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PROCEEDINGS OF THE INDIANA ACADEMY OF SCIENCE

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Cover: Some of the species observed during the 2015 Hills of Gold Biodiversity Survey or Bioblitz, Johnson County, Indiana. (See the article summarizing the results of the bioblitz in this issue of the Proceedings). Upper left: Peromyscus leucopus, White-footed Mouse or Woodmouse. Common throughout the eastern two-thirds of the United States, White-footed mice are omnivorous, eating seeds and insects. (Photo by Tim Carter) Bottom left: Mycena leaiana (Agaricales, Mycenaceae), Orange Mycena. Characterized by its bright orange cap and stalks, this lignicolous (wood rotting) mushroom usually grows in dense clusters on deciduous logs. The pigment responsible for the orange color is thought to have antibiotic properties. (Photo by Steve Russell) Upper right: Terrepene carolina, Eastern Box Turtle, is a special protected species in Indiana. This species gets its common name from the structure of its shell which consists of a high domed carapace (upper shell), and large, hinged plastron (lower shell) which allows the turtle to close the shell, sealing its vulnerable head and limbs safely within an impregnable box. (Photo by John Taylor) Bottom right: Lepomis cyanellus, Green Sunfish. The green sunfish is native to a wide area of North America east of the Rocky Mountains. The body is dark green, almost blue, dorsally, fading to lighter green on the sides, and yellow to white ventrally. (Lepomis, Greek, meaning "scaled gill cover", and cyanellus, Greek, meaning "blue".) (Photo by Brant Fisher)

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Figures 1–4.—Right chelicerae of species of *Centruroides* from Timbuktu. 1. Dorsal view; 2. Prolateral view of moveable finger; 3. *Centruroides* holotype male; 4. *Centruroides* female. Scale = 1.0 mm.

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MECHANICAL AND THERMAL PROPERTIES OF CHITIN FROM DIVERSE SOURCES

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ABSTRACT. Chitin is an abundant polysaccharide that can be found in the exoskeletons and sloughs of many different organisms. Commercially, chitin is extracted from shrimp exoskeletons and used in applications ranging from thickening agents to wound dressing. Previous studies in our group showed that other sources of chitin (lobster, crawfish, and the sloughs of cicada) can be extracted in a similar manner but produce chitin with varying degrees of acetylation and protein content. In this study, chitin from a cicada, lobster, and shrimp source materials was studied to determine their mechanical and thermal properties. The chitin to chitosan ratio of the resulting product also was altered through a reaction with sodium hydroxide at differing temperatures or for differing time periods. The three source materials produced films with similar mechanical strength and thermal properties. Likewise, each responded similarly to changes in the degree of acetylation.

Keywords: Chitin, chitosan, cicada, lobster, shrimp, TGA, mechanical properties

INTRODUCTION

Chitin, a naturally occurring biopolymer, is second only to cellulose in abundance in the biosphere (Zeng et al. 2012). Chitin can be found in the exoskeletons of a number of organisms such as shrimp, lobster, and cicada. Chitin and its deacetylated derivative, chitosan, have many known functions that range from cosmetics (Sahoo et al. 2009) to food (Aranaz et al. 2009) to biomedicines (Ding et al. 2014). Despite the wide variety of uses and wide variety of possible sources, the majority of chitin is derived from two sources, i.e., fungi and shrimp.

In many of its uses, chitin and chitosan are added to other materials as mechanical fillers or thickening agents. Studies have shown that the mechanical properties of these materials can vary greatly depending on the processing of the chitin and/or chitosan and the type of solvent used (Fernandez-Pan et al. 2010). However, even though the processing conditions of chitin have been well-documented in numerous studies, the effect of the source material has not been fully explored. It has even been reported that chitin can be found and extracted from many unique sources ranging from honeybees (Draczynski 2008) to crawfish (Mendez et al. 2015), but evidence for the usefulness of these sources in mechanical applications is lacking. In this study, a comparison of the mechanical and thermal properties of chitin prepared from differing source materials is presented along with the effect of differing ratios of chitin to chitosan.

METHODS

Source material.—Fresh samples of lobster shells and shrimp shells were collected from restaurants in Columbus, IN. Cicada sloughs were collected from the campus of Indiana University – Purdue University Columbus. All fresh source materials were cleaned with deionized water and allowed to dry. After drying, samples were ground into a powder and stored in sealed containers until the extraction process.

Chitin extraction.—The chitin from the raw powder samples was extracted in a multi-step process to remove minerals and other organic materials. The first step was to stir the samples in 2 M sodium hydroxide at reflux for 1 h at a concentration of 40 mg/ml. Once removed from the sodium hydroxide, the sample was brought back to a neutral pH using multiple washes of deionized water and allowed to dry in a desiccator. In the second step, the sample was placed in 2 M hydrochloric acid at room temperature and stirred for 1 h. The sample was returned to a neutral pH using multiple washes of deionized water. The lobster samples were then washed with acetone to remove the astaxanthin, which gives the shell its red

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Table 1.—Temperature and time specifications used to prepare each sample. All samples were stirred at 100 °C while heated and 23 °C when unheated.

Sample source	Degree of acetylation (%)	Stir time (Days)	Heat time (Hours)
Shrimp	32.4	3	5
Shrimp	45.7	7	10
Shrimp	47.6	10	30
Shrimp	54.3	9	20
Lobster	37.7	2	10
Lobster	39.0	1	5
Cicada	39.5	2	10
Cicada	49.8	9	20

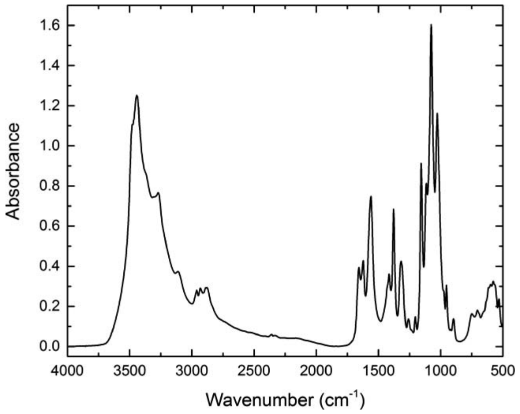

Figure 1.—Representative IR obtained from lobster with a degree of acetylation (DA) of 24.9%.

coloring. The final step for all samples was a wash in 4% sodium hypochlorite for 5 min for discoloration. These samples were dried in a desiccator for 24 h before being stored for future use.

Chitosan conversion.—Each chitin sample (with a concentration of 3 mg of sample per ml of sodium hydroxide) was heated to 100 °C and allowed to stir for a predetermined time. Once the predetermined time was met, the heat was turned off and the samples were allowed to stir at room temperature for another predetermined time (Table 1). The samples were transferred into centrifuge tubes and spun 10 min so that all solid settled to the bottom of the tube. The supernatant was discarded and deionized water was added. Tubes were mixed thoroughly before being placed back into the centrifuge for 10 min. This wash process was repeated until the samples reached a neutral pH. The deacetylated samples were then dried in the desiccator 24 h before being cast into films.

Infrared microscopy.—The selected deacetylated sample was added to enough 1 M acetic acid to reach a concentration of 5 mg/ml. This solution was sonicated for 30 min in a Branson 2800 sonicator to ensure a uniform dispersion. Films were cast overnight under ambient conditions to a thickness of approximately 0.06 mm and then measured utilizing a Nicolet IR100 FT-IR (Fig. 1). The absorbance of the carbonyl peak at 1655 cm^{-1} (only present in chitosan) was compared to the hydroxyl peak at 3600 cm^{-1} (present in both chitin and chitosan) to give an accurate ratio of chitin to chitosan (Czechowska-Biskup et al. 2012).

Young's Modulus.—Films were cut into small strips approximately 5 mm wide with a thickness of 0.06 mm. A single strip was clamped into an Instron Mechanical Tester (model # 2716-020). The maximum load was set at 100 N with a speed of 40 mm/min. The Young's Modulus for each sample was determined by taking the linear slope of the elastic region of the stress/strain curve. A minimum of 10 measurements were taken for each sample to obtain an average value.

Transmission electron microscopy (TEM).—A Tecnai G2 12 Bio Twin transmission electron microscope was used to obtain detailed images of individual chitin fibers. The instrument was run at 80 kV with magnifications ranging from 18,500 to 250,000×.

Thermogravimetric analysis (TGA).—Thermogravimetric analysis was performed on representative chitin samples from each source material on a TA Instruments Q50 TGA instrument. The temperature was ramped from 20° C to 500° C for each sample.

RESULTS AND DISCUSSION

As expected, the three sources in this study produced chitin with very little modification of the extraction process necessary. Chitin fibers appear similar under TEM (Fig. 2) with cicada fibers showing a slightly higher aspect ratio compared to fibers extracted from lobster or shrimp (Table 2).

The thermal stability of chitin did not vary significantly with the source material. Results from the TGA show degradation occurring between 370° C and 390° C for each sample (Fig. 3), consistent with literature sources (Zeng et al. 2010).

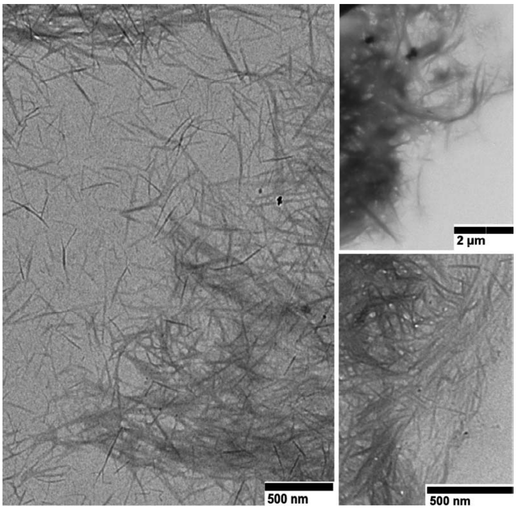

Figure 2.—TEM of chitin from cicadas (left), lobster (top right), and shrimp (bottom right).

Comparing the Young's Modulus of the chitin to chitosan ratio shows a clear relationship between these two properties (Fig. 4) with the median values comparable to other studies (Aklog et al. 2015). However, the source material does not show any significant effect on the Young's Modulus.

Conclusion.—There is always a tradeoff between the chitin to chitosan ratio and mechanical properties. With strong hydrogen bonding between fibers, chitin is a mechanically strong material but these same strong intermolecular forces also decrease solubility. Deacetylating the chitin to chitosan increases solubility but lowers the mechanical properties. The results presented above confirm this and show that the Young's Modulus increases with an increased degree of acetylation (percentage of chitin).

While the degree of acetylation does correlate with Young's Modulus, the source material does not. Additionally, the aspect ratio and thermal stability also show very little relation to source material. Taken together, this lack of significant difference demonstrates the viability of these different materials as potential commercial sources for chitin production.

Table 2.—Aspect ratio and standard deviation of chitin fibers.

	Aspect ratio	Standard deviation
Cicada	15.8	3.7
Lobster	8.3	2.7
Shrimp	7.4	4.4

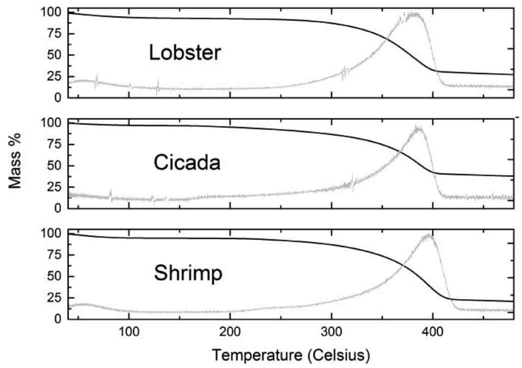

Figure 3.—Thermograms (black with derivative in gray) of chitin from lobster (top), cicada (middle), and shrimp (bottom).

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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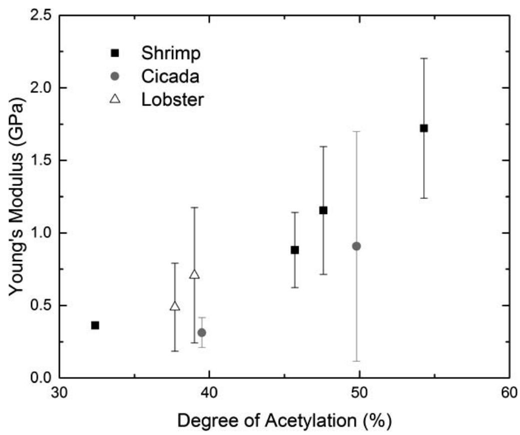

Figure 4.—Young's Modulus of chitin samples from shrimp (black squares), cicada (gray circles), and lobster (white triangles) at various degrees of acetylation.

copy Center at IUPUI for assistance with the TEMs. The authors would also like to thank Emma Parks and Heather Johnson for their assistance in the early stages of the experiment.

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BATS UNDER AN INDIANA BRIDGE

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ABSTRACT. A survey of over 200 bridges and culverts in southwest Indiana was completed in 2004 and 2005. Only a single bridge showed roosting bats, including federally endangered Indiana bats (*Myotis sodalis*) and gray bats (*Myotis grisescens*). Other species present included little brown bats (*Myotis lucifugus*), big brown bats (*Eptesicus fuscus*), and eastern pipistelles (*Perimyotis subflavus*) or now called tri-colored bats. Surveys of this bridge occurred 2006 to 2011. The little brown bat was the most common (6,887) followed by Indiana (878), big brown (774), eastern pipistrelle (29), and gray bat (2). There were more male than female Indiana and little brown bats, especially in the late summer and early fall. The bridge serves as a mating site, day/night roost, and migratory stop-over for little brown bats and Indiana bats. Big brown bats were found throughout the year, while eastern pipistrelles were occasional in winter to early spring. Banding showed many bats have a high fidelity to this bridge, and wing membrane scores did not indicate white-nose syndrome (WNS). Data loggers were placed under the bridge for temperature readings from July 2008 to March 2009 and showed *Myotis* avoiding them (but *Eptesicus* did not) due to ultrasonic noise at about 30 kHz. The bridge acted as a thermal sink at night and throughout most of the day, especially during warmer months. The bridge was warmer and had more constant temperatures than outside temperatures from July to February.

Keywords: Bats, bridges, environment

INTRODUCTION

Bats make extensive use of bridges and culverts for both day and night roosts (Keeley & Tuttle 1999; Whitby et al. 2000; Sandel et al. 2001). In Indiana, most available data indicate that bridges are being used as day and night roosts (Duchamp et al. 2004; Whitaker et al. 2004), although one study emphasized bridge use by bats, specifically the Indiana bat (*Myotis sodalis*), as a thermal sink for night roosting during feeding bouts (Kiser et al. 2002).

Efforts to use bridges and culverts as bat management tools remain rare (Arnett & Hayes 2000; James & Palmer 2007). However, with ongoing bat population declines and habitat destruction, more managers are recognizing and appreciating bridges as important alternative roosting habitat. Bridges can provide day, night, maternity, and migratory roost sites (Adam & Hayes 2000; Lance et al. 2001), while also providing temperature stability, predator protection, and proximity to foraging areas. Thus, with the loss of natural roosts and the ready availability of bridges and culverts, it is not surprising that 24 of 45 bat species in the United States roost in these anthropogenic sites (Keeley & Tuttle 1999). In the United States, there are six federally endangered bat species, two of which (*Myotis sodalis* and *Myotis grisescens*), sometimes roost in bridges (Keeley & Tuttle 1999).

In the US roughly 3,600 highway structures (about 1%) are used by an estimated 33 million bats (Keeley & Tuttle 1999). Features of bridges that correlate with bat use are well known (Davis & Cockrum 1963; Adam & Hayes 2000; Erickson 2002). According to a California Department of Transportation (CALTRANS) study (James & Palmer 2007), major bridges attractive to bats are: (1) built before 1950; (2) located in rural areas; (3) constructed over water ways; and (4) possess girder construction including concrete, timber and steel materials. Keeley & Tuttle (1999) found that bats day roost in expansion joints and crevices where they are protected from predators and inclement weather. They also observed that bats prefer bridges that have roost heights at least 3.1 m above ground, are rain-watered sealed, exhibit full sun exposure, and are not situated over busy roadways (Keeley & Tuttle 1999). In particular, bats gathered in the open areas between support beams to digest food. There the large thermal mass remains warm at night and the

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vertical concrete surfaces between provide protection.

In Indiana, there are over 18,000 state and county-owned bridges (B. Dittrich, INDOT, Pers. Comm.) with INDOT responsible for about a third of them. During the course of a highway study in 2004 and 2005, over 200 bridges and culverts were surveyed for bats. Only one bridge had roosting Indiana bats. This bridge, located in southwestern Indiana, was found to have Indiana bats, little brown bats, and big brown bats; two red bats were mist netted near the bridge (Bryan et al. 2004; Kudlu & Brack 2005). Bridge surveys from 2006 to 2011 showed a limited number of eastern pipistrelles under this bridge. One gray bat was observed in April 2007 and another in September 2012. No bats observed at this bridge showed signs of white-nose syndrome (WNS) which was first reported in Indiana in January 2011 (IDNR 2016).

This paper provides data and observations made at this specific bridge located in Greene County, Indiana. Between October 2006 and April 2011, this bridge was studied to determine what bat species use it seasonally, to learn features of the bridge suitable for roosting, and to collect life history data.

Study area.—The metal bridge spans a large river. The exact location is being withheld at the request of the U.S. Fish and Wildlife Service (USFWS) to prevent potential disturbance to bats by unauthorized visitors. It is located on a two-lane road through a broad open valley with much farmland. Built in 1940, it contains 10 spans and is 300 m long and ranges from 6 to 20 m above the river and floodplain. The north and south reinforced concrete girder spans have full depth concrete sidewalls that are open inside and placed into a hillside which creates the appearance of a cave. The underside of the bridge has cracks and crevices. The bridge span is oriented roughly NNE/SSW 20° with prevailing winds from the southwest (Figs. 1–4).

There is a cleared area about 6 m wide on both sides of the bridge. Beyond that, the tree-lined banks include green ash (*Fraxinus pensylvanica* Marshall), cottonwood (*Populus deltoides* W. Bartram ex Marshall), silver maple (*Acer saccharinum* L.) and sycamore (*Platanus occidentalis* L.). The ground below the north and south ends (concrete) have no vegetation and are sloped uphill from their opening to the back. In this paper, data from both the north and south ends were compiled to represent the bridge as a whole. The middle portion of the bridge over the river included eight metal spans set on concrete piers. No bats roosted on these metal spans.

This bridge is within 24 km of one of the largest Indiana bat hibernacula in its range (n = 49,617 in January 2013); within 40 km of 12 other Indiana bat hibernacula; and about 3 km upstream of known Indiana bat and northern long-eared bat (*Myotis septentrionalis*) maternity colonies. Indiana bats did not use this bridge as a hibernaculum nor have they used other bridges as hibernacula (USFWS 2007). In contrast, Indiana bats frequently are found hibernating in a variety of other man-made structures such as abandoned mines, tunnels, and a dam (USFWS 2007). In Indiana, only natural caves are currently known to serve as hibernacula (Whitaker et al. 2007).

METHODS

Presence of bats (especially the Indiana bat) near concentrations of graffiti and trash under each end of the bridge prompted INDOT, FHWA and the USFWS to install a 6-foot chain-linked fence with a locked gate in April 2006. This fence excluded entry of any unauthorized persons near the roosting bats. In September 2007, signage was erected that stated coordination with INDOT and USFWS was required prior to work on or within 200 feet of this bridge.

In 2004, Hal Bryan and others from Eco-Tech found Indiana bats under this bridge. Early observations of this bridge occurred from April to September 2006, and, with a plan in place, formal bridge inspections began 13 October 2006 and continued to 3 April 2011. Sampling usually occurred between 1100 and 1300 hrs. Data included the number of bats by species, locations, and behaviors. The underside of each end of the bridge was divided into sections and tiers using stringers and cross beams (Figs. 1 & 2). Three tiers were located under the north end, while there were two tiers under the south end.

Generally, sampling was conducted weekly in the fall (September through November) when bats tend to leave for their hibernacula (Bryan et al. 2004) and in spring (March through May) when bats emerge from hibernation and move to their summer habitat. Field surveys in summer (June through August) and winter (December through February) were monthly. In 2008, a 24-hour survey was completed from 1200 on 28 September until 1200 hrs on 29 September at 2 hr intervals.

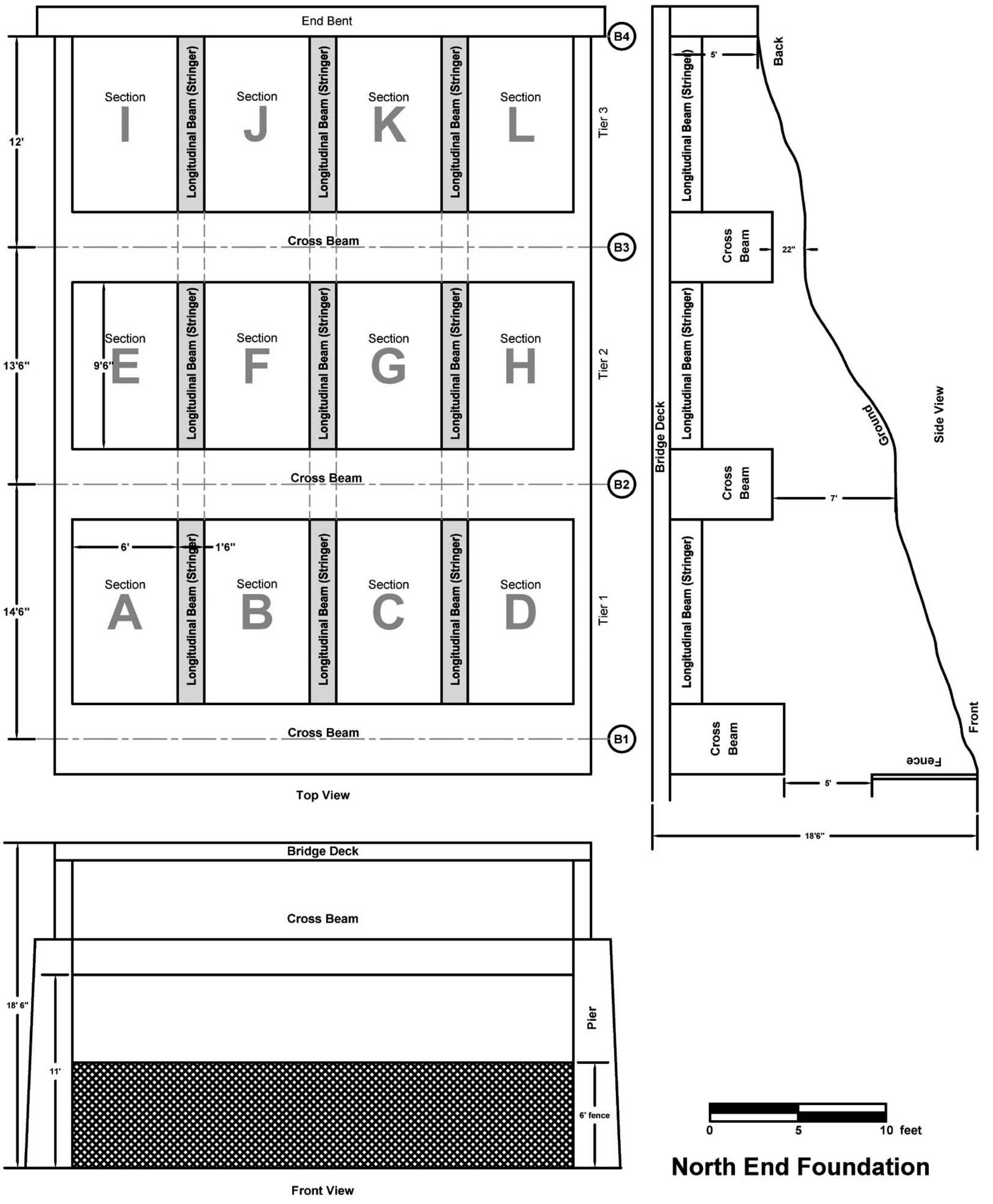

Figure 1.—Front, top and side views of the bridge, north end.

Air and substrate temperatures and relative humidity (specific to the bridge) were measured with an Extech model RH101 infrared thermometer and humidity meter under each concrete end of the bridge with every visit. In 2008 (July through December) and in 2009 (January through March) automated temperature readings were provided by Thermocron iButton dataloggers. Five were secured on a stringer in each tier under the north and south ends of the bridge where bats normally roosted. One datalogger was left outside of bridge for outside air temperatures.

Lighting under the bridge was measured using an Extech light meter. Readings were taken in

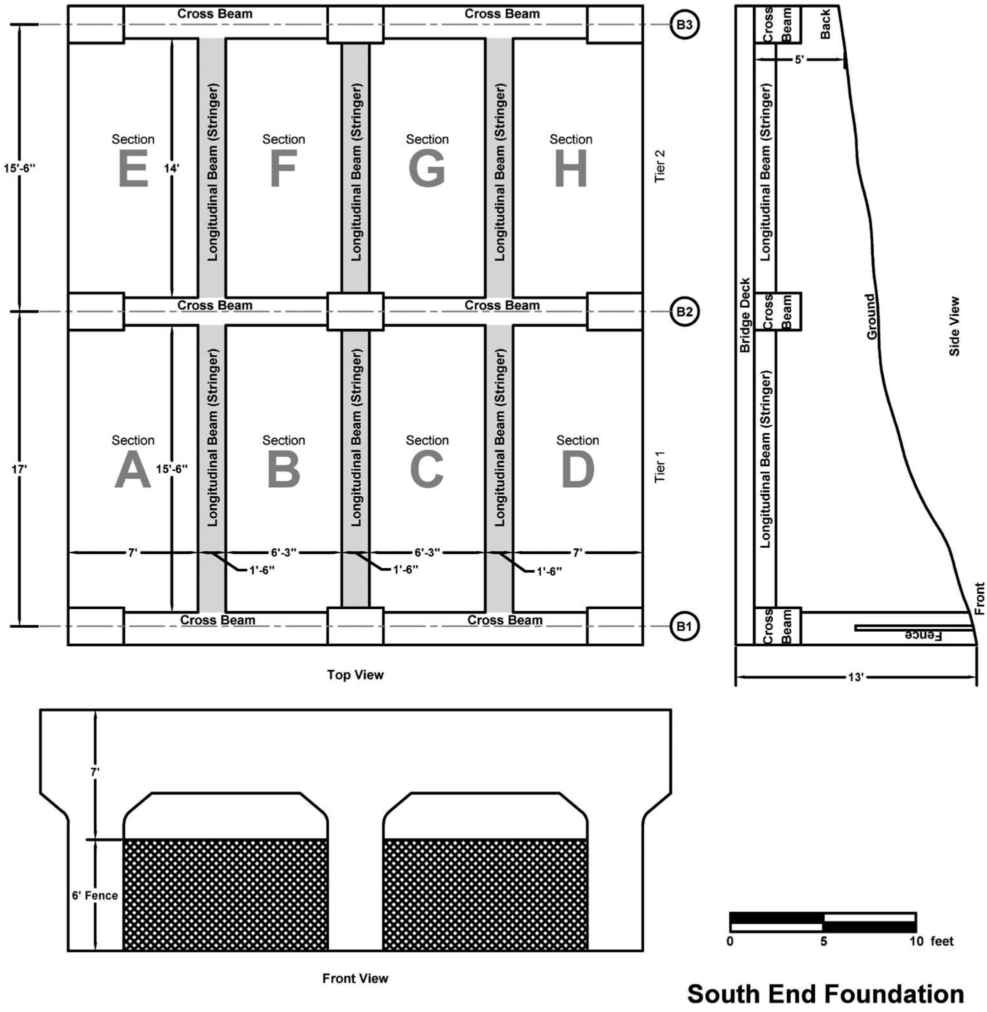

Figure 2.—Front, top and side views of the bridge, south end.

each tier. Sound levels were measured using a Larson-Davis DSP 82 sound level meter (calibrated with a Larson-Davis CAL 200 acoustic calibrator) on the underside and top of the bridge. Wind speed was measured using a Kestrel 1000 Pocket Weather Meter. Calipers were used to measure seam widths in areas associated with bat staining and in areas not stained. Identification of species was by climbing ladders and hand picking bats in order to see species-specific morphological characters.

Banding of bats under the bridge was approved by USFWS under USFWS Federal Permit #TE-179711-0. Banding of 224 bats (60 Indiana bats, 154 little brown bats, 6 big brown bats, and 4 eastern pipistrelles) using silver-colored bands occurred between 29 April and 16 October 2008, while previously, Eco-Tech banded 84 bats (8 Indiana bats, 51 little brown bats, 24 big brown bats and 1 red bat) with orange-colored bands between 26 May and 3 August 2004 (Bryan et. al. 2004). In both efforts, males were banded on the right forearm and females on the left. Data in this paper on sex ratios come from having the bat in hand for identification.

RESULTS AND DISCUSSION

Sound and vibrations.—On 3 May 2007, traffic counts from 1100 to 1200 hrs and from 1330 to 1430 hrs yielded 216 and 252 vehicles,

Figure 3.—Photo of north side of bridge.

Figure 4.—Photo of south side of bridge.

respectively (70% to 80% cars). Sound levels above the bridge were 81.4 to 84.6 dBA, while under the bridge they were 84.1 to 85.0 dBA. Generally, bats did not appear affected by sound or vibrations conducted through the concrete from traffic. However, more intense vibrations caused bats to take to the air, but to ultimately return to roosting. Our results were similar to Keeley & Tuttle (1999) in that the bats appeared to be habituated to vibrations and sounds associated with normal traffic.

Seams and staining.—A seam under the bridge is a groove in the concrete along a stringer or cross-beam with the fillet/ceiling. Average seam width (n = 50) within bat stained areas was 2.9 mm, while average seam width outside stained areas (n = 50) was 2.0 mm. Outside walls (wing walls) did not have seams, but did show some irregular surface areas. A seam is important for bats to get a foothold to roost. Some bats were seen roosting in between loose concrete that had separated from the deck of the bridge. Stains on the concrete were visible year round and tended to be centrally located along stringers (Fig. 5). Staining was not observed within 0.6 m from cross beams and no bats were seen roosting along stringers less than 1.2 m above the ground even though bats had adequate open seams for roosting. Keeley & Tuttle (1999) found bats prefer the highest roost heights. Avoiding predators is likely an explanation in both cases. On one occasion, a domestic cat was observed under the bridge and a black rat snake was observed on the upper end of the fence. Raccoon tracks were routinely seen under the bridge.

Light, wind, temperature, and humidity under the bridge.—On 26 October 2007, light readings (measured in lux units) under the bridge were $\leq$ 162 lx, while above the bridge they were $\geq$ 9,688 lx. Moving to the back of the bridge, each tier had less light. On the north end, bats preferred darker roosting areas: 1,327 bats (45%) roosted in the back, 1,026 (34%) roosted in the middle and 631 (21%) roosted in the front. On 5 December 2007, wind exterior to the underside of the bridge averaged 4.3 kph with wind speeds under the bridge in all tiers measuring 0 kph. Thus, the bridge not only has varying degrees of darkness, it is also windless and protects bats from the outside weather.

Air temperatures at the time of surveys were between 5° C to 32° C in spring and 5° C to 29° C in fall. In summer, temperatures were between 25° C to 33° C and in winter ranged between -1° C to 21° C. The south concrete foundation was significantly warmer than the north in July to September ($p < 0.0001$) and October ($p < 0.0062$), while there was no difference in November to March,

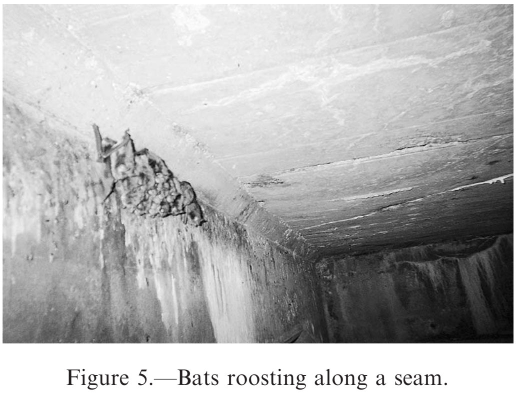

Figure 5.—Bats roosting along a seam.

Table 1.—Monthly data on visits and bat species observed under bridge.

Month	# of Visits	Indiana	Big Brown	Little Brown	Tri-Colored	Gray	Total
January	5	0	33	0	1	0	34
February	5	0	9	0	5	0	14
March	5	3	10	13	1	0	27
April	19	31	16	82	5	1	135
May	9	64	18	440	6	0	528
June	10	8	33	1274	0	0	1315
July	8	55	69	1464	0	0	1557
August	9	39	212	1844	0	0	2050
September	7	85	105	1214	0	1	1405
October	16	449	152	472	0	0	762
November	16	136	50	81	7	0	155
December	7	1	67	3	4	0	62
Totals	118	878	774	6,887	29	2	8,570
% of Total		10.3%	9.0%	80.3%	0.3%	<0.1%	

possibly because of the smaller volume of air in the south than in the north. No readings were taken in April or June 2009 since dataloggers were removed in mid-March 2009, after they were found to emit ultra-sonic sound affecting bat roosting (Willis et al. 2009).

During a 24-hour study on 28–29 September 2008, air temperatures ranged from 12° C to 30° C. Warmest temperatures were from 1200 to 1600 hrs at 26° C to 30° C, respectively. Coolest temperatures were from 0400 to 0800 at 12° C to 14° C, respectively. From 2000 to 0800 hrs, substrate temperatures were warmer than air temperatures. Such data are consistent with the typical pattern of temperature collected under the Mauxferry Road bridge over Nineveh Creek for 36 h, from 1–3 August 2001 (Kiser et al. 2002). The bridge acts as a thermal sink at night and throughout most of the day except possibly in the afternoon. This characteristic is especially notable during warmer months. From July to February the bridge substrate was warmer than outside temperatures and the temperatures changed more slowly and had less overall fluctuation. March did not show such a trend.

Average relative humidity (specific to under bridge) was 48% to 83% in spring, 39% to 80% in fall, 43% to 76% in summer, and 50% to 79% in winter. Relative humidity during a 24-hour study on 28–29 September 2008 ranged from 30% to 83% with the lowest readings from 1200 to 1800 hrs (30% to 50%) and highest readings from 0200 to 0800 (53% to 83%). Lacki (1984) reported greater activity of male little brown bats under conditions of both higher temperature and relative humidity suggesting that these bats alter their flight activity in response to changes in air saturation.

Bat surveys.—There were 118 visits to the bridge in which 8,570 bats were observed comprising five species (Table 1). The little brown bat was the most common (6,887) followed by the Indiana bat (878), big brown bat (774), and to a lesser degree, the eastern pipistrelle (29) and two gray bats. Information on each species follows.

Indiana bats: A total of 878 Indiana bat observations were made under the bridge. They were observed in every month but January and February (Table 1) increasing from three in March (earliest observation was 28 March) to 64 in May and only eight in June. July, August, and September showed a range from 39 to 85. October showed the most at 449 Indiana bats but declined through December when only one Indiana bat was observed. Sex ratios overall were 70 males to 21 females (Table 2). Ratio of male to female Indiana bats in the spring was 13 males to 12 females, while in late summer to fall (during mating season) it was 57 males to 9 females. Two matings were observed in fall, none in other seasons. Females were present in May and from July through September at which time mating occurred. Males were present in April, May and July through October, the times females were most abundant. From such data and the occurrence of this species under this bridge in spring, and late summer through late fall, this bridge may be a migratory stopover. Similar timing of their occurrence has been seen each year in this

Table 2.—Monthly data showing gender and bat species.

	Indiana bats		Big brown bats		Little brown Bats		Tri-colored bats	
Month	Females	Males	Females	Males	Females	Males	Females	Males
January				1				
February				1			1	1
March			1	1	3			
April		1			8	7	2	
May	12	12		2	21	24	1	1
June			2	3	48	64		
July	5	21	3	7	63	91		
August	2	10	17	8	60	102		
September	2	21	2	1	4	56		
October		5	2	7		22		
November			2	4		1		
December				1				
Totals	21	70	29	36	207	367	4	2

investigation. The bridge serves as a day and night roost for this species.

Little brown bats: A total of 6,887 little brown bat observations were made under the bridge. They were the most common bat, present in every month except January and February (Table 1), and increased from 13 in March (earliest observation was 28 March) to 82 in April. In May, adults numbered 440, while the number of little brown bats increased to 1,274 from adults giving birth to pups in June. Pups were observed on 8 June and 13 June. July showed a slight increase to 1,464 with August showing the greatest number of little brown bats at 1,844. This increase may be attributed to recruitment under the bridge from being a migratory stopover. Little brown bats decreased in September to 1,214 and in October to 472 as they left the bridge for hibernation diminishing to only three in December. Sex ratios overall were 367 males to 207 females (Table 2). The ratio of male to female little brown bats in September was 56:4 and 22:0 in October. Mating was observed on 23 August and 28 September 2008 in this species, none in other seasons. Females were present in March through September at which time mating occurred. Males were present primarily in April through November, the times females were most abundant and present after females were gone. This bridge was used by this species as a maternity, nursery, mating, and possibly a migratory stopover. A maternity colony of roughly 300 little brown *Myotis* were found in an Idaho bridge at 44° north latitude (Keeley & Tuttle 1999). Feldhamer et al. (2003) found this species under bridges in southern Illinois.

Big brown bats: A total of 774 big brown bat observations were made under the bridge (Table 1). Although consistently found under the bridge, their numbers were usually five or fewer (55% of the time) or 10 or fewer (78% of the time). On 13 July 2007, there were 35 big brown bats, while on 24 August there were 73. Whether this increase is related to recruitment by young is unknown, but highly probable.

They were present in every month (Table 1) of the year increasing from 9 in February to 212 in August. In September and October, their numbers were 105 and 152 respectively. Sex ratios overall were 36 males to 29 females (Table 2). No mating was observed, but juveniles were observed on 12 July alongside a lactating female. Females and males were most abundant in August (Table 2). Data suggest the bridge may be used as a maternity nursery and for mating. Big brown bats do raise young in bridges and were the second most abundant bridge-dweller (Keeley & Tuttle 1999). The presence of big brown bats in winter is consistent with observations that they often hibernate in buildings and are prone to be active during winter warm spells (Whitaker et al. 2007). Big brown bats were common under bridges in southern Illinois (Felhamer et al. 2003).

Eastern pipistrelles/tri-colored bats: A total of 29 eastern pipistrelle observations were made under the bridge. They were present in winter and early spring, but not observed in summer or fall (Table 1) similar to Sandel et al.

(2001). Ferrara & Leberg (2005) found an increased presence of this species during winter under bridges in Louisiana. Sex ratios overall were two males to four females (Table 2). No mating was observed in this species. From 2006 to 2011, eastern pipistrelles were observed under the bridge from November through May but not in June through October (Table 1). One eastern pipistrelle died in a roosting position sometime in January to April. Data suggest they use the bridge seasonally and it may function as a hibernaculum. Sandel et al. (2001) found the eastern pipistrelle in box culverts in Texas under Interstate Highway 45. They found selection of winter hibernacula in temperate regions may not be dependent on microclimate parameters alone, and the presence of bats in hibernacula varied throughout the year with minimum temperature in winter the only significant microclimate predictor in abundance of bats. Analyses of land-use by Sandel et al. (2001) revealed a significant correlation between number of bats present at each roost and amount of agriculture and forest surrounding each site. There was also a correlation between distances from the opening of the culvert to forest. The bridge studied in this paper was situated in an agriculture and forest matrix.

Gray bats: A gray bat was observed under the bridge on 13 April 2007. The distribution for the gray bat in Indiana is primarily in south central counties bordering the Ohio River (Whitaker & Mumford 2008). This bat is considered an outlier to the main summer distribution of gray bats in Indiana. In September 2012, a gray bat was reported under this bridge (Jared Helms, Pers. Comm.).

Red bats and northern long-eared bats: Two red bats were mist netted and banded on 3 August 2004 next to the bridge (Bryan et al. 2004). During surveys under the bridge in 2006 to 2011, no red bats were observed. The red bat is a solitary species that roosts in foliage (Whitaker et al. 2007).

Even though northern long-eared bats, a federally threatened species, have been found in the vicinity of this bridge, no northern long-eared bats have been observed roosting under this bridge. However, Feldhamer et al. (2003) and USFWS (2014) report this species has been observed roosting under bridges elsewhere.

Roosting behaviors.—Indiana bats roosted singly or in groups of up to 20 individuals. They roosted with little brown bats on occasion and with a big brown bat on a few occasions. Little brown bats also roosted singly or in small groups of up to 30 individuals or occasionally up to 70 bats. Big brown bats usually roosted singly or in pairs and occasionally with little brown bats.

Banded bats.—Indiana bat records from 2008 to 2011 showed nine silver banded recaptures (eight males and one female) and observations of 60 silver banded bats (43 males and 17 females). All Indiana bats were recaptured in 2008 except one female which was recaptured two years later. One orange banded male Indiana bat (banded in 2004) was recaptured in 2006.

Little brown bats showed 14 orange banded recaptures, 49 silver banded recaptures and observations of 90 males and 64 females. Males and females roosted together in this maternity colony which allowed for greater opportunities for them to be recaptured and bands seen from the ground. One male was recaptured four times. He was banded on 29 May 2008 and recaptured 28 June 2008, 8 July 2008, 18 August 2008 and again two years later on 6 August 2010. Keeley & Tuttle (1999) report that bridges and culverts are used by both bachelor and nursery colonies, and as temporary roosts during migration and mating.

On one occasion, a banded lactating big brown bat banded on 3 August 2004 had two slightly smaller big brown bats on each side. Her teats were exposed and no hair was around them. She was recaptured in 2007 and in 2010. Another female big brown bat banded on 3 August 2004 was recaptured approximately five years later in 2009. A male big brown bat banded with a silver band on 31 July 2008 was recaptured again that year and again in 2009.

Four eastern pipistrelles (three females and one male) were banded with silver bands in 2008. No eastern pipistrelles were banded in 2004. There were no recaptures for eastern pipistrelles during this study.

After Indiana State University (ISU) biologists banded bats under the bridge in 2008, they later captured two little brown bats with silver bands at two caves (John Whitaker, Jr. & Brianne Walters, Pers. Comm. 2009–2010). One little brown bat was captured in Wyndotte Cave located approximately 103 km southeast of the bridge, while the other little brown bat was captured in Ray's Cave located approximately 22 km northeast of the bridge. Both of these caves are Priority 1A hibernacula.

Banded bats were also observed on 28–29 September 2008 during the 24-hour study. An orange band was seen on one little brown bat, while a month later, an orange band was seen on a male big brown bat. These recaptures and visual sightings show that bats banded in 2004 were still using this bridge in 2006, 2007, and 2008. During the period 2008 to 2011 the recapture of orange banded bats included two of 24 big brown bats; nine of 51 little brown bats; and one of eight Indiana bats. In addition, two big brown bats and 17 little brown bats were visually observed with orange bands. Such data indicate a high fidelity by bats for this bridge (Table 3).

24-hour study.—During the 24-hour survey of 28–29 September 2008, 1,699 bats were observed including 1,329 little brown bats, 241 Indiana bats and 129 big brown bats. The number of big brown bats stayed fairly constant (mean = 10 $\pm$ 3), while Indiana bats (mean = 19 $\pm$ 15) and little brown bats (mean = 102 $\pm$ 62) varied during the 24-hour period (Fig. 6). Average number of bats between noon and dusk was 217, night time (dark) was 48, and morning (post-dark) was 124. Fifty bats left from under the bridge between 1800 and 2000 hrs but a greater number ($\sim$ 150 bats) did so between 2000 to 2200. Between 0000 and 0600 hrs, the number of bats under the bridge remained fairly constant (mean = 49 $\pm$ 13); by 0800, many bats returned to the bridge ($\sim$ 115); and by 1000 and 1200 there were 130 and 126, respectively.

At the end of the 24-hour study, there were about 90 fewer bats under the bridge than at the start of the survey. Observations included two Indiana bats mating and a movement by bats to higher elevations which may be explained by bats preferring the highest, darkest locations (Keeley & Tuttle 1999). Bats may have moved to higher heights to be away from investigators. However, Ferrara & Leberg (2005) found no support for the hypothesis that surveys of day roosts affected bat use of bridges.

Conclusion.—This seven year study (2004–2011) of this bridge provided considerable data on the Indiana bat, as well as the little brown bat, big brown bat, and the eastern pipistrelle, known today as the tri-colored bat. Because of the large number of visits (118 visits), seasonal patterns on occurrence, density, and behaviors were observed. Prior to this study, it was not known that Indiana bats would be active as early as 28 March and as late as 3 December. There was no previous data indicating this bridge was biologically connected with two Priority 1A caves in south-central Indiana. This study initiated an investigation that concluded iButton dataloggers emitted ultrasonic sound that displaced bats roosting under the bridge. This discovery resulted in a paper (Willis et al. 2009) that recognized such emissions and alerted users to test all dataloggers before use. Lastly, the study disclosed a high fidelity of these bats to this bridge, and no bats showed signs of WNS.

Environmental conditions under this bridge protected bats from predators, wind, rain, snow, and created a favorable environment for roosting and social interaction. From the knowledge gained in studying this bridge and others, FTA and INDOT developed Appendix D: Bridge Assessment Guidance and Form adapted from the INDOT 2010 Bridge Inspection Manual and the Bernardin, Lochmueller and Associates 2007 document. Appendix D is now a part of the Section 7 Consultation and Conservation Strategy for transportation projects (USFWS 2017). The appendix offers favorable characteristics in bridges for bats to roost, provides preliminary indicators of bat presence helpful in bridge inspections, and images helpful for inspectors and biologists. Much attention and interest in bats roosting under Indiana bridges has resulted from studying this bridge in Indiana.

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Table 3.—Banding data for orange (2004) and silver (2008) bands. BB = big brown bat; IB = Indiana bat; LB = little brown bat.

Band color	Species	Band number	Gender	Original date	Recapture date	Recapture date	Recapture date	Recapture date
Orange	IB	1102	Male	5/26/04	10/20/06			
	BB	1957	Female	8/3/04	6/17/09			
	BB	1965	Female	8/3/04	10/31/07	7/12/08	10/16/08	
	LB	668	Male	5/26/04	9/7/07			
	LB	1107	Male	5/26/04	8/29/07	8/30/07		
	LB	1110	Female	5/26/04	6/13/07			
	LB	1114	Female	5/26/04	8/24/07			
	LB	1119	Male	8/3/04	10/5/08			
	LB	1449	Male	5/26/04	10/5/07			
	LB	1450	Female	8/3/04	8/30/07	9/7/07		
	LB	1453	Female	5/26/04	10/30/06	9/14/07	9/25/08	
	LB	1954	Female	8/3/04	8/29/07	6/28/08		
Silver	IB	48	Male	9/25/08	10/5/08			
	IB	501	Male	5/8/08	9/10/08			
	IB	506	Male	5/8/08	8/18/08			
	IB	507	Male	5/8/08	7/8/08			
	IB	513	Male	5/8/08	7/17/08			
	IB	523	Male	7/17/08	9/10/08			
	IB	525	Male	7/17/08	9/25/08			
	IB	528	Male	7/31/08	10/16/08			
	IB	550	Female	7/17/08	5/14/10			
	BB	202	Male	7/31/08	8/13/08	5/28/09		
	LB	501	Male	4/29/08	5/21/08			
	LB	502	Male	4/29/08	5/21/08			
	LB	505	Male	4/29/08	6/5/08			
	LB	506	Male	4/29/08	6/28/08			
	LB	508	Female	4/29/08	6/5/08			
	LB	510	Female	4/29/08	6/5/08			
	LB	518	Female	5/8/08	7/6/08	6/19/10		
	LB	527	Male	5/15/08	10/5/08	8/6/10		
	LB	531	Male	5/29/08	7/8/08			
	LB	534	Male	5/29/08	6/5/08	9/29/10		
	LB	535	Male	5/29/08	6/28/08	7/8/08	8/18/08	8/6/10
	LB	539	Male	7/17/08	8/7/08	8/18/08		
	LB	541	Male	7/17/08	7/31/08			
	LB	551	Male	9/25/08	10/5/08			
	LB	589	Male	9/25/08	6/28/09			
	LB	597	Male	9/25/08	9/25/08			
	LB	958	Male	7/31/08	6/17/09			
	LB	959	Female	7/31/08	8/6/10			
	LB	964	Male	7/31/08	8/13/08	8/18/08	9/25/08	
	LB	969	Male	8/7/08	9/27/09			
	LB	974	Male	8/7/08	9/25/08			
	LB	975	Male	8/7/08	8/18/08			
	LB	977	Male	8/7/08	9/27/09			
	LB	981	Male	8/27/08	10/16/08			
	LB	990	Male	8/27/08	6/17/09			
	LB	6153	Male	7/17/08	6/7/10			
	LB	6159	Male	7/17/08	9/10/08	7/31/08		
	LB	6199	Female	7/17/08	8/27/08			

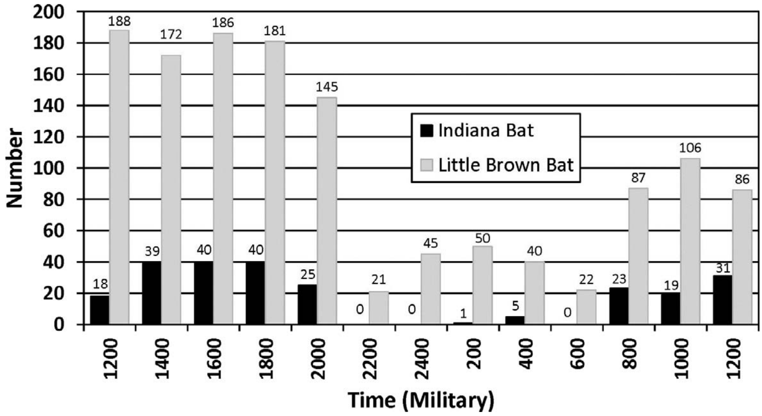

Figure 6.—Number of Indiana bats and little brown bats observed during a 24-hour period on 28–29 September 2008.

field staff (especially Jaime Sias Byerly and Randy Weaver) for their assistance.

This paper is dedicated to Hal Bryan, Eco-Tech, who was instrumental in studying this bridge. He passed away on 15 February 2010. His memory and efforts in studying bats will be remembered. Thank you Hal.

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TREE REGENERATION IN A SOUTHWESTERN INDIANA FOREST: IMPLICATIONS OF LONG-TERM BROWSING BY DEER

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ABSTRACT. Wesselman Woods Nature Preserve (WWNP) has never been subjected to timber harvest. However, deer can completely penetrate WWNP and browse tree seedlings and saplings throughout the forest. In this study, 30 plots (20 × 30 m) were surveyed (1.8 ha total). All trees of every size were identified and categorized into one of four strata based on height—herb layer, shrub layer, midstory, and overstory. Using the Shannon-Weiner Diversity Index, diversity was compared across strata. In the midstory 95% of stems over 150 cm in height and with a dbh < 5 cm) were pawpaws (3841 of 4038 stems). Sugar maples comprised 101 of the remaining midstory trees, and only three other species had more than 10 trees in this stratum. Oak trees had been almost completely lost from the midstory. Given its poor representation of canopy species, the midstory layer had significantly lower diversity compared to other strata. Many tree species (including sweetgum, tulip poplar, blackgum, hackberry, and 12 species of oak) have not transitioned into the midstory stratum, suggesting that regeneration of these species into the overstory is limited. In addition, pawpaw appears to have formed a recalcitrant layer and is anticipated to limit forest regeneration even more. While the patterns observed in this survey suggest that forest regeneration may be constrained by deer browsing at WWNP, an experimental study would be needed to confirm that deer (versus other factors, such as fire suppression or shading conditions of the forest) are responsible for limited regeneration. Placed within a forest management perspective, we discuss one possible experiment to examine concerns related to deer browsing and overabundance of pawpaw trees.

Keywords: *Acer*, *Asimina triloba*, pawpaw, *Odocoileus virginianus*, *Quercus*, deer browsing, Wesselman Woods Nature Preserve

INTRODUCTION

Herbivores alter the composition of plant communities (Augustine & McNaughton 1998; Olff & Ritchie 1998), and mammalian herbivores can have profound effects on their habitats (Augustine & McNaughton 1998; Knapp et al. 1999; Fortin et al. 2005; Pringle 2008; Martin et al. 2010, 2011). White-tailed deer (*Odocoileus virginianus* Zimmerman) populations in the eastern United States have been large enough that browsing by white-tailed deer (hereafter referred to as deer) has degraded the quality of many forest communities (reviewed by McShea et al. 1997). In particular, high deer density threatens tree regeneration (reviewed by Russell et al. 2001; Horsley et al. 2003; Rooney et al. 2004; Comisky et al. 2005; Rossell et al. 2005; Griggs et al. 2006; Long et al. 2007; Goetsch et al. 2011; Kain et al. 2011; Abrams & Johnson 2012; Chollet et al. 2013; Shelton et al. 2014). Browsing alters the physical structure of forests, causing reductions in stem/foliar density, as well as limiting sapling height. While deer browsing suppresses seedling/sapling establishment, preferential browsing also commonly occurs among woody species. By governing changes in woody species diversity in the forest understory, browsing by deer can potentially shift the future canopy forest community.

Deer browse on a wide range of tree species, including both evergreen and deciduous species. Still, oak species, which are commonly dominant/co-dominant canopy species in Midwestern forests (Dyer 2006), are especially at risk because deer greatly prefer to browse on oaks (Rooney & Waller 2003; Rossell et al. 2005; Long et al. 2007; Wakeland & Swihart 2009). While preferential browsing severely limits oak regeneration (Rooney & Waller 2003; Rossell et al. 2005; Belden & Pallardy 2009; Abrams & Johnson 2012), maples often experience browsing only when more preferred species are no longer available, leading to maples becoming more common in forests browsed by deer (Anderson & Katz 1993; Rooney & Waller 2003; Belden & Pallardy 2009).

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While these problematic changes are a concern for all forests in the eastern United States, the negative impact of deer may be of particular concern to virgin forests (i.e., old growth forests that have no history of being logged). Virgin forests can harbor high biodiversity, but such forests are rare (Fischer et al. 2013). In the state of Indiana, the Division of Forests lists only 11 old growth forests (i.e., forests containing trees of 150 years or older) owned by government agencies (IDNR-F 2016), and the Division has designated only three virgin forests (i.e., forests the Division describes as neither touched by human activity nor disturbed by unnatural factors) within the state.

The largest of these virgin forests is Wesselman Woods Nature Preserve (WWNP), which harbors more than 40 woody species (Table 1). At just under 80 ha, WWNP is a small forest tract, even though it is more than twice as large as either of the other two Indiana virgin forests. Nonetheless, small forests, even the size of WWNP, have relatively greater forest edge (Bowen & Burgess 1981) than historic forests of Indiana. Thus, the impact of deer, which favor foraging along forest edges (Alverson et al. 1988; Waller & Alverson 1997; Côté et al. 2004), can be exacerbated in our few remaining virgin forests.

To document the damage associated with high deer populations, studies have utilized exclosures (Alverson et al. 1988; Anderson & Katz 1993; Rossell et al. 2005; Griggs et al. 2006; Long et al. 2007; Goetsch et al. 2011; Abrams & Johnson 2012; White 2012), refuge areas (Comisky et al. 2005; Chollet et al. 2013), and areas with contrasting low deer populations (Horsley et al. 2003; Webster et al. 2005; Tremblay et al. 2007); however, few recent studies have probed the differences among canopy strata that may be caused by deer activity (but see Long et al. 2007). In part, pattern-based surveys are of limited utility because they lack experimental rigor, and so they lack the ability to discriminate among alternative explanations (Swihart et al. 2002). Still, descriptive comparisons between lower woody strata and the overstory can suggest whether constraints on forest regeneration may be associated chronic exposure to intense browsing regimes.

Decades of intensive deer browsing could also lead to the formation of a recalcitrant layer (*sensu* Royo & Carson 2006). Recalcitrant understory layers have been shown to affect regeneration, and deer browsing can facilitate the establishment of a recalcitrant layer (Tighman 1989; Stromayer & Warren 1997; Goetsch et al. 2011; Tanentzap et al. 2009; Kain et al. 2011; Johnson et al. 2015). In turn, forest successional trajectories may be altered, potentially causing a compositional change in the overstory.

Pawpaw (*Asimina triloba* L.) may act as a recalcitrant layer. Pawpaw utilizes annonaceous acetogenins as chemical defenses against herbivores (Ratnayake et al. 1992; Harborne 2001; Arnason & Bernards 2010). While deer will browse other, less-palatable woody vegetation when more-palatable stems are gone, deer avoid browsing pawpaw plants (Wakeland & Swihart 2009; Slater & Anderson 2014). Pawpaw's unpalatable quality, coupled with its shade tolerance (Battaglia & Sharitz 2006) and its vegetative reproduction strategy (Hosaka et al. 2016), may allow pawpaw to form a recalcitrant layer. Slater & Anderson (2014) found that deer browsing led to a dense pawpaw understory as a result of decades of intensive deer browsing. Other studies have suggested that pawpaw may limit canopy tree regeneration (Shotola et al. 1992; Shelton et al. 2014).

To evaluate the possible impact of browsing by deer on the forest composition of WWNP, the tree community among forest strata was compared. If deer browse has been chronic and extensive, tree diversity should be greatest in the overstory (because the canopy is the repository of tree diversity), as well as in the lowest stratum (because of seed production from canopy trees would generate seedling diversity), and diversity should be least in the intermediate strata because preferential browsing by deer would act as a filter, limiting which species could grow beyond sapling height. In addition, the pattern of oak and maple abundance was examined, as well as basal area, among strata to lend support to the argument of preferential browsing by deer. The relative importance of oak was predicted to be greater in the overstory and lowest stratum compared to the two intermediate strata. Finally, pawpaw abundance across strata was examined, comparing its abundance (and basal area) to other woody species. For pawpaw to act as a recalcitrant layer, higher relative abundance of pawpaw should occur in lower forest strata.

METHODS

Study site & species.—Wesselman Woods Nature Preserve (WWNP) is a virgin forest (IDNR-F 2016), having never been harvested for timber. WWNP is designated as a sweet-

Table 1.—Tree species occurring at six sites in Wesselman Woods Nature Preserve. The number of trees observed within each stratum across 30 plots (1.8 ha) is given for each species. * = species not native to Indiana.

Species name	Common name	Herb layer	Shrub layer	Mid-story	Over-story
Acer negundo	boxelder	141	69	4	5
Acer rubrum	red maple	124	0	2	59
Acer saccharum	sugar maple	2027	74	101	277
*Ailanthus altissima**	tree-of-heaven*	0	1	0	0
Asimina triloba	pawpaw	7472	8689	3841	145
Carpinus caroliniana	musclewood	111	9	6	53
Carya cordiformis	bitternut hickory	254	4	3	1
Carya glabra	pignut hickory	1	0	0	0
Carya ovalis	red hickory	1	0	0	0
Carya ovata	shagbark hickory	63	3	2	3
Carya tomentosa	mockernut hickory	2	1	1	1
Catalpa speciosa	northern catalpa	0	0	0	3
Celtis laevigata	southern hackberry	1199	118	4	52
Cercis canadensis	redbud	1	0	0	0
Cornus florida	flowering dogwood	3	1	0	2
Crataegus mollis	downy hawthorn	13	2	1	1
Fraxinus americana	white ash	229	58	6	6
Fraxinus pennsylvanica	green ash	1594	316	14	2
Fraxinus profunda	pumpkin ash	493	30	13	7
*Ilex aquifolium**	English holly*	4	1	0	0
Ilex decidua	possumhaw	3	0	2	0
Juglans nigra	black walnut	0	0	0	1
Liquidambar styraciflua	sweetgum	92	24	2	74
Liriodendron tulipifera	tulip poplar	74	0	1	31
Morus rubra	red mulberry	53	13	2	0
Nyssa sylvatica	blackgum	314	251	8	44
*Paulownia tomentosa**	empress tree*	0	1	0	0
Platanus occidentalis	sycamore	0	0	1	0
Populus deltoides	cottonwood	2	1	0	1
Prunus serotina	black cherry	635	132	1	0
Quercus alba	white oak	236	3	0	6
Quercus bicolor	swamp white oak	2	2	0	1
Quercus falcata	southern red oak	1	0	0	0
Quercus macrocarpa	bur oak	1	0	0	1
Quercus michauxii	swamp chestnut oak	2	0	0	3
Quercus muhlenbergii	chinkapin oak	3	0	0	0
Quercus pagoda	cherrybark oak	19	0	0	0
Quercus palustris	pin oak	3	0	0	1
Quercus prinus	rock chestnut oak	1	0	0	0
Quercus rubra	northern red oak	77	4	1	4
Quercus shumardii	Shumard oak	34	0	0	2
Quercus velutina	black oak	3	0	0	1
Sassafras albidum	sassafras	139	31	3	7
Ulmus americana	American elm	99	3	16	181
Ulmus rubra	slippery elm	147	2	1	13

gum-tulip tree wet mesic lowland forest (*sensu* Jackson 1980) because the forest is a wet, nearly flat lowland forest, with a canopy dominated by sweetgum (*Liquidambar styraciflua* L.) and tulip poplar (*Liriodendron tulipiferad* L.) (Lindsey et al. 1969). WWNP is unique in southwest Indiana because of its exceptional tree diversity and maturity; it is still "representative of Indiana's original ecological conditions prior to human settlement" (Lindsey et al. 1969). While WWNP has one of the highest basal areas of any known forest in the state, the 80 ha preserve is completely surrounded by the city of Evansville, and includes a nature center, one small parcel maintained in lawn, and two small parcels of secondary forest, as well as a trail system (WNS-NRC 2010). Drainage changes have occurred historically, but standing water still occurs in a patchwork of the forest throughout the wetter portions of the year.

While human activities noted above may have altered WWNP, evidence suggests that white-tailed deer (*Odocoileus virginianus)* may have greatly influenced this forest. In the 1990s, censuses estimated the population to be between 30 to 55 deer/km^2 (B. Fichter unpublished data; C.M. Norrick unpublished data; G. Hesselink unpublished data; Ribbens unpublished data). Still, the lack of forest regeneration at WWNP may have been a concern for at least 45 years. The dominant canopy species were already poorly represented in the sapling layer in the 1960s (Lindsey et al. 1969), deer populations have been high in Indiana for decades (IDNR-FW 2015), and deer hunting did not begin in the preserve until 1999 (N. Bogan Pers. Comm.).

Experimental design.—In the summer of 2015, five plots (20 $\times$ 30 m) nested within each of six sites were surveyed (1.8 ha in total). For each of the 30 plots, all individual trees in every forest stratum were identified to species and counted. For stems over 150 cm in height, diameter at breast height (dbh measured at 1.3 m) was recorded. Trees were placed into one of four forest strata based on height. The strata included the herb layer (stems under 50 cm in height), the shrub layer (stems ranging between 50–150 cm in height), the midstory (trees over 150 cm in height and with a dbh $<$ 5 cm), and the overstory (trees with a dbh $\geq$ 5 cm).

Analyses.—For each forest stratum within each plot, diversity was calculated using the Shannon-Weiner Diversity Index (H') (Brower et al. 1990). H' considers the proportion of the total that occurs for each species (evenness), as well as the number of species and total number of individuals (richness). To evaluate whether the four forest strata differed in diversity, a nested, random effects ANOVA was performed using plots as random samples nested within sites, which were treated as random blocks within the forest (JMP 2015). A Tukey post-hoc test was performed to determine differences in diversity among forest strata. Because high pawpaw abundance would create lower evenness (and thereby potentially generate lower H' values) solely due to its high relative abundance and not because of its effects on the other species, the same analysis was performed using a data set where all pawpaws were removed from the analysis. This second analysis evaluated whether diversity differed among strata, ignoring the contribution of pawpaws to richness and evenness.

The primary concern with this approach of evaluating H' is the assumption that H' should be constant across the forest layers in the absence of deer browsing. While other factors can influence tree species diversity, our assumption is conservative; more tree species can colonize as seedlings than can establish in the shrub layer, grow into the midstory stratum, and establish in the canopy. Using this perspective, H' should shift from the largest value to smaller and smaller values as one moves from the lowest strata to the highest one. In contrast, if deer have preferentially browsed certain tree species, H' will be lower in the strata affected by deer and higher in the canopy layer (where the community was established prior to the increase in deer abundance).

For each genus of tree, number of individuals was tallied across all plots (1.8 ha in total), and relative density was calculated for each stratum (Brower et al. 1990). In addition, dbhs were used to calculate basal area (m^2/ha) and relative basal area for the midstory stratum and the overstory. The relative importance of oak and maple abundance and basal area among strata was quantified by dividing the number (or basal area) of oaks by the total number (or basal area) of oaks and maples.

Finally, relative pawpaw abundance was compared across strata. For these comparisons, the shrub layer would be expected to include stems that have escaped browsing by deer just recently (a few years), while stems in the midstory would have grown taller than deer could browse many years ago, and trees in the overstory would have escaped the risk of browsing by deer at a much earlier time.

RESULTS

More than 40 native tree species were identified across the four strata (Table 1). Forty-one species were found in the herb layer, with an average of 13.7 species per plot. In the shrub layer, 27 species were found, with an average of only 5.3 species per plot. The midstory had a very low average of 3.5 species per plot, and only 24 species were identified. Finally, 31 species occurred in the overstory, with an average of 7.2 species per plot.

With pawpaws included in the analysis, the Shannon-Wiener Diversity Index (H') was 1.53 ± 0.07 ($\bar{X}$± SE) in the herb layer, 0.49 ± 0.08 in the shrub layer, 0.26 ± 0.04 in the midstory, and 1.43 ± 0.07 in the overstory. The statistical model was significant ($F_{32,87} = 9.2$; $P < 0.0001$), and significant differences were detected among the four forest strata ($F_{3,87} = 88.3$; $P < 0.0001$). While the overstory was not significantly different from the herb layer, both were significantly higher in diversity than either the shrub layer or the midstory layer (Fig. 1A).

With pawpaws excluded from the analysis, H' was 1.73 ± 0.06 in the herb layer and 1.15 ± 0.10 in the shrub layer. H' was 0.76 ± 0.10 in the midstory stratum and 1.37 ± 0.08 in the overstory. This statistical model was significant ($F_{32,87} = 3.8$; $P < 0.0001$), and significant differences were detected among the four forest strata ($F_{3,87} = 25.3$; $P < 0.0001$). The herb layer had significantly higher diversity than overstory and shrub layers, while both were significantly higher in diversity than the midstory layer (Fig. 1B). Thus, the midstory layer had significantly lower diversity compared to all other strata.

In the herb layer, 380 oaks (including individuals from 13 species) were found across the six areas sampled. Nineteen oak trees (of eight species) were found in the overstory. Still, just nine individual oak trees were observed in the shrub layer, and only a single oak tree was found in the midstory stratum (Table 2). This limited number of oak trees in the shrub layer and midstory stratum prevented statistical comparisons regarding the relative importance of oaks and maples. However, the pattern of their relative abundance is strongly suggestive. When considering the relative number of trees that were oaks (with only oaks and maples included in the calculations), 5% of trees in the overstory were oaks, 6% of trees in the shrub layer were oaks, and 14% of trees in the herb layer were oaks; however, only 1% of trees in the midstory was oaks, while 99% were maples. The comparison between oaks and maples is similarly striking when considering basal area. Oaks constituted only 1% of the basal area in the midstory, whereas maples comprised the other 99%. However, oaks made up 45% of basal area in the overstory, compared to maple, which comprised 55%.

Nearly two-thirds (20,150 of 30,548) of all stems surveyed were pawpaws (Table 2). Pawpaws were the most abundant species in every stratum except the overstory. In contrast, two other traditional sub-canopy specialists (flowering dogwood and redbud) had fewer than five individuals across all plots in all strata combined. Musclewood (*Carpinus caroliniana* Walter), another subcanopy specialist, was reasonably abundant, with 179 individuals across the four strata. Even musclewood, though, had more than 90% of its stems occur in the overstory or herb layer, not in the shrub and midstory strata.

Two genera beside pawpaw were well represented: maple and ash (Table 2). Maple (sugar maple, red maple, and boxelder) was the second most abundant genus, with 2,875 individuals. Sugar maples comprised 2,485 of those stems (Table 1). Ash (green, pumpkin, and white ash)

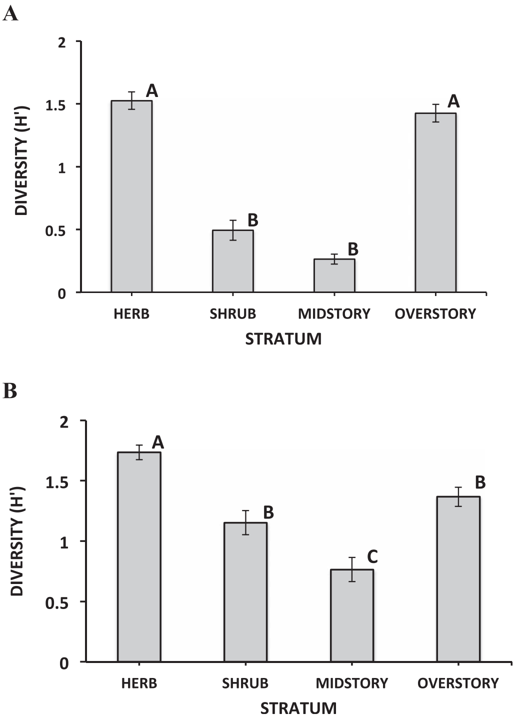

Figure 1.—Bar graph representing mean diversity (± SE) for four forest strata using the Shannon-Weiner Diversity Index (H'). A. When pawpaw was included in the estimate of diversity, and B. When pawpaw was excluded. Strata were designated as herb layer (trees of under 50 cm in height), the shrub layer (trees ranging between 50–150 cm in height), the midstory (trees over 150 cm in height and with a dbh < 5 cm), and the overstory (trees with a dbh ≥ 5 cm). Different letters designate significant differences among strata.

Table 2.—Summary information for genera that contributed to each forest stratum. Number represents the number of individuals within the sampled area of 1.8 ha (30 plots). Relative density was calculated for each species as a percentage of the total number of individuals of all species. Basal area was determined for each species as the cumulative area of all trees of a given species at breast height. Basal area was scaled to m^2/ha. Relative basal area was calculated for each species as a percentage of the total basal area of all species within a given stratum.

	Number				Rel. density				Basal area		Rel. basal area	
Genus	Herb layer	Shrub layer	Mid-story	Over-story	Herb layer	Shrub layer	Mid-story	Over-story	Mid-story	Over-story	Mid-story	Over-story
Asimina	7472	8689	3841	145	47.7%	88.3%	95.1%	14.7%	0.599	0.30	87.1%	0.8%
Acer	2292	143	107	341	14.6%	1.5%	2.6%	34.5%	0.057	6.06	8.2%	15.2%
Quercus	380	9	1	18	2.4%	0.1%	0.0%	1.8%	0.000	4.96	0.0%	12.4%
Liquidambar	92	24	2	74	0.6%	0.2%	0.0%	7.5%	0.001	14.59	0.1%	36.5%
Liriodendron	74	0	1	31	0.5%	0.0%	0.0%	3.1%	0.000	5.20	0.0%	13.0%
Nyssa	314	251	8	44	2.0%	2.6%	0.2%	4.5%	0.003	3.74	0.4%	9.3%
Ulmus	246	5	17	194	1.6%	0.1%	0.4%	19.7%	0.011	1.74	1.6%	4.4%
Fraxinus	2316	404	33	15	14.8%	4.1%	0.8%	1.5%	0.006	1.12	0.9%	2.8%
Celtis	1199	118	4	52	7.7%	1.2%	0.1%	5.3%	0.002	0.90	0.3%	2.3%
Carpinus	111	9	6	53	0.7%	0.1%	0.1%	5.4%	0.004	0.29	0.6%	0.7%
Carya	321	8	6	5	2.0%	0.1%	0.1%	0.5%	0.003	0.16	0.4%	0.4%
Sassafras	139	31	3	7	0.9%	0.3%	0.1%	0.7%	0.000	0.41	0.0%	1.0%
Prunus	635	132	1	0	4.1%	1.3%	0%	0%	0.000	0	0%	0%
Other	79	20	8	8	0.5%	0.2%	0.5%	0.8%	0.002	0.48	0.3%	1.2%

was the third most abundant genus, with 2,768 individuals. Green ash comprised 1,926 of those individuals. These three genera (pawpaw, maple, and ash) encompassed 84.5% of all recorded stems. Still the importance of species varied with forest strata. In the midstory stratum, pawpaws were nearly mono-dominant, with a striking 95% of stems being pawpaws (Table 2). Pawpaws were also extremely important in the shrub layer, at 88%. Pawpaws were less important in the herb layer, with fewer than 50% of stems being pawpaws. Only in the overstory were pawpaws, at 15%, not the most abundant tree.

Pawpaws comprised 87% of the basal area in the midstory (Table 2). In contrast, pawpaw contributed only 0.8% of the basal area to the overstory, even though it was third highest for abundance in the overstory. Only maples and elms were more abundant than pawpaw in the overstory. However, a wide variety of genera had greater basal area than pawpaws in the overstory, including sweetgum, maple, tulip poplar, oak, blackgum, elm, ash, hackberry, and musclewood.

In order of importance for basal area, the five genera that contributed most greatly to the overstory were sweetgum, maple, tulip poplar, oak, and blackgum. When maples were excluded, the remaining four genera comprised 71% of the total basal area in the overstory. Nevertheless, these four genera contributed only 0.5% of the basal area to the midstory. With regard to relative density, these four genera contributed only 5.5% to the herb layer, 2.3% to the shrub layer, and 0.2% to the midstory stratum. Clearly, changes in relative importance among genera across the lower strata have already begun to filter into the forest community found in the overstory.

DISCUSSION

Our findings suggest that deer may have prevented a broad spectrum of tree species from transitioning above the height at which browsing occurs and into the midstory stratum, thereby preventing regeneration into the overstory. These species include sweetgum, tulip poplar, blackgum, hackberry, and 12 species of oak. Five species of hickory were less commonly encountered in the survey, so their patterns across forest strata were less clear. Nevertheless, the perspective that deer negatively impact a wide range of canopy tree species, including hickory, is well supported (Rooney & Waller 2003; Rossell et al. 2005; Long et al. 2007; Wakeland & Swihart 2009).

The near absence of redbud trees and flowering dogwoods in this forest suggests that, in addition to the negative effects deer have on canopy species, browsing by deer may have limited the success of subcanopy tree species at WWNP. In a past survey of WWNP, Lindsey et al. (1969) noted that musclewood, redbud, and dogwood were common in the overstory, but were not regenerating in the herb layer. In that study, redbud and musclewood both contributed to basal area. In our current study, only mature musclewood trees contributed to basal area, while redbud and flowering dogwood have been all but lost from the forest. Indeed, only 15 musclewood stems occurred in the shrub and midstory strata, even though 53 musclewood trees were found in the overstory.

In contrast to the declines seen for most tree species, pawpaws and maples appear to have increased in abundance. Given that pawpaws comprised 88% of the stems in the shrub layer and 95% of the stems in the midstory stratum, this one species has had phenomenal success in regenerating. Historically, pawpaws were reasonably abundant at WWNP. In their survey, Lindsey et al. (1969) observed pawpaw to be common in the herb layer and very abundant in the shrub layer. However, they did not observe pawpaw as components of the midstory nor overstory. In contrast, we observed near mono-dominant status in the shrub and midstory strata. Slater & Anderson (2014) found a similar response to deer in an Illinois forest. In their case, the density of pawpaw stems nearly doubled in a five-year period, while the density of stems declined for seedlings/saplings of most other species. Given the recent and rapid increase in pawpaws, browsing by deer provides a convincing explanation for the increase in pawpaw abundance and decrease of other species.

Maples also appear to have benefitted from browsing pressure by deer, with maple being the most abundant genus in the overstory. When examined at a species level in the midstory, though, only sugar maple was an important contributor, while boxelder and red maple were not (Table 1). In the survey by Lindsey et al. (1969), sugar maple was only a modest component of trees in the overstory and contributed only 1.3% to basal area. However, sugar maple provided 7.6% of the basal area in the current study. Thus, the success of the maple genus was really due to the exceptional regeneration success of sugar maple over the last 40+ years. Sugar maple often experiences browsing only when more preferred species are no longer available, leading to it becoming more common in forests browsed by deer (Strole & Anderson 1992; Anderson & Katz 1993; Rooney & Waller 2003; Belden & Pallardy 2009).

While the patterns discussed suggest that deer may be an important influence on diversity at WWNP, other factors have also been tied to the decline in abundance of tree species. Species differences in shade tolerance can cause variation in regeneration success among tree species. For example, tulip poplar is viewed as a shade-intolerant species that requires large gaps or clearings for successful colonization (Orwig & Abrams 1994; Busing 1995; Kota et al. 2007). Similarly, shade intolerance has been argued to play a role in the failure of oak regeneration (Aldrich et al. 2005). Moreover, both pawpaw and sugar maple are considered to be shade tolerant species (Belden & Pallardy 2009; Slater and Anderson 2014). Fire suppression has also been suggested to reduce the regeneration of canopy dominants species in Eastern forests; Abrams & Nowacki (2008) stated that there exists "a direct link between Indian burning and the widespread distribution of mast species." In contrast to oak and hickory species, which would be favored by burning forests, sugar maple is favored in conditions of fire suppression.

This survey of WWNP was not experimental, so the observations generated in the study cannot discriminate among factors to determine which factor(s) caused the current patterns nor can this study demonstrate which influences are responsible for the changes since the survey by Lindsey et al. (1969). Nevertheless, many remnant forests (including WWNP) may be in situations where action is needed, even in the face of this uncertainty. One such action, deer culling, has been practiced at WWNP for more than a decade, based on the presumption that reducing the deer population would improve conditions for trees species other than sugar maples.

Given the putative shade barrier of pawpaw in the shrub and midstory layers, additional management may be needed beyond hunting deer. Pawpaw can be expected to affect the forest community by acting as a recalcitrant layer (Shotola et al. 1992; Shelton et al. 2014; Slater & Anderson 2014). This putative legacy from decades of intensive deer browsing may prevent forest regeneration from maintaining a highly diverse forest at WWNP. Still, this concern of barriers to regeneration is larger than just this one forest preserve. Given potential plant barriers to regeneration that have been observed for a variety of herbaceous plants and woody species across a range of forest habitats (Tighman 1989; Stromayer & Warren 1997; Goetsch et al. 2011; Tanentzap et al. 2009; Kain et al. 2011; Johnson et al. 2015), this legacy issue may be the primary problem to solve once deer overpopulation concerns have been addressed. The specific problem of pawpaw's expanding range (via sugar maple expansion and mesophication—*sensu* Abrams & Nowacki 2008), combined with continued high deer densities, may make pawpaw the most common recalcitrant layer in old growth forests (Slater & Anderson 2014).

Therefore, we suggest that management actions should take place, and that those actions should incorporate experimental methodology in order to confirm the impact that deer have, both directly through browsing and indirectly by creating a recalcitrant layer. Specifically, we suggest that experimental removal of pawpaw, coupled with protection of vulnerable seedling/sapling species, may be necessary to counter both overabundance due to decades of preferential browsing by deer and the current browsing pressure caused by deer. Such an experiment may also provide valuable information regarding the relative importance of: (a) current deer browsing, (b) constraints associated with canopy tree reestablishment due to pawpaw shading, and (c) the interaction between browsing by deer and shading by pawpaw.

If action is not taken, a wide range of alterations to the forest community can be expected, given the dramatic reduction in the number tree species and concomitant loss of canopy resources. Just from a vegetative structure perspective, deer have been noted to cause the reduction in bird density and diversity by simplifying the understory (Martin et al. 2010; Chollet et al. 2015). However, the greater effect may come from the reduction in tree species. Reduction in oak species, for example, can alter community dynamics in several ways. First, oaks provide resources for 500+ insect species (Marquis & Wheelan 1994; Tallamy 2007), while maples act as host to little more than half that number. Second, shelter-building caterpillars on oaks enhance species richness of other invertebrates (Lill & Marquis 2003). In addition, because abundance and diversity of arthropods is greater on oak trees, greater oak abundance may provide more food resources for more bird species. Still, population and community dynamics of insectivorous birds in response to oak abundance has remained unexamined. Third, leaf litter composition in woodland ponds can influence amphibian success (Rubbo & Kiesecker 2004). Both frog and salamander species had greater survival when reared in a system that used oak leaf litter versus maple leaf litter. Finally, acorns act as a food resource for many mammals, and acorn production can influence mammalian population growth and density (Jones et al. 1998). Thus, deer may reduce trophic complexity of forest communities by altering community structure and composition through selective herbivory (Rooney & Waller 2003), and the effect of deer may be even more exacerbated by their indirect suppression of tree reestablishment if they indirectly create recalcitrant layers.

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PRAIRIE RECONSTRUCTION IN INDIANA: HISTORICAL HIGHLIGHTS AND OUTCOMES

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ABSTRACT. Prairie reconstruction or restoration in Indiana dates at least to 1987 with a demonstration planting at Butler University in Indianapolis. A brief account of this and other tallgrass prairie reconstruction efforts by the Indiana Department of Natural Resources, Taylor University, Newport Chemical Depot, and The Nature Conservancy during the period of 1990 and early 2000 are described. These projects document the rationale behind reconstructing prairies and changes in practices relating to seed mixes. In order to provide an overview of the status and success of Indiana prairie reconstructions, 23 were sampled via a Floristic Quality Assessment (FQA) protocol during the period 2005–2012. Four native prairies were also sampled for comparison. The results indicate that, thanks to the increased availability of more affordable forb rich seed mixes, recent reconstructions may achieve a much higher floristic quality. In fact, certain FQA metrics for some recent prairie reconstructions rival those of native prairies. Species richness per quadrat, however, is always lower in reconstructed prairies. Furthermore, conservative and even some less conservative species are consistently lacking in reconstructed prairies. A resampling of three sites after a lapse of 4 to 5 years showed steady to increasing FQA metrics. The experience in Indiana suggests that restoring and sustaining a tallgrass prairie landscape is possible to a degree, though the efforts are expensive and intensive. Furthermore, planted prairies, as with native prairies, can be vulnerable to repurposing of land.

Keywords: Prairie reconstruction, prairie restoration, Indiana history, floristic quality assessment, restoration flora

INTRODUCTION

Prairie reconstruction and prairie restoration are relatively young ecological disciplines (Packard & Mutel 1997). Reconstructing a prairie most often seeks to establish a prairie planting on former agricultural land, while prairie restoration more narrowly refers to renewing a remnant natural ecosystem that has been taken over to some degree by another plant community (IPN 2017). In response to the soil losses of the Dust Bowl, Aldo Leopold and Norman Fassett, curator of the University of Wisconsin at Madison Arboretum, transformed 11 ha of abandoned pastureland into the world's first prairie reconstruction in 1934 (Pauly 2008). Although this initial effort used sod transplantation from remnant prairies, John Curtis, then a University of Wisconsin graduate student, encouraged direct sowing of seed (Cottam & Wilson 1966; Wegener et al. 2008) that quickly became the accepted method of prairie reconstruction.

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During the 1950s and 1960s Paul Sheppard, George Ward, and later Peter Schramm at Knox College (northwestern Illinois) further developed prairie reconstruction techniques. Schramm would not only champion the return of fire to the prairies but also left his mark through the number and quality of prairie reconstructions he nurtured (Schramm 1970, 1978; Geer et al. 1997).

Prairie reconstruction comes to Indiana.—Indiana's first prairie reconstruction was planted on land that historically was located in the eastern deciduous forest. In 1987, the Holcomb Research Institute, housed at Butler University, selected a site next to newly developed athletic fields in an attempt to display a low maintenance alternative to turf grass (Rebecca Dolan, interview, August 10, 2010; see Appendix A for list of interviewees). Intended to serve as a prairie demonstration more than an actual reconstruction, the proposed prairie was divided into two sections, tall grass and mixed grass, and was planted using seed from Wisconsin. Although there was some concern about using Wisconsin genotype seed, the Institute had no other option. At this time, no vendors in

Indiana produced local seed and the amount of seed required made hand collection unrealistic (Rebecca Dolan, interview, August 10, 2010).

The first sizeable reconstruction in Indiana was at Stoutsburg Savanna in Jasper County. The site supported a rare black oak sand savanna interrupted by swaths of weedy fallow ground (Tom Post, interview, September 17, 2010). When the Indiana Department of Natural Resources (INDR) decided to restore the site in 1990, they called upon Peter Schramm. By this time, Schramm had become one of the most prolific prairie restorationists in the Midwest, planting 25 prairies a year using regional species and genotypes (Tom Post, interview, September 17, 2010; Schramm 1992).

The goal of the Stoutsburg Savanna was unique in the 1990s. In response to the Conservation Reserve Program in 1985, many farmers had started to plant their highly erodible lands with prairie warm season grasses (Schramm 1992). These plantings had few if any prairie forbs and were isolated from remnant prairies, often by many miles. In contrast, the prairie reconstructions of Stoutsburg Savanna were directly adjacent to existing remnant natural areas for the purpose of providing a buffer between the savanna and neighboring agricultural land.

The prairie reconstruction concept had spread sufficiently by 1993 that the earliest homeowner installations began. The oldest may be a 0.8 ha site planted by Phyllis Schwitzer (north of Bloomington, Monroe County, Indiana). The seed mix, from a Wisconsin source, was rich in tall grasses (*Andropogon gerardii* and *Sorghastrum nutans*), but contained over 15 forb species. The planting continues to thrive, especially thanks to the recent use of grass specific herbicide to reduce the dominance by tall grasses.

In 1993, Avis Industrial with assistance from Taylor University in Upland commissioned their own Schramm planting. Planned by Edwin Squiers and Paul Rothrock of Taylor University and Leland Boren of Avis Industrial Corporation, it was an isolated reconstruction planted for both academic and aesthetic purposes. The interior of the planting was dominated by tall grasses with a few forbs, but the edges of the prairie were planted in a dense forb mix (in excess of 40 species) in hopes that the beauty of the prairie flowers could be seen from passing autos (Rothrock & Squiers 2003). In practice, about ten forb species became strongly established and another 25 have persisted somewhere on the 10 ha site.

In the same year, the United States Army participated in prairie reconstruction in Indiana. Phil Cox, the Natural Resource Administrator at Newport Chemical Depot realized that the Depot property still contained a few remnant prairie species (Greninger 2010; Philip Cox, interview, August 25, 2010). In 1993, he met with John Bacone, IDNR Director of the Division of Nature Preserves, and Roger Hedge, an ecologist with the Indiana Natural Heritage Program. As a result, in 1994 the IDNR drafted a report that encouraged the reconstruction of 770 ha of leased agricultural land within the Depot's boundaries. The Mason and Hanger Corporation, the independent contractor responsible for the Depot, hired Peter Schramm to plant a preliminary 3 ha prairie. During the next 10 years (1994–2005) Schramm and Cox expanded the reconstruction to 135 ha, at a cost of $125,000 for seed and maintenance (Phil Cox, interview, August 25, 2010).

For a time the Newport Chemical Depot Prairie became the largest contiguous prairie reconstruction in Indiana. The Depot's lands, including the prairie, were passed to the Newport Chemical Depot Reuse Authority after the Depot's official close in July of 2010. Although plans call for 51% of the complex to remain as "natural areas and open space" (NECDRA 2010), the fertile soils of the prairie reconstruction, further improved and enriched by a prairie cover, could be leased to local farmers and plowed. Indeed, much of this reconstructed prairie acreage has returned to row crop agriculture.

The role of restoration nurseries.—Before the 1990s, no nurseries in Indiana produced prairie seed. Those concerned with importation of nonnative genotypes were forced to collect seed by hand from the scattered remnant prairies (Tom Post, interview, September 17, 2010; Rebecca Dolan, interview, August 10, 2010). This changed in 1994, when the first of three native plant nurseries began producing local genotype seed in bulk.

J.F. New & Associates, Inc. (now Cardno), a major environmental consulting firm in Indiana, founded in 1989, established its native seed nursery in 1994 that originally focused on wetland mitigation seed (Chris Kline, interview, February 6, 2011). Heartland Restoration Services, founded by Eric Ellingson, likewise initially raised seed for wetland mitigation before expanding its operation in 1997 to include prairie species. A year later, Doug Spence and Kevin Tungesvick followed suit and opened Spence Restoration

Nursery in 1998 (Eric Ummel, interview, February 6, 2011; Kevin Tungesvick, interview, September 17, 2010).

Since the late 1990s, the demand for local seed from the non-restoration community increased rapidly. Residential and commercial landscaping companies, private homeowners, and even golf courses, began requesting local genotype seed (Eric Ummel, interview, February 6, 2011). Cardno, Heartland, and Spence, working collaboratively with the ecological community to educate the public on the importance and benefits of local genotypes, were the major force behind this increased demand for local seed. It became a matter of preserving Indiana—not solely its landscape, but its ecological genetics as well (Alan Galbreth, interview, February 8, 2010).

Reconstruction at Kankakee Sands.—In 1996, The Nature Conservancy (TNC) identified three major natural areas in Newton County, Indiana: Conrad Savanna Nature Preserve (a 327 ha black oak sand savanna owned and managed by TNC and the IDNR), Beaver Lake Nature Preserve (a 260 ha IDNR property initially known as the Beaver Lake Prairie Chicken Refuge), and the Willow Slough Fish and Wildlife Area (a IDNR property approximately 4,050 ha) (National Audubon Society 2011). It was an already established postulate of conservation biology that larger populations were more likely to retain ecological integrity (e.g., Wilcox & Murphy 1985; Menges 1991; Noss & Cooperrider 1994). TNC sought to connect the properties to reduce potential problems associated with fragmentation (Chip O'Leary, interview, September 22, 2010). In 1997, TNC purchased 2900 ha from Prudential Insurance for $11 million (Chip O'Leary, interview, September 22, 2010; Ney & Nichols 2010). It became the largest prairie reconstruction effort in the TNC's history with total IDNR and TNC land holdings exceeding 8500 ha (Lucas 2005; Applied Ecological Services 2011).

The Kankakee Sands Restoration Project, headed by Chip O'Leary, initially used hand-collected seed from local remnant prairies, but they soon realized the project was too large to rely on the amount of native seed available. Committed to using local genotype, the reconstruction project established the Kankakee Sands Seed Nursery, an operation that would eventually grow to a 50 ha complex that could produce enough seed to plant 200 ha per year using 130 different species (Chip O'Leary, interview September 22, 2010; Applied Ecological Services 2011).

During the first three years, Kankakee Sands was planted using the traditional tallgrass-heavy seed mix as seen in the Schramm reconstructions and the Butler University site. However, five years into the project, they used a forb-rich mix with only short grasses and continued this practice for the remainder of the reconstruction. They removed *Andropogon gerardii* and *Sorghastrum nutans* entirely from the new seed mixes (Chip O'Leary, interview, September 22, 2010), a seed mix strategy that has now become routine for better quality reconstruction efforts (Kevin Tungesvick, interview, September 17, 2010).

By the early twenty-first century, prairie reconstruction as a concept was firmly established in Indiana as demonstrated by the increased use of native prairie species by landscape architects and homeowners. However, what has been the level of "success" of the reconstruction efforts to date? And what lessons and strategies can be gleaned from the first generation of prairie reconstruction efforts? Intermittently since 2005, we have sought to visit significant reconstructions and apply a standard assessment protocol. The remainder of this paper, summarizes the Floristic Quality Assessment (FQA) of 19 properties, makes comparisons between these and several extant prairie remnants, and records, to the extent possible, planting and management regimes.

METHODS

Twenty-seven prairies (Table 1) were selected for quality sampling across Indiana, including four remnant prairies: Hoosier Prairie, Biesecker Prairie, German Methodist Cemetery, and Smith Cemetery (a degraded remnant recovering from a history of mowing). Reconstructions were located in five of Indiana's ten terrestrial natural regions (Table 1, Homoya et al. 1985), but most were from the Grand Prairie and Central Till Plain Natural Regions. Aside from the prairie reconstructions at Butler University and Christy Woods, the reconstructions utilized land that had experienced recent row crop agriculture. Twelve sites were sampled during July and August of 2005 to 2007. Eight sites were sampled in July and August of 2010, in addition to resampling three previously sampled sites. A final two sites were sampled in 2011 and 2012 (Fig. 1; Table 2).

A total of 39 transects were sampled across all the prairies (Table 2). Twelve sites were sampled using multiple transects based upon site area and

Table 1.—Site characteristics for prairie sampled. With the exception of sites at Butler University and Christy Woods, the reconstructions had recent history of row crop agriculture. Reconstructions marked # buffer adjacent oak savanna. Natural Regions (Homoya et al. 1985) with sample sites were: CTP = Central Till Plain; GP = Grand Prairie; NL = Northern Lakes; NW = Northwestern Moraine; SL = Southwest Lowlands. Those indicated with * were in regions that historically supported extensive oak savanna and prairie communities. The date of planting for certain sites is indicated by a range. This may be a result of uncertainty of the exact year of planting as efforts occurred over several years. Seed mix type refers to the dominance of tall grasses compared to the content of forbs. Due to the mixed nature of seeding at some sites, these labels are approximate.

Site Characteristics				
Site transect	Type	Natural region	Date of planting	Seed mix type
Avis Prairie	Reconstruction	CTP	1993	Tall grass heavy
Biesecker Prairie	Remnant	NM*	NA	NA
Butler University Prairie	Reconstruction	CTP	1987	Tall grass heavy
Christy Woods Prairie	Reconstruction	CTP	1996/extension in 2001	High forbs
Cooper Farm Prairie	Reconstruction	CTP	2002/2003	High forbs
Fisher Oak Savanna	Reconstruction #	GP*	2005	High forbs
German Methodist	Remnant	NM*	NA	NA
Goose Pond 1	Reconstruction	SL	2002	Tall grass heavy
Goose Pond 2	Reconstruction	SL	2002	Tall grass heavy
Hoosier Prairie South Block	Remnant	NM*	NA	NA
Kankakee Sands Dry	Reconstruction	GP*	1999–2003	High forbs
Kankakee Sands Mesic	Reconstruction	GP*	1999–2003	High forbs
Loblolly Prairie	Reconstruction	CTP	1997	Tall grass heavy
Ludwig Prairie	Reconstruction	NL	east in 2000/ west in 2003	High forbs
Merry Lea–Luckey Prairie	Reconstruction	NL	2004	High forbs
Merry Lea–REA Prairie	Reconstruction	NL	2006	High forbs
Newport Chemical Depot	Reconstruction	GP*	post-2000	High forbs
Prairie Border	Reconstruction #	GP*	2005	Tall grass heavy
Prophetstown State Park Bluestem	Reconstruction	CTP-GP*	1998	Tall grass heavy
Prophetstown State Park Farm	Reconstruction	CTP-GP*	2000	Tall grass heavy
Red Tail Nature Preserve	Reconstruction	CTP	1999	Tall grass heavy
Ritchey Woods	Reconstruction	CTP	2001	Tall grass heavy
Smith Cemetery	Remnant (mowing stopped in 1981)	GP*	NA	NA
Stoutsburg Savanna	Reconstruction #	GP*	1991–1995	Tall grass heavy
Taltree Arboretum	Reconstruction	NW*	2000	High forbs
Wapihani Nature Preserve	Reconstruction	CTP	2006	Tall grass heavy
Weiler-Leopold Nature Reserve	Reconstruction	CTP-GP*	1999	Tall grass heavy

notable floristic differences present. In placing transects, areas were selected that seemed representative of overall site quality while avoiding edges and areas unsuitable for successful seedling establishment. Given the scale of the Kankakee Sands project two older plantings were selected that represented distinct moisture regimes. Aside from several urban sites (Ritchey Woods and Wapihani Nature Preserve), each of the reconstructions has had a regular fire management program. For most sites, twenty 0.25 m^2 quadrats, spaced 5 m apart, were sampled along linear 100 m transects. Small sites required parallel 50 m transects. GPS coordinates were recorded for the start and end of transects. For Avis Prairie, data from previous studies (Rothrock & Squiers 2003) were used. These quadrats were from random points along several 15 m transects. For Newport Chemical Depot, direct physical sampling was not possible. Instead a series of high resolution photographs were studied, each of which imaged an area of about 1 m^2 (see below).

Species and their cover were recorded for each quadrat. The Floristic Quality Assessment Com-

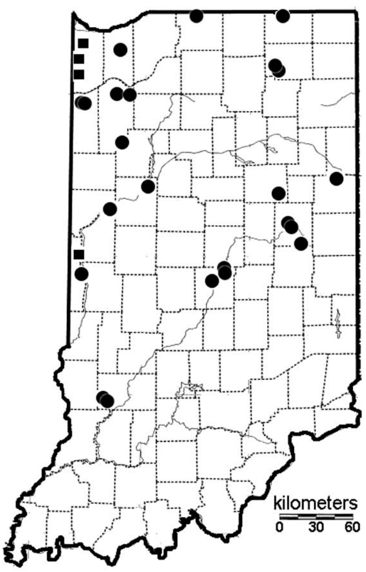

Figure 1.—Map showing locations of remnant prairies (square symbols) and reconstructed prairies (round symbols) used in this study.

puter Program, Version 1.0, was used to calculate mean C values (MC) and native species richness (NR) (Wilhelm & Masters 2000). FQA methodology was originally developed for the Chicago Region, as a standardized, repeatable means of evaluating the quality of a natural area (Swink & Wilhelm 1994), but has found success in evaluation of constructed ecosystems (McIndoe et al. 2008, DeBoer et al. 2011). Care, however, must be taken to consider both MC and NR and to watch for anomalous situations. Metrics were calculated on both transect and quadrat levels. Transect level metrics are based upon the overall checklist of species observed in the 20 sampled quadrats. Quadrat level metrics are the result of calculating FQA metrics for each quadrat and then calculating their mean. As a result, quadrat level analyses are weighted by species frequency. Since Newport Chemical FQA relied upon a slightly larger quadrat size and static images, comparisons will of necessity be tentative, e.g., metrics involving species richness are more tentative than MC and quadrat level more tentative than transect level.

MC and NR were graphed individually from highest to lowest to illustrate the gradient of quality among the sites. The sites were classified using four quality categories: 1) high remnant quality, 2) degraded remnant quality/high quality reconstruction, 3) low quality reconstruction, and 4) poor quality reconstruction. High remnant quality benchmarks were based upon transects from native prairies that lacked obvious degradation (Biesecker, Hoosier, and German Methodist Cemetery). The low quality reconstructions benchmarks were based upon comparison with old field transects (Rothrock et al. 2011). A third benchmark line (that delineates moderate and high quality reconstructions) was positioned midway between the other two lines.

Transects from three prairie reconstructions with high FQA metrics were sampled twice over a 5-year period. These sites included Kankakee Sands, Fisher Oak Savanna, and Ludwig Prairie. The two-tailed t-test was used to determine whether significant changes occurred over that time interval.

RESULTS AND DISCUSSION

The floristic quality of Indiana restored prairies is broad, ranging from conditions similar to an old field (e.g., Ritchey Woods), at the low end, to displaying attributes of a remnant native prairie (e.g., Fisher Oak Savanna). The majority of reconstructions contain ten species, what might be called a tall grass prairie reconstruction flora. The species include grasses such as *Andropogon gerardii, Elymus canadensis, Schizachyrium scoparium,* and *Sorghastrum nutans* and forbs such as *Eryngium yuccifolium, Monarda fistulosa, Parthenium integrifolium, Ratibida pinnata, Rudbeckia hirta,* and *Solidago rigida*. *Silphium* spp. (such as *S. laciniatum*), *Coreopsis tripteris,* and *Symphyotrichum novae-angliae* are also common. The reconstruction flora of more recent installations, such as Fisher Oak Savanna, has little *Andropogon gerardii* and instead may be dominated by grasses such as *Elymus canadensis, E. virginicus,* and *Schizachyrium scoparium*. The reconstruction flora includes some species with high C-values such as *Coreopsis tripteris, Eryngium yuccifolium, Parthenium integrifolium,* and *Silphium* spp. At the same time reconstructed prairies lack a suite of conservative species seen at our reference sites – *Amorpha canescens, Ceanothus americanus, Comandra umbellata, Lithospermum canescens,* and, with few exceptions, *Symphyotrichum oolentangiense*. Surprisingly even some less conservative species (e.g., *Euphorbia corollata* and *Rosa carolina*) were not observed in any of the reconstructions.

Sites with the highest FQA metrics, transect MC in particular, include Fisher Oak Savanna, Kankakee Sands, and Merry Lea. Newport

Table 2.—GPS coordinates recorded for the sites sampled and the year(s) in which the sites were sampled. Those marked with * were later approximated using Google Earth 7.1.5.1557.

GPS Coordinates and Years of Sampling			
Site transect	Beginning	Ending	Year
Avis Prairie Block 2 *	40.453N 85.492W		2005
Avis Prairie Block 3 *	40.453N 85.493W		2005
Biesecker Prairie	41.42039N 87.46778W	41.41982N 87.46866W	2010
Butler University Prairie	39.83990N 86.17533W	39.83966N 86.17641W	2010
Christy Woods Prairie	40.19804N 85.41582W	40.19928N 85.41643W	2010
Christy Woods Prairie (cont.)	40.19917N 85.41572W	40.19917N 85.41573W	2010
Cooper Farm Prairie	40.22729N 85.45512W	40.22812N 85.45506W	2010
Fisher Oak Savanna North	40.84314N 87.04276W	40.84299N 87.04390W	2006/2010
Fisher Oak Savanna South	40.84243N 87.04327W	40.84240N 87.04440W	2006/2010
German Methodist Cemetery	41.34874N 87.46850W	41.34862N 87.46794W	2005
Goose Pond 1	38.96503N 87.14607W	38.96600N 87.14626W	2010
Goose Pond 2	38.99817N 87.20781W	38.99725N 87.20766W	2010
Hoosier Prairie South Block *	41.52171N 87.45315W		2006
Kankakee Sands Dry East	41.08863N 87.41570W	41.08777N 87.41559W	2005/2010
Kankakee Sands Dry West	41.08801N 87.41685W	41.08717N 87.41678W	2005/2010
Kankakee Sands Mesic North	41.10273N 87.43069W	41.10268N 87.43177W	2005/2010
Kankakee Sands Mesic South	41.10203N 87.43051W	41.10189N 87.43163W	2005/2010
Loblolly Prairie *	40.55694N 85.03167W		2010
Ludwig Prairie East	41.74434N 85.88902W	41.74429N 85.89018W	2005/2010
Ludwig Prairie West	41.74441N 85.89138W	41.74435N 85.89250W	2005/2010
Merry Lea–Luckey Prairie North	41.32916N 85.52903W	41.32995N 85.52924W	2010
Merry Lea–Luckey Prairie South	41.32845N 85.52938W	41.32771N 85.52876W	2010
Merry Lea–REA Prairie	41.33854N 85.54662W	41.33722N 85.54645W	2010
Newport Chemical 1 *	39.844N 87.466W	39.844N 87.467W	2011
Newport Chemical 2 *	39.832N 87.475W	39.833N 87.475W	2011
Prairie Border East	41.17798N 86.96605W	41.17706N 86.96598W	2005
Prairie Border West	41.17797N 86.96762W	41.17712N 86.96753W	2005
Prophetstown State Park Bluestem	40.50898N 86.81464W	40.50944N 86.81464W	2007
Prophetstown SP Bluestem (cont.)	40.50865N 86.81448W	40.50906N 86.81445W	2007
Prophetstown State Park Farm	40.49953N 86.82057W	40.49955N 86.83061W	2007
Red Tail Nature Preserve East	40.09871N 85.30035W	40.09961N 85.30055W	2005
Red Tail Nature Preserve West	40.09898N 85.30173W	40.09983N 85.30190W	2005
Ritchey Woods near Entry	39.93880N 86.03394W	39.93966N 86.03403W	2007
Ritchey Woods near Parking Lot	39.93871N 86.03511W	39.93955N 86.03533W	2007
Smith Cemetery	40.02636N 87.45115W	40.02634N 87.45167W	2005
Smith Cemetery (cont.)	40.02633N 87.45109W	40.02619N 87.45165W	2005
Stoutsburg Savanna East	41.17368N 87.09058W	41.17371N 87.09153W	2005
Stoutsburg Savanna West	41.17348N 87.09562W	41.17344N 87.09675W	2005
Taltree Arboretum 1	41.44415N 87.14970W	41.44330N 87.14957W	2012
Taltree Arboretum 2	41.44038N 87.14742W	41.44043N 87.14854W	2012
Wapihani Nature Preserve	39.95360N 86.06495W	39.95352N 86.06386W	2010
Weiler-Leopold Nature Reserve	40.35889N 87.11625W	40.35825N 87.11710W	2010

Chemical Depot is also among these high quality reconstruction sites. MC [native + non-native species] for these sites ranged from 3.9 to 5.2 (Fig. 2), similar or perhaps even slightly exceeding that of three native prairies. Sites with a high quadrat level MC (Fig. 3) include those listed above as well as Taltree Arboretum and Ludwig Prairie. Their quadrat MC ranged from 4.2 to 5.2; all were planted since 2000. Their seed mixes were rich in forb species and deleted or minimized the content of aggressive tall grass species such as *Andropogon gerardii,* a strategy shown to enhance species diversity in prairie plantings (Dickson & Busby 2009). The grass species abundant in some of these mixes, *Elymus canadensis*, acts as a

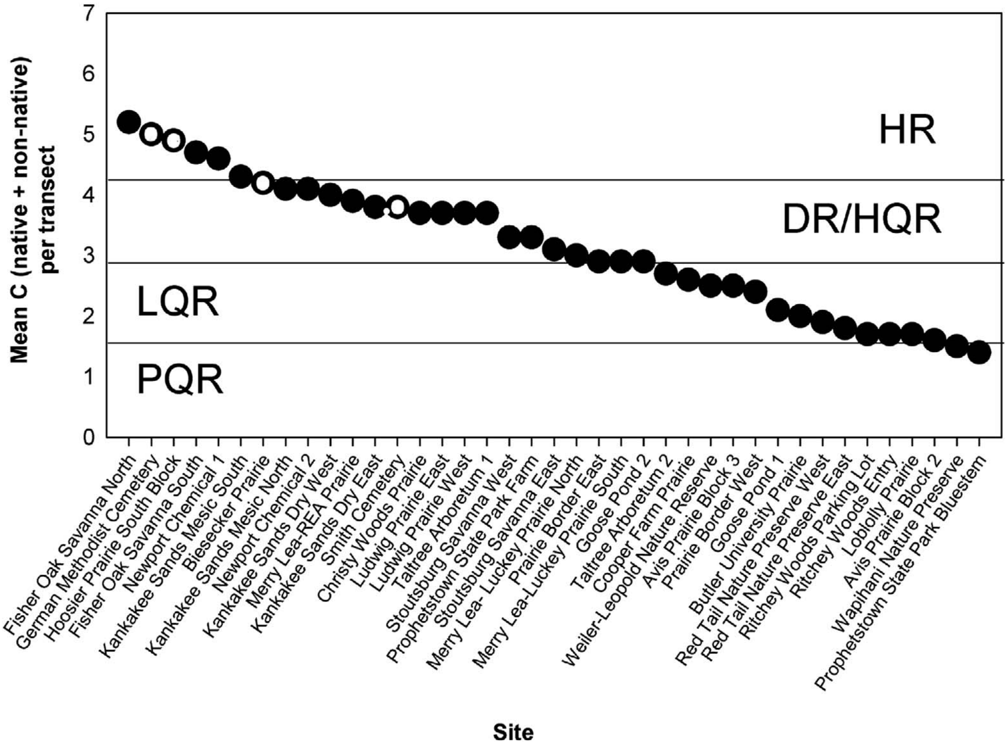

Figure 2.—Transect level mean C (MC) for four remnant prairies (open circles) and 35 transects in reconstructed prairies (solid circles) in Indiana. Prairies with high remnant quality had a transect MC $\geq$ 4.2. The reconstructed prairie at Fisher Oak Savanna and a portion of Newport Chemical Depot and Kankakee Sands (mesic) attained these levels. HR = high remnant quality, DR/HQR = degraded remnant/high quality reconstruction, LQR = low quality reconstruction, and PQR = poor quality reconstruction.

"nurse" species and gradually diminishes in abundance over the first five years of prairie development.

Another strategy that contributed to the high performance of some recent reconstructions is to purge the seed bank of agricultural weeds. Before planting Fisher Oak Savanna the land was prepared using Round-up Ready™ soybeans and applications of glyphosate (Sue Ulrich, interview, August 24, 2010).

Sites with very low MC [transect level, native + non-native] include Prophetstown State Park, Avis Prairie, and Loblolly Prairie, in addition to Ritchey Woods (Fig. 2). These sites have MC ranging from 1.4 to 1.7. Quadrat level results included the same list of sites and the western portion of the Red Tail Conservancy Prairie (Fig. 3). The sites with low FQA metrics frequently shared two attributes. First is their being located on Indiana's Central Till Plain (CTP) outside of the historic prairie and oak savanna region (Table 1). It is difficult to ascertain the importance of location, but one should note that the CTP region tends to have finer silt-clay soils, and soils devoid of prairie mycorrhizae, that may be less conducive to support diverse prairie species. But probably of much greater importance is that these poor to low quality reconstruction sites were planted during the 1990s and, while they support dense cover of native prairie species, they are dominated by tall grasses *Andropogon gerardii*, *Panicum virgatum*, and/or *Sorghastrum nutans*. The interspecific competition with tall grasses reduces forb density at the quadrat level and the resulting MC. Several sites, though, have low MC due to the presence of non-native species (Red Tail Prairie) or an abundance of early successional old field species (Ritchey Woods).

Over time plantings with dominant tall grasses experience a decline in forb species richness (McIndoe et al. 2008), further exacerbating the low species richness of seed mixes used for planting of early reconstructions (or reconstructions with a limited budget). Reconstructions

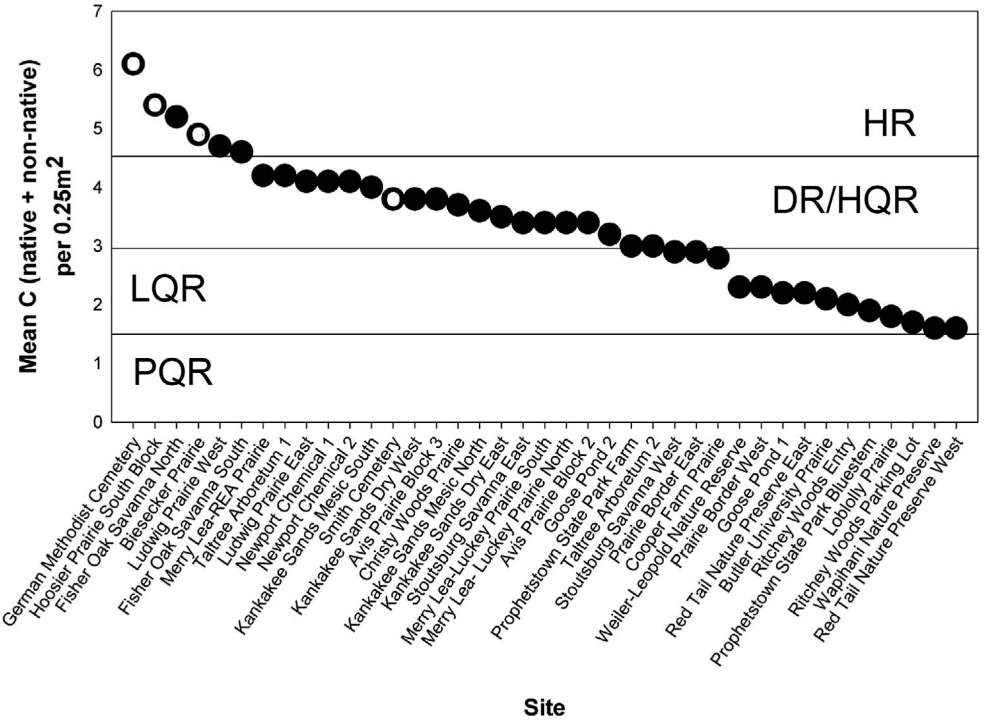

Figure 3.—Quadrat level mean C (MC) for four remnant prairies (open circles) and 35 transects in reconstructed prairies (solid circles) in Indiana. Quadrats were 0.25 m^2 in size. Prairies with high remnant quality had a quadrat MC $\geq$ 4.5. The reconstructed prairie at Fisher Oak Savanna and a portion of Ludwig Prairie attained these levels. HR = high remnant quality, DR/HQR = degraded remnant/high quality reconstruction, LQR = low quality reconstruction, and PQR = poor quality reconstruction.

from the 1990s typically had native species richness (NR) of 2 to 6 species per 0.25 m^2 quadrat and 8 to 24 species per transect (Figs. 4 & 5). Portions of Avis Prairie have particularly low NR since local genotype seed, at the time, had to be hand collected and was costly for large scale plantings. Looking to the future, then, the concern is how to introduce more forbs into these older reconstructions, a question that has become a focus of on-going research (Menges 2008).

If early prairie reconstructions are characterized by lower NR, the more recent efforts do have the highest observed (Fig. 4 & 5). For example, Kankakee Sands and Fisher Oak Savanna as well as portions of Taltree Arboretum, all planted since 2000, have a range of 36 to 44 native species per transect (Fig. 4) and a mean of 8 to 9.4 species per 0.25 m^2 (Fig. 5). However, species richness of the three least disturbed native prairies is notably higher at both scales. These native prairies ranged from 46 to 61 species per transect; they had a mean of 10 to 12.3 species per 0.25 m^2 quadrat.

Peter Schramm installed prairies in Indiana over the period from 1992 to about 2005. His plantings include Stoutsburg Savanna, Avis Prairie, Newport Chemical Depot, and Taltree Arboretum. The seed mix for early plantings included a generous amount of tall grasses. In part this was due to the belief that tall grasses were needed to out compete non-prairie species as well the expense and difficulty of acquiring hand gathered forb seed. The floristic quality of his more recent plantings is clearly higher. Taltree Arboretum (planted in 1996–2000) has 8.5 species per quadrat and 4.2 MC, compared to 5.9 species and 3.4 MC for the best transect at Stoutsburg Savanna. Another recent Schramm planting was at Newport Chemical. The FQA for this site, as noted in the Methods, had to be estimated from photos since physical sampling was not possible. With this limitation in mind, though, this site apparently had a higher MC, especially at the transect level (MC native + non-native = 4.1–4.7), than his earlier efforts (MC = 3.1–3.3).

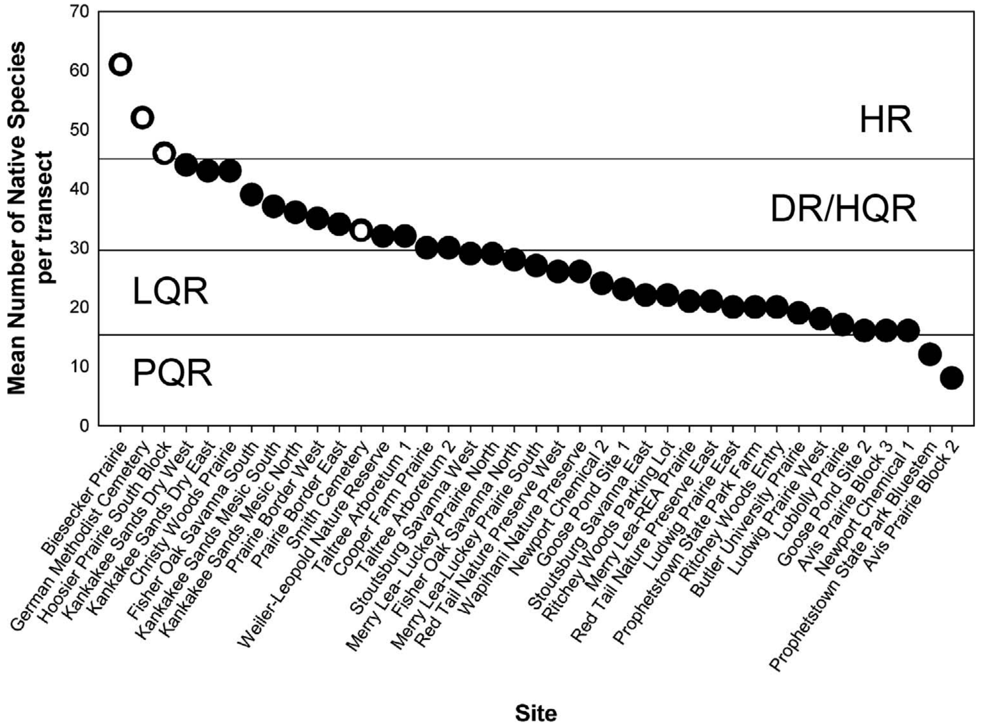

Figure 4.—Transect level native species richness (NR) for four remnant prairies (open circles) and 35 transects in reconstructed prairies (solid circles) in Indiana. Prairies with high remnant quality had 46 or more species per transect. High quality reconstructed prairies reached to 44 species per transect. HR = high remnant quality, DR/HQR = degraded remnant/high quality reconstruction, LQR = low quality reconstruction, and PQR = poor quality reconstruction.

Change in site quality over time.—Three of the higher quality reconstructed prairies were sampled twice over a 4- to 5-year period, in order to evaluate the sustainability of floristic quality. Transects at Kankakee Sands and Fisher Oak Savanna not only performed at relatively high quality for reconstructions, but also maintained their quality as measured by quadrat MC on both transect and quadrat levels.

Fisher Oak Savanna transects and Kankakee Sands Dry transects showed no significant change in quadrat MC ($p > 0.05$) (Table 3). On the other hand, both Kankakee Sands Mesic transects actually showed improvement of quality. In five years, the mean C value for the Mesic North transect increased from 3.10 ± 0.80 to 3.60 ± 0.80 ($p = 0.034$). Kankakee Sands Mesic South increased similarly from 3.50 ± 0.70 to 4.0 ± 0.5 ($p = 0.022$).

Ludwig Prairie, which performed only at a low level in the initial sampling, showed an increase in quality over a five year period (Table 3). At the time of the first sampling Ludwig prairie was 3–5 years of age. The east field, planted in 2000, increased from 3.40 ± 1.00 at the quadrat level to 4.10 ± 0.90 ($p = 0.028$). The west field, planted in 2003, similarly increased in mean C values from 2.90 ± 0.70 to 4.70 ± 0.60 ($p < 0.001$).

Conclusion.—In comparison to the FQA of remnant native prairies and old fields, Indiana prairie reconstructions encompassed a broad quality spectrum: from near remnant quality to low and poor quality. The highest quality transects were clearly those planted since 2000 indicating that important lessons have been learned through our early prairie reconstruction pioneers. The MC of about half of transects sampled fell into the high quality reconstruction level and about 30% also had excellent NR per transect. Thus, human effort over the past 35 years has produced some noteworthy reconstructed prairies. These results should be encouraging news as we seek to rebuild ecosystem function. At the same time,

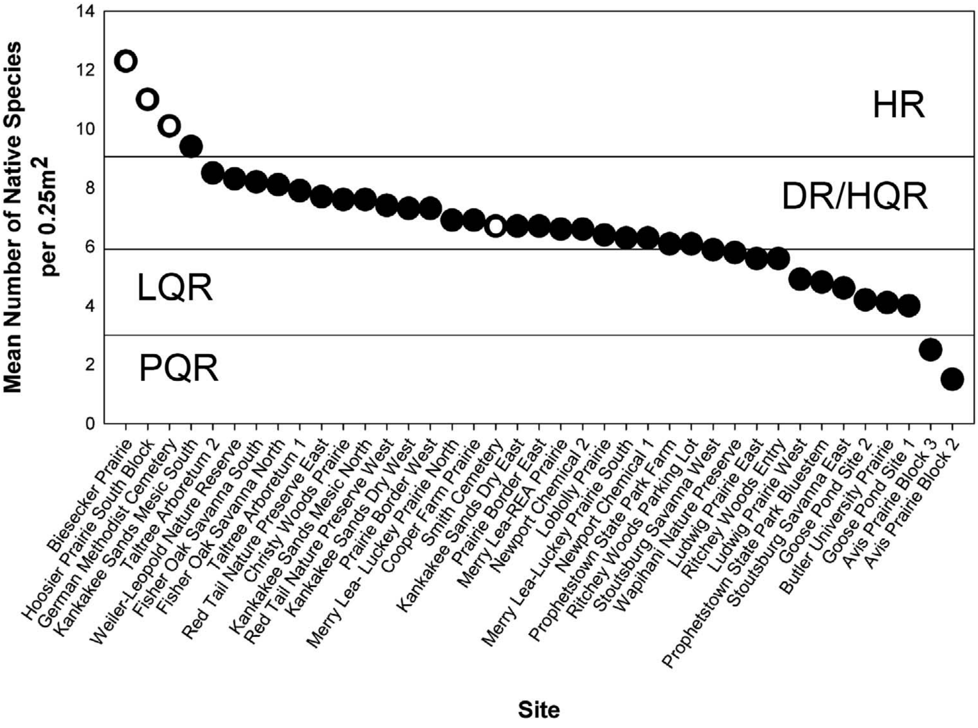

Figure 5.—Quadrat level native species richness (NR) for four remnant prairies (open circles) and 35 transects in reconstructed prairies (solid circles) in Indiana. Quadrats were 0.25 m^2 in size. Prairies with high remnant quality had 10 or more species per quadrat, a level achieved by only one reconstructed prairie. HR = high remnant quality, DR/HQR = degraded remnant/high quality reconstruction, LQR = low quality reconstruction, and PQR = poor quality reconstruction.

Table 3.—Two-tailed t-test results comparing 2005-6 to 2010 mean C. Transects were from Fisher Oak Savanna, Kankakee Sands, and Ludwig Prairies. * means significant at 95% confidence; ** means significant at 99% confidence; df = degrees of freedom.

Fisher Oak Savanna					
Site transect	Mean C 2006	Mean C 2010	t-value	df	*P*-value
Fisher Oak – North	4.80 ± 1.40	5.20 ± 0.80	1.13	38	0.266
Fisher Oak – South	4.30 ± 1.40	4.60 ± 0.90	1.00	38	0.326
Kankakee Sands Transects					
Site transect	Mean C 2005	Mean C 2010	t-value	df	*P*-value
Dry East	3.90 ± 1.10	3.50 ± 1.00	0.98	38	0.333
Dry West	3.40 ± 1.10	3.80 ± 1.10	0.98	38	0.332
Mesic North	3.10 ± 0.80	3.60 ± 0.80	2.20	38	0.034*
Mesic South	3.50 ± 0.70	4.00 ± 0.50	2.38	38	0.022*
Ludwig Prairie Transects					
Site transect	Mean C 2005	Mean C 2010	t-value	df	*P*-value
East	3.40 ± 1.00	4.10 ± 0.90	2.29	38	0.028*
West	2.90 ± 0.70	4.70 ± 0.80	7.53	38	0.000**

what is not regained needs communicating as well. Old-growth grasslands, as described by Veldman et al. (2015), have a mature species composition, endemic species of plants and animals, and high small scale species richness lacking in restored ecosystems. Our floristic assessment supports their observation even for projects that were intense and well-funded.

APPENDIX A.—List of cited interviewees and their positions. A more detailed history of Indiana prairie conservation and reconstruction efforts and additional interviewees is available through the corresponding author or at Pruitt (2011).

Phillip Cox
Former Natural Resources Administrator
Newport Chemical Depot
Vermillion County, IN

Rebecca Dolan
Friesner Herbarium Director
Butler University
Indianapolis, IN

Alan Galbreth
Associate Executive Director
Indiana Crop Improvement Association
Lafayette, IN

Chris Kline
Central Region Director
JFNew
Walkerton, IN

Chip O'Leary
Kankakee Sands Project Director
The Nature Conservancy
Newton County, IN

Tom Post
Northwest Region Ecologist
The Division of Nature Preserves
Medaryville, IN

Kevin Tungesvick
Restoration Ecologist
Spence Restoration Nursery
Muncie, IN

Sue Ulrich
Former President
NICHES Land Trust
West Lafayette, IN

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RESULTS OF THE 2015 HILLS OF GOLD BIODIVERSITY SURVEY, JOHNSON COUNTY, INDIANA

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Linda Cole: Brown County Indiana Naturalist, 635 Oak Run Drive, Nashville, IN 47448 USA

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Jeffrey D. Holland: Department of Entomology, Purdue University, West Lafayette, IN 47907 USA

Marc Milne: Department of Biology, University of Indianapolis, Indianapolis, IN 46227 USA

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ABSTRACT. The Central Indiana Land Trust, Inc. (CILTI) and the Indiana Academy of Science (IAS) hosted a biodiversity survey or bioblitz within the Hills of Gold Conservation Area, Johnson County, Indiana, on 16th and 17th May 2015. The 280 ha (695 acres) bioblitz area incorporated the Laura Hare Preserve at Blossom Hollow to the west, Glacier's End Nature Preserve to the northeast, and a conservation easement connecting the two. Over 65 scientists, naturalists, students, and other volunteers on 13 different taxonomic teams observed and reported 548 taxa during the event. The thirteen taxonomic teams included bats, beetles, birds, fish, freshwater mussels, herpetofauna, small mammals, moths and singing insects, mushrooms/fungi and slime molds, non-vascular plants (mosses), snail-killing flies, spiders, and vascular plants. Three state endangered species were reported, i.e., northern long-eared bat (*Myotis septentrionalis*), Indiana bat (*Myotis sodalis*), and the timid sedge (*Carex timida*). In addition, many state and Johnson County records were reported. This manuscript presents both a brief history of the Hills of Gold Conservation Area and a summary overview of the results. Detailed results are available on the IAS website.

Keywords: Bioblitz, biodiversity survey, Hills of Gold, Blossom Hollow Nature Preserve, Glacier's End Nature Preserve, state endangered, county records, CILTI

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INTRODUCTION

The Indiana Academy of Science's 2015 biodiversity survey or bioblitz was held within the Hills of Gold Conservation Area, as identified by the Central Indiana Land Trust, Inc. (CILTI), Johnson County, Indiana (Fig. 1). The Hills of Gold area is where the last ice sheet from the Wisconsinan Glaciation met the Brown County Hills. Within the site one can find exposed bedrock capped with glacial till, flat bottom streambeds with shale, and chunks of granite. In addition, because the soil of the glacial influenced area is richer and less acidic than unglaciated hills to the south, there is an interesting mix of plants. See the Geology Report by Robert Autio, LPG, Environmental Data & Consulting, LLC, and James Nowacki, LPG, Kayak Lake Tree Farms, for additional details (Hills of Gold Bioblitz Report 2016).

The 280 ha (695 acres) bioblitz area incorporated the Laura Hare Preserve at Blossom Hollow to the west, Glacier's End Nature Preserve to the northeast, and a conservation easement connecting the two (Fig. 2). These areas have always been in private property and, as a result, little historical information concerning the flora and fauna is available. CILTI prides itself on a science-based approach to conservation, and its partnering with the Indiana Academy of Science to sponsor the bioblitz has provided it with considerable data to use in land management and future land protection efforts in the Hills of Gold Conservation Area.

The biodiversity survey in the Hills of Gold Conservation Area was conducted on 16th and 17th May 2015. The event, with over 65 scientists, naturalists, students, and others volunteers, proved an overwhelming success and revealed the area's significant species richness and inherent natural value. This manuscript provides a brief history of the Hills of Gold Conservation Area and a summary of the biodiversity results. For additional details see the Hills of Gold Bioblitz Report (2016).

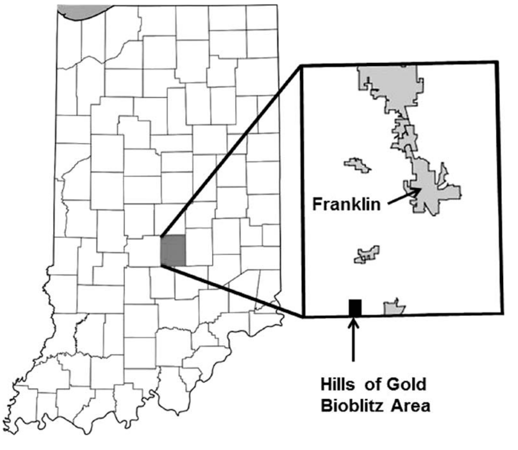

Figure 1.—Location of Johnson County within Indiana (left) and the Hills of Gold Bioblitz Area within Johnson County (right).

BRIEF HISTORY OF THE HILLS OF GOLD CONSERVATION AREA

The area known as Hills of Gold is part of the Brown County Hills extending northward into Johnson County. In fact, they extend farther north than can be easily recognized today, since the northern extension is covered by glacial till. Although Indiana has experienced several ice ages over the millennia, the last one, the Wisconsinan Glaciation, covered the northernmost Brown County Hills around Trafalgar in southern Johnson County. Those last ice sheets that covered much of Indiana extended south of the Hills of Gold area to the east and the west, but the Brown County Hills acted as a cleave that parted the massive ice sheet. In fact, the northern boundary of Glacier's End Nature Preserve is where the ice stopped (Fig. 2).

But that doesn't mean this area is without glacial influence. Loess from a pre-Wisconsin glacial period is found on some ridge tops. While there is exposed Mississippian shale in the creek valleys, glacial erratics, in the form of granite and quartzite chunks, are strewn throughout the area. Weathering has created a mix of soils in the area, some places glacially influenced but others not. This mixture of soils with different pH's supports different plant communities melded together under what appears to be a canopy of homogeneous forest.

Hills of Gold gets its name from another of the glacial erratics once found here. Gold and diamonds are sometimes found in a line across Indiana where the glaciers stopped. Gold was found as small flakes in areas just like Blossom Hollow and Glacier's End Nature preserves in the 19th century and are probably present in minute quantities today.

The portion of the Hills of Gold Conservation Area studied in the 2015 bioblitz is protected as a result of the Central Indiana Land Trust, Inc. (CILTI) and two families. One hundred and thirty-three years after the first Europeans settled near the area in 1825, three Hougham brothers began purchasing land with a dream of developing a lake and residential area in the Blossom and Pitcher Creek drainage. Robert (Bob) Hougham, the son of one of those brothers, continued purchasing and holding land for this purpose. In 1957, Dr. Russell Lamb joined forces with Bob Hougham to develop a larger lake encompassing more of the Pitcher Creek and Indian Creek valley. The two developers also entered into agreements to enable the dam building with several local families, including the Pitchers after whom Pitcher Creek is named.

The first dam was built in 1962 across Callon Hollow, to the west of the bioblitz area. The expense of building this dam precluded work on the main dam, so that preliminary 15 ha (37 acres) lake was sold to Earlham College for a Biological Station. Bob Hougham and Russell Lamb were then able to recruit financial supporters, form a corporation, and build the dam for Lamb Lake in 1966. About 300 families now own homes around and near the lake, said to be the largest privately owned lake in the state.

Much of the shoreline of Lamb Lake has since been developed into a residential area. The bioblitz area, south and east of the lake, was reserved by the lake-builders for future development. However, over time, the Hougham and Lamb families recognized the value of undeveloped and protected land and both families were drawn to natural areas and realized the singular beauty and natural value of the lands they each owned. It was these hundreds of acres of forest that caught the attention of CILTI.

The Central Indiana Land Trust, Inc., formed in 1990, seeks to protect the region's best remaining natural areas. This volunteer-driven organization protected several sites through gifts of land until 1998 when it purchased Burnett Woods Nature Preserve in Hendricks County by writing its first grant to the Indiana Heritage Trust and pooling dozens of small donations. After hiring its first Executive Director in 2001, CILTI began purchasing land on a regular basis, first concentrating on lands along the White River north of Indianapolis utilizing temporary funds specific to that purpose. Also, through the use Indiana Heritage Trust funds as the lead gift, several other land protection projects were successful. However, the board of directors felt this "shot-gun" approach was problematic.

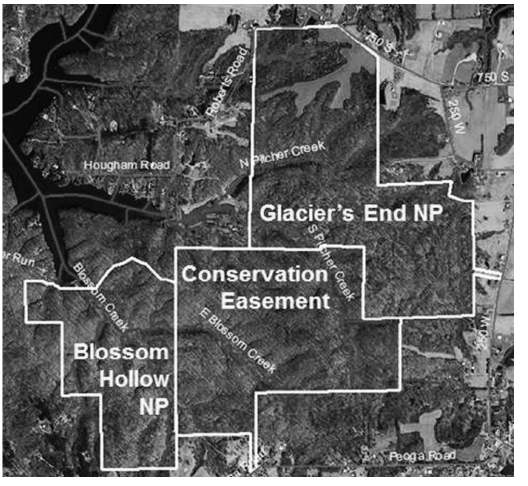

Figure 2.—The 280 ha bioblitz area included Blossom Hollow Nature Preserve to the west, Glacier's End Nature Preserve to the northeast, and a conservation easement connecting the two.

A 2008 strategic conservation plan sought to identify where the best remaining natural areas were as well as where rare and endangered species of plants and animals could still be found. The plan of the Land Trust sought to protect sustainable communities of species.

The Hills of Gold was one of the areas identified in the strategic conservation plan. Field work in 2008 and 2009 revealed large populations of rare birds, such as Worm-eating Warbler, Hooded Warbler, and sightings of the elusive Cerulean Warbler. Beyond those rare birds, forest interior species like Red-eyed Vireo, Acadian Flycatcher, Ovenbird, and Wood Thrush were found in high numbers. Additionally, yearling, juvenile and adult Eastern Box Turtles were found to be commonplace through the area, a sign of a functioning ecosystem with minimal edge effects. The field work was done while the strategic conservation plan was being created. It was finished and adopted by the CILTI Board of Directors in the summer of 2009.

At the same time, Bob Hougham's sons and their families were searching for a way to protect a large portion of their forest holdings into the future. The family attended a CILTI-sponsored workshop on Conservation Easements and within a year, they entered into discussion with CILTI about the details of an easement and the appropriate management of the forest. By December 2009, the first project in the Hills of Gold, the 100 ha (246 acre) Bob's Woods Conservation Easement, was closed.

While working on the Bob's Woods Conservation Easement, the Land Trust staff was introduced to the other family that owned land in the Hills of Gold south of Lamb Lake and began building a relationship with them. The first walk-through of their land occurred in November of 2009, and in 2011, CILTI signed a purchase agreement for 44 ha (109 acres) that became the Laura Hare Preserve at Blossom Hollow; the deal was closed in 2012.

CILTI's plan had designated the Hills of Gold Area one of 13 priority areas in Central Indiana. Although negotiations continued with both families owning land in the area and willing to sell, there wasn't enough cash available. Fundraising for the conservation of 280 ha (700 acres) would have taken many years and been challenging.

With Indiana's Bicentennial on the horizon, outgoing Governor Mitch Daniels decided to make a conservation impact by allocating $20,000,000 into a newly created Bicentennial Nature Trust (BNT) in 2013. The Lilly Endowment added another $10,000,000. The idea was to celebrate the Bicentennial by protecting important natural areas, parks, and trails as a gift to future generations. CILTI adjusted its organizational priorities to leverage the one to one matching dollars of the BNT.

The owners of what is now called Glacier's End Nature Preserve signed a purchase agreement with CILTI for 82 ha (203 acres) in 2014 and an additional 39 ha in 2015. During the same period, the owners of 16 ha (40 acres) south of Blossom Hollow (same owners as Bob's Woods Conservation Easement) signed a purchase agreement with CILTI. By 2016 CILTI completed its largest fundraising campaign in its history and had completed five projects totaling $2,399,000 protecting 280 ha (695 acres) of contiguous habitat.

The 2015 bioblitz took place while most of this land was still in private ownership. This inventory of resources was timely in preparation for management decisions affecting the new preserve.

SUMMARY OF RESULTS AND METHODS

The Hills of Gold bioblitz attracted over 65 scientists, naturalists, students, and others volunteers. Thirteen taxonomic teams and their leaders reported 548 taxa (Table 1). To obtain a complete picture of the biodiversity found at Hills of Gold, long-term seasonal surveys are necessary. This two-day survey provided an initial "snapshot in time" and has revealed the remarkable species richness of this area. An overview of the results from the thirteen taxonomic teams follows. To view the complete results, visit the Indiana Academy of Science website at *http://www.indianaacademyofscience.org/*, lay the cursor over Events at the top of the page and then click BioBlitz Archive.

Bats.—Two survey sites were established in Blossom Hollow Nature Preserve, one on Upper Pitcher Creek and the other in Blossom Hollow. At each site, two double-high mistnet sets and one single high mistnet were deployed. Nets were open for $\sim$ 4 h, beginning at dusk and were checked for bats every 10 min. Captured bats were banded and sex, age, reproductive condition, forearm length (mm), mass (g), and wing damage score for assessing effects of white-nose syndrome (WNS; scores range from 0 to 3) was recorded. A 0.38 gram radio transmitter was attached to an Indiana bat, using non-toxic surgical glue to adhere the transmitter to the skin between the bat's scapulae.

Because it rained steadily for most of the day leading up to a few hours before dusk, only two bats were captured. It is remarkable that both captures were federally protected species, however. One male northern long-eared bat (*Myotis septentrionalis*) was captured, with a healthy weight (7 grams) and no significant wing damage (score = 0), at the Upper Pitcher Creek site. The northern long-eared bat was recently listed as a federally threatened species due to large-scale population declines from the WNS epidemic. One pregnant adult female Indiana bat (*Myotis sodalis*) was captured at the Blossom Hollow site. The Indiana bat is a federally endangered species and also has experienced significant population declines as a result of WNS. This bat was a healthy weight (8.5 g), but had significant wing damage due to WNS (score=2). Using a radio transmitter, the Indiana bat was tracked to two roost trees on nearby private lands over the week following the Bioblitz. She roosted in a large diameter (79.4 cm) cottonwood (*Populus deltoides*) snag (3–5 bats emerged) and a large diameter (60 cm) shagbark hickory (*Carya ovata*) with a snapped off top (24 bats emerged).

Beetles (Coleoptera).—Beetles were collected through the day by sweep-netting vegetation, examining flowers, and hand collecting under

Table 1.—Summary of the 548 taxa reported at the 2015 Hills of Gold Biodiversity Survey, Johnson County, Indiana.

Team	Team leader	Number of taxa and notes
Bats	Joy O'Keefe	Two bats, both endangered; one pregnant adult female Indiana Bat and one adult male Northern Long-eared Bat
Beetles	Jeffrey Holland	17 taxa, 16 species, none of special interest; due to rainy conditions, the list compiled represents a miniscule fraction of the species of the Hills of Gold Area
Birds	Kirk Roth	86 species; 17 migrant species
Fish	Brant Fisher	Three species from one family; no state/federal endangered or special concern species
Freshwater Mussels	Brant Fisher	Evidence (weathered dead shell material) of one species, Paper Pondshell; low diversity expected
Herpetofauna	Bob Brodman	22 species: 16 amphibians and six reptiles; one species of special concern in Indiana; one special protected species in Indiana; four pond-breeding species represent Johnson County records
Mammals	John Whitaker Jr. and Angie Chamberlain	14 taxa. Eight species of mammals were trapped. Four are relatively common, but the other four are relatively uncommon. Of the latter, the Woodland Vole and Southern Bog Lemming are found throughout much of the state. The Pygmy Shrew and Smoky Shrew are found only in forest in the unglaciated hill country of south central Indiana. In addition we had evidence of six other species. They were not caught in traps, but we had evidence of the Eastern Mole (numerous burrows), Gray Squirrel (several observed), Chipmunk (two observed), Southern Flying Squirrel (a dead one observed by staff), Coyote (feces observed), and the White-tailed Deer (numerous tracks).
Moths, etc.	Carl A. Strang	26 taxa total: 20 moth species, two singing insect taxa, and four additional arthropods taxa; none unexpected or particularly uncommon; all moths and singing insects appear to represent county records due to little attention given to Johnson County in the past
Mushrooms	Steve Russell	34 fungal taxa: 31 mushrooms, two plant pathogens, one slime mold; due to the dry weather conditions prior to the bioblitz, the majority of mushrooms were wood rot fungi
Non-vascular Plants	Linda Cole	30 species; species recorded illustrates a healthy biodiversity of a mature mesic woodland environment; 23 potential Johnson County records, two potential state records
Snail-killing Flies	William Murphy	Five species from the subfamily Tetanocerini; two Johnson County records, *Dictya expansa* and *Sepedon pusilla*, the latter rare in Indiana
Spiders	Marc Milne	39 taxa, 33 species; five new distribution records for Indiana; two undescribed species; assemblage of spiders here is diverse, understudied, and unique
Vascular Plants	Donald Ruch	269 species; one state endangered, 4 on the state watch list; 113 potential Johnson Co. records; 31 sedges, 13 ferns, and three orchids

rocks and bark. This was not effective because of the rainy weather. In the evening on Saturday of the survey, a 1000 W metal halide light, a 175 W mercury vapor light and two small UV lights were used to attract and catch beetles on a ridge in the hardwood forests of the conservation easement. This yielded a similarly low number of species because of the constant conditions of drizzle to light rain.

A low number and diversity of beetles were captured (17 taxa, 16 species), undoubtedly because of the rainy conditions. No species of special interest were caught. While some specimens were winnowed from the saturated vegetation or found under bark, the list compiled represents a miniscule fraction of the species that actually are present at the Hills of Gold site. Representatives of all species collected have been accessioned into the Purdue Entomological Research Collection in the Department of Entomology, Purdue University, West Lafayette, Indiana.

Birds.—A total of 86 species, including 18 migrant species, were detected, although ten of these were outside the property boundaries, especially on the trail leading toward the Blossom Hollow Preserve from its parking lot. Several of these species were associated with the large lake adjacent to the trail, such as Canada Goose, Wood Duck, and Great Blue Heron.

Glacier's End Nature Preserve (GENP) had the most species (67) and most individual birds (382) detected, but also had the most acreage and most time spent by researchers. A total of 160 individual birds of 46 species was detected on Blossom Hollow Nature Preserve (BHNP), while in the Conservation Easement (CE) 224 individuals of only 34 species were detected. The differences in diversity and individual bird counts could be influenced by habitat type – the BHNP had more edge habitat to promote diversity, while the CE had a larger tract of forest. This view is supported by the differences in detected birds for each area. BHNP had several species of successional, generalist, or edge specialist species that were not detected in the CE, including Red-shouldered Hawk, Mourning Dove, Least Flycatcher, Warbling Vireo, Eastern Towhee, Song Sparrow, and American Goldfinch (Castrale et al. 1998). The CE had much higher numbers of several mature forest specialists compared to the BHNP, such as Red-eyed Vireo (39 vs. 8), Wood Thrush (22 vs. 5), Worm-eating Warbler (10 vs. 3), Kentucky Warbler (9 vs. 1) and Scarlet Tanager (13 vs. 6).

Twenty-five species occurred in all three sites, including Acadian Flycatcher, Eastern Wood-pewee, Ovenbird, Red-eyed Vireo, Scarlet Tanager, Tennessee Warbler, and Wood Thrush. The three species with the highest number of individuals were Red-eyed Vireo (81), Tennessee Warbler (68), and the Acadian Flycatcher (45).

Fish.—Seining was used to sample the fish diversity present on Hills of Gold properties. Collected fish were identified in the field and returned to the area in which they were collected. The three sites sampled were North Pitcher Creek near the west boundary of Glacier's End Nature Preserve, a pond just south of the intersection of Roberts Road and CR 300W, and Blossom Creek near the north boundary of Blossom Hollow Nature Preserve.

Only three species of fish representing one family were recorded. No state/federal endangered or special concern fish species were collected. The three species, Green Sunfish (*Lepomis cyanellus*), Bluegill (*Lepomis macrochirus*), and Largemouth Bass (*Micropterus salmoides*), are common statewide in a variety of aquatic habitats and are highly tolerant of a wide range of environmental conditions. Green Sunfish was the only fish species collected from the two lotic sites sampled (North Pitcher Creek and Blossom Creek). These streams are intermittent and likely completely dry up each year. The pond sampled at the northern boundary of Glacier's End Nature Preserve contained the two most common pond species (Bluegill and Largemouth Bass) and were likely stocked after the pond's construction.

Freshwater mussels.—Freshwater mussels were sampled using haphazard sampling techniques. Sections of streams and pond located on the properties were visually searched for live freshwater mussels and shell material. Sampling occurred at the same three sites where fish were sampled.

Evidence of only one species of freshwater mussel, Paper Pondshell (*Utterbackia imbecillis*), was found. Paper Pondshell is one of the most tolerant species of freshwater mussels in the state, and can be found statewide in a variety of aquatic habitats. Only weathered dead shell material was collected and only from the pond at the northern boundary of Glacier's End Nature Preserve; it may not still be living at the location. The low freshwater mussel diversity found is expected considering the intermittent nature of the streams.

Figure 3.—The herp team in action. (Photo by Bob Brodman)

Herpetofauna.—Amphibians and reptiles were surveyed by a combination of methods. Terrestrial and wetland habitats were sampled by visual searches and sample cover objects. Calling frogs were identified, and wetlands were sampled for larvae by use of dip nets (Fig. 3). Turtles and larval amphibians were also sampled by use of 10 turtle traps and 21 minnow traps in ponds.

The herpetofauna team found a total of 285 herps of 22 species, including ten reptiles representing six species and 275 amphibians representing 16 species. Blanchard's Cricket Frog (*Acris blanchardi*) is a species of special concern in Indiana having declined throughout the northern half of its geographic range during the last two to three decades. *Acris blanchardi* was common at the pond in the northwest part of Glacier's End. Eastern Box Turtle (*Terrepene carolina*) is a special protected species in Indiana and some were found in Blossom Hollow and the southern part of Glacier's End. Many of the amphibians including Long-tailed Salamander (*Eurycea longicauda*), Southern Two-lined Salamander (*E. cirrigera*), and Slimy Salamander (*Plethodon glutinosis*), were common under cover objects. Four pond-breeding species (Jefferson Salamander (*Ambystoma jeffersonianum*), Spotted Salamander (*A. maculatum*), Wood Frog (*Lithobates sylvaticus*), and Cope's Gray Treefrog (*Hyla chrysosclis*) represent new Johnson County records.

Voucher specimens of *Lithobates sylvaticus* were deposited at the Indiana State Museum. Voucher specimens for *Ambystoma jeffersonianum* (SJCZC A401), *A. maculatum* (SJCZC A402) and *Hyla chrysoscelis* (SJCZC A403) were deposited in the Saint Joseph's College zoological collection in Rensselaer, Indiana. All other species were documented and vouchered by images and retained by Robert Brodman.

Mammals.—Eight species of mammals were trapped. Four are relatively common, but the other four are relatively uncommon. The common species included Masked Shrew (*Sorex cinereus*), Northern Short-tailed Shrew (*Blarina brevicauda*), Prairie Vole (*Microtus ochrogaster*), and the White-footed Mouse (*Peromyscus leucopus*). Of the four uncommon species, the Woodland Vole (*Microtus pinetorum*) and Southern Bog Lemming (*Synaptomys cooperi*) are occasional throughout much of the state. The Pygmy Shrew (*Sorex hoyi*) and Smoky Shrew (*Sorex fumeus*) are found only in forests in the unglaciated hill country of south central Indiana. In addition to these eight species, evidence of six other mammal species was found. Numerous burrows of the Eastern Mole (*Scalopus aquaticus*) were observed, several Gray Squirrels (*Sciurus carolinensis*) and two Chipmunks (*Tamias striatus*) were seen, and one dead Southern Flying Squirrel (*Glaucomys volans*) was seen by a staff member, feces from a Coyote (*Canis latrans*) was observed, and numerous tracks of the White-tailed Deer (*Odocoileus virginianus*) were seen.

Moths, singing insects, and other non-target organisms.—During the day, a transect was walked from the top of the ridge at the southern end of Glacier's End back to the bioblitz headquarters on the north edge of the property. Frequent side excursions were made into the forest and along streams. Singing insects were identified from their songs, moth species flushed into flight were collected, and other invertebrates photographed. In the evening a white sheet with a UV light was set up near the Purdue beetle team's lights on a forested ridge top in the central portion of Blossom Hollow, but facing a different downslope to the east. For ~ 3 hours moths were collected and/or photographed, and some specimens were exchanged with the Purdue group.

Two taxa of singing insects, 20 species of moths, and four additional arthropod taxa were identified in this portion of the bioblitz study. All were identified to species except one singing insect and one additional arthropod; these taxa were identified to genus. None of the species were unexpected or particularly uncommon, though all the moths and singing insects appear to represent county records thanks to little attention given to Johnson County in the past.

Species observed and/or collected are all well within their range, and none are regarded as rare, threatened, or endangered in general references or on the Indiana state lists. Northern Wood Crickets (*Gryllus vernalis*) were scattered thinly in the forest, in low areas not far from streams, as well as high ridge areas. The survey area is just north of the established range boundary for the Southern Wood Cricket (*G. fultoni*), but that species does not appear to have shifted north at this location. Though the Northern Wood Cricket has been found in surrounding counties, this appears to be the first observation of the species in Johnson County. A Shieldback Katydid nymph collected by the Purdue team could be either the Protean Shieldback (*Atlanticus testaceus*) or the Least Shieldback (*A. monticola*). Neither species is listed for Johnson County in the database for the Singing Insects of North America website (*http://entomology.ifas.ufl.edu/walker/buzz/*), the comprehensive source.

The most commonly observed moths in the forest during the day were the Three-spotted Fillip (*Heterophleps triguttaria*) and the Unadorned Carpet (*Hydrelia inornata*). The most common moths to come to the light were the Yellowhorn (*Colocasia flavicornis*), as well as several individuals each of the Porcelain Gray (*Protoboarmia porcelaria*) and the Friendly Probole (*Probole amicaria*). Otherwise, all observed insects were represented by only one or two individuals, mostly in the elevated portion of the forest where the light station was located. According to the assembled records of Mississippi State University's Moth Photographer's Group website (*http://mothphotographersgroup.msstate.edu/*), all of the moth observations represent Johnson County records, but this is more a comment on the lack of attention to Johnson County in the past than it is on the distribution and abundance of these species. All collected specimens were transferred to the Purdue University collection at the West Lafayette campus.

Mushrooms, fungi and slime molds.—Due to the dry weather conditions prior to the bioblitz, the majority of mushrooms were lignicolous (wood rot) fungi, although a number of fleshy species were observed. A total of 34 fungal taxa were listed, including 31 species of mushrooms, two species of plant pathogenic fungi, and one slime mold. All species reported are common and widespread in Indiana. Of particular note is the mushroom *Armillaria tabescens*, as this may be the first documented report of this species in Indiana.

Interestingly, *Lycogala epiderndrum*, a slime mold known as Wolf's Milk, was found. Often mistaken for a fungus/mushroom, especially small puffballs, *L. epiderndrum* is a widespread species of plasmodial slime mold. The fruiting bodies, called aethalia, occur either scattered or in clusters on damp rotten wood, especially on large logs. It may fruit from June to November. The aethalia are small cushion-like globs ranging in color from pink to brown (depending on age). When immature, if the outer wall (or peridium) is ruptured, they may excrete a pink paste. When mature, the color tends to become more brownish.

Non-vascular plants (mosses).—The survey of mosses in Blossom Hollow Nature Preserve revealed a healthy diversity characteristic of a mature mesic woodland environment. Further, the recent rains provided excellent conditions for identifying bryophytes in their most robust, hydrated state, especially important when assessing these overlooked pioneer plants that colonize mature trees (live, dead, and dying) and decaying logs. Our survey sampled from various substrates and habitats including sandstone rock, bark from living and dying trees, tree roots, rotting logs downed for various periods of time, forest soils of mesic slopes, ridges and bottoms, creek beds, and seeps.

Overall, 30 bryophyte species were identified. According to the Flora of North America (FNA 2007) and Welch (1957), 23 species represent potential Johnson County records, and two, *Anomodon viticulosus* (Greater Tongue Moss) and *Weissia controversa* (Pigtail Moss), represent potential state records. Some of the interesting discoveries were both gametophytes and sporophytes of Algal Rock Moss (*Platydictya confervoides*) growing on wet sandstone rather than its usual calcareous rock; a beautiful specimen of the rare Verdigris Mousetail Moss (*Myurella sibirica*); and Cluster Moss (*Rosulabryum capillare*) growing on wet rock. In a sample from a wet rock out of a streambed, there appeared to be a *Selaginella* sp. (Spike Moss, a vascular plant, not a bryophyte) growing within a mat colonization of *Campyliadelphus chrysophyllus* (Bristle Star Moss) and *Rhynchostegium serrulatum* (Beaked Comb Moss). All samples collected for the survey were returned to the environment.

Snail-killing flies (Sciomyzidae).—Fourteen snail-killing flies (Diptera: Sciomyzidae) of five species were recorded from the Hills of Gold Conservation Area. All specimens were collected by use of a sweep net in Glacier's End, the northernmost part of the property, in full sunlight, from sedges and grasses surrounding a small, shallow woodland pond. All five species are members of the sciomyzid subfamily Tetanocerini, the aquatic larvae of which are overt predators of aquatic and semi-aquatic snails in fens, marshes, and even roadside ditches. New for Johnson County were *Dictya expansa* and *Sepedon pusilla*, bringing to eight the number sciomyzid species known from Johnson County.

In Indiana, four of the species (*D. expansa, D. texensis*, *S. armipes*, and *S. fuscipennis*) are common and widespread, whereas *S. pusilla* is decidedly rare, being at both its northern and western limits. Previously it was known in Indiana from only four widely separated counties (Clark, Parke, Tippecanoe, and Union). Of the 14 specimens of *S. pusilla* now known from the state, only five specimens have been collected since 1918, when noted dipterist John M. Aldrich collected extensively in Parke and Tippecanoe counties while living in Lafayette.

Except for *S. pusilla*, all species identified would be expected to occur in suitable habitat anywhere in Indiana. The specimens of *S. fuscipennis* were of the southern form (*S. f. fuscipennis* Loew), which in Indiana is found from approximately the latitude of Indianapolis south; no individuals were of the northern form (*S. f. nobilis* Orth) found from central Indiana north.

Although the Hills of Gold Conservation Area offers limited habitat for sciomyzid species with larvae that require still water, the mature woodlands undoubtedly contain *Euthycera arcuata* (Loew) and *Trypetoptera canadensis* (Macquart). The larvae of these two common and widespread species prey on land snails and are found throughout Indiana in forested habitats. In North America, *E. arcuata* 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 (Foote & Keiper 2004). Both species of sciomyzids are rarely collected by use of a sweep net. They are most often captured in Malaise traps, which were not used in this study.

Spiders.—The surveying methodology included the use of Berlese funnels, litter sifting, and hand collecting. (For details of these methods, see the final bioblitz report on the IAS website.)

For the low amount of sampling conducted at the Hills of Gold Conservation Area (28 person-hours in one day), the spider diversity was relatively high. In total, 39 different taxa (33 species) of spider were found in 32 different genera from 15 different families. The number of seemingly-monotypic genera found likely indicates a vast under sampling of the area. The most diverse family was linyphiidae (11 species in 9 genera) and the most diverse genus was *Agyneta* (3 species; within linyphiidae). In addition to the high diversity relative to the sampling regime conducted, the number of rare species found in this sampling period was high. The discovery of *Agyneta evadens*, *A. parva*, *Styloctetor purpurescens*, *Scotinella redempta*, and *Neon nelli* was unexpected since all of these species represented new distribution records for the state of Indiana. Moreover, our sampling of this area uncovered two undescribed species of linyphiid: *Oreonetides* sp. and *Agyneta* sp. In summary, the Hills of Gold Conservation Area holds an assemblage of spiders that is diverse, understudied, and unique.

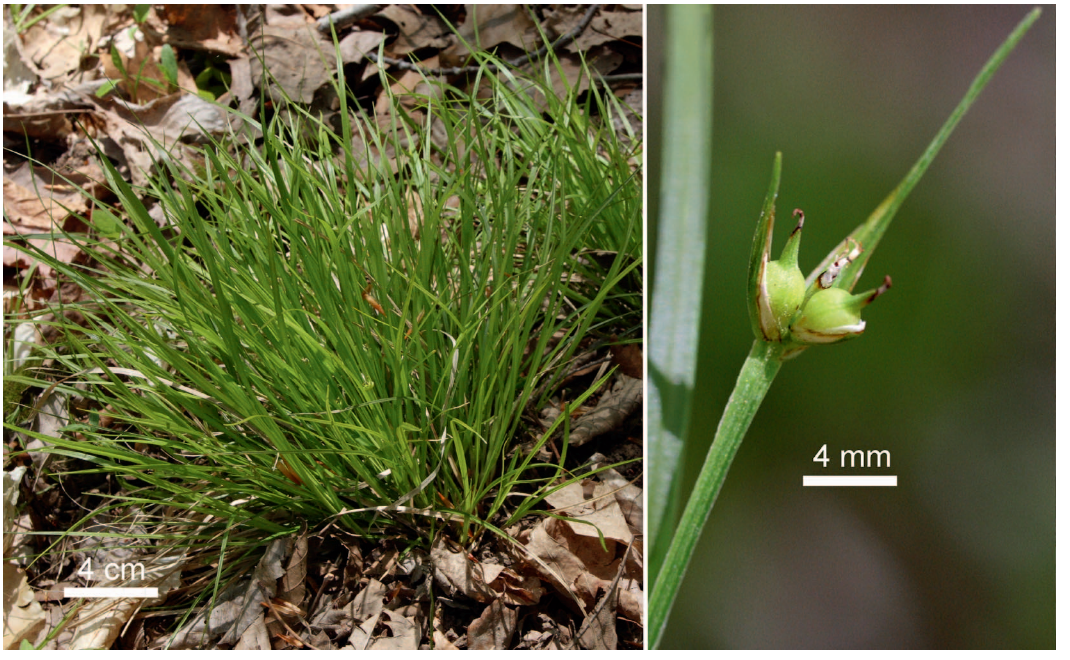

Figure 4.—*Carex timida*, timid sedge, is a state endangered species. (Photo by Paul Rothrock)

All vouchered specimens were sent to Indiana State University.

Vascular plants.—A total of 269 taxa, 251 native and 18 non-native, all identified to at least species, was reported from the bioblitz area. From each of the three separate sites the following was reported: Blossom Hollow Nature Preserve (187 taxa, 179 native), the Conservation Easement Property (177 taxa, 171 native), and Glacier's End Nature preserve (201 taxa, 189 native). The distribution across the three sites and the relative abundance of each species was determined. (See final bioblitz report.) Of the 269 species reported, 114 occurred in all three sites and 113 represent potential Johnson County records. According to the February 12, 2016 list of Endangered, Threatened, Rare and Extirpated Plants of Indiana (Nature Preserves 2016), one species is listed as state endangered, *Carex timida* (Fig. 4), and four species are listed on the state watch list, *Huperzia lucidula*, *Hydrastis canadensis*, *Panax quinquefolius*, and *Viola pubescens*. (State endangered means that the species has less than five occurrences in the state.)

The thirteen families with the most species were, in order, Cyperaceae (31), Asteraceae (28), Poaceae (15), Rosaceae (14), Ranunculaceae (9), Brassicaceae and Lamiaceae (7 each), Apiaceae and Rubiaceae (6 each), and the Boraginaceae, Caryophyllaceae, Fagaceae, and Violaceae (5 each). These 13 families represent 53% of the 269 species reported.

For the native plants from the three sites combined, the FQI (Floristic Quality Index) was 74.2 and the mean C (Coefficient of Conservatism) was 4.7. These numbers clearly indicate that The Hills of Gold Conservation Area has "remnant natural quality and contains some noteworthy remnants of natural heritage of the region" (Swink & Wilhelm 1994). Clearly, this area should be considered of paramount importance and should be conserved. Floristic quality is also reflected in the species present. Within the Core Conservation Area, there were three species of orchid, i.e., *Aplectrum hyemale* (Putty-root Orchid), *Galearis spectabilis* (Showy Orchis), and *Goodyera pubescens* (Downy Rattlesnake Plantain). In addition, the presence of ferns is an excellent indicator of the quality of and lack of disturbance of a site. To this point, the Core Conservation Area of the bioblitz included twelve species of ferns and the fern ally *Huperzia lucidula* (Shining Clubmoss). Lastly, the Coefficient of Conservatism, or C-value, which range from zero to ten, is an index of the fidelity of an individual species to undisturbed plant communities characteristic of the region prior to European settlement. The higher the C-value the more conserved the species is to an undisturbed habitat. A close examination of the 251 native plants reported during the bioblitz revealed that 64 species (25.5%) have $C \geq 7$. Within this group there were 37 species with $C = 7$, 24 species with $C = 8$, three species with $C = 9$ (*Carex careyana*, *Carex timida*, and *Diplazium pycnocarpon*), and one species with $C = 10$ (*Cynoglossum virginianum*).

Lastly, a physiognomic analysis of the vascular flora observed in all sites combined reveals that 67 species (25%) were woody (trees, shrubs and woody vines), 144 species (53.5%) were herbaceous (herbaceous vines and forbs), 45 species (16.7%) were graminoids (grasses and sedges), and 13 (4.8%) were ferns and their allies. Overall, these numbers represent the composition of high quality woodland in south-central Indiana. Fortunately, the future preservation of the Hills of Gold Conservation Area is assured under the guidance of the Central Indiana Land Trust, Inc.

ACKNOWLEDGMENTS

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LOADS OF NITRATE, PHOSPHORUS, AND TOTAL SUSPENDED SOLIDS FROM INDIANA WATERSHEDS

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ABSTRACT. Transport of excess nutrients and total suspended solids (TSS) such as sediment by freshwater systems has led to degradation of aquatic ecosystems around the world. Nutrient and TSS loads from Midwestern states to the Mississippi River are a major contributor to the Gulf of Mexico Hypoxic Zone, an area of very low dissolved oxygen concentration in the Gulf of Mexico. To better understand Indiana's contribution of nutrients and TSS to the Mississippi River, annual loads of nitrate plus nitrite as nitrogen, total phosphorus, and TSS were calculated for nine selected watersheds in Indiana using the load estimation model, S-LOADEST. Discrete water-quality samples collected monthly by the Indiana Department of Environmental Management's Fixed Stations Monitoring Program from 2000–2010 and concurrent discharge data from the U. S. Geological Survey streamflow gages were used to create load models. Annual nutrient and TSS loads varied across Indiana by watershed and hydrologic condition. Understanding the loads from large river sites in Indiana is important for assessing contributions of nutrients and TSS to the Mississippi River Basin and in determining the effectiveness of best management practices in the state. Additionally, evaluation of loads from smaller upstream watersheds is important to characterize improvements at the local level and to identify priorities for reduction.

Keywords: Nutrient loads, mass transport, Indiana, nitrate, phosphorus, suspended solids

INTRODUCTION

Many factors can influence the concentration of nutrients and total suspended solids (TSS) in streams including climate, basin size, land use, and hydrological management practices (Meybeck et al. 2003; Domagalski et al. 2008). Excess nutrients (primarily nitrogen and phosphorus) can lead to eutrophication which degrades the structure and function of aquatic food chains (Dodds & Welch 2000). Excessive TSS, such as sediment, can also alter aquatic habitats through sedimentation (Bilotta & Brazier 2008). Eutrophication and sedimentation can lead to economic losses in recreational water usage and waterfront real estate, as well as increased spending on recovery and drinking water treatment (Carpenter et al. 1998; Bilotta & Brazier 2008; Dodds et al. 2009). The impacts of excessive nutrient and TSS loading in streams may not just be local; they can lead to degradation of water quality and habitat in waterbodies far downstream, and can contribute to large scale ecological effects in coastal areas (Diaz & Rosenberg 2008). The world's second largest hypoxia zone is located in the Gulf of Mexico in the shallow waters of the Louisiana shelf (Rabalais et al. 2002). Transport of dissolved nutrients and nutrients bound to suspended solids from Midwestern states to the Mississippi River has been identified as one of the main contributors to this hypoxia (Alexander et al. 2008, Robertson & Saad 2013, Robertson et al. 2014).

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The Mississippi River drains all or portions of 31 states. The U.S. Geological Survey's Spatially Referenced Regressions On Watershed attributes (SPARROW) model (based on data from 1992 to 2002 and detrended to 2002) was used to estimate that of those 31 states, nine states (including Indiana) contributed 75% of the total nitrogen and total phosphorus delivery to the Gulf of Mexico (Alexander et al. 2008). Those nine states, however, constitute only 33% of the drainage area. The model identified corn/soybean row crop as the main contributor of total nitrogen loads, while phosphorus loads were linked to non-recoverable manure from pasture/rangelands. The SPARROW model results indicated that Indiana contributed 10–17% of the nitrogen and 5–10% of the phosphorus mass transported to the Gulf of Mexico from 1999 to 2002. A recently updated SPARROW model indicated that watersheds in Indiana contribute the third highest amount of nitrogen and the seventh highest amount of phosphorus to the Gulf of Mexico in comparison to other Midwest states (Robertson

Table 1.—Sites used for loads analysis and their associated USGS stream gages. Map ID for Figure 1. All sites are in Indiana.

Map ID number	Site name	Watershed	Drainage area (km^2)	USGS gage number
1	Wabash River at Montezuma	Lower Wabash	28,775	03340500
2	Wabash River at Lafayette	Middle Wabash	19,384	03335500
3	Wabash River at Peru	Upper Wabash	6,952	03327500
4	White River at Petersburg	White	28,796	03374000
5	West Fork White River near Centerton	West Fork White	6,436	03354000
6	East Fork White River at Seymour	East Fork White	6,056	03365500
7	Kankakee River at Shelby	Kankakee	4,604	05518000
8	Iroquois River near Foresman	Iroquois	1,404	05524500
9	Patoka River near Winslow	Patoka	1,682	03376300

et. al. 2014). Robertson et al. (2014) estimated that Indiana contributed 155,742,615 kg/yr of nitrogen and 6,767,868 kg/yr of phosphorus to the Gulf of Mexico, based on model applications with data from 1971 to 2006 with results made applicable to 2002. When loads were divided by watershed area to compute yields for each state, Indiana had the highest yield (kg/km^2) of nitrogen, 1,804 kg/km^2, and the seventh highest yield of phosphorus, 78.4 kg/km^2.

Evaluation of nutrient loads from select individual watersheds within Indiana may provide insight about the overall contribution of nutrients to the Mississippi River. Within Indiana, the Indiana Department of Environmental Management (IDEM) has collected monthly stream-water samples as part of their Fixed Station Monitoring Program (FSMP) since 1957 and the U.S. Geological Survey (USGS) has operated a network of stream gages that provides continuous stream discharge records for many sites and streams since the 1930's (Jian et al. 2012). The objective of this study was to use these two long-term data sets to calculate loads of nitrate, phosphorus, and TSS for select Indiana watersheds for the period 2000 to 2010; and compare loads to previously modeled contributions from Indiana.

METHODS

Site selection.—Nine sites from the IDEM FSMP and their associated USGS stream gages were selected and used to estimate loads of nitrate plus nitrite as nitrogen (referred to as nitrate in this paper), total phosphorus (referred to as phosphorus in this paper), and total suspended solids (referred to as TSS in this paper) from Indiana (Table 1, Fig. 1). There are 163 sites across the state in IDEM's FSMP (as of 2012). The nine IDEM FSMP sites chosen for load calculations were those located on the furthest downstream reaches of large watersheds within Indiana that drain to the Mississippi River system. For the two largest Indiana watersheds, the Wabash River and White River, two additional sites located upstream were also chosen to further examine

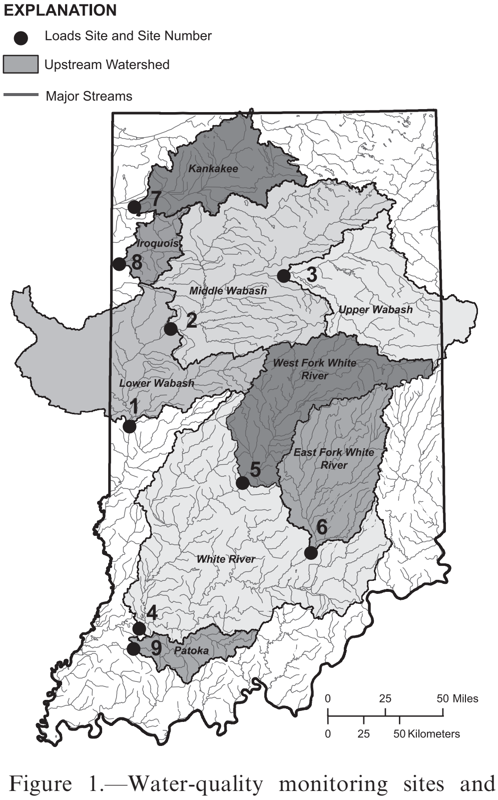

Figure 1.—Water-quality monitoring sites and upstream drainage areas used in load models. Map ID number is associated with Table 1.

Table 2.—Load model number and equations for predefined models ranked for model fit used in S-LOADEST to estimate loads for Indiana watersheds from 2000-2010. [a_0, intercept; $a_{\#}$, coefficient; ln, natural log; ln*Q*, ln(discharge) - median of ln(discharge); *dtime*, decimal time - decimal time adjustment; sin, sine; cos, cosine]

Model number	Regression model equation
1	$a_0+a_1\ln Q$
2	$a_0+a_1\ln Q+a_2\ln Q^2$
3	$a_0+a_1\ln Q+a_2 dtime$
4	$a_0+a_1\ln Q+a_2\sin(2\pi dtime)+a_3\cos(2\pi \mathrm{dtime})$
5	$a_0+a_1\ln Q+a_2\ln Q^2+a_3 dtime$
6	$a_0+a_1\ln Q+a_2\ln Q^2+a_3\sin(2\pi dtime)+a_4\cos(2\pi dtime)$
7	$a_0+a_1\ln Q+a_2\sin(2\pi dtime)+a_3\cos(2\pi dtime)+a_4 dtime$
8	$a_0+a_1\ln Q+a_2\ln Q^2+a_3\sin(2\pi dtime)+a_4\cos(2\pi dtime)+a_5 dtime$
9	$a_0+a_1\ln Q+a_2\ln Q^2+a_3\sin(2\pi dtime)+a_4\cos(2\pi dtime)+a_5 dtime+a_6 dtime^2$

loads within those larger watersheds. These sites had a complete water-quality record of monthly samples from 2000–2010 and were co-located at or within a reasonable distance from a USGS streamflow gage with a complete record of discharge data from 2000–2010. ArcGIS 10.1 geographic information system (ESRI 2012) was used to identify USGS stream gages that were co-located or on the same stream reach as a FSMP site. All sites had a drainage basin difference of less than 7% between the water-quality site and the USGS streamflow gage, except the Iroquois River at Foresman, IN which had an 18% difference.

Water-quality and streamflow data.—Data for monthly water-quality samples analyzed for nitrate, phosphorus, and TSS were obtained from IDEM's Assessment Information Management System (IDEM 2013) database. Laboratories and analysis procedures changed and reporting limits for phosphorus and TSS changed over time so the highest reporting limit was used to determine censored values. Nitrate had no censored values. Streamflow data for USGS gages associated with FSMP sites were obtained from the USGS National Water Information System (NWIS) (U.S. Geological Survey 2013a-i) database for each site from 2000–2010. The program waterData (Ryberg & Vecchia 2012) was used to screen and standardize zero and missing values and to mathematically assign probable streamflow values for missing values.

Load models.—Loads were calculated for nitrate, phosphorus, and TSS with the program S-LOADEST, an adaptation of LOADEST developed by Runkel et al. (2004). S-LOADEST was written for S-Plus statistical software (TIBCO 2008, any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government). This program calibrates load models using time-series streamflow and constituent concentration data. The regression models relate the concentration of nitrate, phosphorus, or TSS from monthly water-quality samples to the daily mean discharge on the day of sampling. In addition to streamflow and concentration, the program considers various functions of discharge, seasonality, and time over the 11 year period to calibrate models.

The S-LOADEST program has three methods that can estimate coefficients of the dependent variables in load models: Adjusted Maximum Likelihood Estimation (AMLE), Maximum Likelihood Estimation (MLE), and Least Absolute Deviation (LAD) (Runkel et al. 2004). The AMLE and MLE methods are most appropriate when data are normally distributed. LAD can be used when data are not normally distributed; however, it cannot be used with censored data. The AMLE method can be used with censored data; if the AMLE method is selected and no censored data are present, the method converts to MLE (Dempster et al. 1977; Wolynetz 1979; Cohn 1988; Cohn 2005). Because censored data were present, AMLE was used for estimating loads for this study. The AMLE method corrects for transformation bias in the regression-model coefficients.

Using the AMLE method, S-LOADEST software ranked nine predefined models (Table 2) for each site and constituent. Models were ranked and the best defined model was identified on the basis of Akaike Information Criterion

Table 3.—Annual loads, standard error of prediction and annual yields of nitrate plus nitrite as nitrogen, total phosphorus, and total suspended solids for Indiana watersheds.

Wabash River at Montezuma, IN									
	Nitrate plus Nitrite as Nitrogen			Total phosphorus			Total suspended solids		
Year	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)
2000	24,127	7.58	838	1,082	0.25	38	377,684	189	13,125
2001	73,970	22.31	2,571	2,614	0.53	91	706,642	263	24,557
2002	78,513	23.18	2,729	2,548	0.47	89	841,810	309	29,255
2003	61,407	15.46	2,134	3,037	0.65	106	990,941	426	34,438
2004	64,812	15.45	2,252	2,529	0.45	88	744,401	260	25,870
2005	61,517	18.65	2,138	2,656	0.70	92	541,988	215	18,835
2006	61,815	14.11	2,148	2,228	0.34	77	638,479	181	22,189
2007	65,577	18.27	2,279	2,576	0.46	90	664,677	211	23,099
2008	67,578	20.52	2,348	3,397	0.70	118	1,021,398	370	35,496
2009	58,372	17.81	2,029	3,114	0.63	108	1,051,483	403	36,542
2010	37,329	11.83	1,297	2,382	0.61	83	981,318	495	34,103
Wabash River at Lafayette, IN									
	Nitrate plus Nitrite as Nitrogen			Total phosphorus			Total suspended solids		
Year	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)
2000	15,816	3.97	816	815	0.08	42	271,770	121	14,020
2001	38,959	9.63	2,010	1,895	0.24	98	597,620	234	30,831
2002	36,594	9.04	1,888	1,615	0.21	83	551,351	212	28,444
2003	36,438	8.04	1,880	2,672	0.52	138	1,476,687	871	76,181
2004	32,821	6.45	1,693	1,882	0.25	97	678,319	291	34,994
2005	37,181	9.65	1,918	2,152	0.41	111	503,475	209	25,974
2006	35,745	6.79	1,844	1,884	0.22	97	499,412	148	25,764
2007	40,530	9.83	2,091	2,262	0.33	117	552,029	193	28,479
2008	43,058	11.41	2,221	2,600	0.44	134	683,567	253	35,264
2009	35,724	9.76	1,843	2,214	0.34	114	621,995	255	32,088
2010	23,760	6.19	1,226	1,518	0.18	78	475,807	216	24,546

Table 3.—Continued.

Wabash River at Peru, IN									
	Nitrate plus Nitrite as Nitrogen			Total phosphorus			Total suspended solids		
Year	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)
2000	9,787	3.60	1,408	408	0.08	59	93,302	28	13,421
2001	14,932	4.34	2,148	923	0.18	133	214,769	61	30,893
2002	12,664	3.33	1,822	681	0.13	98	186,179	59	26,781
2003	16,431	4.20	2,363	1,508	0.30	217	484,582	189	69,704
2004	9,713	2.26	1,397	743	0.12	107	194,793	56	28,020
2005	11,966	3.42	1,721	1,080	0.22	155	292,131	108	42,021
2006	11,095	2.54	1,596	905	0.14	130	236,182	66	33,973
2007	13,015	3.50	1,872	1,158	0.23	167	339,607	113	48,850
2008	12,915	3.51	1,858	1,038	0.21	149	333,655	113	47,994
2009	11,496	3.25	1,654	796	0.16	114	259,939	85	37,391
2010	9,826	3.33	1,413	619	0.12	89	204,445	62	29,408
White River at Petersburg, IN									
	Nitrate plus Nitrite as Nitrogen			Total phosphorus			Total suspended solids		
Year	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)
2000	22,656	7.26	787	1,803	0.32	63	915,881	198	31,806
2001	28,929	8.74	1,005	2,491	0.48	87	1,131,904	310	39,308
2002	34,660	11.39	1,204	3,204	0.59	111	2,346,413	834	81,484
2003	30,946	6.78	1,075	3,115	0.45	108	1,769,853	456	61,462
2004	27,073	6.92	940	2,725	0.40	95	1,376,605	352	47,805
2005	29,746	12.29	1,033	3,192	0.63	111	1,541,229	531	53,522
2006	39,781	11.64	1,381	4,263	0.70	148	2,153,985	590	74,802
2007	31,153	10.29	1,082	2,918	0.53	101	1,366,626	399	47,459
2008	41,105	16.15	1,427	4,674	0.96	162	3,159,257	1113	109,712
2009	32,272	8.82	1,121	3,452	0.62	120	1,870,784	494	64,967
2010	23,987	8.00	833	2,280	0.43	79	1,255,475	336	43,599

Table 3.—Continued.

West Fork White River near Centerton, IN									
	Nitrate plus Nitrite as Nitrogen			Total phosphorus			Total suspended solids		
Year	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)
2000	4,302	0.36	668	433	0.06	67	57,316	17	8,906
2001	7,380	0.84	1,147	756	0.11	117	220,484	113	34,258
2002	7,866	0.99	1,222	906	0.19	141	655,117	778	101,789
2003	10,083	1.51	1,567	1,423	0.45	221	1,951,766	3140	303,258
2004	7,229	0.79	1,123	800	0.11	124	315,372	236	49,001
2005	9,062	1.36	1,408	1,246	0.39	194	828,691	1197	128,759
2006	9,993	1.30	1,553	1,047	0.14	163	335,683	174	52,157
2007	8,449	1.19	1,313	981	0.20	152	434,467	370	67,506
2008	9,963	1.44	1,548	1,198	0.28	186	1,003,170	1176	155,869
2009	7,626	0.88	1,185	764	0.11	119	311,435	189	48,390
2010	6,486	0.71	1,008	651	0.12	101	397,036	383	61,690
East Fork White River at Seymour, IN									
	Nitrate plus Nitrite as Nitrogen			Total phosphorus			Total suspended solids		
Year	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)
2000	10,471	2.41	173	414	0.13	7	204,070	137	3,370
2001	11,407	2.39	188	679	0.32	11	269,804	240	4,455
2002	12,459	3.03	206	1,127	0.72	19	736,552	1003	12,162
2003	11,691	1.94	193	611	0.18	10	334,247	248	5,519
2004	7,976	1.43	132	704	0.49	12	270,774	328	4,471
2005	10,341	2.62	171	1,380	1.11	23	466,598	706	7,705
2006	13,358	2.78	221	1,113	0.55	18	469,714	433	7,756
2007	8,855	2.26	146	803	0.45	13	266,720	279	4,404
2008	12,437	3.66	205	2,208	1.93	36	1,525,883	3038	25,196
2009	10,510	2.15	174	1,107	0.76	18	858,391	1408	14,174
2010	9,353	2.27	154	761	0.39	13	610,067	703	10,074

Table 3.—Continued.

Kankakee River at Shelby, IN									
	Nitrate plus Nitrite as Nitrogen			Total phosphorus			Total suspended solids		
Year	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)
2000	1,875	0.42	407	94	0.01	20	27,841	7	6,047
2001	3,761	0.69	817	137	0.02	30	33,466	7	7,269
2002	3,528	0.59	766	137	0.02	30	38,686	10	8,403
2003	2,333	0.31	507	105	0.01	23	29,844	7	6,482
2004	3,055	0.43	664	124	0.01	27	32,385	7	7,034
2005	3,358	0.60	729	115	0.01	25	25,881	5	5,621
2006	2,825	0.39	614	120	0.01	26	29,602	6	6,430
2007	4,090	0.59	888	173	0.02	38	43,910	11	9,537
2008	5,163	0.92	1,121	232	0.03	50	58,748	16	12,760
2009	3,980	0.75	864	204	0.03	44	54,372	14	11,810
2010	1,925	0.40	418	128	0.02	28	36,239	9	7,871
Iroquois River near Foresman, IN									
	Nitrate plus Nitrite as Nitrogen			Total phosphorus			Total suspended solids		
Year	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)
2000	1,347	0.33	959	21	0.005	15	5,452	1.7	3,883
2001	3,929	0.91	2,798	55	0.013	39	10,008	3.2	7,128
2002	3,710	0.88	2,642	55	0.014	39	12,105	5.1	8,622
2003	2,988	0.71	2,128	130	0.051	93	22,755	16.0	16,207
2004	3,296	0.61	2,348	56	0.013	40	12,348	4.7	8,795
2005	2,365	0.56	1,684	38	0.011	27	6,707	2.5	4,777
2006	3,571	0.62	2,543	53	0.009	38	12,137	3.6	8,645
2007	3,249	0.72	2,314	54	0.012	38	10,962	4.0	7,808
2008	3,656	0.86	2,604	111	0.034	79	16,679	7.3	11,880
2009	2,858	0.72	2,036	80	0.025	57	14,278	6.9	10,170
2010	1,840	0.45	1,311	43	0.012	31	10,025	3.5	7,140

Table 3.—Continued.

	Patoka River near Winslow, IN								
	Nitrate plus Nitrite as Nitrogen			Total phosphorus			Total suspended solids		
Year	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)	Annual load (Metric Tons)	Standard error of prediction (Metric Tons)	Annual yield (kg/km^2)
2000	1,176	0.33	699	145	0.03	86	52,446	16	31,181
2001	1,064	0.32	633	133	0.03	79	39,043	15	23,212
2002	1,475	0.42	877	194	0.04	115	100,068	50	59,493
2003	1,188	0.27	706	124	0.02	74	49,108	13	29,196
2004	1,309	0.30	778	155	0.02	92	61,212	19	36,392
2005	1,031	0.29	613	110	0.02	65	35,263	11	20,965
2006	1,935	0.58	1,150	291	0.05	173	111,725	41	66,424
2007	1,202	0.35	715	124	0.02	74	38,319	11	22,782
2008	1,611	0.60	958	229	0.05	136	110,809	57	65,879
2009	1,517	0.34	902	202	0.04	120	80,652	22	47,950
2010	1,072	0.24	637	106	0.02	63	39,318	9	23,376

(AIC). The model with the lowest AIC score for each site and constituent was further evaluated with diagnostic plots for each model factor and residual plots to determine if residuals had equal statistical variance and were evenly distributed.

Once the best model was determined, USGS daily mean discharge data from associated stream gages for the period 1 January 2000 to 31 December 2010 were used with the regression model to estimate daily loads of nitrate, total phosphorus, and TSS for each site. Annual loads were estimated as the sum of the daily loads for each year. For each watershed, mean and median annual load for each constituent were calculated for the 11 year period.

Yields.—To compare loads for sites with varying drainage basin sizes, yields were calculated by dividing the load at each site by the watershed area. This allowed the comparisons between sites in units of tons per square kilometer per year.

RESULTS

Annual loads for nitrate, phosphorus, and TSS varied by year for each site (Table 3). For most sites and constituents, the lowest annual load values occurred in 2000 (Table 3). In general, standard error of prediction seen in the models was higher in years where the load was higher. For median annual loads for the 11 year period, sites with larger drainage areas (Lower Wabash, White River) tended to have higher loads than those with smaller drainage basins (Table 4, Figs. 2A-C). For yields, the values were more similar and in some instances sites with smaller watersheds had higher yields than those with larger watersheds (Table 4, Figs. 2D-F). Most notably the Iroquois River near Foresman, the site with the smallest watershed area, had the highest median annual yield for nitrate.

Nitrate.—Median annual nitrate loads for 2000–2010 vary across the state (Fig. 3). Median annual nitrate loads range from 1,202 metric tons per year at the Patoka River near Winslow, IN to 61,815 metric tons per year at the Wabash River at Montezuma, IN (Table 4, Fig. 2A). The Iroquois River near Foresman, IN had the largest median annual nitrate yield at 2,314 kg/km^2 per year, and the Patoka River near Winslow, IN had the lowest nitrate yield at 715 kg/km^2 per year (Table 4, Fig. 2D).

Phosphorus.—Median annual phosphorus loads for 2000–2010 vary across the state of Indiana (Fig. 4). Median annual phosphorus

Table 4.—Median annual loads and yields from 2000 to 2010 for Indiana watersheds. Bold number indicates highest value. All site are in Indiana.

Site Name	Watershed	Drainage area (km^2)	Median annual load (Metric Tons)	Median annual yield (kg/km^2)
			Nitrate plus Nitrite as Nitrogen	
Wabash River at Montezuma	Lower Wabash	28,775	**61,815**	2,148
Wabash River at Lafayette	Middle Wabash	19,384	36,438	1,880
Wabash River at Peru	Upper Wabash	6,952	11,966	1,721
White River at Petersburg	White	28,796	30,946	1,075
West Fork White River near Centerton	West Fork White	6,436	7,866	1,222
East Fork White River at Seymour	East Fork White	6,056	10,510	1,735
Kankakee River at Shelby	Kankakee	4,604	3,358	729
Iroquois River near Foresman	Iroquois	1,404	3,249	**2,314**
Patoka River near Winslow	Patoka	1,682	1,202	715
			Total Phosphorus	
Wabash River at Montezuma	Lower Wabash	28,775	2,576	90
Wabash River at Lafayette	Middle Wabash	19,384	1,895	98
Wabash River at Peru	Upper Wabash	6,952	905	130
White River at Petersburg	White	28,796	**3,115**	108
West Fork White River near Centerton	West Fork White	6,436	906	**141**
East Fork White River at Seymour	East Fork White	6,056	803	133
Kankakee River at Shelby	Kankakee	4,604	128	28
Iroquois River near Foresman	Iroquois	1,404	55	39
Patoka River near Winslow	Patoka	1,682	145	86
			Total Suspended Solids	
Wabash River at Montezuma	Lower Wabash	28,775	744,401	25,870
Wabash River at Lafayette	Middle Wabash	19,384	552,029	28,479
Wabash River at Peru	Upper Wabash	6,952	236,182	33,973
White River at Petersburg	White	28,796	**1,541,229**	53,522
West Fork White River near Centerton	West Fork White	6,436	397,036	61,690
East Fork White River at Seymour	East Fork White	6,056	466,598	**77,046**
Kankakee River at Shelby	Kankakee	4,604	33,466	7,269
Iroquois River near Foresman	Iroquois	1,404	12,105	8,622
Patoka River near Winslow	Patoka	1,682	52,446	31,181

loads ranged from 55 metric tons per year at the Iroquois River near Foresman, IN to 3,115 metric tons per year at the White River at Petersburg, IN (Table 4, Fig. 2B). The West Fork White River near Centerton, IN had the largest median annual phosphorus yield at 141 kg/km^2 per year, and the Kankakee River at Shelby, IN had the lowest phosphorus yield at 28 kg/km^2 per year (Table 4, Fig. 2E).

TSS.—Median annual TSS loads for 2000–2010 vary across the state of Indiana (Table 4, Fig. 5). Median annual TSS loads ranged from 12,105 metric tons at the Iroquois River near Foresman, IN to 1,541,229 metric tons per year at the White River at Petersburg, IN (Table 4, Fig. 2C). The East Fork White River at Seymour, IN had the largest median annual TSS yield at 77,046 kg/km^2 per year, and the Kankakee River at Shelby, IN had the lowest TSS yield at 7,269 kg/km^2 per year (Fig. 2F).

DISCUSSION

Stream loads are influenced by transport of the constituents to the stream and instream processes. Excess precipitation and associated runoff can increase the transport of constituents to streams

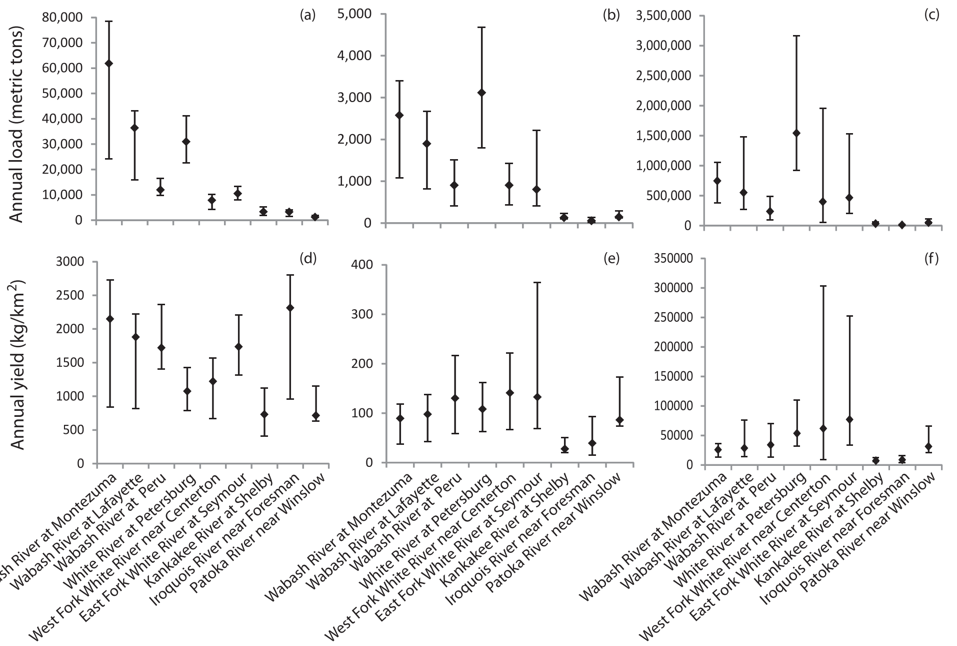

Figure 2.—Median annual loads for a) nitrate plus nitrite as nitrogen, b) total phosphorus, and c) total suspended solids, and annual yields for d) nitrate plus nitrite as nitrogen, e) total phosphorus, and f) total suspended solids. Diamond represents the mean annual load from 2000–2010. Whiskers represent the range of values for annual load (min and max) for each of the nine Indiana watersheds.

as well as increase the amount of water flowing in a stream. In addition, high stream flows can increase stream constituent transport levels through resuspension of constituents that may have settled to the stream bed or bank during periods of low discharge. On the other hand, prolonged time periods of little precipitation and runoff will lead to lower stream loads of constituents because of reduced flow and transport. During 2000, Indiana precipitation accumulation totals were below normal causing a moderate-to-severe drought through mid-June. The drought led to very low stream discharge at sites around the state (Stewart et al. 2001). Consequently, during the study period, six of the nine sites had their lowest annual load for nitrate, and eight of the nine sites had their lowest annual load for phosphorus and TSS in 2000.

Annual loads for TSS and phosphorus were correlated ($R^2 = 0.77$, Root mean-squared error (RMS) = 548; Fig. 6A); when TSS load was high, phosphorus load tended to be high and when TSS load was low, phosphorus load tended to be low. Annual nitrate loads were not as strongly correlated to annual loads of TSS ($R^2 = 0.30$, RMS = 16,400; Fig. 6B). Phosphorus binds to suspended sediment particles that are included in the TSS measurements. Though measurements of TSS can under estimate sediment concentrations, especially when sand-sized material exceeds 25% of the sediment mass (Gray et al. 2000), the correlation between phosphorus and TSS is still apparent. Nitrate did not follow this pattern because it is soluble in water and not influenced by the presence of sediment (Baker 1980).

Median annual yields for phosphorus and TSS were the lowest at Kankakee River at Shelby, IN and the Iroquois River near Foresman, IN. The northwestern part of Indiana, which includes the Iroquois and Kankakee River basins, is dominated by sandy soils, whereas the rest of the state has soils dominated by silt and clay (Clark & Larrison 1980). When sand concentrations are high in water, measurements of TSS are biased low (Gray et al. 2000). Also, sand particles have a lower surface area to mass ratio than silt and clay, reducing the area for phosphorus binding (Kaiserli et al. 2002). The high quantity of sand in soils in northwestern Indiana may help explain why the yield for both TSS and total phosphorus in the Iroquois and Kankakee River basins were lower than in streams elsewhere in the state.

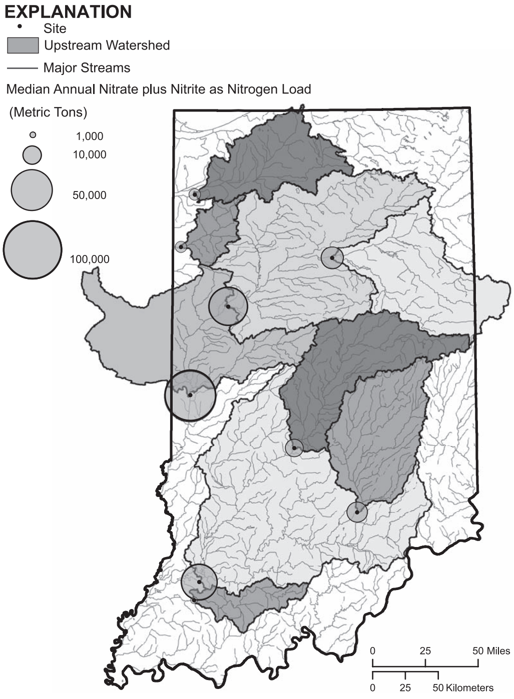

Figure 3.—Median annual loads of nitrate plus nitrite as nitrogen in metric tons per year in Indiana watersheds for the period 2000–2010. Size of circle is proportional to the load.

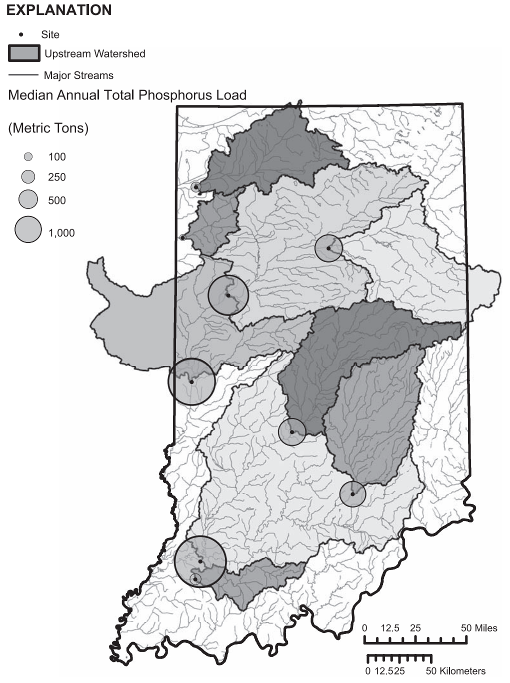

Figure 4.—Median annual loads of total phosphorus in tons per year in Indiana watersheds for the period 2000–2010. Size of circle is proportional to the load.

Instream dynamics, such as resuspension, storage, or nutrient uptake, make it difficult to pin-point the source of nutrients and TSS loads in streams. When a load is measured at a site it does not mean that that watershed contributes that full amount to the Gulf of Mexico; it only means that flux is moving past that point in the basin at the given time. Alexander et al. (2000, 2008) found that the proximity of a source to a large stream or river is strongly correlated with the fraction of its nitrogen or phosphorus load that is delivered to the Gulf of Mexico. Delivery was found to increase with stream size; however, reservoirs tended to reduce the amount of phosphorus delivered downstream due to sediment trapping. Loads estimated at smaller upstream sites, though important in understanding the dynamics of the smaller watershed, may not give much insight into the load that ultimately reaches an estuary system.

During 2003, the annual load of TSS at sites in the West Fork White River basin and the Upper Wabash River basin were higher than the annual loads at the downstream sites, i.e., the White River at Petersburg and the Wabash River at Montezuma. During this year, there were severe isolated rainstorms in both July and September in northern Indiana that resulted in floods in the West Fork White River basin and in the Upper Wabash River basin. The flooding in these upstream basins caused the daily discharge values above the 99th percentile seen for the period of record at these sites (U.S. Geological Survey 2004). Flood events can cause large amounts of TSS to be transported due to increases in erosion (Charlton 2008). Large amounts of constituents were moving through the upstream sites (West Fork White River basin and Upper Wabash River), but the lower portion of these watersheds (White River at Petersburg and the Wabash River at Montezuma) did not experience the high runoff and constituents likely settled out along the course of the water moving downstream as stream flow energy decreased. This illustrates that the loads measured at smaller upstream watersheds may not indicate what is delivered to the Gulf; however, they are important for measuring improvements or identifying priorities for reduction at a local level.

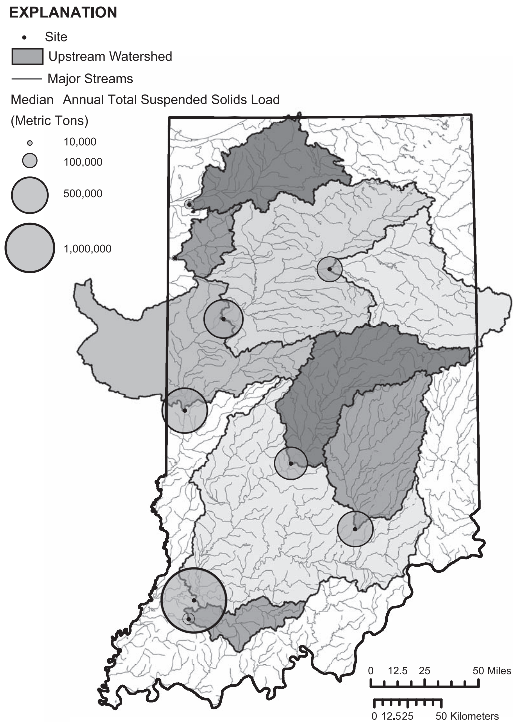

Figure 5.—Median annual loads of total suspended solids in tons per year in Indiana watersheds for the period 2000–2010. Size of circle is proportional to the load.

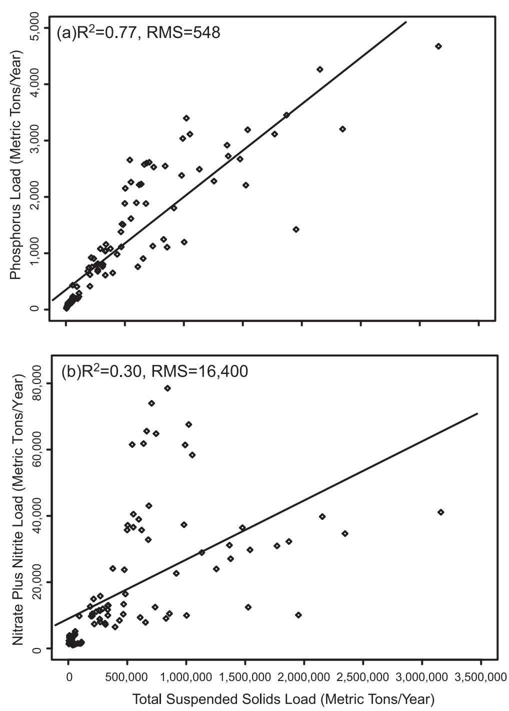

Figure 6.—Comparison of estimated annual loads for 10 years (2000–2010) at nine stations in Indiana for a) total phosphorus and total suspended solids and b) nitrate plus nitrite as nitrogen and total suspended solids, with R^2 and root-mean-square error (RMS). Line represents the regression for each graph.

There are multiple ways to estimate mass transport in rivers and streams. A simple one-to-one comparison of loads or yields calculated from differing methods and models may be inappropriate because of differences in assumptions, time scales, and target watersheds. However, simple comparisons of the general results between the different methods may be useful in evaluating qualitatively the results of the models. This study uses a simple qualitative comparisons between the results from using LOADEST (based upon discrete sampling and discharge data from 2000–2010) and the results from previous SPARROW applications (based upon long-term mean-annual loads (made applicable to 2002)). The median annual loads from the two most downstream sites (Lower Wabash at Montezuma, IN and White River at Petersburg, IN) were combined to estimate a "total" annual load delivered from Indiana. The Robertson et al. (2014) SPARROW model predicted 155,744 MT/yr of total nitrogen was delivered to the Gulf of Mexico from Indiana, the "total" annual load delivered of nitrate delivered from Indiana in this study was 92,761 MT/yr. The two models had similar yields, the SPARROW model estimated a total nitrogen yield of 1,800 kg/km^2 and the LOADEST model from this study estimated a nitrate yield of 1,600 kg/km^2. Even though this study uses nitrate concentrations and SPARROW models use total nitrogen, these results are comparable since in streams draining to the Gulf of Mexico it has been shown that the majority of total nitrogen is in the form of nitrate (Goolsby et al. 2001). Total phosphorus loads from Indiana to the Gulf of Mexico estimated from SPARROW models were 6,768 MT/yr, and the "total" annual load delivered for phosphorus from this study was 5,691 MT/yr. The phosphorus yields were estimated to be 78 kg/km^2 by the SPARROW model and 90 kg/km^2 for this study. Robertson et al. (2014) used all watersheds draining into the Mississippi to make their model (86,337 km^2); while this study only evaluated loads from the two largest river basins in Indiana, the Wabash and the White (57,570 km^2), to estimate loads. The similarities in delivered loads and yields from the Robertson et al (2014) SPARROW models and those calculated in this study illustrate that the Wabash and White River are likely the main sources for nutrients to the Gulf of Mexico from Indiana. Monitoring loads at these sites is important for assessing how Indiana is influencing Mississippi River loads and to determine if management practices are helping to reduce loads from the state.

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131ST ANNUAL ACADEMY MEETING[1]

Presidential Plenary Address by Michael A. Homoya[2]

"INDIANA 1816 – CONNECTING WITH OUR PAST, PRESERVING FOR OUR FUTURE"

Although some text has been added, the following generally adheres to the outline and content of Michael Homoya's presidential address given at the 131st Annual Academy Meeting held in Indianapolis on March 26, 2016. Citations have been added.

INTRODUCTION

In 1916 the annual meeting of the Indiana Academy of Science (IAS) occurred during the centennial anniversary of Indiana's statehood. The IAS president reported on the general advancements in science that had occurred worldwide (Bigney 1917), while others addressed advancements made in Indiana during the previous 100 years, e.g., Evermann (1917) and Coulter (1917) on zoology and botany, respectively. They all make for interesting reading, but it was a presentation given in 1895 by the principal founder of the IAS, Amos Butler, that intrigued me most (Butler 1896). His presidential address, titled *Indiana: A Century of Change in the Aspects of Nature,* was also a centennial piece of sorts, but not one regarding the 100th anniversary of statehood. It was rather a description of landscape changes starting with the influx of settlers beginning in the late 1700s.

For him to know that those changes had occurred require knowledge of what existed previously. By the time Butler was born in 1860, much of Indiana's aboriginal landscape was already cleared and developed, so while he hadn't seen first-hand the conversion that had taken place prior to his life, he did have access to settlers still living that did. He also had available written accounts provided by explorers, pioneers, and government surveyors from as early as the 1600s. From those resources he was able to contrast his contemporary landscape to that which had occurred a century earlier.

[1] J.W. Marriott, Indianapolis, IN, 26 March 2016.
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This year, 2016, is Indiana's bicentennial, and I would like to return to the topic, to remember a land of vast forests, expansive wetlands, clear flowing waterways and lakes, and prairies stretching across the horizon as far as the eye could see; to remember a time when herds of bison traveled ancient paths, passenger pigeons darkened the skies, and wolves, bears and panthers roamed the land.

Join me as we turn the clock back more than 300 years and begin with what may be the earliest first-person written account of Indiana's landscape.

THE FRENCH CONNECTION

In December 1679, while traveling in a canoe with La Salle and others down the Kankakee River, Fr. Louis Hennepin described floating through a seemingly endless expanse of treeless wetlands (Shea 1880). "It [the river] takes its course through vast marshes, where it winds about so, ..." He also described travelling on that tortuous river for a whole day and had "not advanced more than two leagues [ca. 8 kilometers (5 miles)] in a straight line." It was expansive, as he said that "As far as the eye could reach nothing was to be seen but marshes full of flags and alders." They were traveling through what later became known as the Grand Kankakee Marsh, an area of vast marshland estimated to be over 200,000 hectares (≈ 500,000 acres) in extent. So vast it was that some have referred to it as the "Everglades of the North."

THE BRITISH ARE COMING!

In subsequent years many others followed Hennepin, entering the state mostly via a variety of water courses, particularly the Ohio and Wabash rivers. One trip resulted in what is thought to be the earliest known painting of the Indiana landscape (Fig. 1). It was made during a trip down the Wabash River by British military lieutenant Henry Hamilton on his way to Fort Sackville in Vincennes. During one of his encampments he took time to illustrate a limestone promontory jutting into the Wabash. Indicated on today's USGS

Figure 1.—"View of Wabash." This 1778 sketch by Henry Hamilton is a site now known as Rock Island near Logansport, Indiana. It is believed to be the earliest depiction of Indiana's landscape still extant. (Original: MS Eng 509.2 (32), Houghton Library, Harvard University. This image was obtained from http://oasis.lib.harvard.edu/oasis/deliver/~hou00125.)

topographic maps as Rock Island, Hamilton's painting shows a cliff shaped somewhat differently than today and an open understory in the forest (compared to the rather dense understory present now). This is possibly the result of artistic license, but very possibly it depicts reality, as some natural breakdown of the cliff has likely occurred since then as well as succession of plant growth. Regarding the latter, the openness of the understory could be attributed to thinning by fire. Indigenous peoples were known to have used fire as a means of "cleaning" out brush and trees.

PUBLIC LAND SURVEY

Approximately 8 million hectares ($\approx$ 21 million acres) of Indiana's 9 million hectares ($\approx$ 23 million acres) is believed to have been forested. Forests extended statewide except in the northwestern part of the state where prairie prevailed. We know this in great part due to the work conducted by surveyors of the U.S. Government's Public Land Survey (PLS).

Beginning in the late 1700s in the southern half of the state and into the 1840s in the north, practically the entire state was marked off into square mile sections as part of a system forming townships. The surveyors took notes regarding the land's suitability for farming (e.g., 1st rate, 2nd rate, etc.), kinds of trees present (if in timber) and presence of prairie or other natural features, including rock outcrops, water features, and even pigeon roosts. The notes, available for viewing at the Indiana State Archives in Indianapolis, are the best source of information that exists anywhere for specific locations of natural features on the ground.

I find the notes' main value helps us know the original plant composition of a site, at least as it existed 200 years ago. For example, save for a few planted trees and exotic ornamentals no natural vegetation presently exists at the state capitol campus in Indianapolis. There's no clue what grew there naturally. Information provided by PLS surveyors provides a good idea however.

In 1820 surveyor W. Laughlin wrote about a section line within a half of a block of the Capitol noting: "Land 1st Rate, Ash, Walnut, Sugar, &c., undergrowth Spicewood, Prickly Ash, &c." (notes for the line between sections 2 and 11, Township 15 North, Range 3 East ; Fig. 2). At the point where section lines 1, 2, 11, & 12 intersect, Circle Centre Mall now stands. It was there in 1820 that the surveyor noted a black walnut (*Juglans nigra*) with a diameter 12 inches at breast height (dbh) and an elm (*Ulmus* sp.) 24 inches at dbh. These trees are known as bearing or "witness" trees, ones used by subsequent surveyors and settlers to help locate the section corner.

Those trees weren't particularly large, but there were larger ones in the area (1/2 mile away the surveyors recorded a black walnut with a diameter of 44 inches; Fig. 2). Elsewhere in the state surveyors recorded trees of greater diameter. Of particular note was an approximately 8 foot diameter American chestnut (*Castanea dentata*) located in southern Perry County. American chestnut was once common in southern Indiana and the eastern United States but is now practically absent due to chestnut blight.

The species mentioned most often in the literature as the largest tree in the early Indiana landscape was American sycamore (*Platanus occidentalis*). In the early 1800s several travelers, including Michaux (1805), James (1823) and Maximilian (see Witte & Gallagher 2008) noted large sycamore trees over 12 feet in diameter occurring in the Wabash and Ohio River floodplains. The largest tree known to me, at least in terms of diameter, was one measured in the Ohio River floodplain of southern Harrison County (Pickett 1828). It was almost 21 feet in diameter! Trees of that size, of any species, are no longer present in Indiana.

Also gone from the Hoosier landscape are the various natural grassland types. Most of the early writers referred to them as prairies, or barrens, the latter typically containing a mixture of grasses and forbs that characterize prairie as well as hosting an

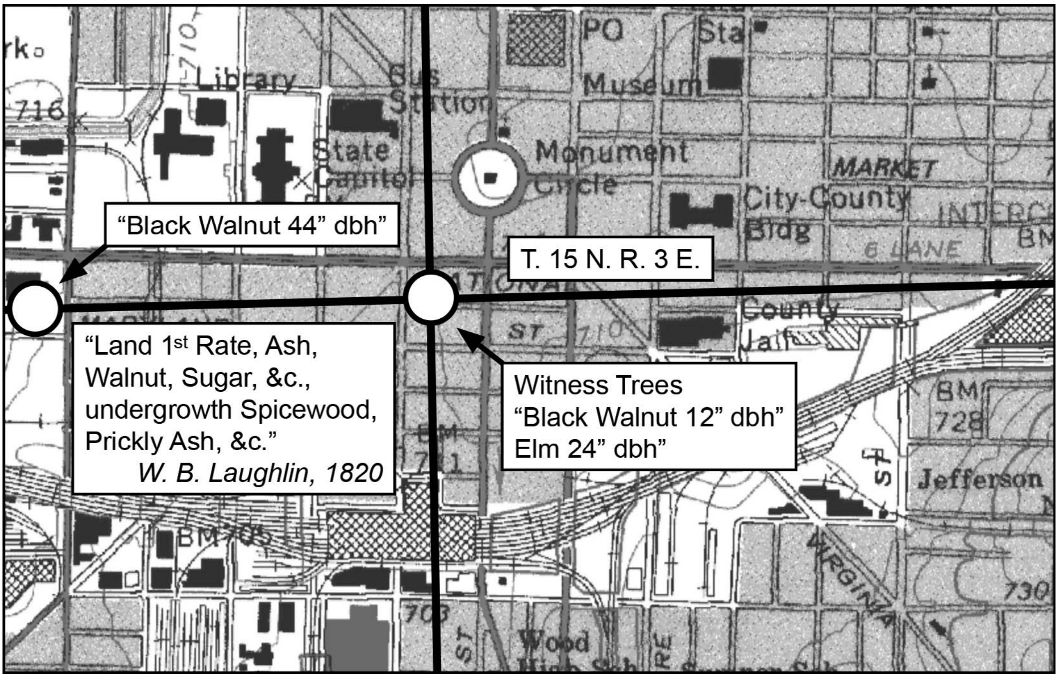

Figure 2.—Notes on the vegetation of land now occupied by downtown Indianapolis as recorded in 1820.

assortment of scattered shrubs and trees. Occurrences of native grasslands existed throughout the state, albeit rather sparingly in most areas except in the far northwestern counties. In those counties prairie was dominant, especially in what is now Benton County, where one could travel for tens of miles through prairie and see few if any trees save for an occasional prairie grove. Today, because of conversion to agriculture, almost none of the land formerly occupied by prairie retains native vegetation.

GRAND PRAIRIE AND THE BIG BARRENS

The vastness of the prairie land in northwestern Indiana and adjacent Illinois caused some people to refer to it as the Grand Prairie. Modern day remnants, however so small, indicate that it must have been beautiful beyond imagination. Several early observers confirm such an impression. “Its surface was undulating like the waves of the sea after a storm, and covered with luxurious grass interspersed with wildflowers of every hue. Around and completely inclosing and seemingly protecting it, stood the forest. I have seen since then many parks of great natural and artistic beauty, but none so charming as was the rolling prairie on that bright morning in June.” (McCulloch 1889). McCulloch, who became Secretary of the Treasury under Abraham Lincoln and two subsequent presidents, was reflecting about a particular prairie in La Porte County.

At the other end of the state was another large area of natural grassland. Occupying over 32,000 hectares ($\approx$ 80,000 acres) in the karst plain of central Harrison and Washington counties (Keith 1983), this “Big Barrens” region was similar to the northwestern prairie in its diversity of species. The physician and botanist Asahel Clapp of New Albany wrote that the barrens had, “A much greater number of species . . . than I have observed in any other place. It is indeed like a botanic garden but much more interesting.” (Clapp 1836).

The barrens region was crossed by the Buffalo Trace, a trail connecting Vincennes and Clarksville that was thought to have been created by bison herds moving across the state to and from salt licks in Kentucky. Bison were certainly seen on the trace, whether they created it or not. On 4 October 1787, Joseph Buell wrote while travelling the Trace on day three after leaving Vincennes for the Falls of the Ohio: “In our march to day, came across five buffaloes. They tried to force a passage through our column. The general ordered the men to fire on them. Three were killed, and the others wounded.” (Hildreth 1848). Although there are reports of

Figure 3.—"Confluence of the Fox River and the Wabash." In 1832 Karl Bodmer painted this landscape located near New Harmony, Indiana. Note the Carolina Parakeets (*Conuropsis carolinensis*) on the vines attached to a large American sycamore (*Platanus occidentalis*). (Original is housed at the Joslyn Art Museum in Omaha, Nebraska. This image was obtained from https://commons.wikimedia.org/wiki/File:Confluence_of_the_Fox_River_and_the_Wabash._Watercolor_by_Karl_Bodmer_1832.jpg)

bison in the state after 1800 most if not all were likely gone by then. Some of the other prominent animals once established in Indiana but now absent include elk, mountain lion, Greater Prairie Chicken, American black bear, gray wolf, Common Loon, and the now extinct Carolina Parakeet and Passenger Pigeon.

BIRDS OF A FEATHER

There are several firsthand accounts of Carolina Parakeet observations in Indiana, most made in the early 1800s. Of particular interest is a painting that includes them made by Karl Bodmer. The scene is of the mouth of the Fox River as it enters the Wabash River near New Harmony and it depicts Carolina Parakeets perched on vines draped on branches of a sycamore tree (Fig. 3). Prince Maximilian, a scientist with whom Bodner was travelling, wrote in his journal that on 25 October 1832 "Mr. Bodner sketched on the [Fox] island where he saw the parakeets." (Witte & Gallagher 2008). Also in southern Indiana, near French Lick, William Blane (1824) reported, "I saw a large flock of beautiful green and yellow parroquets. These were the first I had met with; and they are very tame, and allowed me to come close to them. I got off my horse, and stopped a short time to admire them." It appears that the last record of a Carolina Parakeet sighting made in Indiana was in 1859 (Butler 1898). The last known individual of the species died in 1918 at the Cincinnati Zoo.

Flocks of Passenger Pigeons harbored billions of individuals and were described by many observers as darkening the sky by their numbers. Famed ornithologist and artist John James Audubon , while on the left bank of the Ohio River across from Harrison County wrote that in the autumn of 1813 he observed that "The air was literally filled with

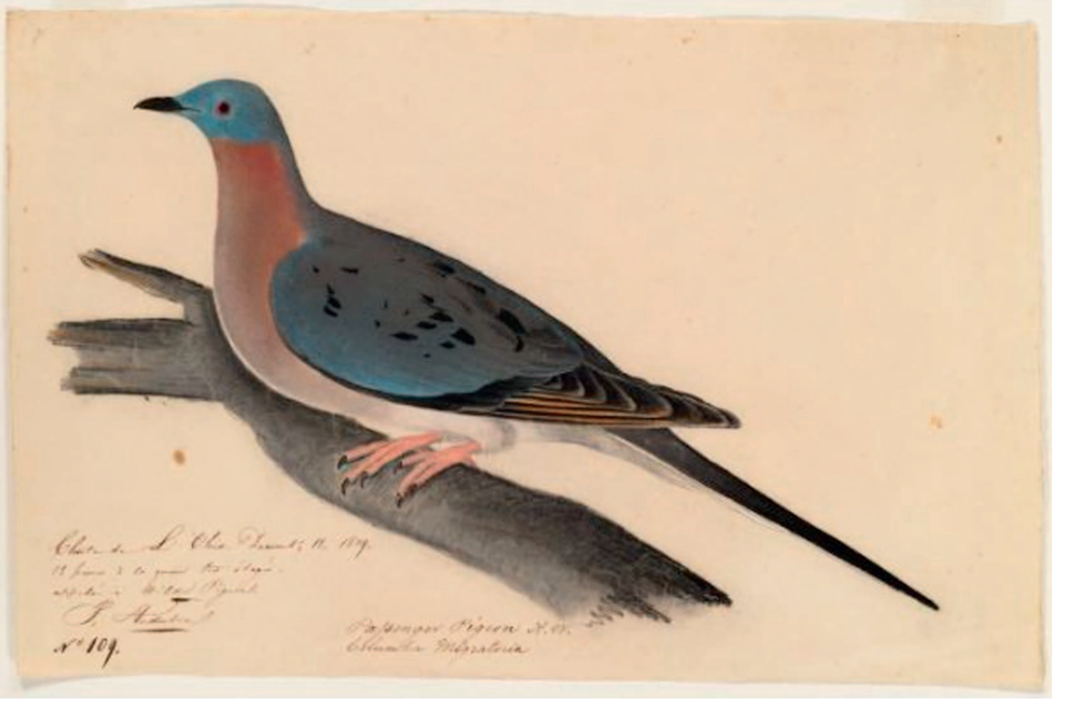

Figure 4.—Passenger Pigeon painted by J.J. Audubon in 1809 from the Falls of the Ohio near Clarksville, Indiana. (Original: MS Eng 509.2 (49), Houghton Library, Harvard University. This image was obtained from http://oasis.lib.harvard.edu/oasis/deliver/deepLink?_collection=oasis&uniqueId=hou00007.)

Pigeons; the light of noon-day was obscured as by an eclipse…." (Audubon 1831) (Fig. 4). The Passenger Pigeon has been considered by some to have been the most numerous bird in North America if not the world. It is almost unfathomable that a species once so abundant would become extinct – and in such a short time. Almost exactly a century after Audubon's observation, in a zoo not far from where he observed that phenomenal flight, the last known Passenger Pigeon drew its final breath. Interestingly, the last verified wild Passenger Pigeon, both in Indiana and the world, was taken from near Laurel in 1902 (Butler 1913; Greenberg 2014).

Famed Hoosier novelist Gene Stratton Porter also wrote about Passenger Pigeons and many other birds that lived in the swamplands near her home. She was somewhat of an anomaly for her time, writing passionately about swamps and the organisms they harbored while others were vigorously draining them. In her book titled *The Moths of the Limberlost* (Stratton-Porter 1912), she wrote: "To me [the swamp] has been of unspeakable interest, [a source of] unceasing work of joyous nature…." She was certainly one of the early residents of our state to recognize that our natural landscape was rapidly disappearing and in her own way made the call to appreciate and protect it.

Others were too, including prominent members of our Indiana Academy of Science.

PRESERVING FOR OUR FUTURE: AWARENESS, THE ASK, AND ACTION

Since its existence, the Academy of Science has been at the forefront in proclaiming the importance of preserving Indiana's natural features. I will mention three people in particular that have played important roles in this regard. All were presidents of the IAS and it is from each of their presidential addresses that I draw the following.

AWARENESS

Amos Butler, a founding father of our organization, was one of the first to talk about the diminishing existence of our state's natural land and associated biota. It is quite clear that he was very aware of the consequences caused by the growth and development that had taken place in the previous century. I quote his 1895 address given at the 11th IAS Annual Meeting entitled: *Indiana: A Century of Change in the Aspects of Nature:*

"Removing the timber and breaking the ground began to show their effects upon the springs and water courses. Many became dry during the warm season. All life, be it salamanders, fishes, mollusks, insects or plants, that found therein a home, died." He continues: "The birds that lived among the reeds and flags, mingling their voices with the frogs, disappeared, and the land reclaimed, tells, in its luxuriant growth of corn, no story to the casual passer-by of the former population which occupied it." (Butler 1896). As Butler and others became aware of the tradeoffs that came with a growing civilization, they began contemplating ways to save the remnants of aboriginal Indiana.

But who would get it started?

THE ASK (OR THE COMMAND?)

Charles Deam, Indiana's preeminent botanist of the early 20th century and the state's first state forester, was one of those people lamenting the degradation and destruction of Indiana's natural areas. In his presidential address during the 40th IAS Annual Meeting, Deam makes a clear plea for protection by stating: "Further I would urge, that the state purchase tracts in our botanical areas and preserve them as laboratories for the study of our native flora and fauna. . . ... This generation has no idea how much these [natural] areas will be worth in the future, and we should present them these areas as a gratuity, if not from a sense of moral obligation. The total cost of such wild life laboratories would not equal the cost of a mile of concrete road, and their educational and economic value will more than justify the expense." (Deam 1925).

But would it happen?

ACTION

Deam's urging was somewhat satisfied by the establishment of Indiana's system of state parks starting in 1916 with the creation of McCormick's Creek State Park, but it became apparent that more land was in need of protection. By the 1960s there was a ground swell of interest in the environment, including protection of natural areas that didn't necessarily fit the criteria used for the creation of state parks, e.g., expanded recreational use or large size. In 1967 Alton Lindsey, Damian Schmelz, and Stanley Nichols embarked on an extensive inventory of the state to locate the best of the variety of natural community types known to exist. That work resulted in the monumental publication *Natural Areas in Indiana and Their Preservation* (Lindsey et al. 1969). It was an impetus, among other things, that led to the creation of a state nature preserves system. Lindsey, in his presidential address to the membership at the 83rd IAS Annual Meeting, outlined it all under the title: "Indiana's New System of Scientific Areas and Nature Preserves." (Lindsey 1968).

The system he referred to was the result of the Nature Preserves Act, a law passed by the state legislature in 1967 (IC 14-31-1 – 1967 Nature Preserves Act) with bi-partisan support to protect high quality natural areas. This spawned the Indiana Division of Nature Preserves, and from its humble beginnings with a two person staff and one preserve (Pine Hills Nature Preserve) the Division has grown to oversee a program that now numbers 274 state dedicated nature preserves protecting over 50,000 acres.

Thanks to the work of many, especially by certain members of the Indiana Academy of Science and the organization itself, there has been meaningful progress in preserving our natural heritage for future generations. It is my hope that such progress will continue and that all of you will be part of it in some way.

Thank you.

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Michael A. Homoya, M.S., 2015–2016 Indiana Academy of Science President. Michael has been botanist and plant ecologist for the Indiana Department of Natural Resources Division of Nature Preserves and Natural Heritage Program since 1982. His primary responsibilities include discovery and assessment of natural areas for possible designation as state dedicated nature preserves, conducting field surveys for rare species and state significant natural communities, and updating the official Indiana list of rare, threatened, and endangered vascular plants. His current efforts have focused on monitoring and recovery of Indiana's rarest plants. Homoya has been a member of IAS since 1982 and in 1992 he was inducted as a Fellow. In 2015 he was the recipient of the Academy's Distinguished Scholar Award. A selection of Homoya's publications includes "The Natural Regions of Indiana" (Proceedings of the Indiana Academy of Science 1985) and *Orchids of Indiana* (Indiana Academy of Science and Indiana University Press 1993). Others of note include "The Diversity of Indiana's Flowering Plants" (in M. Jackson, Ed.), *The Natural Heritage of Indiana* (Indiana University Press 1997) and *Wildflowers and Ferns of Indiana Forests: A Field Guide* (Indiana University Press 2012).

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INDIANA ACADEMY OF SCIENCE
2016 Year End Financial Report

	Balance 1-Jan-16	Revenues	Expenses	Balance 31-Dec-16
OPERATING FUND				
Dues		24,565.00		
Interest		10.82		
Misc. Income		556.80		
Contributions		10.00		
Annual Meeting		44,720.00		
Foundation Support		232,008.73		
Officer's Expenses			121,313.81	
Operating Expenses			30,958.70	
Financial Expenses			2,939.14	
Newsletter Expenses			0.00	
Annual Meeting			77,379.30	
Academy Store		0.00	0.00	
Web Site Expenses			47,528.14	
Operating Funds Total	**37,468.35**	**301,871.35**	**280,119.09**	**59,220.61**
RESTRICTED FUNDS				
Proceedings	21,681.06	17,966.89	17,916.89	21,731.06
Publications	(49,292.24)	66,836.34	68,496.16	(50,952.06)
Research Grants*	10,511.31	64,098.73	65,394.73	9,215.31
Lilly Library	6,756.47	0.00	0.00	6,756.47
Welch Fund	6,108.56	0.00	0.00	6,108.56
Life Member's Fund	14,343.61	0.00	0.00	14,343.61
Past President's Fund	8,599.17	0.00	0.00	8,599.17
Special Projects	1,040.55	16,000.00	10,400.00	6,640.55
Total Restricted Funds	**19,748.49**	**164,901.96**	**162,207.78**	**22,442.67**
TOTAL FUNDS	**57,216.84**	**466,773.31**	**442,326.87**	**81,663.28**
FUNDS ON DEPOSIT				
Checking Account	18,423.92	571,191.98	527,699.42	61,916.48
Money Market Savings Account	41,170.45	65919.61	104,021.00	3,069.06
Cert. of Deposit	13,523.76	31.06		13,554.82
TOTAL FUNDS DEPOSITED	**73,118.13**			**78,540.36**

*** Provided support for 28 senior member grants and 3 high school grants**

ACADEMY FOUNDATION FUNDS		
TOTAL ACADEMY FOUNDATION FUNDS	**8,543,914**	**$8,715,040.31**

Foundation Funding Used For

Operating Fund	232,008.73
Proceedings	3,838.27
Publications	60,886.03
Grants	60,462.60
Special Projects	16,000.00
Total	**373,195.63**

Michael S. Finkler
Treasurer

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