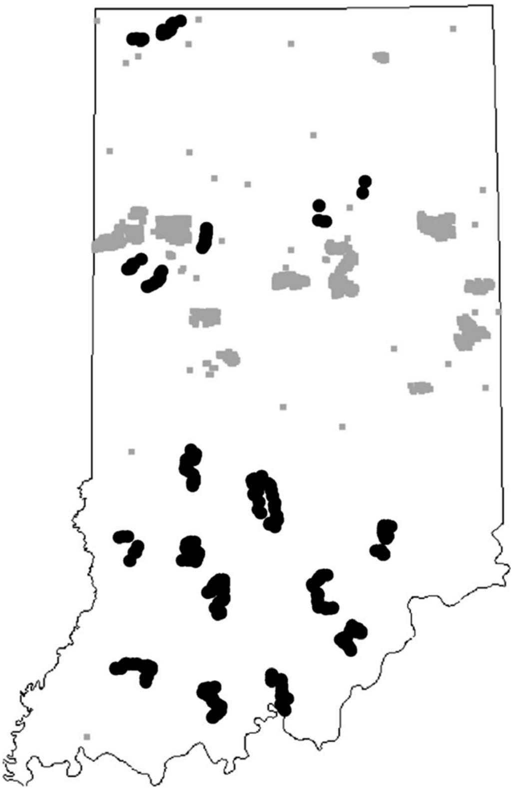

Figure 1.—Presence records of the eastern red bat (black circles) and wind turbines (gray squares) in Indiana, USA.

group present was calculated. All map manipulations were conducted in ArcMap 10.2.2.

RESULTS

Presence data.—A total of 4,649 echolocation calls was obtained from 47 surveys conducted across the state of Indiana. Figure 1 shows the 455 echolocation calls identified by EchoClass (v2) as red bats. Because red bats were relatively common, this map closely resembles the entire area surveyed. Spatial autocorrelation analysis resulted in a calculated ANN value of 639.58 m. When compared to the 500 null models created, the ANN of red bat occurrences showed no difference from random ($p = 0.058$), thus occurrences were considered independent and free from spatial autocorrelation. In addition 1678 wind turbine records were obtained (Fig. 1).

Empirical models.—The optimum model for red bats had the lowest AICc and a regularization parameter multiplier value of 3. The southern portion of the state had the highest predicted suitability for red bats (Fig. 2a). The training AUC for the red bat model was 0.705, while the AUC for the evaluation data set was 0.615 (SD = 0.025) with a max AUC of 0.671 (maximum AUC is calculated based on using total MaxEnt distribution and, in practice, training and evaluation AUC values may exceed this maximum; Philips et al. 2006). AUC values were significantly greater than those of null models ($p < 0.002$). The variables that contributed the most to the predicted suitability of this model were proportion of forest within 500 m, proportion of area with forest edge within 5 km, and proportion of forest within 1 km. The first two variables showed a strong positive effect on suitability while the final variable showed a strong negative effect on suitability (Fig. 3). It should be noted that the response curves of covariates assume all other environmental variables are held at mean values (Table 1). Thus, the seemingly contradictory results of optimal habitat suitability with complete forest cover within 500 m but no forest within 1 km is neither possible nor the actual conclusion of the model.

The optimum model for wind turbines had the lowest AICc and a regularization parameter multiplier value of 3. The predicted suitability for wind energy occurred mostly in the central portion of the state (Fig. 2b). The training AUC for the wind energy development model was 0.896 and the evaluation AUC was 0.890 (SD = 0.006) with a maximum AUC of 0.883. AUC values were significantly greater than those of null models ($p < 0.002$). The highest contributing variables were wind power at 50 m, land cover type, and wind speed at 100 m/s. Suitability peaked around 300 W/m^2 for wind power at 50 m and showed a strong positive effect between approximately 250 W/m^2 and this peak (Fig. 4a). Above 300 W/m^2, suitability dropped dramatically. Wind speed at 100 m showed a strong positive effect approximately between 8.0 m/s and 8.6 m/s with suitability plateauing at greater wind speed (Fig. 4b). Land cover types were treated categorically. The most positively associated land cover types were "cultivated crops" and "hay/pasture" (Fig. 4c).

Conflict potential.—For red bat and wind turbine suitability maps, max SSS threshold values of 37.26 and 14.15, respectively, were used to categorize each map into suitable and

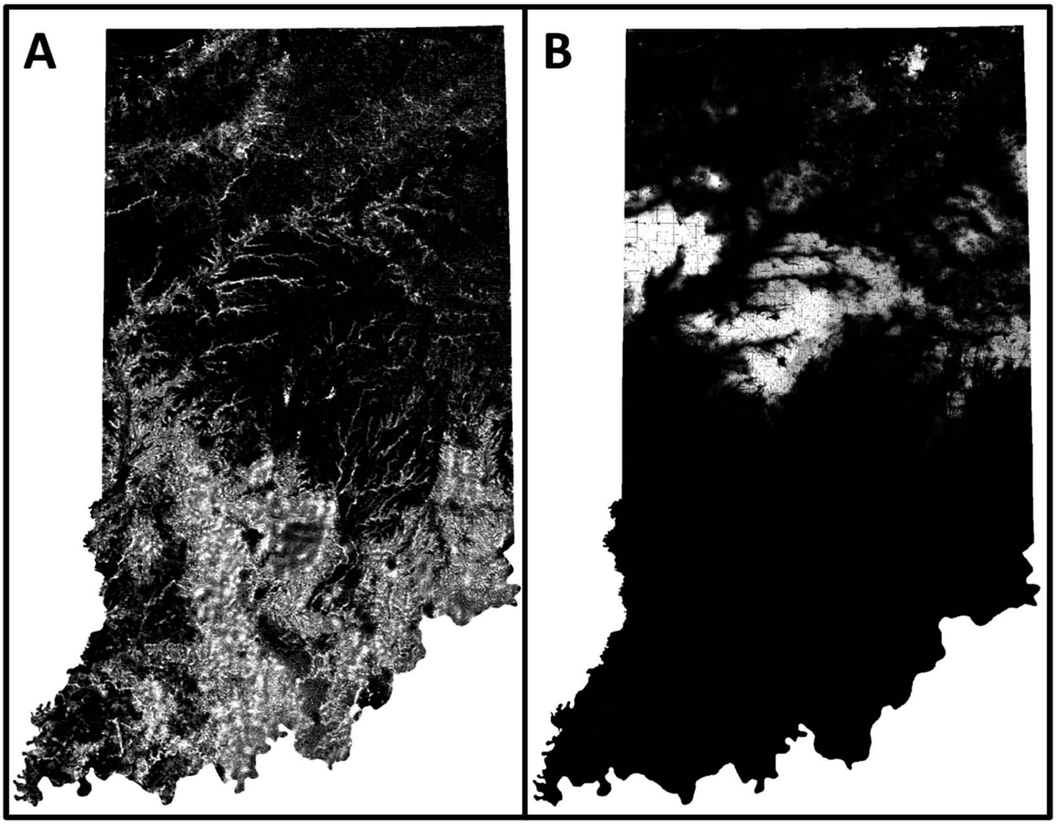

Figure 2.—Raw output maps showing (A) red bat habitat suitability and (B) wind development habitat suitability. For both maps, lighter colored areas, or areas with a greater value, represent greater suitability.

unsuitable regions. When maps were overlain, the majority of the state exhibited a low conflict potential between wind energy development potential and suitable red bat habitat (Fig. 5). Approximately 73.8% of the state was unsuitable for both wind energy development and red bats (Table 2), constituting an area of about 69,554 km^2. The majority of the remaining areas were regions with suitable habitat for either wind energy development or red bats, but not both. Less than 1% of the entire state represented areas suitable for both wind turbine development and red bats. The areas of high conflict were located in the northwestern and west-central portions of the state and comprised approximately 4 km^2. Areas where cropland is adjacent to deciduous forest patches dominate much of the conflict. This is particularly evident in rural areas (i.e., areas not highly or moderately developed). A portion of the area along Lake Michigan, where there is a high density of forest, also revealed a high conflict potential. This area has a relatively high wind power (> 250 W/m^2) at 50 m, as well.

DISCUSSION

With a rapidly changing landscape, identifying areas that may support potentially threatened species but that may put such species at risk from human development is of upmost importance (Manel et al. 2001; Roscioni et al. 2013; Santos et al. 2013). Wind energy has the potential to provide a sizable portion of Indiana's energy needs (AWEA 2018), but establishing a coexistence of this clean energy source and maintaining habitat for wildlife populations is a growing management concern (Baerwald & Barclay 2009; Arnett & Baerwald 2013). Due to their ecological importance as consumers of insects (Boyles et al. 2011), temperate bats are of particular concern (Mickleburgh et al. 2002). Additionally, bats are long-lived and have relatively low reproductive rates (Barclay & Harder 2003), so the effect of fatalities due to wind energy development may have a disproportionate impact on bat populations. Quantifying the potential for conflict between wind energy development and wildlife may be an efficient way to reduce bat mortality from wind energy development (Roscioni et al. 2013; Santos et al. 2013). Models produced by SDMs have the potential to be useful tools aiding in the siting of wind energy facilities in areas to reduce the risk of bat fatalities (Roscioni et al. 2013; Santos et al. 2013.)

While MaxEnt has been shown to produce reliable and informative models, several criticisms of presence-only modeling exist (Royle et al. 2012; Yackulic et al. 2013). Particularly, it is important to emphasize that the results from this study provide only an index of relative habitat suitability and not quantitative estimates of occupancy. Additionally, while the detection probability of red bats was not directly measured, we acknowledge that detection probability may have varied throughout the sampled areas (Yackulic et al. 2013). For example, differences in structural complexity near or above the roads used to survey bats may have affected the ability of the acoustic detectors to identify bats at various intervals along the road (Patriquin et al. 2003; Broders et al. 2004; Yates & Muzika 2006). In addition, while the sampling area of this study was considered to be within a 30 m buffer area along roads, our models predicted suitability across the entire state of Indiana. Although the sampling area represents much of the state of Indiana, this should be taken into consideration when interpreting results.

Red bat suitability models.—While previous studies have investigated habitat selection of red bats, our red bat model estimates the potential for suitable habitat by quantifying features of the habitat and projecting those predictions across a broad area. Furthermore, this model represents nocturnal activity of resident red bats. The habitat needs of foraging red bats may be very different from roosting red bats (Pauli et al. 2015), and these needs likely differ between resident and migrant red bats. The three most important variables for the red bat model were the proportion of forest within 500 m, forest edge within 5 km, and the proportion of forest within 1 km. The proportion of forest within 500 m showed a positive relationship with suitability across Indiana. Forest edges within 5 km also had a positive relationship with suitability, but forest within 1

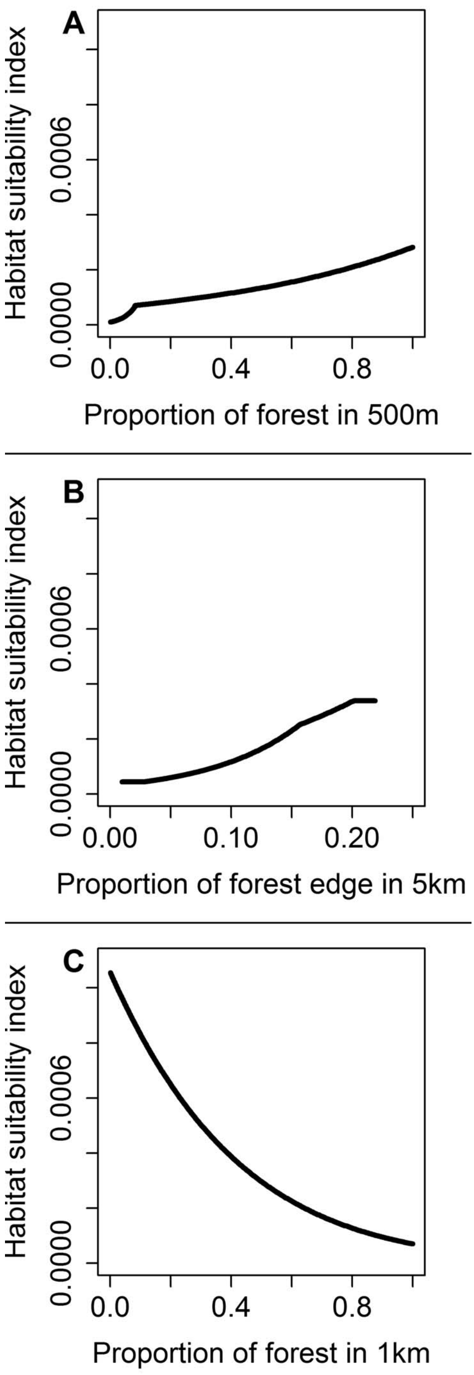

Figure 3.—The response curves of the top three most influential variables on the red bat model. The trend line represents how habitat suitability varies as the following variables change while all other variables are kept constant: (A) proportion of forest within 500 m, (B) proportion of forest edge within 5 km, and (C) proportion of forest within 1 km.

Table 1.—Mean values of predictor variables at sample locations used for the red bat habitat suitability model and wind energy development model. Generating the response curves from the MaxEnt models involved setting all variables, except for the variable of interest, to this constant mean value. The most common landcover type, which was used in the wind energy development model, was cultivated crops.

	Predictor variable		Mean
Red bat model	Forest edge (%)	500 m	19.15
		1 km	13.27
		3 km	12.77
		5 km	13.03
	Forest (%)	500 m	68.51
		1 km	68.64
		3 km	66.32
		5 km	61.36
	Developed area (%)	500 m	0.10
		1 km	0.11
		3 km	0.19
		5 km	0.27
	Streams in forest (%)	500 m	5.17
		1 km	5.08
		3 km	4.75
		5 km	4.48
	Stream length (m)	500 m	47.28
		1 km	196.61
		3 km	1727.12
		5 km	4649.54
Wind energy model	Wind power (watts per m^2)	50 m	300.75
		100 m	470.94
	Wind speed (m/s)	50 m	6.68
		100 m	7.75
	Slope (%)	-	1.10

km was a negative relationship. Forest edges can be particularly important to foraging red bats and other insectivorous bats (Mager & Nelson 2001; Law & Chidel 2002; Morris et al. 2010), but too much "clutter" (i.e., obstacles) within the foraging area may impede flight and echolocation (Fenton 1990; Elmore et al. 2005). This suggests that although forests, particularly forest edges or openings within forest, may be important for roosting, traveling, and some foraging opportunities within a small spatial scale, contiguous forests at a scale of 1 km may not provide optimal foraging habitat. Southern Indiana is composed of relatively intact forests (Jenkins 2012), and forest edges likely provide highly used foraging and traveling habitats.

While our model had somewhat low AUC values (Swets 1988; Araujo & Guisan 2006), comparisons with null models show that the potential to provide valuable information on the habitat preferences of red bats is significant (Raes & ter Steege 2007). However, the ability of our model to correctly discriminate between a presence location and a random site, based on AUC values, is still rather low. This could be explained by the fact that species with a broad geographic range and generalized habitat preferences provide models of relatively low predictive power (Kadmon et al. 2003; Hernandez et al. 2006). Red bats appear to be habitat generalists with an ability to use a variety of habitat types (Furlonger et al. 1986; Elmore et al. 2005; Ford et al. 2005). Furthermore, modeling nocturnal activity combines both foraging and commuting detections. This aggregation of locations that bats might select for different activities may dilute some of the precision of habitat selection models. Additionally, the lower AUC of the evaluation data compared to the training data may indicate that overfitting occurred, even though we attempted to account for overfitting (Merckx et al. 2011; Warren & Seifert 2011). Nonetheless, this model is valuable as a method for delineating areas likely to be favored by red bats in Indiana.

Wind development models.—In contrast to the habitat preferred by foraging red bats, sites suitable for wind energy development in Indiana are generally in very open habitats with flat terrain. The main consideration when assessing areas for wind energy development is wind resource availability (Brower et al. 2010). Two factors that greatly influencing wind resource availability are wind power and wind speed. Our model indicated that wind power at 50 m and wind speed at 100 m were the most influential variables predicting suitable areas for wind development. Generally, wind power greater than 400 W/m^2 and wind speed greater than 7.0 m/s at 50 m is suitable for most wind development applications (Bailey et al. 1997). Our results indicated that wind power at 50 m peaked around 300 W/m^2 (Fig. 4a) corresponding to the minimum requirement. Although our results represent wind speed at 100 m, the wind speed minimum requirement at 50 m can be extrapolated to this height using a form of the power function (Bailey et al. 1997) that accounts for wind shear at varying heights. The resulting extrapolated minimum value for wind speed at a height of 100 m is 7.1 m/s. Our

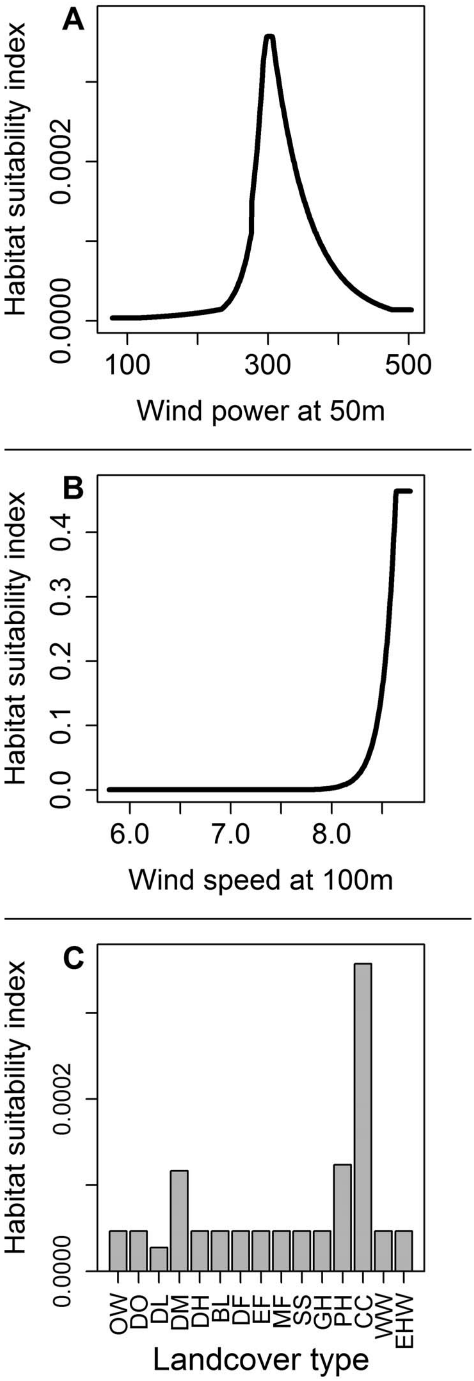

Figure 4.—The response curves of the top three most influential variables on the wind energy development model. The trend line represents how habitat suitability varies as the following variables change while all other variables are kept constant: (A) wind power (W/m^2) at 50 m, (B) wind speed (m/s) at 100 m (note the different scale used for y-axis), and (C) land cover type [OW = open water, DO = developed open space, DL = developed low intensity, DM = developed medium intensity, DH = developed high intensity, BL = barren land, DF = deciduous forest, EF = evergreen forest, MF = mixed forest, SS = shrub/scrub, GH = grassland/herbaceous, PH = pasture/hay, CC = cultivated crops, WW = woody wetlands, EHW = emergent herbaceous wetlands].

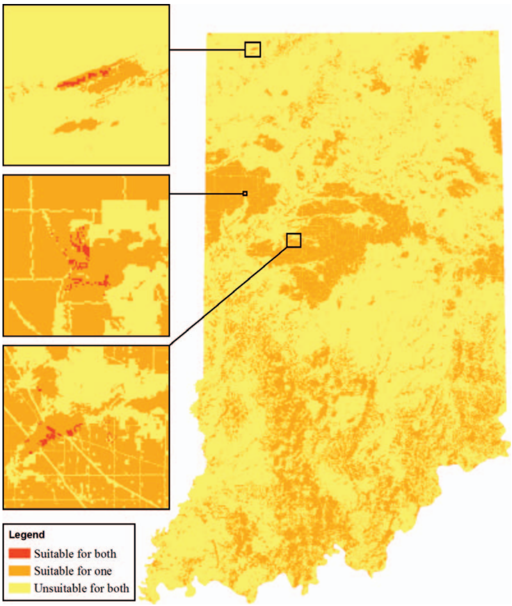

Figure 5.—A representation of the conflict potential between habitat suitability for the red bat and wind energy development describing areas that are unsuitable for both, suitable for one, or suitable for both. The three insets are included to make areas of conflict visible. They contain 57% of the identified areas of conflict on the map.

results indicated that wind speed at 100 m plateaus at 8.6 m/s and greater (Fig. 4b) exceeding this minimum requirement.

Land cover type was also an important variable in predicting suitable wind energy development areas. In particular, cultivated crops and hay/pasture had the most influence on suitability than any other cover type. Indeed, most of the utility-scale wind farms currently in operation are located in agricultural, grassland, and desert habitats (Kunz et al. 2007; Arnett et al. 2008; Denholm et al. 2009).

For these two factors our results coincide with the industry standards used to assess areas for

Table 2.—The area (km^2) and percentage of each combination of suitable and unsuitable cells for the habitat suitability for red bats and the habitat suitability for wind energy development.

Red bat suitability	Wind energy development Unsuitable	 Suitable
Unsuitable	69554 (73.8%)	9676 (10.3%)
Suitable	14961 (15.9%)	4 (0.004%)

wind development in Indiana and other Midwestern states. Moreover, much of the best wind resource availability in Indiana is located in the northern part of the state (GEC 2005), and supported by our results

Compared to the predictive power of our red bat model, our wind model showed better predictive power overall (Swets 1988; Raes & ter Steeg 2007), indicating that it is likely sufficient for identifying suitable wind energy development locations within this region based on the given environmental variables and parameters (Pearce & Ferrier 2000). While using SDMs to predict potential development by humans is in its infancy, it has great potential for the prediction of future wind energy developments (Pocewicz et al. 2013; Petrov & Wessling 2015).

Potential conflict.—Our conflict potential map represents an alternative to assessing wind energy impacts on bats during siting analyses conducted before construction of facilities begins (Roscioni et al. 2013; Santos et al. 2013). Modeling future anthropogenic development to determine possible impacts on wildlife can be a useful and relatively quick approach to identifying conflicts (Copeland et al. 2013; Pocewicz et al. 2013). With such contrasting habitat requirements, it was not unexpected that there would be little conflict for suitable locations between bats and wind turbines in the state. Our conflict analysis did show a low potential for conflict between suitable summer habitat for red bats and suitable habitat for wind energy development in Indiana. Presumably, this indicates that summer resident red bats are not likely to occur where wind turbines might be present. However, a small proportion of the state showed a high potential for conflict, particularly in areas where large-scale wind energy projects already exist, and of potential concern is the influence these and future wind projects may have on bats.

For this study, several variables seemed to be of particular influence on high conflict potential. The presence of high conflict areas showed a pattern along areas where rural habitat (i.e., cultivated crops and hay/pasture fields) was adjacent to forest. Red bats readily utilize forest edge and open areas for foraging (Mager & Nelson 2001; Walters et al. 2007; Morris et al. 2010), yet as the distance from edge or forests increases foraging activity decrease (Johnson et al. 2004). Red bats have been observed foraging over agricultural lands (Walters et al. 2007), but generally, when foraging over terrestrial habitat, they prefer foraging over or near areas with some degree of woody vegetation (Furlonger et al. 1986; Hart et al. 1993). At a wind energy facility in Minnesota, Johnson et al. (2003) observed that the majority of bat activity recorded at wind turbines was located at turbines near woodlands. In our case, the wind turbines surveyed are located within rural areas with flat and relatively non-forested terrain. Nevertheless, this pattern of high suitability for wind development in these areas where agricultural fields meet forest edges suggests that wind development could potentially be problematic to foraging bats.

In summary, there is little risk for resident red bats at current wind energy facilities in Indiana except where high quality foraging habitat is situated near wind energy facilities. Because there appears to be little foraging opportunity for bats at wind energy facilities within farmland, conflict may not be great. Nonetheless, our model showed that there is a potential of conflict in areas where forest edge, which can provide quality foraging opportunities, exists near agricultural land. Thus, perhaps the risk for resident bats would be when they are commuting between roosts and foraging areas (Arnett et al. 2005). Furthermore, there is concern for migrating bats, as the peak of bat fatalities is generally during the fall migration period and migratory tree bats comprise a majority of the fatalities in most regions (Arnett & Baerwald 2013), but this warrants additional study in Indiana. Additionally, future studies should consider utilizing more than mobile acoustic surveys, such as stationary acoustic surveys, to detect bats.

Our examination of suitability models and conflict potential using MaxEnt are tools that may be useful for identifying areas that are preferred by red bats but that may be susceptible to development, particularly in states that utilize similar habitats for wind energy development as Indiana. With the rapid increase of wind energy development, a means of securing optimal habitat for bats before the construction of future facilities could be both economically efficient and biologically beneficial.

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MICROPLASTIC POLLUTION IN INDIANA'S WHITE RIVER: AN EXPLORATORY STUDY

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ABSTRACT. Plastic material is now a ubiquitous source of aquatic pollution. Microplastics, tiny plastic pieces often not visible to the naked eye, are a growing environmental concern in both marine and freshwater ecosystems. While many studies have documented the abundance and danger of microplastics in global oceans, little research is available on microplastic presence and impact in riverine ecosystems. This exploratory study aims to build on the findings of recent freshwater microplastic studies by reporting on the occurrence and types of microplastic pollution found in the West Fork White River in central Indiana. Fifteen surface water samples were collected from three bridge sites along the river over a four-month period (August - November 2015) and analyzed using established NOAA laboratory methods. Analysis revealed various microplastic particle types in the White River, with synthetic fibers being the predominant type collected. A total of 146 plastic pieces were collected across all sites and collection periods, with an average microplastic concentration of 0.71 items m^{-3}. Surprisingly, there were no significant differences in microplastic concentrations among sites of differing population density. Further, a local wastewater treatment plant had no effect on particle type or abundance. These findings contribute to current gaps in microplastic research on freshwater, especially fluvial, environments. This contribution may guide researchers in better understanding the extent to which these synthetic particles are polluting U.S. surface waters as a whole.

Keywords: Microplastics, White River, freshwater ecosystems, microbeads

INTRODUCTION

Microplastics are primary- and secondary-sourced plastics smaller than 5 mm in size (Arthur et al. 2008; Ivar do Sul & Costa 2014). Primary microplastics are those manufactured to be microscopic in size for industrial and domestic use. Secondary microplastic debris can be derived from various classes of plastics that come from land-based sources, especially plastic packaging (including disposable single-use items), as well as fishing industry litter (Andrady 2011). Primary microplastics can come from microplastics used in air-blasting technology to remove rust and paint from machinery, boat hulls, and engines (Browne et al. 2007; Cole et al. 2011). Primary microplastics also include plastic beads (or spherules) from exfoliating facial cleansers, body washes, hand soaps, and toothpastes. Polyethylene pieces, or "scrubbers," have been utilized in personal care products to replace natural exfoliates, such as pumice or apricot husks (Zitko & Hanlon 1991; Gregory 1996; Fendall & Sewell 2009). After product use, these plastic pieces wash down the drain with the product and end up in city wastewater systems. Here, they can bypass removal by the initial coarse treatment screens (Derraik 2002; Vesilind 2003), potentially making their way into final effluent and sewage sludge (Fendall & Sewell 2009; Cole et al. 2011).

Synthetic textiles can contribute to microplastic pollution concerns due to possible release of fibers into sewage systems when laundered. Synthetic fibers, such as nylon, Orlon, dacron, and spandex, were first used by the textile industry over 50 years ago to supplement natural fibers such as wool, cotton, and linen (Habib et al. 1998). Aerobic or anaerobic bacteria used in sewage treatment do not readily decompose synthetic fibers, allowing them to concentrate in sewage sludge or be discharged with effluents (Habib et al. 1998). Rivers are a likely source of transport for these synthetic fibers through the aquatic environment. The application of digested and dewatered sludge as a low-grade fertilizer (a common agricultural practice), in addition to atmospheric deposition, are also likely sources of synthetic fibers throughout a watershed.

While Habib, et al. (1998) found a prevalence of synthetic fibers in sewage sludge, Rochman et al.

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(2015) conservatively estimates that approximately 8 trillion microbeads per day are emitted into U.S. aquatic habitats. As the issue has become more publicized by the media, especially with the recent discovery of microbead abundance in the Great Lakes (Eriksen et al. 2013; Baldwin et al. 2016; Mason et al. 2016b), some companies have pledged to remove these plastics from their "rinse-off personal care products" (Rochman et al. 2015). To ensure their removal, President Obama signed a bill, the Microbead-Free Waters Act of 2015, which required manufacturers to eliminate the pollutant from their products by 2017. However, this does not solve the accumulation of microplastics from other sources.

Despite being a likely source for microplastic flow to the ocean, the literature on riverine microplastic concentrations is minimal (Gaspari et al. 2014; Lechner et al. 2014; McCormick et al. 2014; Mani et al. 2015; Baldwin et al. 2016). With recent studies indicating microplastics in the Great Lakes (Eriksen et al. 2013; Baldwin et al. 2016; Mason et al. 2016a), it is timely to quantify microplastic types and concentrations in unstudied riverine systems, such as those within the White River in Indiana. The objectives of this exploratory study were to (i) identify and (ii) compare the abundance and types of microplastics at three sites along the West Fork of the White River in central Indiana. Microplastics were identified as fragments (broken down larger plastics), beads (spherules from personal care products, bead blasting, etc.), fibers (from synthetic textiles), films (plastic wrapping and bags), foam (foam packaging and cups), and pellets (preproduction pellets 5 mm and larger). The findings could provide information on spatial differentiation of plastics between locations along a river with varying watershed characteristics, in addition to characterizing the extent of this pollutant in an unexamined freshwater system. We hypothesized that (i) microplastics at three sites along the West Fork of Indiana's White River differed in abundance, and (ii) microplastic concentrations downstream from a waste water treatment plant and those sampled in areas with greater population density were greater than those upstream or from areas with lower population density.

METHODS

Site description.—The White River of central Indiana was chosen because there are currently no studies that have sampled for microplastics.

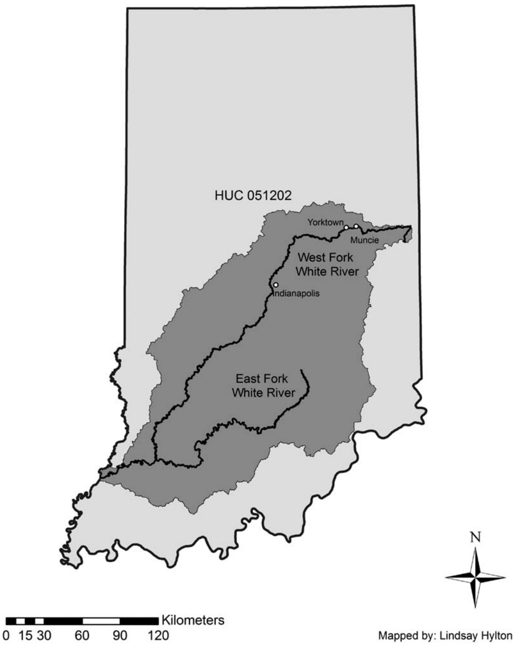


Figure 1.—Site locations within the White River watershed are shown along the Indiana White River. Site 1 (Muncie), Site 2 (Yorktown), and Site 3 (Indianapolis) are marked with white points.

This study was conducted at three locations within the watershed of the West Fork White River basin in Delaware and Marion Counties, Indiana (Fig. 1). Sampling took place from August to November of 2015. Sites 1 and 2 were chosen based on their relative locations upstream and downstream, respectively, of the Muncie Water Pollution Control Facility, and Site 3 was chosen for its more urban and highly populated watershed. Feasibility and safety also played a role in choosing the specific bridges used for the sampling locations. All three sampling locations are located along the West Fork White River, which fall within the Upper White (River) Watershed (HUC 05120201). This watershed has a drainage area of 7055 km^2 and 573 km of flowing water. Its land use is approximately 60% agriculture and 25% developed land (US Census Bureau Data 2015; USGS StreamStats 2017). Further, the three sites fall within two subwatersheds (HUC-12). Sites 1 (Muncie) and 2 (Yorktown) are located in York Prairie Creek-White River, which has 36 km^2 of developed land, 53% of its total area. Site 3 (Indianapolis) is located in Pogues Run-White River, which has 60 km^2 of

Table 1.—Watershed characteristics (US Census Bureau Data 2015; USGS StreamStats 2017).

Site	Population	Sampling point drainage area (km^2)	Drainage % urban development
Muncie	70,087	632.7	4.4
Yorktown	11,231	636.9	4.8
Indianapolis	853,173	4,228.7	10.7

developed land, contributing to 99% of its total area (Wikiwatershed 2017) (Fig. 1).

Sampling locations.—The first sampling site is the furthest upstream, located at the S. Nichols Avenue Bridge in Muncie, IN (40° 11′ 6″ N 85° 24′ 42″ W) (Fig. 1). According to USGS StreamStats data, the drainage area contributing flow to the point sampled is 4.4% urban and drains 632.7 km^2 (Fig. 1; Table 1). The water here is generally shallow, consisting of riffle, glide, and some pool habitats, and is divided by a sand bar. The substrate appears to be sandy silt with cobble. Site 1 is located 2.4 km upstream of the Muncie Water Pollution Control Facility.

The second sampling site, located at the S. Nebo Road Bridge in Yorktown, IN (40° 11′ 9″ N 85° 27′ 43″ W), is located 1.8 km downstream of the Muncie Water Pollution Control facility. The total drainage basin area at this point is 4.8% urban and drains a total of 636.9 km^2 (Fig. 1; Table 1; USGS StreamStats 2017). There is a large rocky island in the middle of the river and the water is generally shallow, with silt and cobble substrate. The habitat consists of riffles, runs, and pools.

The third sampling site is located at the Oliver Avenue Bridge in Indianapolis, IN (39° 45′ 30″ N 86° 10′ 25″ W) (Fig. 1), making it the site furthest downstream. At this sampling point, the total drainage basin area is 10.7% urban and drains 4,228.7 km^2 (Fig. 1; Table 1; USGS StreamStats 2017). The water is significantly deeper than at the first two sites, consisting of primarily slow glide and run habitats. During the study period, Indianapolis had an estimated population of 853,173 (2015), making it the most densely populated watershed of the three sampling sites (US Census Bureau 2015; Table 1).

Sampling procedure.—At each of the three sites, surface water samples were collected on five dates between August through November 2015 ($N = 3$ sites $\times$ 5 events $=$ 15 total events). During each event, two sequential (duplicate) 10 min surface water samples were collected from the same point from the site bridge, the values from these duplicate samples were later averaged to provide one value for each site for each sample event. (While 30 samples were collected, duplicates were averaged, thus $N = 15$). All samples were collected during daylight hours and not within 48 h of a runoff event (an event resulting in combined sewer overflow). Samples were collected using a Wildco stationary stream drift net (99.06 cm length, 45.72 cm wide, and 30.48 cm tall, 363μm mesh) with a detachable mesh dolphin bucket (368μm mesh). This mesh size falls within a commonly used size range in other microplastic studies (Hidalgo-Ruz et al. 2012; Baldwin et al. 2016). The net was modified to be deployed from a bridge and for flotation.

A digital mechanical flowmeter (2030R, General Oceanics, Miami, FL) was attached across the mouth of the net to measure the velocity of the water entering. The total volume of water being filtered through the net was calculated using the width and height of the net, the duration of the sample, and the velocity of flow (Lechner et al. 2014; Baldwin et al. 2016). Due to low velocity ($<$ 10 cm s^{-1}) at all three sites, a low-flow rotor was used. After 10 min, the net was rinsed with a pressure sprayer into a 200 ml dolphin bucket at the end of the net (Baldwin et al. 2016). The plastics and organic debris collected were rinsed from the bucket into a sealed glass jar with deionized water, and then placed on ice for transport to the laboratory (McCormick et al. 2014). This process was immediately repeated to obtain the duplicate sample. A water sample also was collected at each sampling event using a standard grab sampler deployed from the bridge to assess water temperature at the time of sampling. Air temperature was noted and estimated river discharge for each location were collected from USGS steam flow measurements at sites 03347000 (White River at Muncie) and 03353000 (White River at Indianapolis) (http://waterdata.usgs.gov/nwis/rt).

Laboratory analysis.—Processing and laboratory analyses of samples were completed using a modified version of the methods developed by the National Oceanic and Atmospheric Administration (NOAA; Masura et al. 2015; Baldwin et al. 2016). Samples were first wet sieved through two stacked stainless steel sieves (mesh sizes 500 µm and 250 µm). These sizes were chosen to highlight the smaller spectrum of microplastic pollution (250–500 µm) and to adhere to a commonly used sieve size (500 µm; Hidalgo-Ruz et al. 2012). In this study a sieve to exclude plastics greater than the upper microplastic size limit (> 5 mm) was not utilized, in order to decrease processing time and avoid overall plastic loss by the use of an additional sieve.

After sieving, mass of dried solids was determined (Masura et al. 2015). Wet peroxide oxidation was then used to degrade any organic material prior to density separation (Masura et al. 2015). Floating solids and plastics were drained into either 250 µm or 500 µm mesh size custom-made nylon sieves (Masura et al. 2015). After a 24 h drying period, visual sorting of the samples was conducted with the use of a stereoscope (dissecting microscope) at 40× magnification or higher (Hidalgo-Ruz et al. 2012; McCormick et al. 2014; Masura et al. 2015). Tweezers were used to remove all identifiable plastics from the sieves for both size categories and transfer them to labeled glass vials for storage. Each sample was examined under the microscope using the Marine & Environmental Research Institute (MERI) identification guidelines (Hidalgo-Ruz et al. 2012; MERI 2015). Plastic particles were counted and categorized into six categories based on their morphology: fragments (broken down larger plastics), beads (spherules from personal care products, bead blasting, etc.), fibers (synthetic textiles), films (plastic wrapping and bags), foam (foam packaging and cups), and pellets (preproduction pellets 5 mm and larger) (Lechner et al. 2014; Zbyszewski et al. 2014; Baldwin et al. 2016). Additionally, the "hot needle test" (*sensu* De Witte et al. 2014) was used in distinguishing between plastic and non-plastic particles, especially for fibers. The total plastic count for each sample was recorded, along with type (from one of the six categories mentioned previously), and color.

Quality assurance and control.—Precautions were taken during this study to avoid contamination. Samples were processed under a fume hood and always remained covered when not in use. Other equipment and tools used in the laboratory also were washed and covered after use. Further, samples were collected and analyzed in duplicates to increase precision.

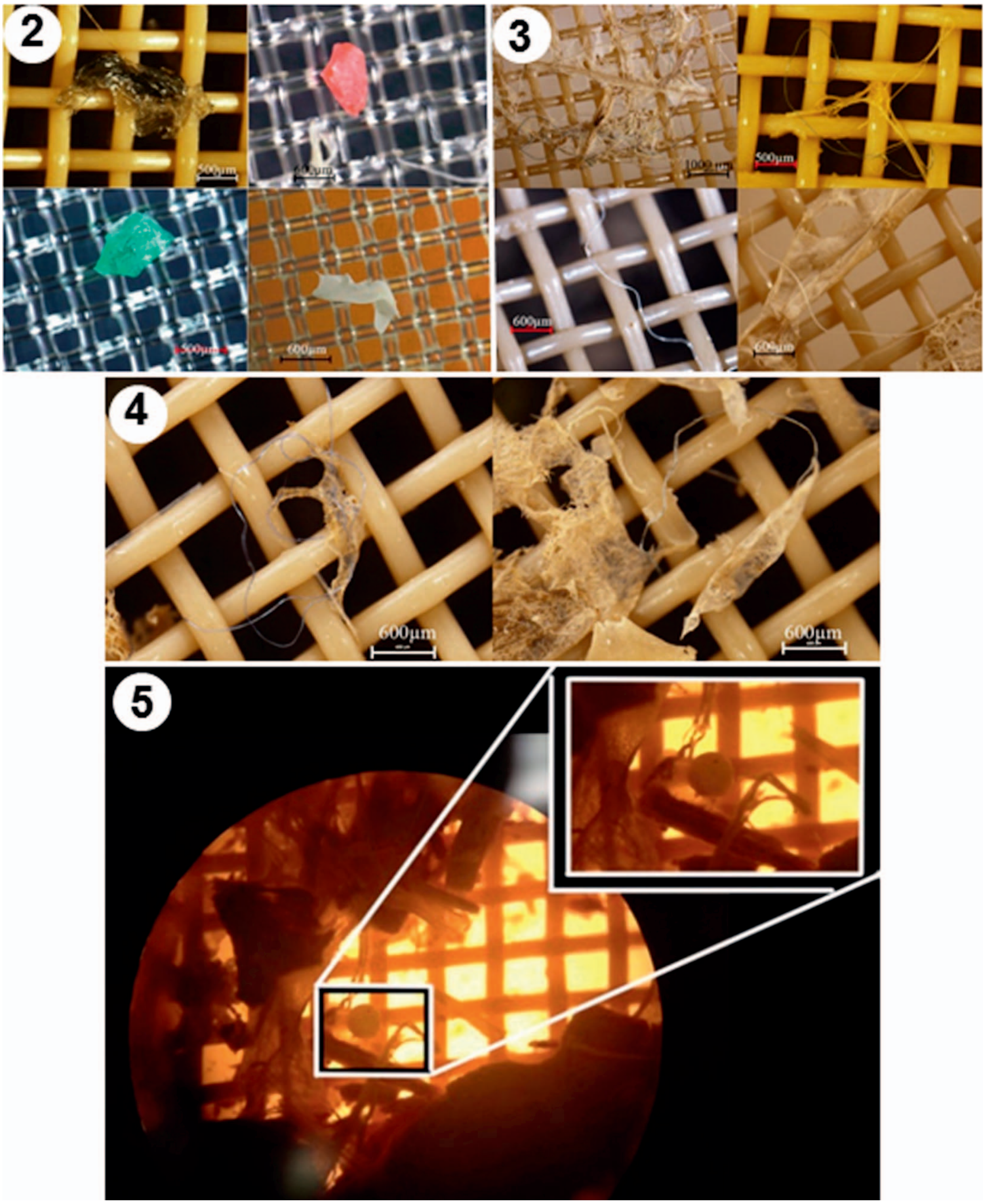
Statistical data analysis.—Plastic concentrations were reported in particles, or items, per cubic meter (item m^{-3}) (Hidalgo-Ruz et al. 2012; Baldwin et al. 2016). Data analyses were conducted using SPSS software (IBM Corp., Armonk, NY) with statistical significance reported at $\alpha = 0.05$. Because of the limited sample size ($N = 15$), a Kruskal-Wallis test by ranks (equivalent to a non-parametric one-way ANOVA test) was used to evaluate differences in plastic concentrations among sites. Kruskal-Wallis was also used to compare concentrations among months. All analyses were completed for plastics collected only on the 250 µm sieve (the smaller particles) and for plastics collected on both the 250 and 500 µm sieves combined (total plastics). This study aimed in part to capture microplastics on the smaller end of their size range (250–500 µm), which are often underestimated (Baldwin et al. 2016).

RESULTS

Total pieces and types collected.—Microplastics of numerous types, colors, and sizes were collected from all three sites sampled (Figs. 2–5). Translucent, white, black, and red plastics were the most prevalent colors collected (Fig. 2). Across all samples, a total of 146 plastic pieces were collected from the White River over the duration of this study. Of those pieces, 40 (27 %), were in the 250–500 µm size range (Figs. 6 & 7).

The 146 microplastic pieces collected in this study were comprised of 16 fragments, one spherule, 112 fibers, three films, 13 foamed plastics, and one pellet (Fig. 7). Synthetic fibers were the predominant plastic type collected (~80 % of the total plastics collected) (Fig. 6). Fragments and foamed plastics constituted the next largest plastic type (11–13% and 4–9%, respectively; Fig. 6), while films, beads, and pellets ranged from 1–2% of items collected (Fig. 6).

Average concentrations.—Site 1 (Muncie) and Site 3 (Indianapolis) resulted in very similar average concentrations of smaller microplastics (0.24 and 0.23 items m^{-3}, respectively; Fig. 8). However, for the average *total microplastic* concentration, Site 1 (Muncie) had an average of 0.75 items m^{-3}, compared to Site 3 (Indianapolis) which had the highest of the three sites (0.93 items m^{-3}; Fig. 8). Site 2 (Yorktown) had the lowest average concentra-



Figures 2–5.—Various microplastics, fibers, and spherules collected. 2. Microplastic fragments of various colors and sizes. 3. Various synthetic fibers. 4. Non-synthetic fibers (which did not melt during the hot needle test) and are likely made of cotton or rayon. 5. Spherule (microbead) among organic debris.

tion for smaller microplastics, with 0.15 items m^{-3} (Fig. 8). Site 2 (Yorktown) also had the lowest average *total microplastic* concentration, with 0.44 items m^{-3} (Fig. 8). The overall average *total microplastic* concentration for the White River, based on the three sites sampled, was 0.71 items m^{-3} (Fig. 8).

Concentration differences between sites.—There was no difference in plastic concentration among sites for either small plastics ($p =$

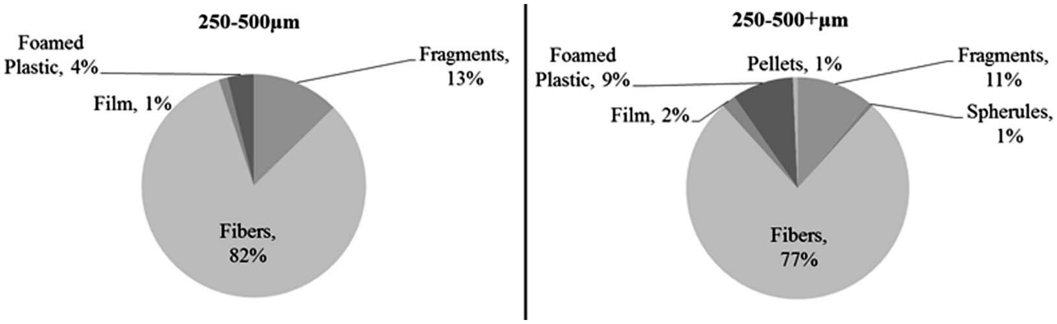


Figure 6.—Graph of average plastic concentration percentages by microplastic type across all three sites for 250–500 µm (left) and 250–500+ µm (right).

0.961) or total plastics ($p = 0.395$) concentrations. Further, there was no difference in plastic concentration among sampling month for either the smaller ($p = 0.849$) or total ($p = 0.753$) plastics.

DISCUSSION

Total pieces and types collected.—The lack of beads (spherules) and pellets found in the White River, along with the prevalence of fibers (~80% of the average particles collected; Fig. 6) and fragments (11% of the average particles collected; Fig. 6) are relatively consistent with the results of recent fluvial/tributary studies, but not of lacustrine/lake studies. Pellets and beads also were scarce in recent tributary Great Lake samples, despite large portions of discoveries in lake samples (Baldwin et al. 2016). Studies along the Danube and European Rhine rivers that found large concentrations of plastic pellets (as well as beads) were attributed to their proximity to vast plastic manufacturing facilities along these rivers. It also should be noted that those watersheds were much larger in scale (Lechner et al. 2014; Mani et al. 2015). The prevalence of fibers in this study (Fig. 6) mirrors the large-scale study on 29 Great Lake tributaries, where Baldwin et al. (2016) observed a similar dominance of fibers and fragments (71% and 17% on average, respectively). Other fluvial studies done on the Seine River and Chicago's North Shore Channel found fibers were the most abundant plastic type collected (Gasperi et al. 2014; McCormick et al. 2015), followed by fragments (McCormick et al. 2015). Fragments were also in high quantities in the Rhine, while fibers were not accounted for in the Danube studies (Lechner et al. 2014; Mani et al. 2015).

Other tributary samples from freshwater, non-fluvial, lacustrine/lake studies (namely the Great Lakes) found a variety of particle types but consistently show substantially higher abundance of fibers (Free et al. 2014; Baldwin et al. 2016). However, in non-tributary Great Lake samples, only 2% of particles were fibers compared to 20% in Mongolian lakes (Baldwin et al. 2016). The authors attribute this difference in fiber contents between fluvial and lacustrine environments in part to analytical methods, but also to the actual physical properties of different plastic types and

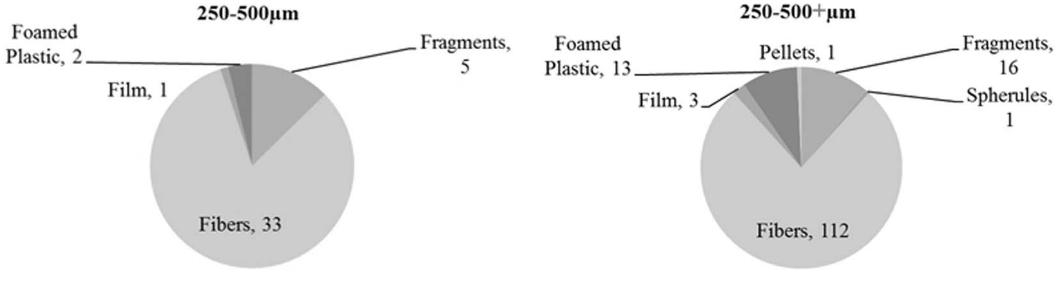


Figure 7.—Graph of average total microplastics counted across all three sites by type for 250–500 µm (smaller plastics; left) and 250–500+ µm (total plastics; right).

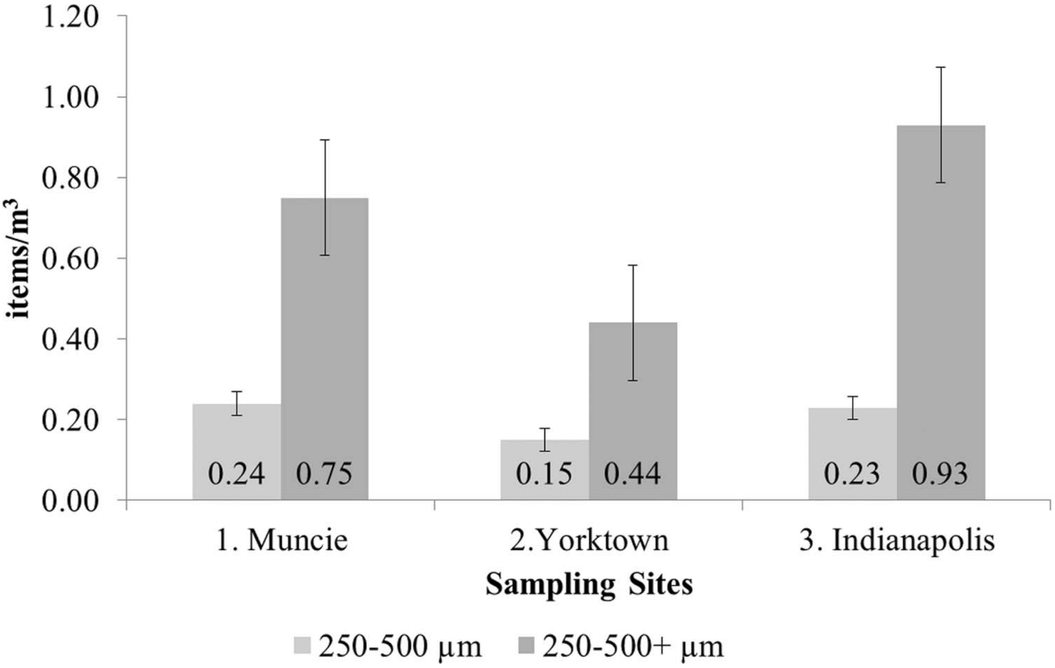


Figure 8.—Graph of average plastic concentration by site (items m^{-3}).

the unique hydraulics of river systems versus those of the Great Lakes (Baldwin et al. 2016). Calmer lake currents allow for easily sunk fibers (i.e., rayon, polyester, nylon, and cellulose acetate) to settle into the sediment, while more tumultuous river currents may keep these fibers in suspension where they are easier to capture during surface sampling events (Baldwin et al. 2016; Ballent et al. 2016). This may explain the lower fiber quantities found in lakes compared to tributaries. Conversely, most films, pellets, or foams are made of polymers that tend to float (polypropylene, polystyrene, and polyethylene) until changes in density, due to biofouling, cause them to sink. This increases the likelihood of finding them in higher concentrations in lake systems compared to other plastic types (Baldwin et al. 2016; Ballent et al. 2016). This relative lack of fibers found in lakes, due to settling, suggests the need for further research into microplastic abundance within lakebed sediments and the possible effects on the organisms living in that habitat.

Average concentrations.—The plastic concentrations measured from three sites on the White River (0.44–0.93 items m^{-3}, mean 0.71 items m^{-3}; Fig. 8) are comparable to the limited literature available for river microplastic studies. Baldwin et al. (2016) summarized notable studies on Chicago's North Shore Channel and Paris' Seine River with mean concentrations of 1.9 to 17.9 and 0.28–0.47 pieces m^{-3}, respectively, while Great Lakes tributaries reported a mean of 4.2 items m^{-3}.

Concentration differences between sites.—Although plastic concentrations in this study did not differ significantly by sampling site (Fig. 8), the quantities and types of plastics found are consistent with those of other recent fluvial studies (Baldwin et al. 2016) and contribute to the overall understanding of microplastic abundance and behavior in this unique environment (Figs. 2–5). Additionally, the results build on the understanding of the effect of watershed attributes, such as urban development, on microplastic pollution. Although not statistically significant, the average total plastic concentration at the Indianapolis site was the highest of all three sampling locations (0.93 items m^{-3}; Fig. 8), likely attributable to a densely populated, urban subwatershed (Yonkos et al. 2014). Muncie had the next highest, followed by Yorktown (0.75 and 0.44 items m^{-3}, respectively; Fig. 8). Greater quantities of impervious surfaces and combined storm sewers in urban watersheds enhance prevalence and mobility of plastic litter into receiving water bodies (Baldwin et al. 2016).

Another point of interest in this study was in determining whether samples taken at Site 2 (Yorktown), which is just downstream of the Muncie Water Pollution Control Facility (a waste water treatment plant, or WWTP), would have higher microplastic concentrations than those taken at Site 1 (Muncie), which is just upstream. Since microplastic concentrations were not found to differ significantly between any of the sites and the Yorktown site showed the lowest microplastic concentrations (Fig. 8), there was no evidence that this WWTP was discharging microplastic pollution at higher rates. The Muncie site had nearly doubled the average total plastic concentration as the Yorktown site (0.44 vs. 0.75 items m^{-3}; Fig. 8). Some recent studies have suggested that WWTPs can be relatively efficient at microplastic removal and can ultimately act as both a sink and a source for this pollutant (Carr et al., 2016; Mintenig et al. 2017).

WWTP processes can be effective at removing microbeads (Carr et al. 2016; Murphy et al. 2016) but less so with other forms of microplastics such as fibers or fragments (Mason et al. 2016a). The lack of beads (spherules) found in this study (only 1 total; Fig. 7) is consistent with the findings of Murphy et al. (2016), who explain that most microbeads in face washes contain positively-buoyant polyethylene and tend to float on the surface of wastewater, where they are easily skimmed off during grease removal. Conversely, Mason et al. (2016a), while studying wastewater effluent from 17 different U.S. wastewater treatment facilities, found fibers (59%) and fragments (33%) to be the most common microplastic types, which is consistent with the current study (Figs. 6 & 7). Likewise, McCormick et al. (2014) who sampled in Chicago's North Shore Channel, both upstream and downstream of a WWTP, found a high abundance of microplastic downstream of the facility, in comparison with the upstream site.

Heavy precipitation events represent another mechanism through which WWTPs could contribute microplastic pollution, even microbeads, to waterways (Murphy et al. 2016). Many Midwestern states, including Indiana, have numerous combined sewer overflow outfalls along waterways, where untreated combined waste and stormwater is discharged when the volume of influent to a facility exceeds the treatable volume. This untreated wastewater has potential to greatly affect the amount of microplastic entering the environment (Murphy et al 2016), yet no studies have investigated stormwater overflow as it relates to microplastic pollution.

Considerations for future microplastic studies.—This study aimed to capture microplastics on the smaller end of their size range (250–500 μm). These are often underestimated since one finds an inverse relationship between particle size and plastic concentration (Baldwin et al. 2016). Our results from limited sampling suggest that Indiana's White River contains quantities of microplastic particles, especially of synthetic fibers, comparable to other fluvial studies. The widespread use and laundering of synthetic clothing and the land application of treated sewage sludge, in addition to atmospheric deposition and overland runoff, are all likely sources of synthetic fibers found in the White River and similar systems.

Overall, the prevalence of microplastics in aquatic systems worldwide suggests a considerable source of pollution with unknown long-term consequences. In both marine and freshwater environments, they can easily be mistaken for food by aquatic life, or, if heavy enough, eventually make their way into sediment. Here, they could become available to benthic feeders or remain in the environment for years to come. Future studies on freshwater organisms and sediments will help researchers gain a better understanding of the fate of increasing microplastic pollution.

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TARGETED rDNA SEQUENCE DETERMINATION FROM GEOGRAPHICALLY ISOLATED POPULATIONS OF *PROTEROMETRA MACROSTOMA* (TREMATODA: AZYGIIDAE)

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ABSTRACT. *Proterometra macrostoma*, a digenetic trematode, was described to have eight morphological variants from cercariae specimens collected from North Elkhorn Creek, Scott County, Kentucky. We have identified and characterized a new population of *P. macrostoma* collected from Clear Creek, Wayne County, Indiana, through examining shed cercariae. The objectives of this study were to conduct rDNA sequence comparisons among individual cercarial phenotypic variants from both populations to (1) determine the partial 28S and complete internal transcribed spacer 2 (ITS2) ribosomal DNA sequence for *P. macrostoma*, (2) demonstrate that Clear Creek samples are indeed *P. macrostoma*, and (3) phylogenetically place *P. macrostoma* among related trematodes. First, we identified and collected *Pleurocera semicarinata livescens* from Clear Creek and monitored these gastropod intermediate hosts for patent trematode infections. Of the four different species of trematodes observed, the characteristic furcocystocercous *P. macrostoma* cercariae were identified. Examination of the cercariae demonstrated phenotypic variation in spined and/or unspined papillae as established in the prior North Elkhorn Creek study. Genomic DNA was isolated and purified from individual cercariae, representing different morphological variants, in both populations. PCR was successful in amplifying across three rDNA loci (partial 5.8S, complete ITS2, and partial 28S) and multiple recombinant clones were independently sequenced. This study represents the first sequence determination for these rDNA regions in *P. macrostoma*. Subsequent rDNA sequence analysis confirmed 100% identity independent of population or phenotypic variant and phylogenetic analyses placed *P. macrostoma* within a monophyletic clade of the *Proterometra* genus. Ongoing specimen acquisition, sequence analysis, and phenetic studies should be conducted to further resolve *Proterometra* phylogeny.

Keywords: Parasitology, trematode, cercaria, rDNA, Wayne County

INTRODUCTION

Trematodes are a globally diverse and highly successful class of flatworm. *Proterometra macrostoma* is a digenetic trematode requiring a freshwater gastropod intermediate host and ingestion of the shed cercariae by a centrarchid fish, the definitive host (Dickerman 1934, 1945; Uglem & Aliff 1984). The geographic distribution of *P. macrostoma* comprises at least 12 states within the Great Lakes and Mississippi River drainages (Riley & Uglem 1995). In this study, we identify a putative population of *P. macrostoma* in Clear Creek (CC), Wayne County, Indiana.

Prior studies have categorized *P. macrostoma* into variant strains based on the presence and organization of spined papillae on the anterior and middle sections of the characteristic furcocystocercous cercariae (Dickerman 1945; Riley & Uglem 1995). In particular, Riley & Uglem (1995) recognized eight strains (I-VIII) of *P. macrostoma* from multiple locations, and initially characterized from North Elkhorn Creek (NEC), Scott County, Kentucky (38.18333°N; -84.48861°W). In addition, these putative strains demonstrated contrasting (1) shedding rates that seemingly coincided with the presence/absence of migrant centrarchid hosts, (2) cercarial swimming patterns within the water column, and (3) infectivity into varied sunfish definitive hosts (Riley & Uglem 1995).

A logical progression of this work, explicit from the Riley & Uglem (1995) study, called for DNA sequence analysis to elucidate the conspecific nature of morphological variants within this population. Additionally, Womble et al. (2016) highlight the need for additional *Proterometra* rDNA sequence to be elucidated, particularly *P. macrostoma*, in order to resolve sequence-constrained azygiid phylogenies. To date, the only *P. macrostoma* DNA sequences available in Gen-

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Bank consist of partial sequences of the 18S small subunit ribosomal RNA and cytochrome oxidase subunit 1 genes (Van Steenkiste et al. 2015).

Sequence analyses of the 28S ribosomal large subunit gene and the internal transcribed spacer 2 (ITS2) region have been used in crafting numerous trematode phylogenies and species identifications, including *Proterometra* (Olson et al. 2003; Womble et al. 2015, 2016). The objectives of this study were to use targeted rDNA sequence analysis to (1) determine the 28S ribosomal large subunit and ITS2 rDNA sequences for *P. macrostoma*, (2) verify the identity of recently isolated CC samples of *P. macrostoma*, and (3) use targeted rDNA sequencing to investigate the strain variants of *P. macrostoma* among CC and NEC parasite populations. Our novel findings would provide insight into the intraspecies phylogeny and taxonomy of these geographically separated populations of trematodes and in doing so validate their phylogenetic relationships among other digeneans. Thus, this study would provide the first report examining the relationship between rDNA sequence and *P. macrostoma* phenotypic variants as justified by Riley & Uglem (1995).

MATERIALS AND METHODS

Study sites and specimen acquisition.—In June 2014, our research team was the first to identify *Pleurocera semicarinata livescens* (formerly *Elimia semicarinata*), a known intermediate host of *P. macrostoma*, in Clear Creek (CC), Wayne County, Indiana and purportedly the same intermediate host species examined in the original NEC report (Riley & Uglem 1995). CC is a tributary of the Whitewater River in Wayne County, Indiana. In June 2015 and 2016, over 300 *Pleurocera semicarinata livescens* snails were collected from CC (39.81851°N; -84.91737°W) and subsequently taxonomically verified (R.T. Dillon, pers. comm.). Individual snails were maintained in 40 ml of filtered creek water at 18° C with a 12 hr light/12 hr dark photoperiod and surveyed for patent infections via cercarial shedding. Live individual cercariae were collected and immediately processed for genomic DNA isolation and purification. Infected and uninfected snails were properly returned to the original collection site. To date, four species of trematode cercariae have been observed emerging from this new population including the characteristic furcocystocercous cercariae of *P. macrostoma*. This new population of putative *P. macrostoma* exhibits the spined and unspined variant cercarial phenotypes as described in NEC. NEC is a tributary of the Ohio River in Scott County, Kentucky. Freshly emerged and isopropanol-preserved NEC (38.18333°N; -84.48861°W) *P. macrostoma* cercariae, shed from *Pleurocera semicarinata livescens* snails, were provided by Dr. Ronald Rosen. Recently shed (< 24 hr) cercariae were imaged using an EVOS FL Cell Imaging System (Thermo Fisher Scientific, Waltham, MA) and categorized into strains based on Riley & Uglem (1995).

Identification of this new CC population allowed for preliminary comparative studies with the previously described NEC *P. macrostoma* population (Riley & Uglem 1995; Rosen et al. 2013). Phenotypic *P. macrostoma* variants, based on the pattern or absence of spined papillae, were confirmed in both NEC and CC cercariae shed from *Pleurocera semicarinata livescens* snails. First, in June 2015, snails ($n = 118$) were collected from NEC and 37% were infected with *P. macrostoma*. Phenotypic variants I, III, IV, V, and VIII were classified from these NEC samples based on Riley & Uglem (1995). In June 2016, the new population of putative *P. macrostoma* in CC was identified (Fig. 1) and shed cercariae were classified as type IV and VI variants. The characteristic large furcocystocercous cercariae demonstrated dark emergence patterns typical of *P. macrostoma* (Lewis et al. 1989). While the frequency of total trematode infections from CC was 37% (from > 300 snails sampled), only 3.5% were infected with *P. macrostoma.* Trematode co-infections in the CC population were relatively abundant which might validate the lower *P. macrostoma* incidence. This reduced overall infectivity has been witnessed in prior trematode co-exposure studies. Both reduced overall infectivity in the intermediate host and cercarial productivity was observed in a schistosome model system with co-exposure of parasite strains (Thiele & Minchella 2013).

DNA extraction and PCR amplification.—Total genomic DNA from individual *P. macrostoma* cercariae was extracted and purified using a DNeasy Blood and Tissue Kit according to the manufacturer's (Qiagen, Valencia, CA) instructions. The elution volume was reduced (50ul) and eluted twice to increase the final concentration of the genomic DNA. Three universal trematode primer sets

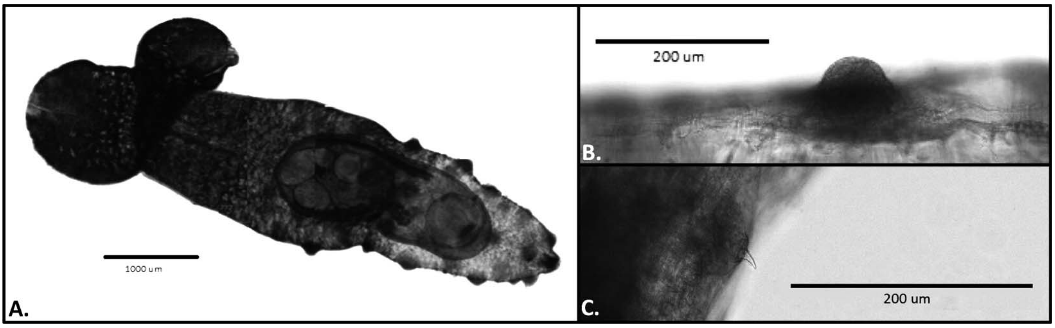

Figure 1.—Microscopic image of the furcocystocercous *Proterometra macrostoma* cercaria isolated from Clear Creek, Richmond, Indiana. A. Composite image of representative variant type IV cercaria. B. Higher magnification image indicating smooth, unspined papillae characteristic for the anterior and middle third sections of type IV cercariae. C. Higher magnification of spined papillae characteristic of the middle third section of type IV cercariae. Respective scale bars (μm) are indicated in each panel.

targeting the rDNA region (Fig. 2) of the *P. macrostoma* genome were used in this study: (1) LSU5 5′-TAGGTCGACCCGCTGAAYTTAAGCA-3′) and 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′); (2) 3S (5′-GGTACCGGTGGATCACTCGGCTCGTG-3′) and A28 (5′-GGGATCCTGGTTAGTTTCTTTTCCTCCGC-3′); and (3) OphetF1 (5′-CTCGGCTCGTGTGTCGATGA-3′) and OphetR1 (5′-GCATGCARTTCAGCGGGTA-3′). The LSU5/1500R primer set was used for initial confirmation of *P. macrostoma* species identification producing an amplicon within the 28S large ribosomal locus. This region and this primer set was previously used in the formation of robust trematode phylogenies (Barker et al. 1993; Olson et al. 2003). PCR cycling parameters for the LSU5/1500R primer set were as follows: 3 min at 94° C; 35 total cycles of 94° C for 30 sec, 56° C for 30 sec, 72° C for 2 min; and a 7 min extension at 72° C. The 3S/A28 and OphetF1/OphetR1 primer sets amplify the internal transcribed spacer 2 (ITS2) region of rDNA and have been influential in determining several helminth phylogenies (Bowles et al. 1995; Womble et al. 2015, 2016; Sherrard-Smith et al. 2016). PCR cycling parameters for the ITS2 primer sets were as follows: 5 min at 95° C; 35 total cycles of 94° C for 30 sec, 53° C for 1 min, and 72° C for 1 min; and a 7 min extension at 72° C. Appropriate positive and negative PCR controls were used throughout the study to safeguard resulting sequence accuracy and to account for potential PCR errors. All PCRs were performed using the premixed 2× PCR Master Mix (Promega, Madison, WI) and all amplicons were sepa-

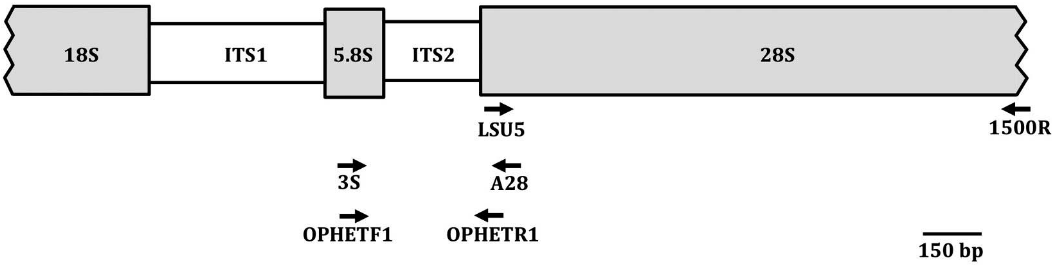

Figure 2.—Partial schematic of the *Proterometra macrostoma* rDNA locus and location of universal trematode primer sets used in this study. Positions of the three primer sets are indicated by name directly below directional arrows. Schematic is to scale (1 cm = 150 bp) with the exception of arrow lengths. Internal transcribed spacer 1, upstream from the 18S region, is not shown. To assist with scaling, the 18S and 28S regions have been truncated as indicated (jagged edges). Best estimations of the ITS1 and 5.8S region sizes were based on prior trematode rDNA studies.

rated and visualized on a 1% agarose gel stained with SYBR Safe.

Recombinant cloning, sequencing, and sequence determination.—Remaining PCR amplicons were cloned using the TOPO TA Cloning™ Kit for Sequencing (pCR™4-TOPO™ Vector and One Shot™ TOP10 Chemically Competent *E. coli*) following manufacturer's (Thermo Fisher Scientific, Waltham, MA) protocols. Multiple recombinant colonies were selected from each cloning reaction and independently grown overnight in 4 ml of LB broth plus ampicillin. Recombinant plasmids were purified using the QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA) and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Size and purity of recombinant clones was verified by *Eco*RI restriction digest and gel electrophoresis. Approximately 700 ng per sample of purified recombinant plasmid DNA was sequenced at the Yale University DNA Analysis Facility on Science Hill using M13 Forward and Reverse sequencing primers. The plasmid vector allowed for sequencing calls to have settled prior to calling the amplicon bases allowing for sequencing precision at the PCR product termini. Recombinant sequences were trimmed, aligned (ClustalW and Geneious Alignment) using default settings, and analyzed using Geneious (v.10.2.3). Consensus sequences were determined from sequencing both strands of independent recombinant clones. Consensus sequences have been deposited into GenBank with the accession numbers MF927953-MF927958.

Phylogenetic analysis.—Phylogenetic analysis was independently performed on the partial 28S rDNA and the ITS2 rDNA sequence. Since the rDNA sequences of *P. macrostoma* from each variant and population shared 100% sequence identity, only the consensus was used in subsequent analyses. First, the overlapping sequences from the 3S/28 and LSU5/1500R amplicons allowed for the formation of a 1,758 bp contig. NCBI BLASTn of this contig retrieved related azygiid rDNA sequences for subsequent phylogenetic analysis: *Azygia longa* (KC985234.1), *Proterometra* sp. (KC985237.1), and *Otodistomum cestoides* (AY222187.1). Together with our *P. macrostoma* contig and *Diplodiscus mehrai,* the designated outgroup, these sequences were trimmed to between 1,259–1,262 bp which represented the approximate 28S rDNA region amplified by the LSU5/1500R primer set. These five sequences were aligned using the MUSCLE algorithm (Geneious v.10.2.3) set to eight for the maximum number of iterations (Edgar 2004; Kearse et al. 2012). The alignment was exported to MEGA6.06 and assessed for optimal phylogenetic test using the Find Best DNA/Protein Models (ML) analytic (Tamura et al. 2013). The general time reversible plus gamma (GTR+G) yielded the lowest BIC (7102.431). A phylogenetic tree was constructed using the GTR+G model on the MrBayes plugin in Geneious v.10.2.3 (Huelsenbeck & Ronquist 2001).

Based on this scarcity of total 28S rDNA sequence data from related taxa, the ITS2 region alone was subject to phylogenetic analysis and followed Womble et al. (2016) using trimmed *Azygia longa* (KT808319.1)*, Leuceruthrus micropteri* (KT808320.1), *Proterometra ariasae* (KT808317.1), *Proterometra epholkos* (KM503118.1), *P. macrostoma* (MF927955), and *Transversotrema borboleta* (JF412524.1), set as the outgroup. Briefly, the ITS2 region was aligned (ClustalW, default conditions, in MEGA6.06), manually reviewed/edited, and subject to Kimura's 2-parameter and gamma distributed (K2+G) model (Kimura 1980). The K2+G model had yielded the lowest BIC (2268.782) from the best model predictor in MEGA6.06.

RESULTS AND DISCUSSION

Identification of *P. macrostoma* and the variant phenotypes in the NEC and CC populations was imperative to meet the objectives of this study. The subsequent targeted rDNA sequence elucidation allowed for: (1) the first determination of the partial 28S ribosomal large subunit and complete ITS2 rDNA sequences for *P. macrostoma*, (2) sequence comparisons among the CC and NEC variants, and (3) the phylogenetically grouping of *P. macrostoma* among CC and NEC parasite populations and other azygiid trematodes.

The universal LSU5/1500R primer sets (Fig. 2) yielded amplicons for all samples in the expected size range (1,392 bp) within the 28S ribosomal large subunit gene locus. No difference in band size was evident following agarose gel imaging. PCR fragments were subsequently cloned and sequenced yielding an identical 1,346 bp primer trimmed sequence independent of the strain or study site. The resulting sequence represents an

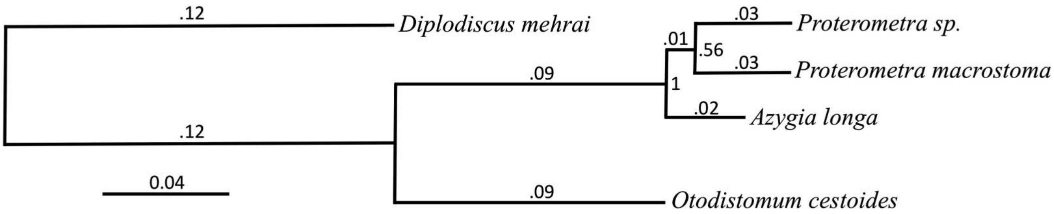

Figure 3.—Bayesian phylogenetic tree of *Proterometra macrostoma* among other digenean trematodes based on partial 28S rDNA. Posterior probabilities are placed to the right of nodes and substitutions per site are indicated above each branch. GenBank sequence accessions used are as follows: *Proterometra* sp. (KC985237.1), *Proterometra macrostoma* (contig of MF927953/MF927955 or MF927954/MF927956), *Azygia longa* (KC985234.1), *Otodistomum cestoides* (AY222187.1), and *Diplodiscus mehrai* (KX506857.1). *Diplodiscus mehrai* was assigned as the outgroup because of its phylogenetic relationship to the Azygiidae. The total characters used in the inference were 1,268. A scale bar is indicated.

estimated 35% of the entire 28S large ribosomal subunit rDNA (Lockyer et al. 2003; Blair 2006). Consensus alignments for each strain were readily achieved and used to resolve the rare ambiguous bases. NCBI nucleotide BLAST results of the consensus sequence produced two 95% identity matches: *Azygia longa* 28S ribosomal RNA gene, partial sequence (GenBank ID: KC985234.1) and *Proterometra* sp. SSC-2013 28S ribosomal RNA gene, partial sequence (GenBank ID: KC985237.1) (Calhoun et al. 2013). The relatedness of these taxa demonstrated novel authentication of the partial 28S large subunit rDNA sequence for *P. macrostoma*. The 1,346 bp sequence was 100% identical between sites (CC and NEC) and morphological variants (CC: IV, and VI; NEC (III, IV, V, VIII). For each site, the consensus *P. macrostoma* partial 28S large subunit rDNA sequence has been submitted to GenBank (MF927953 and MF927954).

To further validate the rDNA conservation of the two populations and morphological variants, the ITS2 region was selected for amplification and targeted sequencing. In prior studies, ITS2 sequence analysis has been used as a phylogenetic determinant of trematode species delineation (Morgan & Blair 1998; Nolan & Cribb 2005) , including a novel species, *Proterometra ariasae* (Womble et al. 2016). The A28/S3 and OPHETF1/OPHETR1 primer sets (Fig. 2) successfully amplified products of 476 bp and 423 bp respectively, independent of population or morphological variant. ITS2 sequence comparisons yielded 100% identity regardless of population or phenotypic variant. ITS2 sequences for variants IV and VI from CC and variants IV and VIII from NEC were used in the analysis. Resulting ITS2 consensus sequences have been submitted to GenBank (MF927955- MF927958). BLAST comparisons of consensus sequences yielded a top hit with 96% identity to *Proterometra ariasae* isolate MW-PS-134 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence (KT808318.1) (Womble et al., 2016). These findings strongly indicate that both populations are indeed *P. macrostoma*. The fact that the phenotypic variants, independent of population, lack DNA polymorphisms suggests genetic conservation among morphological strains.

The Bayesian phylogenetic inference representing partial 28S rDNA resulted in the placement of *P. macrostoma* within a monophyletic clade with the related taxa *Proterometra sp.* (KC985237.1) (Fig. 3). This *Proterometra* clade then formed a sister clade with the azygiid fluke, *Azygia longa* (KC985237.1). Even with the limited number of comparative 28S rDNA sequences available, this represents the first phylogenetic comparison able to include *P. macrostoma*. The ITS2 phylogenetic analysis placed *P. macrostoma* within a monophyletic clade exclusive to the *Proterometra* genus (Fig. 4). These findings were consistent with and provide additional support for the Womble et al. (2016) phylogenetic models.

In summary, the perfect sequence conservation of the 1,758 bp rDNA region among morphological variants and geographically separated populations (CC and NEC) makes the case against classifying the *P. macrostoma* variant strains based on rDNA sequence alone. Ultimately, this study provides novel and relatively extensive baseline rDNA sequence information for *P. macrostoma*, identifies a new Indiana population of *P. macrostoma*, and suggests the morphological

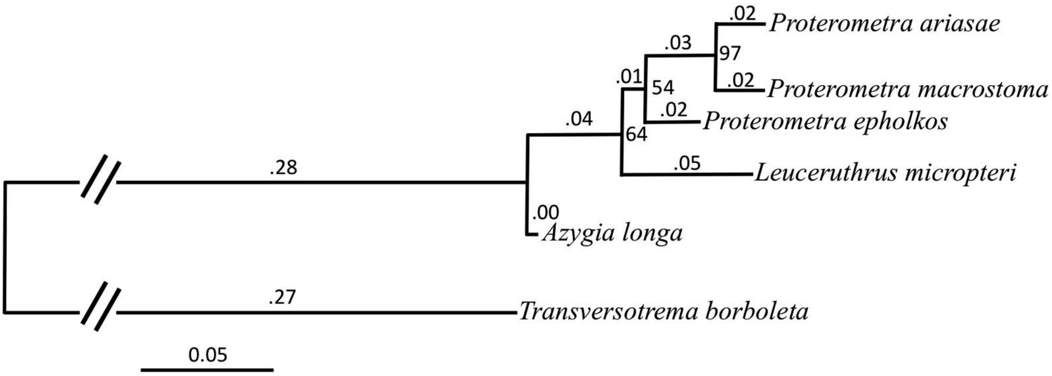

Figure 4.—Maximum Likelihood phylogenetic tree placing *Proterometra macrostoma* among other azygiid trematodes inferred from internal transcribed spacer 2 (ITS2) rDNA sequences. The percentage of trees yielding clustered taxa is indicated to the right of nodes and substitutions per site are indicated above each branch. GenBank sequence accessions used are as follows: *Proterometra ariasae* (KT808317.1), *Proterometra macrostoma* (MF927955), *Proterometra epholkos* (KM503118.1), *Leuceruthrus micropteri* (KT808320.1), *Azygia longa* (KT808319.1), and *Transversotrema borboleta* (JF412524.1). *Transversotrema borboleta* was assigned as the outgroup because of its phylogenetic relationship to the Azygiidae. The total characters used in the inference were 361. A scale bar is indicated.

phenotypes, present in both populations, lack sequence variation within the rDNA region examined. Our findings also contribute to a developing *Proterometra* phylogeny. Future phenetic studies to examine spined and unspined papillae structure and gene expression/regulation in another Indiana population are warranted and could provide additional insight into this system (Krist 2000).

ACKNOWLEDGMENTS

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GENETIC IDENTITY OF THE LEAST BROOK LAMPREY (*LAMPETRA AEPYPTERA*) IN INDIANA

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ABSTRACT. The Least Brook Lamprey (*Lampetra aepyptera*) is a common inhabitant of small streams throughout the southeast United States and reaches its northern-most extent near the boundary of the glacial till plains of southern Indiana, Ohio, and western Pennsylvania. Previous genetic studies found that populations from eastern Kentucky and Ohio were distinct from other populations of *L. aepyptera*, suggesting that these populations from the upper Ohio River basin were isolated in their current locations well before the Pleistocene. However, samples from Indiana (or elsewhere in the lower Ohio River basin) were not included in these studies. As the modern Ohio River system was established in the late Pleistocene (or after), samples from Indiana will be critical to our understanding of the historical factor(s) giving rise to the distribution of *L. aepyptera* in the Ohio River basin. Sequence variation of the mitochondrial NADH dehydrogenase subunit 3 gene from specimens of *L. aepyptera* collected from across its distribution were examined to better understand the phylogeographic position of the Indiana populations. Specimens collected from southern Indiana, Illinois, and the Green River of Kentucky (the lower Ohio River basin) formed a well-supported monophyletic group with specimens collected from the upper Ohio River basin. Deeper relationships within the species remain unresolved. The Ohio River clade shows evidence of reduced genetic heterogeneity relative to more southerly populations, consistent with an assemblage of populations that has recently expanded. Our results suggest that the contemporary distribution of *L. aepyptera* in the Ohio River basin was established after the integration of the modern Ohio River system in the late Pleistocene.

Keywords: Ohio River drainage, *Lampetra aepyptera*, biogeography, mtDNA, Pleistocene

INTRODUCTION

The Least Brook Lamprey, *Lampetra aepyptera* (Abbott 1860), is a non-parasitic species that occurs in headwater streams of the southeastern United States and reaches its northern-most extent in the Ohio River basin (Rhode & Jenkins 1980). The species was originally described as '*Ammocoetes aepyptera*' from a single specimen from 'the Ohio River' near Meigs, Ohio (Abbott 1860; see species account in Trautman 1981), and was first reported in Indiana by Jordan (1918) from Griffith's Creek (Monroe County) and by Creaser (1939) from Lick Creek (Orange County). Although subsequent surveys have established the presence of *L. aepyptera* in southern Indiana (viz. Simon 2011), no other information regarding its natural history is available for the populations that occur in the State.

The life history of the *L. aepyptera* was documented in Maryland (Seversmith 1953) and Kentucky (Walsh & Burr 1981), and is known to include a filter-feeding larval (ammocoete) stage and a short lived (non-feeding) adult stage that dies after spawning in the spring. Other information regarding the natural history of *L. aepyptera* includes descriptions of its karyotype (Alabama: Howell & Denton 1969), demographic structure and sex ratios (Alabama, Delaware, Kentucky, Maryland, and Tennessee: Docker & Beamish 1994), and its phylogenetic relationships with other lamprey species (Docker et al. 1999; Lang et al. 2009). Finally, Martin & White (2008) inferred the phylogeographic structure of *L. aepyptera* from mitochondrial DNA (mtDNA) variation, but did not include samples from Indiana (see also White & Martin 2009).

MtDNA-based studies such as Martin & White (2008) have revealed evidence of Pleistocene vicariance and post-Pleistocene expansion of many North American fishes (e.g., Strange & Burr 1997; Near et al. 2001), and thereby provide important insights into the processes that gave rise to contemporary patterns of biodiversity. A major Pleistocene event that may have influenced the distribution of *L. aepyptera* was the formation of the modern Ohio River basin by the integration of components of the ancient Teays River system to the east (including the modern tributary streams in eastern Kentucky and southern Ohio)

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with the Old Ohio River system to the west (including the modern Wabash River of Indiana, the Green River of Kentucky, and the smaller Ohio River tributaries in southern Indiana and Illinois (Melhorn & Kempton 1991; Hoagstrom et al. 2014)). Martin & White (2008) found that lamprey populations from the upper Ohio River basin (eastern Kentucky and southern Ohio) were genetically divergent from other populations and conjectured that this 'Ohio River Clade' was a relict of a pre-Pleistocene distribution in the ancient Teays River system. However, the distinctiveness of their 'Ohio River Clade' may be an artifact of incomplete sampling, as samples from Indiana, Illinois, and central Kentucky (the lower Ohio River basin = Old Ohio) were not available for Martin & White's (2008) analysis (Fig. 1). Thus, samples from Indiana will provide a better understanding of the biogeographic history of *L. aepyptera* in the Ohio River basin.

Herein, we supplement the mitochondrial data set collected by Martin & White (2008; available on GenBank) with new samples from southern Indiana, Illinois, and central Kentucky to assess the historical biogeography of *L. aepyptera*. The primary objective of this study was to determine the genetic identity of the populations that occur in Indiana and assess the roles of pre-Pleistocene vicariance and/or post-Pleistocene dispersal in shaping its present distribution within the Ohio River basin. In particular, we test whether populations of *L. aepyptera* from Indiana form a monophyletic group with those from the upper Ohio River (consistent with a post-Pleistocene dispersal), or if the Indiana populations are more closely related to more southerly populations (consistent with pre-Pleistocene distributions in both the Old Ohio and Teays systems).

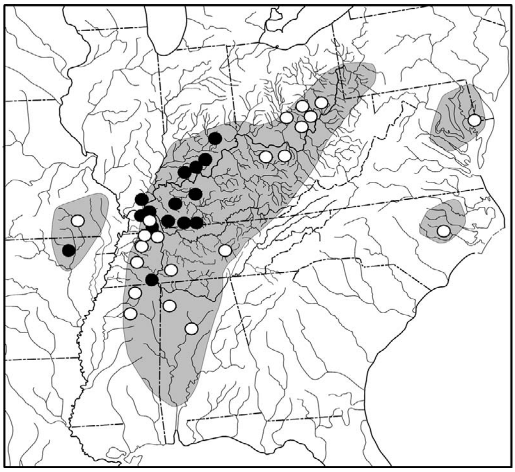

Figure 1.—Distribution of *Lampetra aepyptera* (shaded areas) following Rhode & Jenkins (1980). White spots represent sample localities from Martin & White (2008) and black spots represent sample localities collected for this study.

METHODS

Adult lampreys were collected by seine and ammocoetes by electrofishing from various tributaries of the lower Ohio River basin, including streams in southern Illinois, Indiana, and Kentucky (Table 1). Specimens of *Lethenteron appendix* (formerly *Lampetra appendix*) were collected for outgroup comparison in the phylogenetic analyses (below). Tissues (fin and muscle) were fixed in the field with ethanol and brought to the laboratory for processing. Voucher specimens were formalin-fixed and deposited in the Natural History Museum of the University of Southern Indiana. Whole genomic DNA was extracted from the ethanol-fixed tissues by a standard phenol-chloroform extraction procedure.

The polymerase chain reaction (PCR) was used to amplify the mitochondrial NADH dehydrogenase subunit 3 (ND3) gene with the primers ND3-F and ND3-R originally developed by Docker et al. (1999). PCR reactions consisted of a 25µl volume with concentrations of 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1.0 µM of each primer, and 1.0 unit of *Taq* polymerase. An initial denaturation at 94° C for 2 min was followed by 35 cycles of denaturation (94° C, 1 min), annealing (52° C, 1 min), and polymerase extension (72° C, 1 min). A final extension at 72° C for 7 min was included to reduce the number of partial strands. Amplification products were purified using spin-columns (Qiagen), and resuspended in ddH_2O prior to automated sequencing on an ABI 3700 genetic analyzer.

Trace files for all sequences were edited using BioEdit (Hall 1999) and initial alignments were made with CLUSTALX (Thompson et al. 1997). Final alignments included ND3 sequences from Martin & White (2008) and Docker et al. (1999; Table 1). Phylogenetic analyses were performed on the DNA sequence data with a combination of parsimony and likelihood approaches. Maximum Parsimony (MP) analyses were performed using PAUP* (Swofford 2002). All characters were treated as unweighted, and searches were heuristic with starting trees obtained by stepwise addition,

Table 1.—Collection localities and GenBank accesson numbers for NADH dehydrogenase subunit 3 (ND3) sequences from *Lampetra aepyptera*. Samples new for this study are indicated by asterisks (*).

Locality	System/Drainage	GenBank
Big Creek, Hardin Co., IL*	Lower Ohio	MH177976
Anderson River, Perry Co., IN*	Lower Ohio	MH177977
Stinking Fork, Crawford Co., IN*	Little Blue/Lower Ohio	MH177978
Patoka River, Orange Co., IN*	Wabash/Lower Ohio	MH177979
Vernon Fork, Jackson Co., IN*	White/Wabash/Lower Ohio	MH177980
West Fork, Ohio Co., KY*	Green/Lower Ohio	MH177981
W. Fork Pond River, Christian Co., KY*	Green/Lower Ohio	MH177982
Donaldson Cr., Trigg Co., KY*	Cumberland/Lower Ohio	MH177983
Big Sinking Creek, Carter Co., KY	Little Sandy/Upper Ohio	DQ532792
Big Caney Creek, Elliott Co., KY	Little Sandy/Upper Ohio	DQ532798
Strouds Run, Athens Co., OH	Hocking/Upper Ohio	DQ532801
Camp Creek, Pike Co., OH	Scioto/Upper Ohio	DQ532788
M. Branch Shade River, Athens Co., OH	Shade/Upper Ohio	DQ532800
Spring Cr., Todd Co., KY*	Red/Cumberland	MH177984
L. Whippoorwill Cr., Logan Co., KY*	Red/Cumberland	MH177985
Cane Creek, Putnam Co., TN	Caney Fork/Cumberland	AF177965
Trace Creek, Graves Co., KY*	Clarks/Tennessee	MH177986
Panther Creek, Graves Co., KY*	Clarks/Tennessee	MH177987
Wildcat Creek, Calloway Co., KY*	Blood/Tennessee	MH177988
McCollough Fork, Calloway Co., KY	Blood/Tennessee	DQ532795
Bear Creek, Henry Co., TN	Big Sandy/Tennessee	DQ532793
Weatherford Creek, Wayne Co., TN	Indian/Tennessee	DQ532790
Robinson Creek (#1), Hardin Co., TN*	Tennessee	MH177989
Robinson Creek (#2), Hardin Co., TN*	Tennessee	MH177990
Little Bear Creek, Franklin Co., AL	Bear/Tennessee	DQ532789
Little Black River, Ripley Co., MO	Black/White	DQ532799
Mill Creek, Sharpe Co., AR*	Strawberry/White	MH177991
Terrapin Creek, Graves Co., KY	Obion/Mississippi	DQ532803
Middle Fork Obion Creek, Henry Co., TN	Obion/Mississippi	DQ532794
Tar Creek, McNairy Co., TN	Forked Deer/Mississippi	DQ532802
Gaylor Creek, Hardeman Co., TN	Hatchie/Mississippi	DQ532785
Yellow Leaf Creek, Lafayette Co., MS	Yazoo/Mississippi	DQ532786
Kettle Creek, Lafayette Co., MS	Yazoo/Mississippi	DQ532787
Schultz Creek, Bibb Co., AL	Cahaba/Mobile	DQ532796
Davis Mill Creek, Dorchester Co., MD	Chesapeake/Atlantic	DQ532797
Neuse River, Johnston Co., NC	Atlantic	DQ532791
Lethenteron appendix (outgroup)		
Driftwood River, Bartholomew Co., IN*	White/Wabash/Lower Ohio	MH177992

1000 random addition sequence replicates, and TBR branch swamping. Support for nodes was assessed by bootstrap resampling (Felsenstein 1985) with 1000 pseudoreplicates using the same parameters as for the parsimony analysis. For the Maximum Likelihood (ML) analysis, the best-fitting model of nucleotide substitution was chosen with jModelTest (Darriba et al. 2012) following Akaike's (1974) information criterion. PHYML 3.0 (Guindon & Gascuel 2003) was then used with the specified optimal model to infer the most likely set of phylogenetic relationships. Branch support for the ML analysis was estimated by bootstrap resampling with 100 pseudoreplicates.

Finally, the patterns of nucleotide diversity and mismatch distributions were examined to evaluate evidence of recent population expansion with tests implemented in DNASP (Librado & Rozas 2009). Nucleotide diversity (π) is the average number of nucleotide differences between sequences within a sample and is analogous to heterozygosity at the nucleotide level (Nei 1987); recently founded populations are expected to have lower levels of nucleotide diversity than older populations (Avise 2000). The frequency distribution of pairwise differences (mismatches) between haplotypes is another estimate of population history, wherein historically stable populations are expected to exhibit multimodal mismatch distributions, while those that have undergone a recent expansion should show unimodal distributions (Slatkin & Hudson 1991; Rogers & Harpending 1992). Results of the mismatch analysis were assessed with Tajima's *D*, wherein positive values represent a decrease in population size, negative values represent a recent population expansion, and a value of '0' is consistent with a population in mutation-drift equilibrium (Tajima 1989).

RESULTS

The aligned data set consisted of 351 bp of the mitochondrial ND3 gene, with 72 polymorphic sites, 49 of which were parsimony-informative; the remaining sites were invariant. Among the polymorphic sites, 17 (23.61%) were at the first, 9 (12.50%) at the second, and 46 (63.89%) were at the third codon position. Base composition was similar to that previously reported for lamprey mitochondrial sequences (e.g., Caputo et al. 2009; Lang et al. 2009; Strange et al. 2016), with a low guanine content (13.11%) relative to the proportions of adenine (25.94%), cytosine (28.03%), and thymine (32.92%) residues. All sequences passed the X^2 test for homogeneity of nucleotide composition ($X^2 = 6.97$, df = 108, $p > 0.99$) and showed no evidence of transition or transversion saturation. Average sequence divergence between all *L. aepyptera* ND3 haplotypes was 3.74% (range 0.3–6.3%).

Both the MP and ML analyses of the ND3 sequence data yielded similar phylogenetic topologies within *L. aepyptera*, although both analytical methods failed to resolve deeper relationships within the species (Fig. 2). Parsimony analysis resulted in 766 equally parsimonious trees with 140 steps each (CI = 0.614; RI = 0.784). Likelihood analysis (using the GTR model identified by jModelTest) identified a single phylogenetic tree with a negative log likelihood score of -1222.925 and 144 parsimony steps. The Ohio River Clade (previously identified by Martin & White 2008) was well supported by both analyses (> 92% bootstrap support) and included samples from eastern Kentucky and southern Ohio as well as our samples from Indiana, central Kentucky (Green River system), southern Illinois, and one sample from the lower Cumberland River system (Donaldson Creek). Samples from the Tennessee River drainage formed a monophyletic group in all of the equally parsimonious trees and likelihood analysis, but received < 50% bootstrap support in both MP and ML analyses. Samples within the Tennessee River Clade fell into two subclades, one corresponding to samples from the lower Tennessee River system and another from tributaries of the middle Tennessee River.

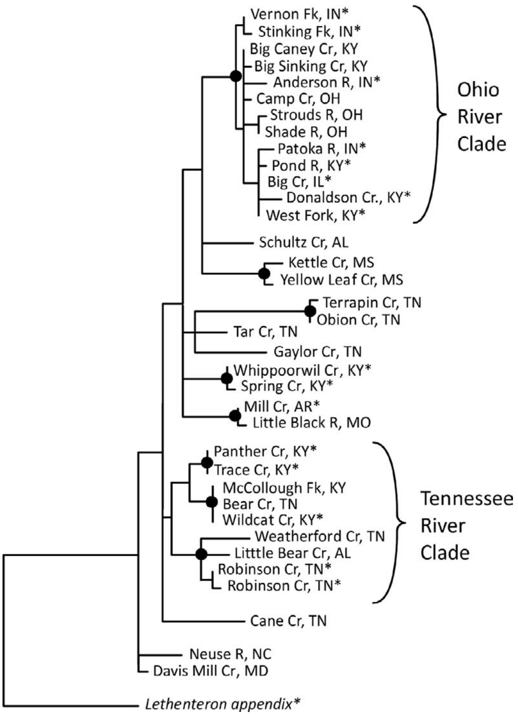

Figure 2.—Phylogenetic relationships of NADH dehydrogenase subunit 3 (ND3) sequences from *Lampetra aepyptera* as inferred from parsimony and likelihood analyses. Filled circles at nodes represent bootstrap support greater than 90% in both analyses; branch lengths are proportionate to the likelihood estimates of the number of substitutions per site. Samples new for this study are indicated by asterisks (*).

Demographic data from the Ohio River Clade (which is distributed along the glacial boundary) and the Tennessee River Clade (which occurs in unglaciated portions of western Kentucky and Tennessee) revealed very different patterns. Haplotypes of the Ohio River Clade exhibit lower nucleotide diversity ($\pi = 0.012$) than do sequences of the Tennessee River Clade ($\pi = 0.020$). Within the Ohio River Clade, nucleotide diversity among the samples from the lower Ohio River basin was higher ($\pi = 0.012$) than that of the samples from the upper Ohio River basin ($\pi=0.006$). Mismatch distribution plots for the entire sample (species-wide) showed a multimodal distribution (Fig. 3A), consistent with a model of non-expanding populations at mutation-drift equilibrium (Tajima's D = -0.899; $p > 0.10$). Similarly, the Tennessee River Clade also exhibited a multimodal distribution with no significant difference from expectations of a stable set of populations (Fig. 3B; Tajima's D = 0.123; $p > 0.10$). In comparison, mismatch distribution for the Ohio River Clade was unimodal, as expected for a recent population expansion (Fig. 3C). Although Tajima's D was negative (-0.879) for the Ohio River Clade, the value did not differ significantly from zero ($p > 0.10$).

DISCUSSION

Our samples from southern Indiana, Illinois, and central Kentucky demonstrate that the 'Ohio River Clade' is not restricted to southern Ohio and eastern Kentucky (= Teays River System), but is broadly distributed throughout the Ohio River basin above the Cumberland and Tennessee rivers. The close relationship between lampreys from the lower and upper Ohio River basin is further reflected in low levels of nucleotide diversity and a unimodal mismatch distribution analysis (Fig. 3B), consistent with a recent (post-Pleistocene) range expansion of the Ohio River Clade following the integration of the modern Ohio River system. Tajima's D for the Ohio River Clade did not differ significantly from zero ($p >$ 0.10), but this may have been the result of the small sample size (n = 13 haplotypes). In contrast, samples collected from the Tennessee River system and elsewhere show deeper divergences with unresolved relationships among drainages, higher levels nucleotide diversity, and a multimodal mismatch distribution, as expected for resident populations of drainage systems that presumably predate the glacial activities of the Pleistocene. In short, mtDNA variation in *L. aepyptera* is consistent with a recent dispersal within the Ohio River system after the establishment of the modern drainage pattern.

Criteria for recognizing glacial refugia and the paths of post-Pleistocene dispersal of fishes typically include patterns of monophyly among mtDNA haplotypes and relative levels of genetic diversity in putative source and dispersant populations (Avise 2000). We postulate that the Pleistocene refugium from which the northern clade emerged was located in the Old Ohio River system rather than the Teays River system for two reasons. First, although there is little phylogenetic structure within the Ohio River Clade, samples from the lower Ohio River basin show evidence of greater nucleotide diversity (π = 0.012) than samples collected from the upper Ohio River basin ($\pi = 0.006$). Second, tributaries of the lower Ohio River basin are adjacent to the remainder of the species' distribution and it seems likely the Old Ohio River was part of a historically contiguous

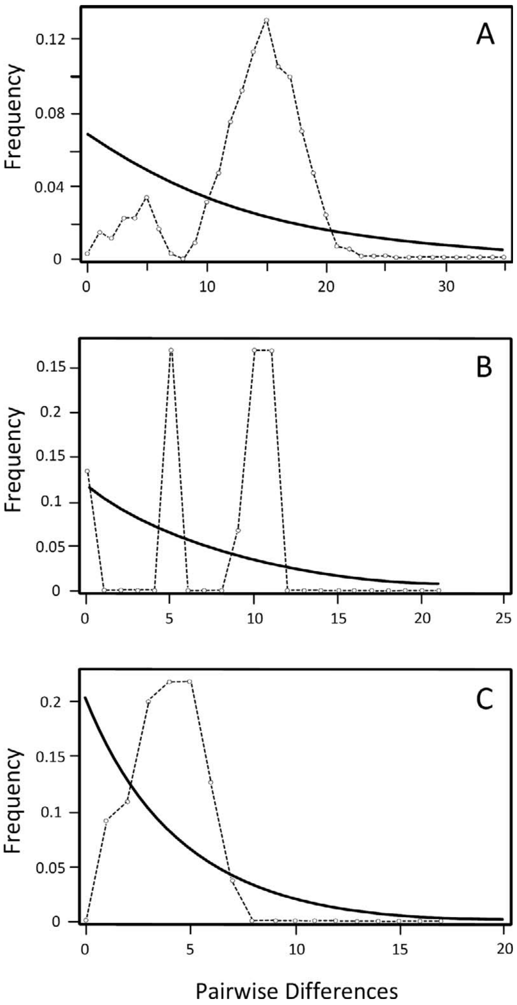

Figure 3.—Mismatch-distribution of pairwise differences of NADH dehydrogenase subunit 3 (ND3) haplotypes of *Lampetra aepyptera*. Shown are observed (dashed lines) frequencies for (A) the entire sample, (B) the Tennessee River Clade, and (C) the Ohio River Clade. Expected frequency distributions under a model of population expansion are shown by solid lines.

distribution. Although the data presented here do not falsify the hypothesis that *L. aepyptera* was part of an ancient Teays fauna, dispersal from the lower Ohio River basin (Old Ohio system) into the upper Ohio River basin appears to be the most parsimonious explanation for the origin for the Ohio River Clade.

Other stream fishes native to southern Indiana show similar phylogeographic patterns as reported here for *L. aepyptera*. For example, Strange & Burr (1997) examined mtDNA variation in the Streamline Chub, *Erimystax dissimilis* (Cyprinidae), and found evidence for a Pleistocene refugium in the Green River and Tennessee River drainages, followed by a post-Pleistocene dispersal into the newly integrated Ohio River (see also Simons 2004). Likewise, Berendzen et al. (2003) hypothesized that the Northern Hog Sucker, *Hypentelium nigricans* (Catostomidae), dispersed from the Old Ohio into the upper Ohio River system following the retreat of the glaciers. Thus, our conclusions regarding the history of *L. aepyptera* in the Ohio River system are consistent with other fishes, yet differ from that of Martin & White (2008). Given the large hiatus between their collection localities and the unresolved relationships among major drainage populations, it is understandable that Martin & White (2008) interpreted the upper Ohio River Clade as a relict of the pre-Pleistocene Teays River fauna.

In conclusion, it is clear that populations of *L. aepyptera* that occur in Indiana are closely related to other populations in the Ohio River basin. Although the use of a single (and relatively short) genetic marker makes any assessment of the deeper relationships among drainage populations premature, our analysis (and that of Martin & White 2008) suggests that *Lampetra aepyptera* represents a species complex with more taxonomic diversity to be described outside of the Ohio River basin (Boschung & Mayden 2004). Future investigations into the deeper divergences within *L. aepyptera* should include longer (> 1000 nucleotides) regions of the mitochondrial genome and/or nuclear genes (viz. Espanhol et al. 2007; Caputo et al. 2009; Docker et al. 2012).

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