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Proceedings
of the
Indiana Academy of Science

VOLUME 122 **2013** **NUMBER 2**

PROCEEDINGS OF THE INDIANA ACADEMY OF SCIENCE

The *PROCEEDINGS* OF THE INDIANA ACADEMY OF SCIENCE is a journal dedicated to promoting scientific research and the diffusion of scientific information, to encouraging communication and cooperation among scientists, and to improving education in the sciences.

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Cover: The Munsee Woods Nature Preserve is owned by the Red-tail Land Conservancy (RTLC), Munsee Woods Nature Preserve (MWNP) is an ~19 ha (~ 45.5 acres) woodland located 9.5 km southeast of downtown Muncie, Indiana, and just west of the northern end of Prairie Creek Reservoir in Delaware County. Formerly known as Camp Munsee, the site was owned by the Girl Scouts of Central Indiana. The name of the camp came from the Munsee clan of the Delaware Indians. "Munsee" means a place where stones are gathered together. Camp Munsee was operated as a residence camp from 1937 until 1964. Following the movement of the residence camp to Camp Windigo in northern Delaware County in 1965, Camp Munsee experienced a steady decline in its use. The site was purchased and renamed by the RTLC in 2010. A portion of the funds to purchase the site were donated by the Indiana Heritage Trust, Indiana Department of Natural Resources. Munsee Woods Nature Preserve was designated a state nature preserve in 2014, and is the only state nature preserve in Delaware County. Upper left: The west side of Lycoming Lodge which serves as the nature center and meeting facility for RTLC events at the site. Lycoming Lodge is one of the buildings remaining from the former Girl Scout camp. Upper right: Image of the young successional woodland that covers the northern half of the site. Through much of the 20th century, the northern half of MWNP was open fields. Lower left: Inflorescences of *Solidago altissima*, tall goldenrod. Lower right: Inflorescences of *Symphyotrichum pilosum*, hairy white old-field aster.

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Publication date: 3 February 2015

This paper meets the requirement of ANSI/NISO Z39.48-1992 (Permanence of Paper).

ISSN 0073-6767

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2013. Proceedings of the Indiana Academy of Science 122(2):55–61

A PRELIMINARY COMPUTATION STUDY OF COUNTER ION EFFECT ON TRANSITION STATE MODELS IN ETHYNYL SUBSTITUTION OF FLUORINE IN *F*-BODIPY.

Matthew Vincent, Jeff Seyler and **Priyadarshine Hewavitharanage**: Department of Chemistry, University of Southern Indiana, Evansville, IN 47712 USA

ABSTRACT. Substitution of the fluorine atoms bonded to the boron center in 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (*F*-BODIPY) compounds with ethynyl or aryl linkages can be achieved through either Grignard or lithium organometallic reagents. Use of Grignard reagents allows for the isolation of monosubstituted and disubstituted products, whereas use of organolithium reagents allows for the isolation of only the disubstituted product. Using SPARTAN modeling software and quantum chemical calculations, preliminary theoretical S_N2 transition state energies were determined for both the mono- and disubstituted transition states using both Grignard and lithium trimethyl silyl protected ethynyl reagents. These models were used to calculate differences in theoretical activation energies between the mono- and disubstitution processes, $\Delta\Delta E = -11.3$ kJ/mol for the lithium reagent and $\Delta\Delta E = 35.9$ kJ/mol for the Grignard reagent. The lower second substitution activation energy for our lithium reagent (negative $\Delta\Delta E$) and the higher second step for our Grignard reagent (positive $\Delta\Delta E$) supports the experimental findings where organolithium reagents are only capable of producing the disubstituted product.

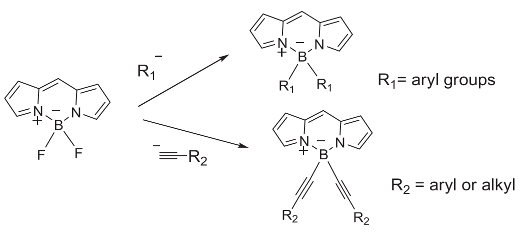
Keywords: BODIPY, quantum chemical calculations, semi-empirical, density functional theory, transition states

INTRODUCTION

The ever accelerating growth of modern technology has created a profound demand for more efficient fluorophores which possess qualities such as a large Stokes' shift, high molar absorption coefficients, high fluorescence quantum yields, high photo stability, high emission peak intensities, and low intersystem crossing (Loudet & Burgess 2007; Ulrich et al. 2008). New fluorescent compounds are being developed for applications in fields such as sensors, light emitting devices, and biological labeling (Saki et al. 2006; Ziessel et al. 2009; Ju et al. 1995). The 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (*F*-BODIPY) dyes are a family of highly investigated dyes. *F*-BODIPY dyes possess many of the previously mentioned properties paramount to modern fluorophores, including extremely high quantum yields, high molar absorption coefficients, and high emission peak intensities. However, they have a very small Stokes shift (Goze et al. 2007). Fluorophores with small Stokes shifts are susceptible to self-quenching of fluorescence (Moerner, W. E., 2007). This problem has been addressed by substituting the two fluorines bonded to boron in *F*-BODIPY with chromophoric molecules, such as pyrene, anthracene, phenanthracene, and biphenyl. These aromatic molecules absorb photons at a shorter wavelength than *F*-BODIPY and transfer their energy to the BODIPY core, which can then release the energy through fluorescent decay (Ulrich et al. 2005; Goze et al. 2006; Goze et al. 2007).

Substitution of fluorine with various chromophores can be done by using aryl nucleophiles or ethynyl nucleophiles (Scheme 1) to create a large, virtual Stokes shift, anywhere in the range of 8,000 cm^{-1} to 16,000 cm^{-1} (Ulrich et al. 2005; Goze et al. 2006; Goze et al. 2007; Nzeata & Hewavitharanage 2012).

It is important to note that substitution of fluorine has little effect on the λ_{max} of absorbance, which remains in the range of 515 cm^{-1} to 526 cm^{-1} because substituted chromophores are not in conjugation with the



Scheme 1.—Synthesis of BODIPY derivatives.

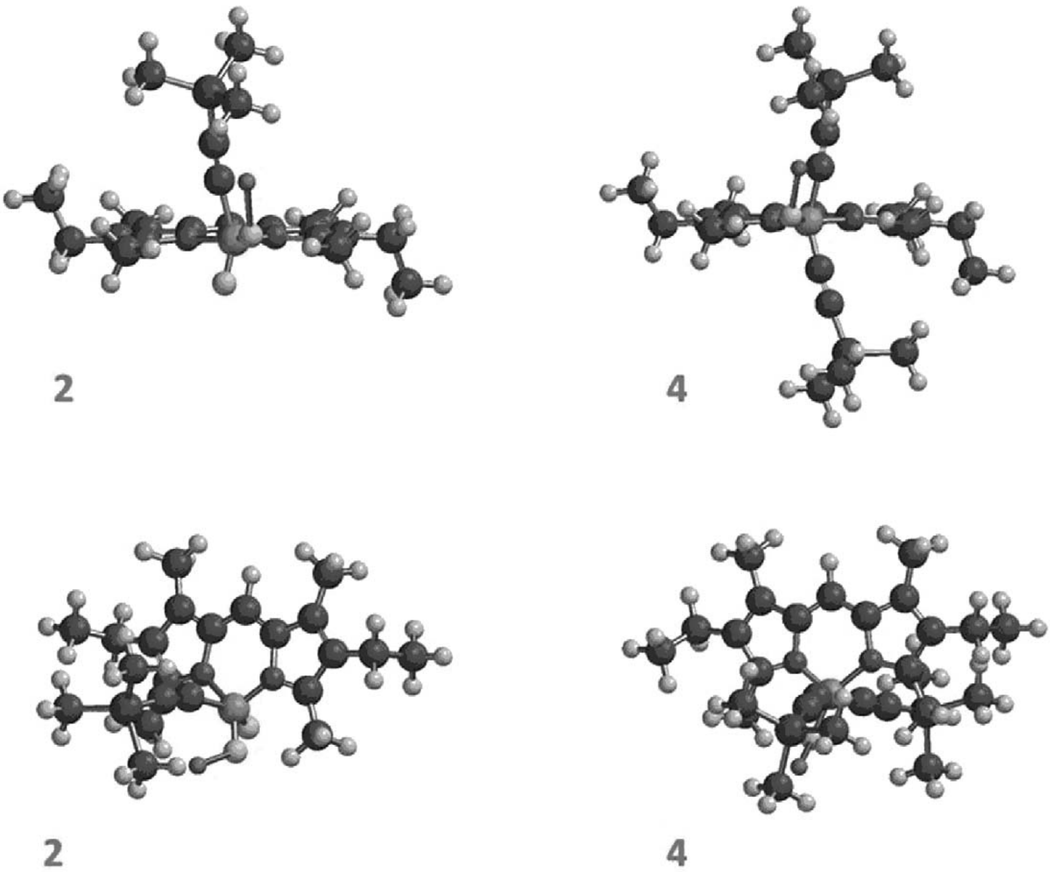
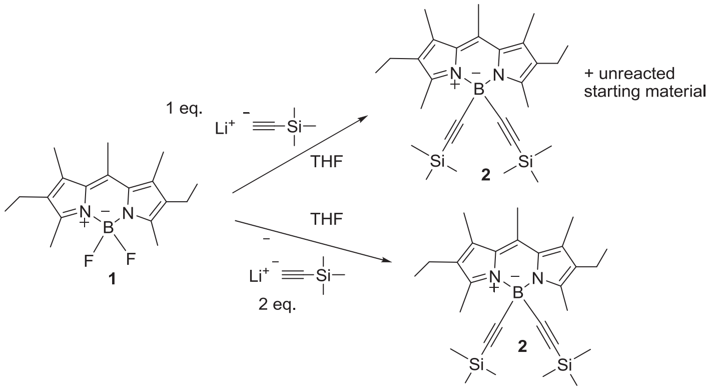


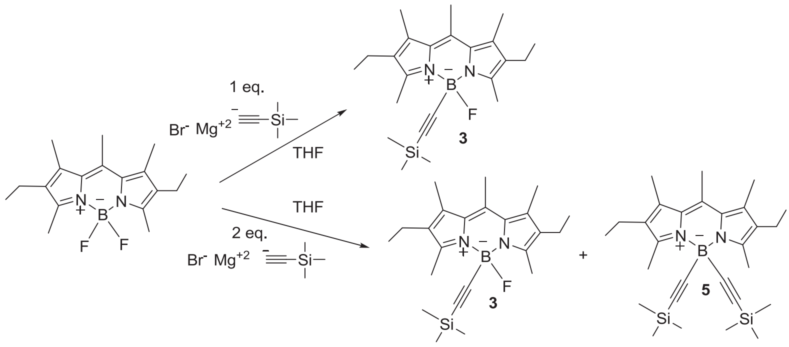
Figure 1.—Transition state models with lithium cation(2 for monosubstitution and 4 for disubstitution).

BODIPY core (Goze et al. 2006; Goze et al. 2007). *E*-BODIPY compounds offer the potential for further modification when the substituting nucleophile is an ethynyl group, including nucleophilic addition to and polymerization of the compound at the alkyne site (Goze et al. 2006; Nagi et al. 2008, Nzeata & Hewavitharanage 2012).

The substitution of fluorine with an ethynyl anion can be accomplished by using a lithium organometallic reagent, Scheme 2, or a Grignard reagent, Scheme 3.



Scheme 2.—Synthesis of E-BODIPY using ethynyl anion generated with n-butyllithium.



Scheme 3.—Synthesis of E-BODIPY using ethynyl anion generated with a Grignard reagent.

According to our results (Nzeata & Hewavitharanage 2012; Hewavitharanage et al 2013) and those of others (Goze et al. 2006; Goze & Ziessel 2007), ethynyl nucleophiles reveal that only the disubstituted product can be produced when using a lithium reagent (Scheme 2), while either the monosubstituted or disubstituted product can be produced when using a Grignard reagent (Scheme 3).

In an attempt to understand why this would happen with such similar reagents and identical nucleophiles, preliminary semi-empirical and density functional quantum chemical calculations of theoretical S_N2 transition state energies

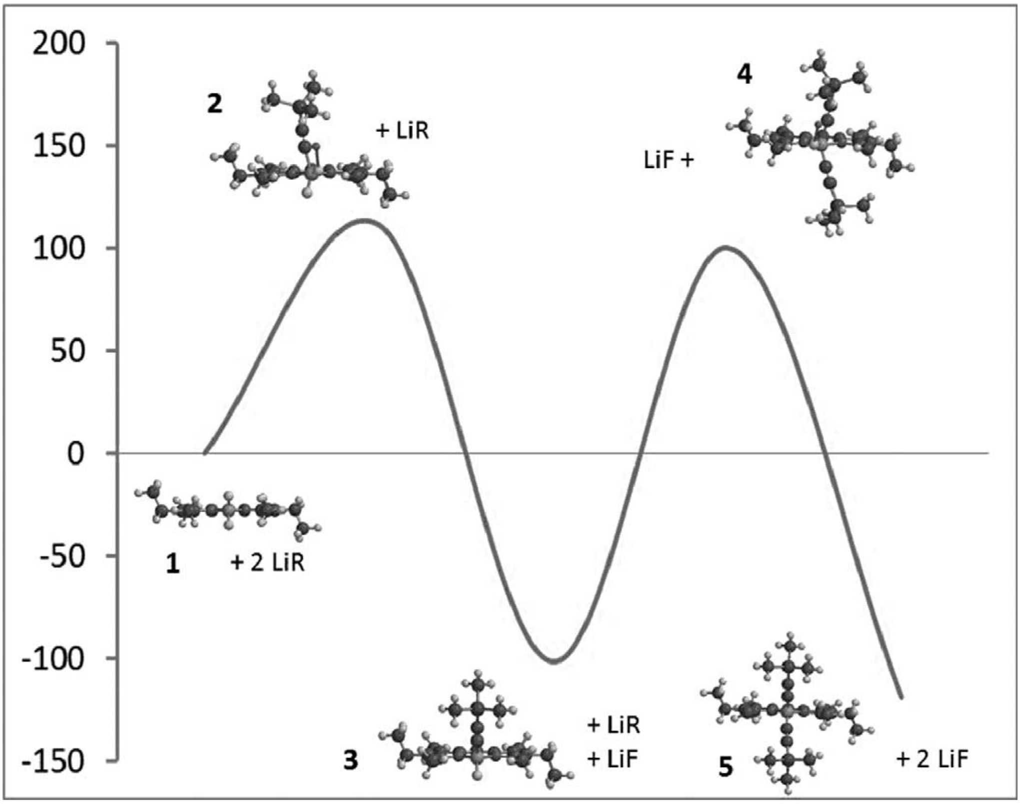


Figure 2.—Energy profile for the substitution of fluorine on F-BODIPY with lithium ethynyl anion. R = trimethylsilylacetylide anion.

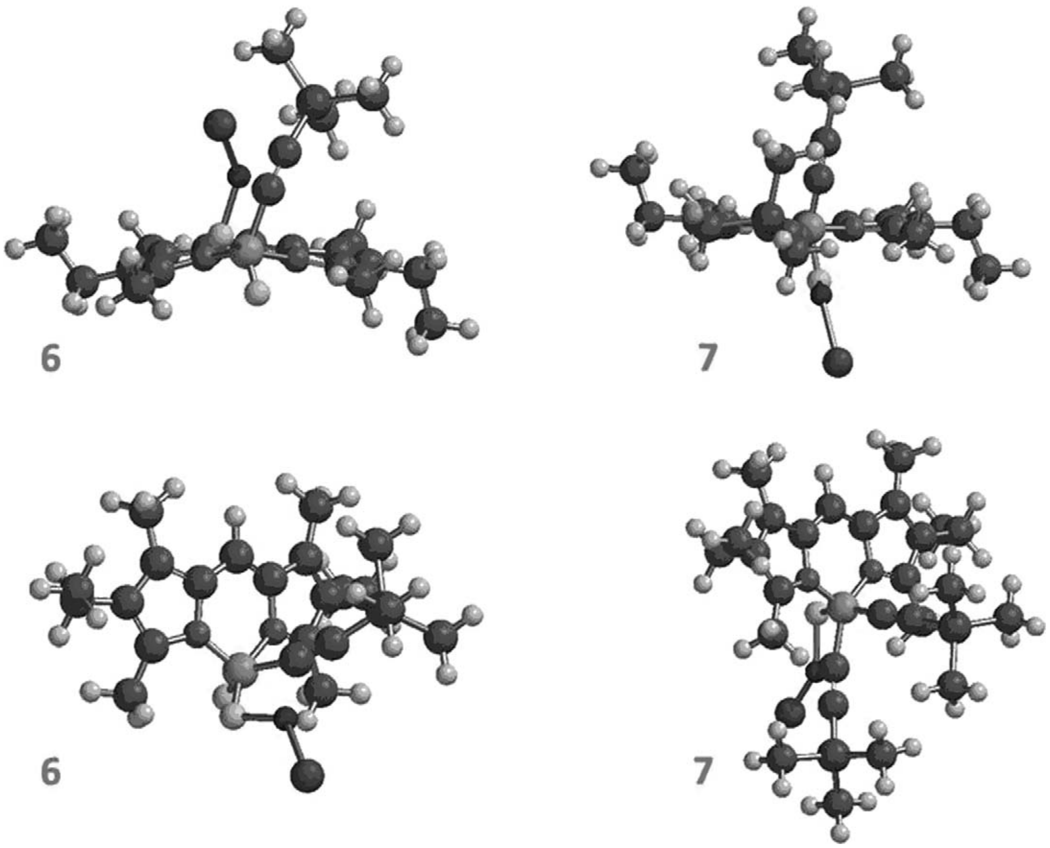


Figure 3.—Transition state models with Grignard reagent (6 for monosubstitution and 7 for disubstitution).

for the mono- and disubstituted BODIPY derivatives were performed. Models of the reagents, products, and reaction intermediates were also constructed in order to estimate the overall reaction energy profiles.

METHODS

All calculations were performed using SPARTAN '08 modeling software (Wavefunction, Inc). The transition state (TS) models were developed by full transition state geometry optimizations using semi-empirical PM3 calculations. Theoretical S_N2 transition states were chosen for the models because both the reagents in question are strong nucleophiles and it aligns with the geometry of the reaction center. Additional IR frequency calculations were performed on each transition state, and each model showed only one imaginary frequency corresponding to ethynyl anion attack and fluoride departure. The reagents, products, and intermediates necessary to develop reaction energy diagrams were obtained using full equilibrium geometry optimizations, also using semi-empirical PM3 calculations. Gas phase single point energy calculations were performed on all semi-empirical optimized transition states, reagents, products, and intermediates using density functional theory B3LYP 6-31G* method (Laird et al., 1996; Salahub & Zerner, 1989; Elstner, 2007). These DFT values were used to determine activation energies and create reaction energy diagrams.

RESULTS AND DISCUSSION

Including the ethynyl anion's respective counter cation, either lithium or magnesium bromide, allowed for more realistic TS models with a coordination between the fluoride leaving group and the nucleophile's respective cation. Although inclusion of dimeric organometallic reagents and solvent molecules has been demonstrated both experimentally and

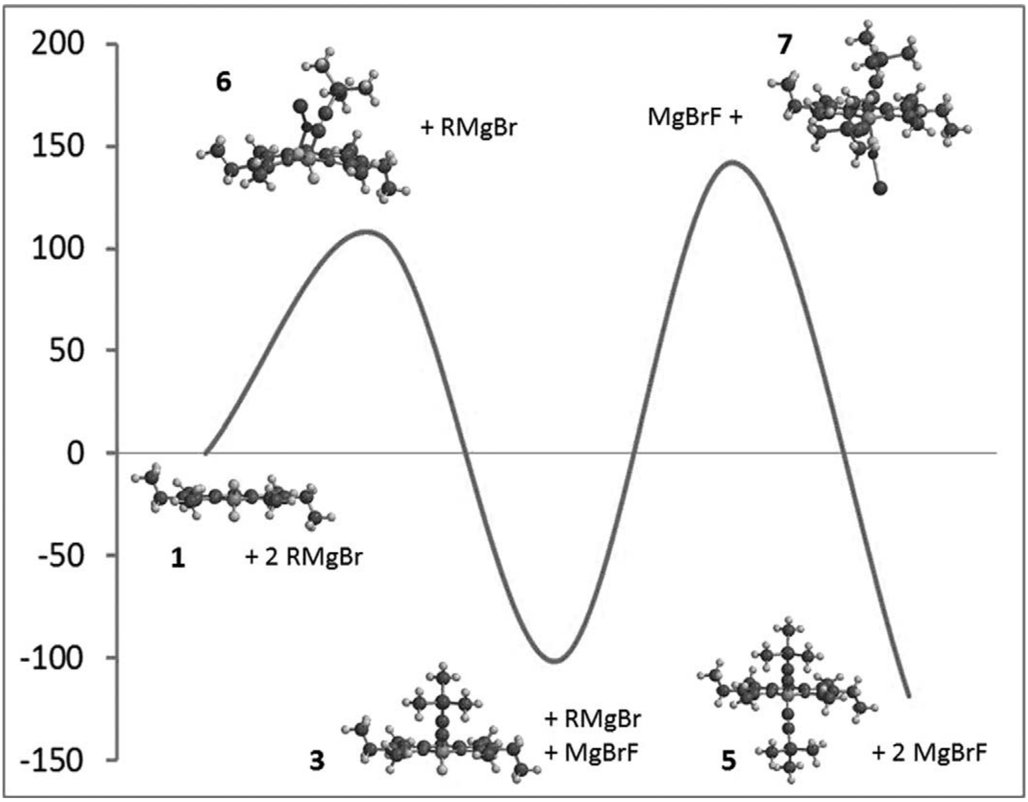


Figure 4.—Energy profile for the substitution of fluorine on F-BODIPY with magnesium bromide ethynyl anion. R = trimethylsilylacetylide anion.

computationally (Anders et.al., 1996, Barbosa et.al., 2011 and Mongin & Harrison-Marchand, 2013) to be important in reactions, we have opted for a more simplistic view in this preliminary study.

The lithium transition states (Figure 1) and energy profile (Figure 2) are considered first followed by the Grignard transition states (Figure 3) and energy profile (Figure 4).

Lithium organometallic reagent.—Both the monosubstituted and disubstituted transition states, **2** and **4** respectively, for the ethynyl substitution of fluorine with a lithium counter cation displayed unconventional geometry at the trigonal bipyramidal boron center. The ethynyl anion was found to attack in the axial position while the leaving fluoride ion, coordinated with a lithium cation, was in the equatorial position. All attempts to construct a conventional S_N2 transition state were unsuccessful.

The reaction energy profile, along with transition state, reactant, and product models can be viewed in Figure 2. All reported activation energies are based on single point DFT values and are relative to the starting materials, which were considered 0 kJ/mol. Transition states **2** and **4** have relative activation energies of 111.3 kJ/mol and 100.0 kJ/mol, respectively. Since the activation energy of the second substitution is lower than that of the first, $\Delta\Delta E = -11.3$ kJ/mol, then there is necessarily enough energy in the system to support the second substitution. Also, the disubstituted product, **5**, has a lower relative energy than the *F*-BODIPY starting compound, **1**, and the monosubstituted product, **3**, implying that it is the most thermodynamically stable of the three compounds. This theoretical evidence supports the finding that only the disubstituted product is formed when using lithium organometallic reagents.

Grignard reagent.—Unlike the substitution of fluorine by an ethynyl anion with a lithium counter ion, the use of an ethynyl anion with a magnesium bromide counter ion displays "textbook" transition state geometry. The monosubstituted and disubstituted transition states, **6** and **7** respectively (Figure 3 and Figure 4), had the ethynyl anion attacking the trigonal bipyramidal boron center in the axial position and the leaving group fluorine, coordinated with magnesium bromide, in the second axial position. All attempts to construct an unconventional S_N2 transition state model were unsuccessful.

Figure 4 depicts the reaction energy profile, along with the transition state, reactant, and product models. The relative activation energies of **6** and **7** are 106.0 kJ/mol and 141.9 kJ/mol, respectively. Since the activation energy of the second substitution is considerably higher than that of the first substitution, $\Delta\Delta E$ = 35.9 kJ/mol, then there must be extra energy added to the system in order to encourage the formation of the disubstituted product. The formation of **3** requires significantly less energy than the formation of **5**. The relative energy of **3** is much lower than that of **1**, and the relative energy of **5** is only slightly lower than **3**. This means that the products are indeed more stable than the starting compound, but the activation energy required to form the transition state 7 is greater than that of 4. Thus, the formation of the monosubstituted product prevails when using a Grignard reagent at 0°C. Goze et al. (2006) have shown that the addition of energy by raising the reaction temperature will allow the formation of the disubstituted product; this is supported by the data we have gathered.

In summary, the density functional theory single point energy calculations we performed on the semi-empirical PM3 optimized S_N2 transition state models provided data that is consistent with our experimental results and those of others. That is, use of a lithium reagent to substitute for fluorine at the boron center in *F*-BODIPY will yield only the disubstituted product and use of a Grignard is capable of yielding both the mono- and disubstituted products. In the case of the lithium reagent, this is due to lower activation energy for the second substitution. This means that if the first substitution can occur, then there is necessarily enough energy in the system to support the second substitution. In the case of the Grignard reagent, the monosubstituted product is achievable at 0 °C because the activation energy for the second substitution is higher than that of the first. However, when the reaction is performed at room temperature the increased amount of energy present in the system due to thermal excitation the disubstituted compound is the major product. From these calculations, it appears that the nucleophile dependent counter cation has an impact on the transition state energies and course of the ethynyl substitution of *F*-BODIPY. This is an indication that full DFT geometry optimizations and perhaps inclusion of solvent molecules, are worth pursuing using theoretical S_N2 transition states in order to get more realistic values for the reaction energy profiles.

ACKNOWLEDGEMENTS

This research was supported by The Indiana Academy of Science as well as The Faculty Research and Creative Work Award of University of Southern Indiana.

LITERATURE CITED

Elstner, M., T. Frauenheim, J. McKelvey, & G. Seifert. 2007. Density Functional Tight Binding: Contributions From the American Chemical Society Symposium. The Journal of Physical Chemistry A 111:5607–5608.

Goze, C., G. Ulrich, L. Mallon, B. Allen, A. Harriman, & R. Ziessel. 2006. Synthesis and photophysical properties of borondipyrromethene dyes bearing aryl substituents at the boron center. Journal of the American Chemical Society 128:10231–10239.

Goze, C., G. Ulrich, & R. Ziessel. 2006. Unusual fluorescent monomeric and dimeric dialkynyl dipyrromethene-borane complexes. Organic Letters 8:4445–4448.

Goze, C., G. Ulrich, & R. Ziessel. 2007. Tetrahedral boron chemistry for the preparation of highly efficient "cascatelle" devices. Journal of Organic Chemistry 72:313–322.

Henriques, A.M. & A.G.H. Barbosa. 2011. Chemical Bonding and the Equilibrium Composition of Grignard Reagents in Ethereal Solutions. 115:12259–12270.

Ju, J., C. Ruan, C. Fuller, A. Glazer, & R. Mathies. 1995. Fluorescent energy transfer dye-labeled primers for DNA sequence analysis. Proceedings of the National Academy of Sciences, U.S.A. 92:4347–4351.

Koch, R., B. Wiede, & E. Anders. 1996. Theoretical Study of the Deprotonation of Nitriles, RCH2CN: Ab Initio and PM3 Calculations of Intermediate Aggregates and Transition States. Journal of Organic Chemistrty 61:2523–2529.

Laird, B., R. Ross, & T. Ziegler. 1996. Density Functional Methods in Chemistry: An Overview.

In Chemical Applications of Density Functional Theory. 2–17. Washington, D. C.

Loudet, A. & K. Burgess. 2007. BODIPY dyes and their derivatives: syntheses and spectroscopic properties. Chemical Reviews 107:4891–4932.

Moerner, W.E. 2007. New Directions in Single-Molecule Imaging and Analysis. Proceedings of National Academy of Science U.S.A. 104: 12596–12602.

Mongin, F. & A. Harrison-Marchand. 2013. Mixed AggregAte (MAA): A Single Concept for All Dipolar Organometallic Aggregates. 2. Syntheses and Reactivities of Homo/HeteroMAAs. Chemical Reviews 113:7563–7727.

Nzeata, P. & P. Hewavitharanage. 2012. Synthesis of an *E*-BODIPY based fluorescent Co-polymer containing organoboron quinolate units. 3:13–16.

Saki, N., T. Dinc & E. Akkaya. 2006. Excimer emission and energy transfer in cofacial boradiazaindacence (BODIPY) dimmers built on a xanthene scaffold. Tetrahedron 62:2721–2725.

Salahub, D. & M. Zerner. 1989. Quantum Chemistry Throughout the Periodic Table. *In* The Challenge of d and f Electrons. 1–15. Washington, D. C.

Ulrich, G., C. Goze, M. Guardigli, A. Roda, & R. Ziessel. 2005. Pyrromethene dialkynyl borane complexes for ‘‘cascatelle’’ energy transfer and protein labeling. Angewandte Chemie International Edition 44:3694–3698.

Ulrich, G., A. Harriman, & R. Ziessel. 2008. The chemistry of fluorescent BODIPY dyes: versatility unsurpassed. Angewandte Chemie International Edition 47:1202–1219.

Ziessel, R., G. Ulrich, A. Harriman, M. Alamiry, B. Stewart, & P. Retailleau. 2009. Solid-state gas sensors from functional difluoroboradiazaindacene dyes. Chemistry – A European Journal 15:1359–1369.

Vincent, M., E. Beabout, R. Bennett, & P. Hewavitharanage. 2013. Synthesis and properties of amphiphilic BODIPY derivatives bearing hydroxyl groups. Tetrahedron Letters 54:2050–2054.

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IDENTIFYING LOCAL SCALE FOOD WEB VARIATION USING STABLE $\delta^{13}C$ AND $\delta^{15}N$ ISOTOPES IN A CENTRAL INDIANA RESERVOIR AND DOWNSTREAM RIVER

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ABSTRACT. We used stable isotopes of $\delta\ ^{13}C$ and $\delta\ ^{15}N$ were measured from fish and macroinvertebrate consumers, terrestrial and aquatic primary producers, and detritus to describe food webs for a reservoir and downstream riverine habitat. Measurements of $\delta\ ^{13}C$ indicated that reservoir energy sources were primarily autochthonous while the riverine habitat was primarily a result of allochthonous carbon sources. Measurements of $\delta\ ^{15}N$ indicated that terrestrial primary producers were basal to organic matter, aquatic primary producers, macroinvertebrates, and fishes, respectively. We found significant differences between riverine and reservoir $\delta\ ^{13}C$ and $\delta\ ^{15}N$ levels among seven fish taxa representing Cyprinidae, Centrarchidae, Percidae, Ictaluridae, and Clupeidae families. Riverine and reservoir overall $\delta\ ^{13}C$ and $\delta\ ^{15}N$ levels differed by an average of 7.9% and 7.7% by taxa, respectively. Reservoir fish exhibited lower intra specific variation in $\delta\ ^{13}C$ and $\delta\ ^{15}N$ values compared to river fish indicating less variation in diet. Reservoir fish were also found to feed at more extreme trophic positions (high and low) compared to riverine fish. This evidence suggests the presence of habitat-induced variability in local scale food webs and has implications for understanding aquatic ecosystem diversity and organization.

Keywords: Food web variation, stable δ 13C and δ15N isotopes, reservoir and river habitat, fish ecology

INTRODUCTION

Variation in food web structure has been linked to a variety of trophic level and productivity patterns associated with freshwater communities (Carpenter et al. 1987; Polis & Strong 1996; Hoeinghaus et al. 2008). However, the majority of community food web studies utilize relatively large geographic areas that do not specifically address the potential influence that local habitats may have on food webs (Finlay et al. 2002; Roach et al. 2009). Furthermore, less is understood about the role that habitat may play in driving local variation in food webs among similar or identical taxa within assemblages. Ultimately, describing the role that habitat plays in structuring food webs can serve as a tool for aquatic conservation.

Previous studies have indicated that alterations in habitat (micro- and macro-) can influence food web variation as a result of secondary mechanisms linked with flow, temperature, and nutrient availability (see Boecklen et al. 2011 for a review). Alteration of flow regime is the primary impetus of aquatic habitat modification globally (Poff et al. 1997; Pringle et al. 2000). Flow regime alteration has been shown to reduce community complexity, homogenize habitats, and shift functional organization (Poff & Ward 1990; Poff & Allan 1995). Impoundment of lotic ecosystems is one of the primary mechanisms of flow regime alteration (Petts 1984). Consequently, two common aquatic ecosystems in North America are reservoirs and subsequent altered downstream riverine segments. Impoundments have been linked with changes in assemblage structure (Taylor et al. 2008) and abiotic attributes of lotic ecosystems (Ward & Stanford 1983) in both the downstream and upstream reach surrounding riverine impoundments (Pringle 1997). However, the study of isotope variation among altered freshwater ecosystems is relatively recent (Diebel & Vander Zanden 2009; Doi 2009). Identification of local scale food web variation in trophic structure and energy sources that occurs due to the presence of impoundments can provide useful information for conservation and restoration by providing additional information to assess the influence

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of habitat alteration on aquatic communities (Hoeinghaus et al. 2008).

Naturally occurring stable isotopes can be used to quantify food web structure (Peterson & Fry 1987; Fry 1988; Doi 2009). Stable isotopes have been used across a wide range of spatial (Fry 1991; Doi 2009) and temporal (Wainwright et al. 1993) aquatic communities (Finlay et al. 2002) and taxa (Genner et al. 2003) to understand trophic and energy dynamics. Compared to classic approaches for understanding trophic relationships (i.e., stomach content analysis, visual observation) isotope analysis allows detailed identification of trophic position (δ^{15}N), energy origin (δ ^{13}C), and food web complexity (Vander Zanden et al. 1997; Melville & Connolly 2003). Specifically, carbon^{13} facilitates detection of autotrophic sources in individuals which may not be readily observable (such as in upper trophic levels) using other diet study approaches (Melville & Connolly 2003). Additionally, nitrogen^{15} concentrations increase in organisms with increasing trophic level revealing informative trophic patterns (Cabana and Rasmussen 1996). From a conservation or management perspective, variation in isotopic signatures (Fry 2002) can be used to function as a covariate of habitat heterogeneity or 'quality'. However, application on a local scale to infer effects of habitat differences on trophic dynamics has been limited, particularly in the study of Indiana fishes.

Our study objective was to use stable isotopes to describe local scale variation in trophic position and energy sources and test for variation between riverine and reservoir aquatic communities. We selected taxa that occurred in both habitats to compare food webs by habitat using producers and consumers. Our null hypotheses were that trophic position (δ^{15}N) and energy origin (δ ^{13}C) would be similar between habitats and among species.

METHODS

The study area was the West Fork of the White River and Prairie Creek Reservoir in Delaware County, Indiana (Fig. 1). The White River watershed has a drainage area of 712 km^2 in Delaware County (Hoggatt 1975). Prairie Creek Reservoir is a 515 ha impoundment of a headwater tributary of the White River. We collected consumers (fishes and macroinvertebrates), producers (algae, terrestrial plants, and macrophytes), and detritus (course particulate organic material, CPOM) in September 2009 (fishes) and 2010 (macroinvertebrates, producers, detritus). Samples were collected by seine, hoop net, electrofishing, d-frame kick net, or visual searching.

Three individuals of each fish species (Table 1) were collected from each habitat. River sampling included one wadable site approximately 200 m in length and 15 m in width. Reservoir sampling included approximately 600 m of wadable shoreline within 10 m of shore. All target adult individuals were collected and three individuals from each habitat were randomly selected for tissue samples. To avoid differences in isotope signature due to intraspecific allometric size variation (Genner et al. 2003) only adult individuals of similar body size were used. Sex of individual fish was not determined. A small sample of white muscle tissue was removed from the dorsal / caudal region of each individual fish immediately following collection for isotope analysis. Although our samples did not include the complete assemblage from either the reservoir or riverine habitats, we selected our study taxa based on co-occurrence in both habitats. We collected a minimum of three individuals for each macroinvertebrate taxon from each habitat. Macroinvertebrate individuals were starved for 48 hours prior to processing to reduce confounding effects of retained gut contents (Jardine et al. 2005). Macroinvertebrates were not selected to represent all prey items of the sampled fishes. Rather, the sampled bivalve, gastropod, dragonfly and midge larvae were selected to represent various possible trophic levels within the macroinvertebrate assemblage. The primary producers collected included leaves from overhanging riparian terrestrial trees (*Fagus* sp.), periphytic algae, and the dominant aquatic macrophytes (primarily watermilfoil *Myriophyllum spicatum*) that were present in both habitats. Detritus was collected along shorelines concurrent with consumer sampling and was primarily composed of woody debris and unidentifiable humic material. A minimum of three individual samples for each producer and detritus group were collected from each habitat.

All samples were separated and individually desiccated at 60° C, ground into a homogenous powder using a mortar and pestle, and packed

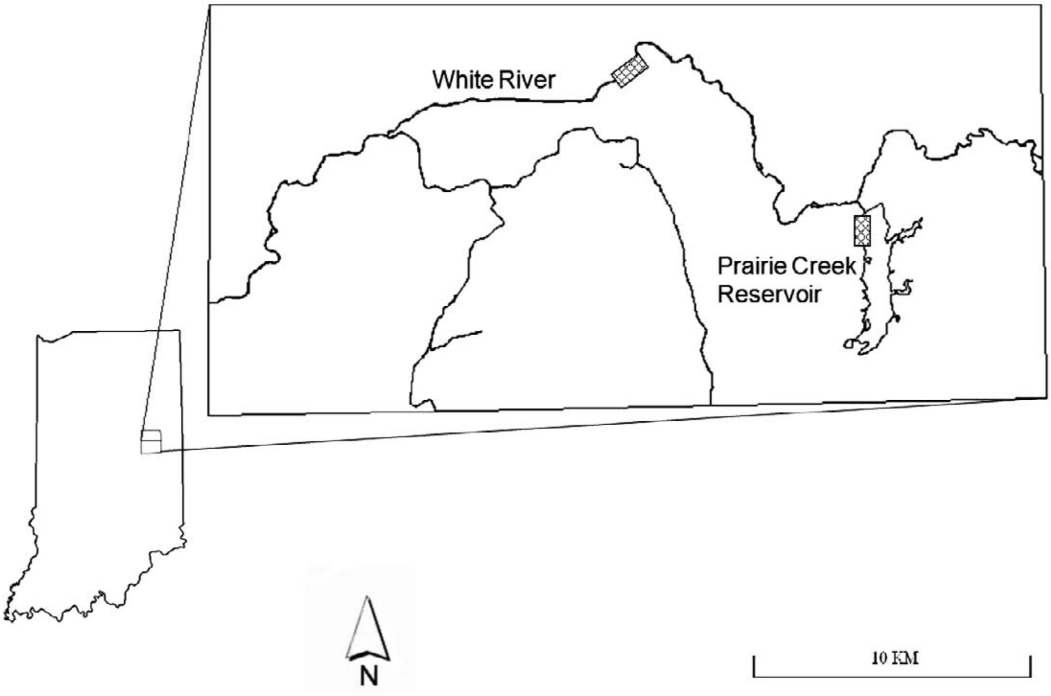

Figure 1.—West Fork of the White River and Prairie Creek Reservoir located in Delaware County, Indiana, USA. Sampling areas are shaded.

into separate tins (Pinnegar & Polunin 1999). Samples were analyzed for ^{13}C and ^{15}N enrichment using a continuous flow mass spectrometer (University of Arkansas Stable Isotope Laboratory). Mean δ ^{13}C and δ ^{15}N levels were compared between reservoir and riverine fish species using two-sample t-tests. In an effort to reduce false discovery rates inherent in multiple pairwise comparisons based on traditional p values we used Q values for tests of significance (Storey 2002). Alpha was set at 0.05 for all tests of significance. To examine variation in δ ^{13}C and δ ^{15}N for the reservoir and river we calculated and compared coefficient of variation (CV) for all collected fish individuals from each habitat.

Several caveats to this study design are a result of limited resources: multiple collecting years and overall sample size. Fish were collected during summer 2009 and primary producers and macroinvertebrate consumers were collected during summer 2010. Samples were analyzed immediately following processing and were not stored during the interim time period. Variation between 2009 and 2010 may introduce bias when directly comparing fish to primary producers, primary consumers, detritus, etc. However, direct comparisons of taxa within years (e.g. reservoir fish to riverine fish, etc.) are applicable.

RESULTS

The fish species collected were smallmouth bass (*Micropterus dolomieu*), white crappie (*Pomoxis annularis*), johnny darter (*Etheostoma nigrum*), spotfin shiner (*Cyprinella spiloptera*), yellow bullhead (*Ameiurus natalis*), gizzard shad (*Dorosoma cepedianum*) and common carp (*Cyprinus carpio*) (Table 1). Macroinvertebrate taxa were filter feeding Asian clam (*Corbicula fluminea*), a grazing gastropod (*Physa acuta*), predatory dragonfly larvae (*Odonata*), and omnivorous non-biting fly midge larvae (*Chironomidae*). Only target species of fish and macroinvertebrates were collected and noted. For a full list of fish and macroinvertebrate species richness and assemblages see archived fish and macroinvertebrate annual reports compiled by the City of Muncie, IN, Bureau of Water Quality (http://www.munciesanitary.org/departments/bureau-of-water-quality/).

Trophic position.—The mean δ ^{15}N sample values of all sample groups ranged from 4.4 to

Table 1.—Mean δ ^{13}C and δ ^{15}N values (standard deviation in parentheses) and results of two sample *t* test comparisons by location (Prairie Creek Reservoir - PCR and White River - WR for each fish species. Q values are in parentheses and abbreviations are for Fig. 1 and Fig. 2.

Species	Mean total length (mm)	PCR mean δ ^{13}C	WR mean δ ^{13}C	*t*-value δ ^{13}Cdf = 4	PCR mean δ ^{15}N	WR mean δ ^{15}N	*t*-value δ ^{15}Ndf = 4
Spotfin shiner (*Cyprinella spiloptera*) SFSHR	84.7	−25.0 (0.9)	−28.0 (0.5)	5.1 ($q < 0.01$)	13.3 (0.3)	15.4 (0.2)	−9.1 ($q < 0.01$)
Gizzard shad (*Dorosoma cepedianum*) SHAD	272.8	−27.0 (0.5)	−31.2 (0.2)	14.0 ($q < 0.001$)	14.4 (0.5)	14.1 (0.1)	NS
Carp (*Cyprinus carpio*) CARP	549.8	−27.0 (0.9)	−26.1 (1.2)	NS	14.6 (0.7)	12.0 (0.7)	4.4 ($q < 0.01$)
Yellow bullhead (*Ameiurus natalis*) YBLHD	239	−26.3 (0.2)	−25.0 (0.3)	−6.0 ($q < 0.01$)	15.1 (0.6)	14.5 (0.1)	NS
Johnny darter (*Etheostoma nigrum*) JDRT	58.5	−23.4 (0.3)	−27.1 (0.6)	9.9 ($q < 0.001$)	16.0 (0.0)	15.9 (0.3)	NS
Smallmouth bass (*Micropterus dolomieu*) SMB	347	−24.4 (1.0)	−25.9 (1.0)	NS	16.2 (0.2)	15.4 (0.3)	3.1 ($q < 0.01$)
White crappie (*Pomoxis annularis*) WCRP	230.5	−26.7 (0.3)	−26.2 (0.6)	NS	17.1 (0.3)	16.2 (0.2)	4.7 ($q < 0.01$)

17.1 in the river and 4.4 to 16.2 in the reservoir habitats (Fig. 2). Terrestrial leaves occupied the lowest trophic position, while white crappie occupied the highest trophic position in both habitats. We did not detect a significant difference in the basal mean levels (CPOM, algae, aquatic macrophytes) of the food webs for the reservoir and river (two sample *t ^{15}N [mean CPOM, algae, aquatic macrophytes]* = −0.95, $q = 0.36$). Given similar basal δ ^{15}N values consumer trophic positions were estimated from relative δ ^{15}N values (see Vander Zanden et al. 1997 for trophic position formula). Common carp, smallmouth bass, and white crappie occupied a significantly higher trophic position in the reservoir than in the riverine habitat (Table 1). Spotfin shiner in the river had a higher trophic position than in the reservoir. Gizzard shad, johnny darter, and yellow bullhead did not differ significantly in trophic position between habitats. Coefficient of variation for all fish δ ^{15}N from the river was 9.4‰, comparably higher than the CV for all sampled reservoir fish (8.3‰).

Energy sources.—The range for δ ^{13}C values in the river was −32.0 for Asian clam to −20.1 for aquatic macrophytes (Fig. 2). The ranges for δ ^{13}C values in the reservoir were −38.0 for CPOM to −15.2 for aquatic macrophytes (Fig. 2). Mean δ ^{13}C values for the fish community (except yellow bullhead) were significantly more positive in the reservoir than the river habitats (Table 1; Fig. 2). The separation of δ ^{13}C among fishes corresponded with increasingly negative values of terrestrial primary producers (leaves) and more positive aquatic primary producers (algae). Gizzard shad, johnny darter, and spotfin shiner had higher δ ^{13}C levels in the reservoir than in the river habitat. Yellow bullhead had higher δ ^{13}C levels in river habitat. Common carp, smallmouth bass, and white crappie δ ^{13}C did not differ significantly among habitat types. Coefficient of variation for all fish δ ^{13}C from the river was 7.5‰, comparably higher than the CV for all sampled reservoir fish (5.7‰).

DISCUSSION

Trophic relationships.—Fishes are typically the top consumers in aquatic food webs (Pinnegar & Polunin 1999). Our results support this and identified the same top consumer (white crappie) for reservoir and riverine habitats. However, the hierarchical order of mean δ ^{15}N values for the other fish taxa

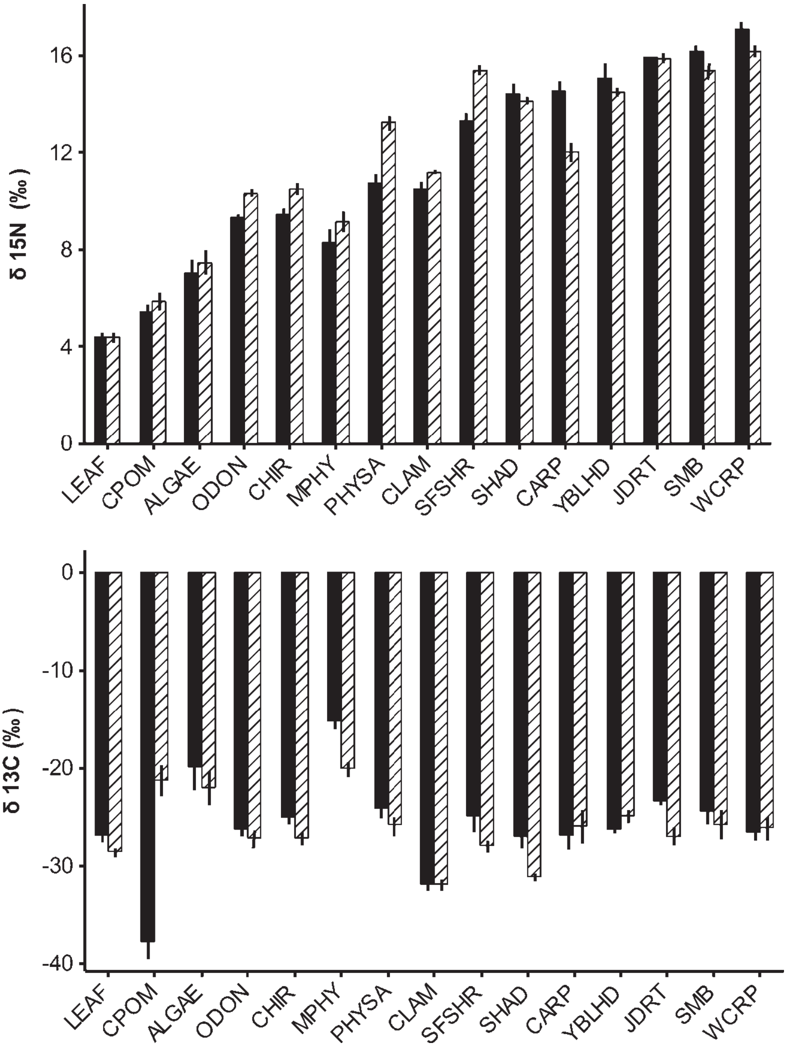

Figure 2.—Mean δ ^{13}C and δ ^{15}N for White River (striped bars) and Prairie Creek Reservoir (solid bars) taxa (error bars are one standard deviation). Abbreviations are *FISH:* spotfin shiner (SFSHR), gizzard shad (SHAD), common carp (CARP), yellow bullhead (YBLHD), johnny darter (JDRT), smallmouth bass (SMB), and white crappie (WCRP). *PRODUCERS:* algae (ALGAE), submerged macrophytes (MPHY), course particulate organic matter (CPOM), and terrestrial leaves (LEAF). *MACROINVERTEBRATE CONSUMERS*: grazing *Physa acuta* snail (PHYSA), non-biting midge larvae (CHIR), dragonfly larvae (ODON), and Asian clam *Corbicula* (CLAM). Note: fish were collected September 2009 and detritus, primary producers, and macroinvertebrates were collected September 2010.

differed between reservoir and riverine food webs. This reorganization is likely a reflection of habitat induced differences in diet and energy assimilation of these taxa. These results may also reflect decreased competition in the reservoir habitat and a more simplified web of interaction. Interestingly, the observed variation in δ ^{15}N trophic numbers paralleled habitat associations as inferred by previously published regional studies such as Trautman (1981). For example, fish taxa that are generally considered more lentic specialists (white crappie and gizzard shad) had greater δ ^{15}N variation in reservoir habitats compared with fishes that are generally considered lotic specialists (johnny darter and smallmouth bass) which exhibited greater variation in riverine habitats (Table 1).

Contribution of energy sources.—The reservoir and downstream riverine ecosystems contained contributions from both terrestrial and aquatic producers. However, the reservoir food web δ ^{13}C values suggested increased importance of autochthonous energy sources compared to the river that used a greater proportion of allochthonous sources as demonstrated through increasingly positive δ ^{13}C consumer values (Table 1; Fig. 2). This pattern is expected and has been demonstrated across multiple systems and habitats (Kling et al. 1992). Hoeinghaus et al. (2008) found that trophic web length increased in impounded systems as a function of primary autochthonous productivity. One exception to this pattern is the yellow bullhead individuals from the river habitat resulted in lower δ ^{13}C values. In addition, several outliers in the δ ^{13}C values of gizzard shad and *Corbicula* clam samples likely indicate the absence of several important biotic components from the riverine foodweb, such as zooplankton and phytoplankton. Overall, we attribute the intra- and interspecific differences in δ ^{13}C energy sources to be the result of habitat differences. However, some overlap in energy sources for the ecosystems are expected due to their spatial proximity (Hoeinghaus et al. 2008; Doi 2009).

Role of habitat degradation on isotope variation.—Reduced variation in isotope signatures (Fry 2002) and reduced variation in food chain length can indicate that habitats are homogenized and degraded. We suggest that further study of habitat degradation as a source of increased variation in local isotope signatures of aquatic communities is necessary. Further, we suggest that reduced variation in isotope signatures and modified consumer position and variation in food webs in a reservoir is a result of the altered reservoir habitat and is an indicator of loss of ecosystem stability (Hoeinghaus et al. 2008). These results provide additional evidence of negative impacts of altered natural hydrologic regimes. Future studies of food web variation at multiple spatial scales to examine additional impacts including agriculture, urbanization, and channelization are warranted.

ACKNOWLEDGEMENTS

This work was funded through an internal Ball State University Department of Biology grant provided by an anonymous donor. The authors wish to thank L. Etchison, M. Allen, A. Schmelzel and J. Jacquemin for field assistance and M. Bernot for use of laboratory equipment. All fishes were collected under Indiana scientific permit # 09-0233.

LITERATURE CITED

Boecklen, W.J., C.T. Yarnes, B.A. Cook & A.C. James. 2011. On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics 42:411–440.

Cabana, G. & J.B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences USA 93:10844–10847.

Carpenter, S.R., J.F. Kitchell, J.R. Hodgson, P.A. Cochran, J.J. Elser, M.M. Elser, D.M. Lodge, D. Kretchmer & X. He. 1987. Regulation of lake primary productivity by food web structure. Ecology 68:1863–1876.

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

Doi, H. 2009. Spatial patterns of autochthonous and allochthonous resources in aquatic food webs. Population Ecology 51:57–64.

Finlay, J.C., S. Khandwala & M.E. Power. 2002. Spatial scales of carbon flow in a river food web. Ecology 83:1845–1859.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnology and Oceanography 33:1182–1190.

Fry, B. 1991. Stable isotope diagrams of freshwater food webs. Ecology. 72:2293–2297.

Fry, B. 2002. Stable isotopic indicators of habitat use by Mississippi River fish. Journal of the North American Benthological Society 21:676–685.

Genner, M.J., S.J. Hawkins & G.F. Turner. 2003. Isotopic change throughout the life history of a Lake Malawi cichlid fish. Journal of Fish Biology 62:907–917.

Hoeinghaus, D.J., K.O. Winemiller & A.A. Agostinho. 2008. Hydrogeomorphology and river impoundment affect food-chain length of diverse Neotropical food webs. Oikos 117:984–995.

Hoggatt, R.E. 1975. Drainage area of Indiana streams. Department of the Interior, U.S. Geological Survey, Indianapolis, Indiana.

Jardine, T.D., R.A. Curry, K.S. Heard & R.A. Cunjak. 2005. High fidelity: isotopic relationship between stream invertebrates and their gut contents. Journal of the North American Benthological Society 24:290–299.

Kling, G.W., B. Fry & W.J. O'Brien. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology 73:561–566.

Melville, A.J. & R.M. Connolly. 2003. Spatial analysis of stable isotope data to determine primary sources of nutrition for fish. Oecologia 136:499–507.

Peterson, B.J. & B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Reviews in Ecology and Systematics 18:293–320.

Petts, G.E. 1984. Impounded Rivers: Perspectives for Ecological Management. John Wiley and Sons, New York, USA.

Pinnegar, J.K. & N.V.C. Polunin. 1999. Differential fractionation of 13C and 15N among fish tissue: implications for the study of trophic interactions. Functional Ecology 13:225–231.

Poff, L.N. & J.V. Ward. 1990. The physical habitat template of lotic systems: recovery in the context of historical pattern of spatio-temporal heterogeneity. Environmental Management 14: 629–646.

Poff, L.N. & J.D. Allan. 1995. Functional organization of stream fish assemblages in relation to hydrological variability. Ecology 76:606–627.

Poff, L.N., J.D. Allan, M.B. Bain, J.R. Karr, K.L. Prestegaard, B.D. Richter, R.E. Sparks & J.C. Stromberg. 1997. The natural flow regime: A paradigm for river conservation and restoration. Bioscience 47:769–784.

Poff, L.N., J.D. Olden, D.M. Merritt & D.M. Pepin. 2007. Homogenization of regional river dynamics by dams and global biodiversity implications. Proceedings of the National Academy of Sciences USA 104:5732–5737.

Polis, G.A. & D.R. Strong. 1996. Food web complexity and community dynamics. American Naturalist 147:813–846.

Pringle, C.M. 1997. Exploring how disturbance is transmitted upstream: going against the flow. Journal of the North American Benthological Society 16:425–438.

Pringle, C.M., M.C. Freeman & B.J. Freeman. 2000. Regional effects of hydrologic alterations on riverine macrobiota in the New World: tropical-temperate comparisons. Bioscience 50:807–823.

Roach, K.A., J.H. Thorp & M.D. Delong. 2009. Influence of lateral gradients of hydrological connectivity on trophic positions of fishes in the Upper Mississippi River. Freshwater Biology 54:607–620.

Storey, J.D. 2002. A direct approach to false discovery rates. Journal of the Royal Statistics Society Serial B Statistics Methodology 64:479–498.

Taylor, C.M., D.S. Millican, M.E. Roberts & W.T. Slack. 2008. Long-term change to fish assemblages and the flow regime in a southeastern U.S. river system after extensive aquatic ecosystem fragmentation. Ecography 31:787–797.

Trautman, M.B. 1981. The Fishes of Ohio. Ohio State University Press, Columbus, Ohio.

Vander Zanden, M.J., G. Cabana & J.B. Rasmussen. 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ15N) and literature dietary data. Canadian Journal of Fisheries and Aquatic Science 54:1142–1158.

Wainwright, S.C., M.J. Fogarty, R.C. Greenfield & B. Fry. 1993. Long-term changes in the Georges Bank food web: trends in stable isotopic compositions of fish scales. Marine Biology 115:481–493.

Ward, J.V. & J.A. Stanford. 1983. The serial discontinuity concept of lotic ecosystems. Pp. 29–42. *In* Dynamics of Lotic Ecosystems (T.D. Fontaine III & S.M. Bartell, eds.). Ann Arbor Science, Ann Arbor, Michigan.

Manuscript received 17 August 2013, revised 13 February 2014.

2013. Proceedings of the Indiana Academy of Science 122(2):69–77

DECOMPOSITION OF NITROGLYCERIN IN SMOKELESS POWDERS BY AEROBIC THERMOPHILIC COMPOSTING

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ABSTRACT. The reported study evaluated the disappearance of nitroglycerin as applied in commercial smokeless powder (SP), in compost prepared from vegetative matter. Double base smokeless powder was applied to mesocosms at rates of 0, 1 and 5% (w/w) and mesocosms were incubated for 70 d. Compost was prepared at two moisture levels, *viz.*, −0.5 bar and −0.1 bar (*wet* and *moist*, respectively). Amendments to microcosms included agricultural limestone and alkaline powerplant fly ash. Determination of free NG was performed using gas chromatography with electron capture detection. In wet compost, NG concentrations decreased by 72% from Day 7 to Day 35. In contrast, NG in moist compost exhibited significantly ($p < 0.05$) less NG solubilization and decomposition. Compost nitrate levels increased markedly after 28 d incubation; however, no correlation was determined between NG concentrations and NO_3 levels or compost pH. Incorporation of limestone or fly ash had no marked impact on NG disappearance. Destruction of NG appears to be substantially more rapid via thermophilic composting compared with incubation in soil, and should serve as a low-cost, viable technology for NG-contaminated media.

Keywords: Composting, nitroglycerin, smokeless powder

INTRODUCTION

Nitroglycerin (NG; glycerol trinitrate; $C_3H_5N_3O_9$) is commonly employed by military forces as an ingredient in artillery and rocket propellants (Accashian et al. 1998). It is also used extensively by private firearms enthusiasts, as NG is a component of smokeless powders (Ahlner et al. 1991; Halasz 2010). Nitroglycerin has been documented as a significant contaminant of soil, surface water, and groundwater resulting from both military conflict and munitions manufacturing and testing (Jenkins et al. 2002; 2001; Pennington et al. 2003, 2002; 2001). It has been detected in soils at concentrations as high as 14,000 mg/kg (Jenkins et al. 2007; Hewitt et al. 2004). Thiboutot et al. (2004a, 2004b) measured soil NG concentrations at 6,560 mg/kg. Nitroglycerin occurs in soil at Indiana military bases and private firing ranges.

In recent decades a range of chemical, physical and biological methods have been applied for remediation of NG and other energetic materials in soil and groundwater, including sorption to activated carbon, reduction with inorganic chemicals, Fenton reaction, alkaline hydrolysis and bioremediation (Kalderis et al. 2011; Accashian et al. 1998). Many of the above chemical treatment processes, however, employ hazardous chemicals, require specialized training by operators, and tend to adversely affect soil biological and physical quality.

In the United States, environmentally-friendly remediation technologies such as bioremediation are encouraged by federal and state agencies (USEPA 1993a; 1993b). Degradation of NG has been documented under both aerobic and anaerobic conditions using mixed or pure strains of bacterial species (Marshall and White 2001; Meng et al. 1995; White et al. 1996; Wendt et al. 1978; Pesari & Grasso 1993). Recent research has revealed the existence of NG-degrading bacteria in activated sewage sludge, river water, and soils (White et al. 1995; Wendt et al. 1978; Zhang et al. 1997).

Various studies have investigated composting as a means to treat energetics-contaminated soils; however, virtually all have addressed treatment of TNT (2,4,6-trinitrotoluene), RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) (Pennington & Brannon 2002; Williams et al. 1992; Griest et al. 1990; Lowe et al. 1989; Isbister et al. 1984). Few have studied decomposition of propellants. During 45-day incubations using bench-scale compost reactors, no biodegradation of triple (M3 IAIEl) and

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double base (NOSIH-AA2) propellants was observed by Adrian (1996).

Both limestone and powerplant fly ash have been used as additives in composting food waste and yard waste (Wong et al. 2009; Koivula et al. 2004). According to the American Coal Ash Association (2011), 59.9 million tons of fly ash were generated in the US in 2011. Beneficial uses of fly ash include as a bulking material and buffering agent in composting. Wong et al. (2009) found that fly ash buffered pH during composting of food waste and enhanced decomposition efficiency. When ash was mixed with agricultural lime, the composting period was shortened to ~28 days compared with ~42 days in 3% lime. The addition of ash to compost increased the porosity of the feedstock, and also increased concentrations of macro- and micronutrients of the final product (Koivula et al. 2004).

Limited work has been documented regarding the decomposition of NG, and even less is known with respect to the reactions of smokeless powder and consequent release of NG. In the reported study, the feasibility of aerobic thermophilic composting was assessed for decomposition of NG in smokeless powder. Specifically, the objectives were to compare the efficiency of smokeless powder-derived NG decomposition in vegetable composts: (1) of two moisture regimes; and (2) treated with liming agents (agricultural limestone and powerplant fly ash).

MATERIALS AND METHODS

Characterization of compost feedstock and fly ash.—Compost feedstock consisting of fruit and vegetable scraps and yard waste (leaves, grass clippings) was prepared, with paper scraps added as a bulking agent. The feedstock was incubated for approx. four weeks and aerated via physical mixing every two days. Bulk samples of fly ash were collected from air pollution control devices at the Breed power station (Fairbanks, IN).

Solids (compost and fly ash) pH was measured using a standardized AB15 Accumet pH meter on a 1:5 solids:deionized H_2O slurry. Total organic carbon (TOC) and total nitrogen (N) were analyzed on a Perkin Elmer Series II CHNS/O Analyzer 2400 (Shelton, CT). Acetanilide was the standard used. Water soluble nitrate was measured using Szechrome reagents (Polysciences, no date) in a BioteK PowerWave XS2 microassay system® (Winooski, VT).

Potassium concentrations were determined after extraction by 1.0 N ammonium acetate, pH 7.0, followed by analysis using a Perkin Elmer AAnalyst 2000 flame atomic absorption spectrometer (FAAS) set in emission mode (Knudsen 1982). Phosphorous was measured using Bray-1 extractant combined with a microplate method (PowerWave XS2 Microplate Spectrophotometer) (Olsen 1982). Metal (Cd, Cr, Cu, Fe, Ni, Pb, and Zn) concentrations were determined using DTPA (diethylene triamine pentaacetic acid) extraction followed by FAAS. Samples were extracted with DTPA solution (0.05 M) for 2 h on an oscillating shaker. The mixtures were filtered through Whatman no. 2 filter paper and analyzed using FAAS (Sposito 1982). All glassware was washed with Alconox^{TM} detergent and rinsed with deionized water prior to use.

Nitroglycerin incubation.—Smokeless powder was added at a rate of 1% (w/w) and 5% each to four nylon mesh bags (500g) of compost and mixed manually with a stainless steel spatula. The mesh bags were subsequently placed into 20-l compost-filled mesocosms, which were transferred to a Model 815 Freas Incubator set at 55°C. There were four replications for each treatment.

Compost treatments included two moisture regimes, i.e., wet versus moist. The wet treatment was set to approx. −0.5 bar moisture, and the moist treatment to −0.1 bar as measured using an Irrometer® tensiometer.

A second set of mesocosms (−0.1 bar moisture only) was treated with either agricultural limestone or fly ash at 1% or 5% (w/w). One treatment received 2.5% fly ash plus 2.5% limestone. The compost feedstock was aerated by mixing with a steel spatula every 7 d. No supplemental nutrients were added to the mesocosms. Four replicates were prepared for each treatment.

Compost solids were collected weekly during 70 days of incubation. Samples were collected using a stainless steel rod and transferred immediately to plastic bags. Solids (5 g) were extracted by shaking with 25 ml 92% ethanol for 30 min on a reciprocating shaker (Asbaghi & Pichtel 2012). The suspensions were filtered using Whatman no. 2 filter paper and stored at 4°C until analysis. A sterile, autoclaved sample was also collected at each date as a control.

Gas chromatographic (GC) analysis of extracts was conducted using a Perkin Elmer Clarus 500 gas chromatograph with an electron capture detector (ECD) and a Programmed on-Column (POC) Inlet System. The system included a 6 m Perkin Elmer fused silica capillary column measuring 0.53 mm ID with a 1.5 µm film thickness. Samples measuring 1 µl were injected into the column. The GC oven was temperature programmed as follows: 130°C for 1 min, 10°C/min ramp to 160°C, 30°C/min ramp to 285°C hold for 1 min. The carrier gas was He at a 7.0 ml/min flow rate. The ECD temperature was set to 300°C and the makeup gas was N_2 at a 30 ml/min flow rate. A 1000 mg/L nitroglycerin (NG) standard in ethanol was obtained from AccuStandard, Inc., New Haven, CT. The TotalChrom® Navigator Application (v. 6.3) (Perkin Elmer, Shelton, CT) was used to process, record and report the chromatographic results.

At each sampling date, the pH and soluble nitrate content of the compost mass was determined as described above.

Statistical analysis.—Data obtained for NG decomposition as a function of treatment were tested for statistical significance using analysis of variance (ANOVA). Tests showing significance at $\alpha = 0.05$ were analyzed using a Pairwise Comparisons Test. SPSS^{TM} and MS Excel were used on a Windows-based PC for all statistical analyses.

RESULTS AND DISCUSSION

Characterization of compost, fly ash and smokeless powder.—The compost feedstock was alkaline (pH = 7.9). Total C and N measured 31.3 and 2.5%, respectively, resulting in a C:N ratio of 12.6:1. The feedstock contained low concentrations of P and extractable metals (Table 1). The fly ash was highly alkaline (pH = 11.8) and contained low concentrations of total C and N (1% and < 0.1%, respectively). Concentrations of P and extractable metals were low. Mean NG concentration in the SP was 255,150 mg/kg (data not shown). This value is comparable to those for other commercial smokeless powders (Western 2010, 2007).

Nitroglycerin incubation.—In the wet compost containing 1% SP, soluble NG was detected at 1382 mg/kg on Day 7 and attained the highest level, 2023 mg/kg, at Day 14 (Fig. 1). By Day 63 soluble NG concentrations had declined to 36 mg/kg. In the 5% SP treatment, soluble NG was measured at 13,192 mg/kg on Day 7, following which it declined to 5438 on Day 14 and 1523 on Day 35 (Fig. 1), a 72% decrease over 28 d. Nitroglycerin concentrations declined gradually from Day 42 through Day 70 (2950 to 2303 mg/kg, respectively).

Soluble NG was significantly ($p < 0.05$) higher in the wet compost compared with moist compost (Fig. 2), implying the enhanced participation of anaerobic microorganisms. At Day 14, NG concentrations in the moist compost measured 66 mg/kg, 97% less than that measured in wet compost.

For both moisture regimes, composting SP resulted in significantly ($p < 0.05$) more rapid release and decomposition of NG as compared with decomposition in soil. Asbaghi (2012) found that NG occurring in smokeless powder persisted in soil in significant quantities (152 mg/kg at a 1% SP rate) after 60 d. The current findings also contrast those of Adrian (1996), where negligible biodegradation of triple and double base propellants was observed during 45 d of composting. Double base smokeless powders typically contain nitrocellulose, dibutyl phthalate, diphenylamine, ethyl centralite, waxes and other hydrocarbon-based additives (Western 2007). All should be amenable to beta-oxidation as carried out by heterotrophic microorganisms present in compost. As these compounds are acted upon by microorganisms, the NG is released.

For both the limestone- and fly ash-amended compost, NG was released at 56 d (Figs. 2–3), following which the concentrations returned to baseline.

The nascent NG should be readily amenable to microbial decomposition. Mixed microbial cultures from aeration tank sludge were capable of metabolizing NG (Accashian et al. 1998; Wendt et al. 1978; Zhang et al. 1997). Aerobic microbial cultures have been shown to have the capacity to remove NG rapidly even in the absence of a supplemental carbon source. Most studies, however, have shown the benefits of additional carbon sources (Christodoulatos et al. 1997; Wendt et al. 1978).

Substantial N losses were detected in the compost mixtures (Fig. 4). Most of the losses occurred during Days 28–70 of composting and maturation. Soluble nitrate concentrations increased throughout the study; from Day 35 to

Table 1.—Selected chemical and physical properties of the compost and fly ash amendment.

Parameter	Compost	Fly ash
pH	7.9	11.8
EC, dS/m	0.23	2.4
C, %	31.3	1.0
N, %	2.5	< 0.1
C:N ratio	12.6:1	n/a
Bray-1 P, %	2.8	1.0
Total metals, mg/kg		
Cd	< 0.1	16
Cu	0.2	90
Fe	4290	122500
Zn	160	890

day 70, a 63% increase in nitrate was noted for the 1% SP rate (2.6 to 7.0 mg/kg, respectively). It is not known, however, whether the nitrate originated from the NG or the compost feedstock. Eklind & Kirchmann (2000) found that net N losses were 43–62% in various mixtures of vegetable compost. All recent work is in agreement as regards a single NG denitration pathway under both aerobic and anaerobic conditions in which NG is used as a nitrogen source. Biodegradation occurs via successive denitrations to glycerol dinitrates (GDNs) and glycerol mononitrates (GMNs). Nitroglycerin metabolism produces glycerol 1,2- and 1,3-dinitrate (1,2-GDN and 1,3-GDN), and glycerol 1- and 2-mononitrate (1-GMN and 2-GMN) (Wendt et al. 1978), while nitrate is lost from the parent molecule.

Compost pH trends were similar for both the limestone and fly ash treatments (Figs. 5–6).

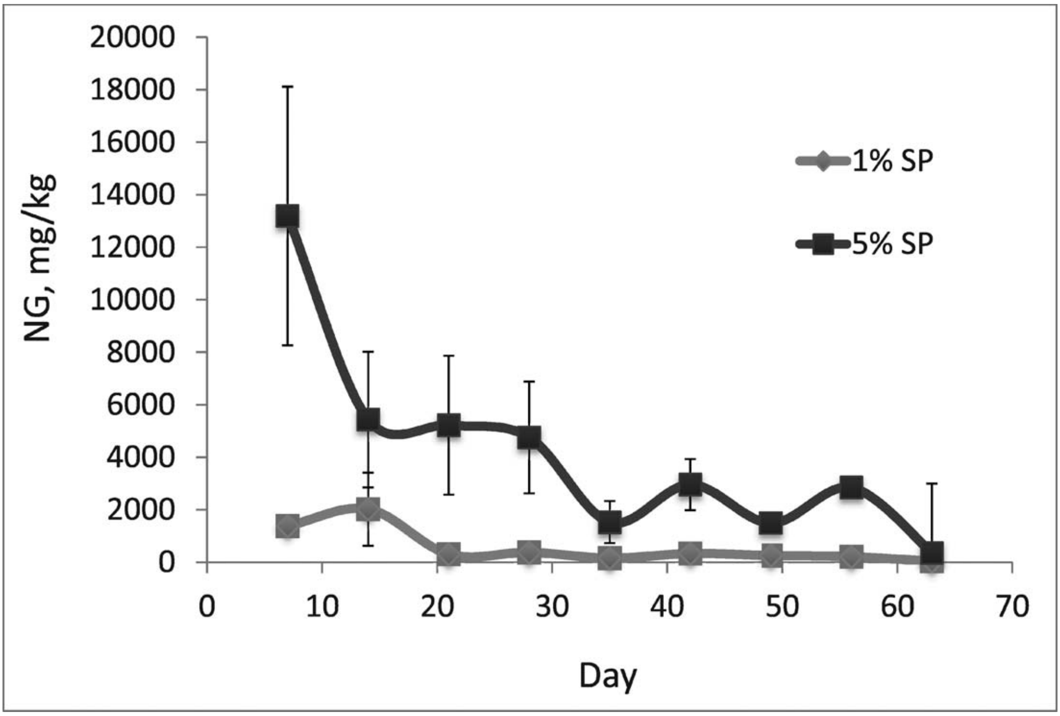

Figure 1.—Nitroglycerin occurring in the wet compost at two rates of smokeless powder, 63 d.

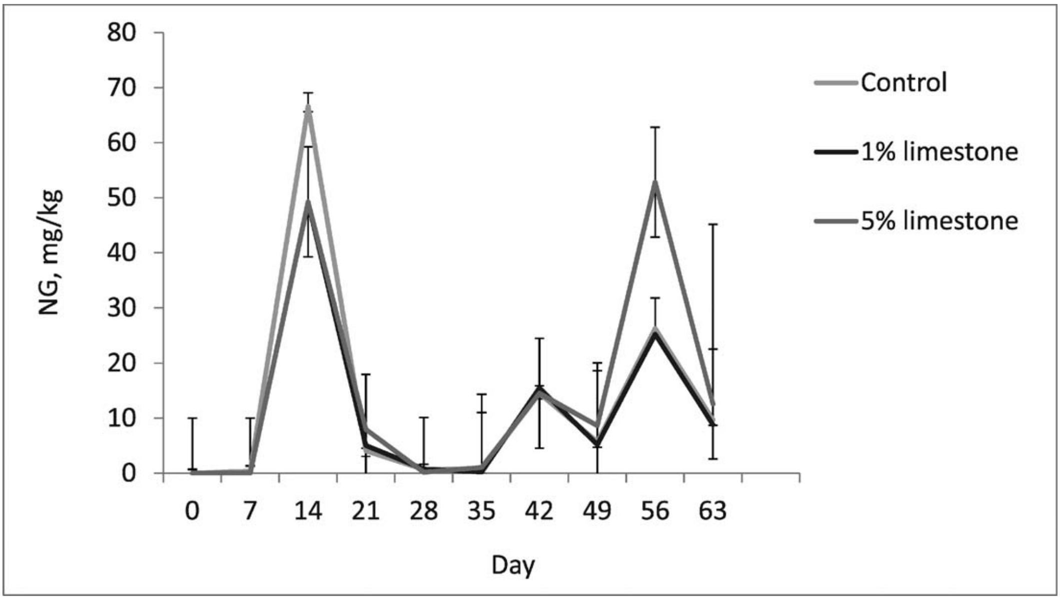

Figure 2.—Nitroglycerin occurring in compost amended with two rates of limestone, 63 d.

Initial pH values ranged between 7.5–7.8. By Day 14, pH climbed to 9.1 and subsequently declined in all treatments. By Day 70, pH values ranged from 7.9 for the 5% limestone treatment, to 8.6 for the 5% fly ash treatment. Such pH values are fairly typical for compost originating from vegetable wastes. Eklind & Kirchmann (2000) measured pH values of 8.2 in household waste compost after 600 d. Incorporation of limestone or fly ash had no marked impact on NG disappearance (Figs. 5–6).

Compost pH measurements did not correlate with nitroglycerin solubilization or decomposition. Nitroglycerin decomposition does not result in the release of basic compounds;

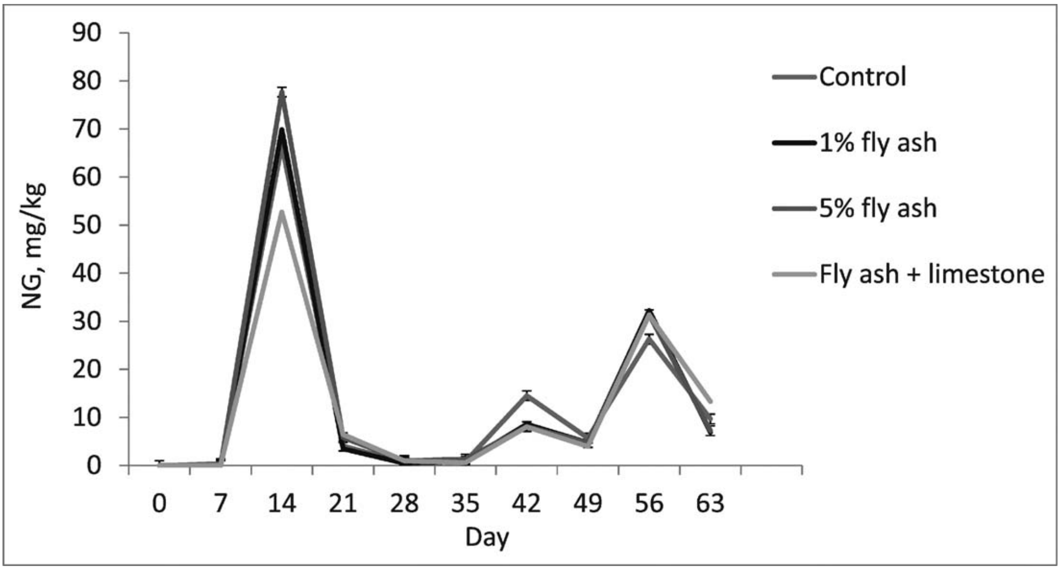

Figure 3.—Nitroglycerin occurring in compost amended with two rates of fly ash, and fly ash + limestone, 63 d.

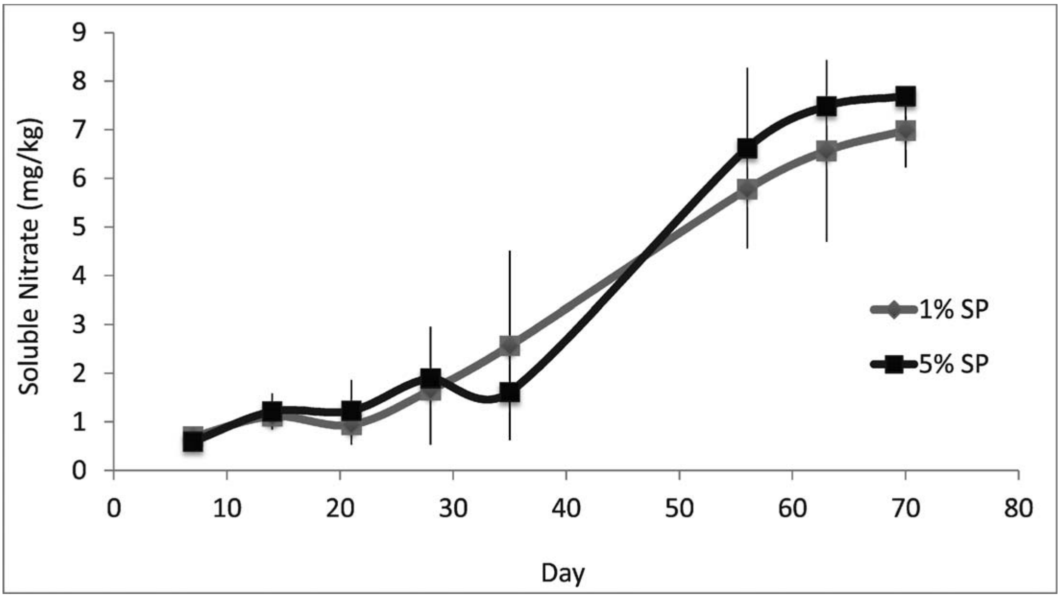

Figure 4.—Soluble nitrate levels in compost treated with two rates of smokeless powder, 63 d.

however, when NO_3^- is released from the NG molecule there is the potential for biological reduction to ammonium (NH_4^+) (Brady & Weil, 2010). In a study of smokeless powder decomposition in soil, Trensey (2013) found that ammonium concentrations ranged from 6.4 to 16.3 mg/kg.

Destruction of NG appears to be substantially more rapid via thermophilic composting compared with incubation in soil, and should serve as a low-cost, viable technology for NG-contaminated media. Studies are currently underway in our laboratory attempting to isolate and enumerate the various microbial

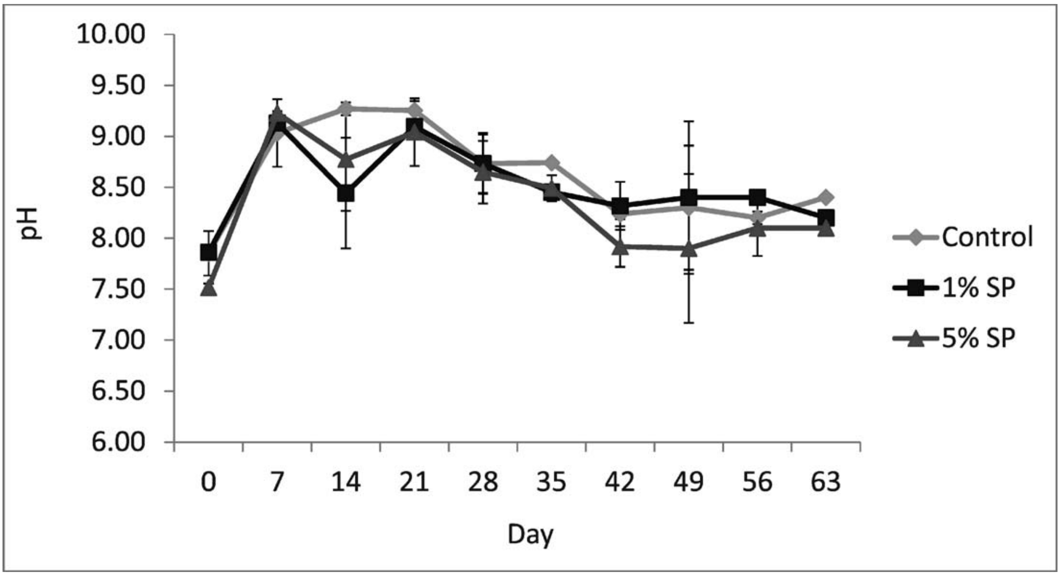

Figure 5.—pH trends in compost amended with limestone, 63 d.

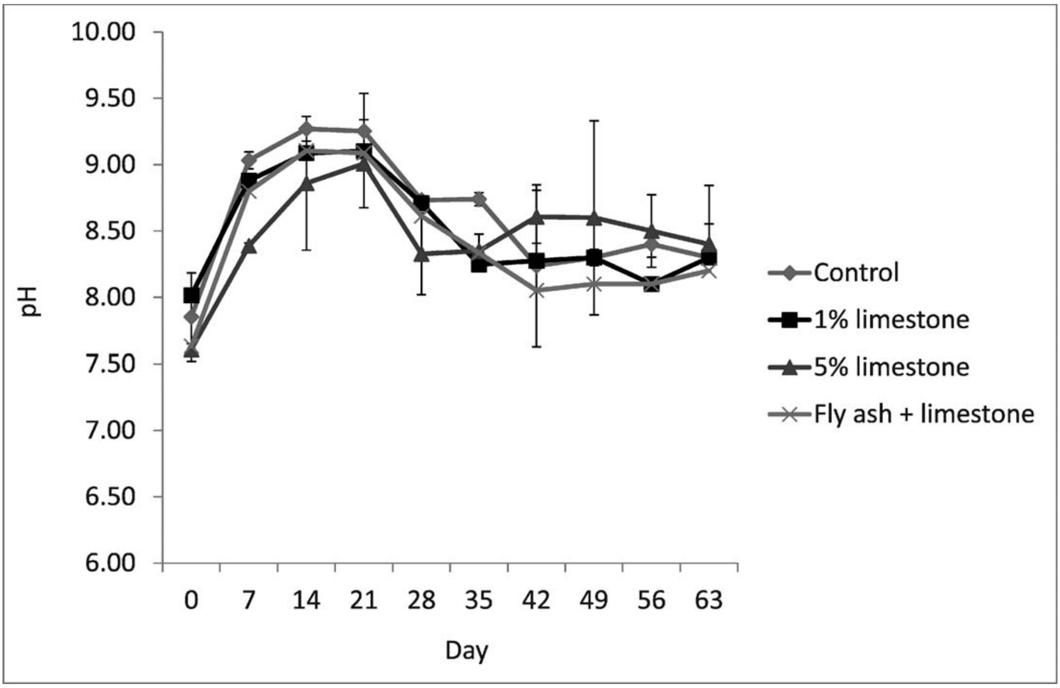

Figure 6.—pH trends in compost amended with fly ash, and fly ash + limestone, 63 d.

populations that are active during microbial decomposition of nitroglycerin.

REFERENCES

Accashian, J.V., R.T. Vinopal, B.J. Kim & B.F. Smets. 1998. Aerobic growth on nitroglycerin as the sole carbon, nitrogen, and energy source by a mixed bacterial culture. Applied and Environmental Microbiology 64:3300–3304.

Ahlner, J., R.G. Andersson, K. Torfgard & K.L. Axelsson. 1991. Organic nitrate esters: Clinical use and mechanisms of actions. Pharmacological Reviews 43:351–423.

Asbaghi, N. & J. Pichtel. 2012. Phytoremediation of nitroglycerin in smokeless powders. Environmental Biotechnology 8(2):45–54.

Asbaghi, N. 2012. Phytoremediation of nitroglycerin in smokeless powders. Doctoral Dissertation. Ball State University, Muncie, Indiana.

Brady, N.C. & R.R. Weil. 2010. Elements of the Nature and Properties of Soils, 3rd Ed. Pearson Publishing. Upper Saddle River, New Jersey.

Brannon, J.M., D.D. Adrian, J.C. Pennington, T.E. Myers & C.A. Hayes. 1992. Slow release of PCB, TNT, and RDX from soils and sediments. Technical Report EL-92-38, US Army Corps of Engineers, Waterways Experiment Station, Vicksburg, Mississippi.

Christodoulatos, C., S. Bhaumik & B.W. Brodman. 1997. Anaerobic biodegradation of nitroglycerin. Water Research 31:1426–1470.

Halasz, A., S. Thiboutot, G. Ampleman & J. Hawari. 2010. Microwave-assisted hydrolysis of nitroglycerine (NG) under mild alkaline conditions: New insight into the degradation pathway. Chemosphere 79:228–232.

Hewitt, A.D., T.F. Jenkins, T.A. Ranney, D. Lambert & N. Perron. 2004. Characterization of energetic residues at firing ranges: Schofield barracks and training area. Distribution and fate of energetics on DOD test and training ranges. Interim report 4 ERDC TR-04-4, US Army Engineer Research and Development Center, Vicksburg, Mississippi.

Jenkins, T.F., J.C. Pennington, G. Ampleman, S. Thiboutot, M.R. Walsh, E. Diaz, K.M. Dontsova, A.D. Hewitt, M.E. Walsh, S.R. Bigl, S. Taylor, D.K. MacMillan, J.L. Clausen, D. Lambert, N.M. Perron, M.C. Lapointe, S. Brochu, M. Brassard, R. Stowe, R. Farinaccio, A. Gagnon, A. Marois, D. Gilbert, D. Faucher, S. Yost, C. Hayes, C.A. Ramsey, R.J. Rachow, J.E. Zufelt, C.M. Collins, A.B. Gelvin & S.P. Saari. 2007. Characterization and fate of gun and rocket propellant residues on testing and training ranges: Interim report 1. Technical report ERDC TR-07-01, Strategic Environmental Research and Development Program, Vicksburg, Mississippi.

Jenkins, T.F., M.E. Walsh, P.H. Miyares, A.D. Hewitt, N.H. Collins & T.A. Ranney. 2002. Use of snow covered ranges to estimate explosives residues from high-order detonations of army munitions. Thermochimica Acta 384:173–185.

Kalderis, D., A.L. Juhasz, R. Boopathy & S. Comfort. 2011. Soils contaminated with explosives: Environmental fate and evaluation of state-of-the-art remediation processes (IUPAC Technical Report). Pure Applied Chemistry 83(7): 1407–1484.

Knudsen, D., A. Peterson & P.F. Pratt. 1982. Potassium. *In* Methods of Soil Analysis, Part 2 (A.L. Page, R.L. Miller & D.R. Keeney, eds.). American Society of Agronomy, Madison, Wisconsin.

Marshall, S.J. & G.F. White. 2001. Complete denitration of nitroglycerin by bacteria isolated from a washwater soakaway. Applied and Environmental Microbiology 67:2622–2626.

Meng, M., W.-Q. Sun, L.A. Geelhaar, G. Kumar, A.R. Patel, G.F. Payne, M.K. Speedie & J.R. Stacy. 1995. Denitration of glycerol trinitrate by resting cells and cell extracts of *Bacillus thuringiensis/cereus* and *Enterobacter agglomerans*. Applied and Environmental Microbiology 61:2548–2553.

Olsen, S.R. & L.E. Sommers. 1982. Phosphorus, Pp. 403–430. *In* Methods of Soil Analysis, Part 2 (A.L. Page, R.L. Miller & D.R. Keeney, eds.). American Society of Agronomy, Madison, Wisconsin.

Polysciences, Inc. No date. Szechrome reagents. Technical Data Sheet 239. Warrington, Pennsylvania

Pennington, J.C. & J.M. Brannon. 2002. Environmental fate of explosives. Thermochimica Acta 384:163–172.

Pennington, J.C., T.F. Jenkins, G. Ampleman, S. Thiboulet, J.M. Brannon, J. Lewis, J. DeLaney, J. Clausen, A.D. Hewitt, M.A. Hollander, C.A. Hayes, J.A. Stark, A. Marois, S. Brochu, H.Q. Dinh, D. Lambert, A. Gagnon, M. Borchard, R.B. Martel, P. Patrick, M. Nancy, R. LeFebure, W. Davis, T.A. Ranney, C. Ganther, S. Taylor & J.M. Ballard. 2003. Distribution and fate of explosives on DoD test and training ranges: Interim report 3. Technical Report ERDC TR-03-2. US Army Engineer Research and Development Center. Vicksburg, Mississippi.

Pennington, J.C., J.M. Brannon & J.E. Mirecki. 2002. Distribution and fate of energetics on DoD test and training ranges: Interim Report 2. Technical Report ERDC TR-02-8. US Army Engineer Research and Development Center, Vicksburg, Mississippi.

Pennington, J.C., T.F. Jenkins, J.M. Brannon, J. Lynch, T.A. Ranney, T.E. Berry, C.A. Hayes, P.H. Miyares, M.E. Walsh, A.D. Hewitt, N. Perron & J.J. Delfino. 2001. Distribution and fate of energetics on DoD test and training ranges: Interim Report 1. Technical Report ERDC TR-01-13. US Army Engineer Research and Development Center, Vicksburg, Mississippi.

Pesari, H. & D. Grasso. 1993. Biodegradation of an inhibitory nongrowth substrate (nitroglycerin) in batch reactors. Biotechnology and Bioengineering 41:79–87.

Sims, G.K., T.R. Ellsworth & R.L. Mulvaney. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. Communications in Soil Science and Plant Analysis 26(1–2):303–316.

Spanggord, R.J., R.W. Mabey, T.W. Chou, P.L. Alferness, D.S. Tse & T. Mill. 1980. Environmental fate studies of HMX, phase II, Detailed studies, Final Report, SRI International, Menlo Park, California.

Sposito, G., L.J. Lund & A.C. Chang. 1982. Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Zn, Cd and Pb in solid phases. Soil Science Society of America Journal 46:260–264.

Thiboutot, S., G. Ampleman, A. Marois, A. Gagnon, M. Bouchard, A.D. Hewitt, T.F. Jenkins, M. Walsh, K. Bjella, C. Ramsey & T.A. Ranney. 2004a. Environmental conditions of surface soils, CFB Gagetown training area: delineation of the presence of munitions-related residues (phase III, final report). Technical report DREV-TR-2004-205, Defence Research and Development Canada-Valcartier, Quebec, Canada. http://cradpdf.drdc.gc.ca/PDFS/unc57/p522641.pdf

Thiboutot, S., G. Ampleman & A. Marois. 2004b. Environmental condition of surface soils and biomass prevailing in the training area at CFB Gagetown, New Brunswick. DRDC Valcartier TR 2003-152, Defence Research and Development Canada-Valcartier, Quebec, Canada.

US Environmental Protection Agency. 1993a. Guide for Conducting Treatability Studies Under CERCLA: Biodegradation Remedy Selection. EPA/540/R-93-519b. Office of Emergency and Remedial Response, Washington, DC.

US Environmental Protection Agency. 1993b. Biogenesis^{TM} Soil Washing Technology. Innovative Technology Evaluation Report. EPA/540/R-93/510. Office of Research and Development. Cincinnati, Ohio.

Wendt, T.M., J.H. Cornell & A.M. Kaplan. 1978. Microbial degradation of glycerol nitrates. Applied and Environmental Microbiology 36:693–699.

Western Powders, Inc. 2010. Material Safety Data Sheet. Ramshot Double-base Smokeless Powders. Miles City, MT. [Online]. Available at http://www.ramshot.com/wp-content/uploads/2010/12/msds1.pdf, Accessed 04 March 2014.

Western Powders, Inc. 2007. Material Safety Data Sheet. Accurate Double-base smokeless powder, propellant. Miles City, MT. Online]. Available at http://207.65.1.181/data/AccurateDoubleBasePropellant.pdf, Accessed 04 March 2014.

White, G.F., J.R. Snape & S. Nicklin. 1996. Bacterial biodegradation of glycerol trinitrate. International Biodeterioration and Biodegradation 38:77–82.

White, G.F., J.R. Snape & S. Nicklin. 1995. Bacterial degradation of nitrate ester explosives. In Proceedings of the 26th International Annual Conference of ICT, Pyrotechnics: Basic principles, technology, application. DWS Werbeagentur und Verlag GmbH, Karlsruhe, Germany.

Wong, J.W.-C., S.O. Fung & A. Selvam. 2009. Coal fly ash and lime addition enhances the rate and efficiency of decomposition of food waste during composting. Bioresource Technology 100:3324–3331.

Zhang, Y.Z., S.T. Sundaram, A. Sharma & B.W. Brodman. 1997. Biodegradation of glyceryl trinitrate by *Penicillium corylophilum* Dierckx. Applied and Environmental Microbiology 63:1712–1714.

Manuscript received 18 September 2013, revised 18 March 2014.

2013. Proceedings of the Indiana Academy of Science 122(2):78–92

THE INFLUENCE OF COPPER, LEAD AND IRON ON STREAM SEDIMENT NITRIFICATION IN CENTRAL INDIANA STREAMS

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ABSTRACT. Copper, lead, and iron have frequently been detected throughout Indiana freshwaters. Since microbial activity is a holistic measure of ecosystem function, changes in microbial activity in response to metal concentrations may indicate potential areas of concern. Metal concentrations in seven streams of the Upper White River watershed of central Indiana were measured during spring (May) and summer (August) in conjunction with measurement of sediment nitrification rates using the nitrapyrin-inhibition technique. Additionally, the influence of copper, lead, and iron on microbial nitrification was measured using *in vitro* mesocosms inoculated with stream sediment. Sediment metal concentrations ranged from 654–1,985 mg Fe/kg and 1.00–2.91 mg Cu/kg sediment. Dissolved metal concentrations ranged from below detection to 0.10 mg Fe /L and 0.01–0.02 mg Cu/L. Stream sediment nitrification rates were positively correlated to sediment copper concentrations. Metal concentrations of 127 mg/L may reduce stream sediment nitrification although stream physiochemical characteristics and history of metal exposure also influence microbial response. Further, stream sediment metal concentrations may affect nitrifying microbes more than dissolved metal concentrations.

Keywords: Copper, iron, lead, nitrification, metal concentrations, stream

INTRODUCTION

The rate of nitrification in the environment is dependent on multiple factors including the biological community, dissolved organic carbon (DOC), temperature, dissolved oxygen (DO), and pH (Kemp and Dodds 2001; Strauss et al. 2002; Earl et al. 2006). Nitrification is primarily limited by the concentration of ammonia and nitrite in an environment which can be influenced by stream organisms (Jones and Hood 1980; Villaverde et al. 1996; Lee et al. 1997; Ciudada et al. 2007) as well as surrounding land use (Galloway 1998). Nitrification is secondarily limited by physiochemical characteristics of the environment. Although much research has documented variation in nitrification rates, there is limited understanding of how anthropogenic contaminants may influence nitrification rates. Specifically, increased urbanization and industrial activities have raised concerns regarding metal pollution in freshwaters and their potential influence on microbial activity.

The effect of metal concentrations on nitrification rates is related to the type and concentration of metal and the species of nitrifying microbes present (Mertoglu et al. 2008). Metals are found naturally in the environment but can also enter an ecosystem via human activity including following fossil fuel combustion and leaching from disposed items including batteries and other metal products (Guinee et al.1999; Rimmer et al. 2006). Even as recycling efforts increase, the mining of many metals continues to grow, leading to an increase of metal concentrations in the environment (Guinee et al.1999). Landscape remediation and construction activities can cause metals trapped within soil to more enter aquatic ecosystems causing the sediment to act as a metal sink (Blake et al. 2007). Depending on water flow, sediment-bound metal may diffuse into the water column. The rate of diffusion is a function of water chemistry as well as stream discharge yielding variable metal concentrations over time. Freshwater metal concentrations and their potential

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effects on the ecosystem must be more comprehensively assessed to protect freshwater integrity.

Certain metal compounds are known to influence biological processes, including microbial activity. Microbial nitrification processes are typically not affected by low metal concentrations; however, as concentrations increase they can inhibit activity (Hu et al. 2004). Toxicity is related to free metal ion concentration, rather than total metal concentration, so metals that dissociate in water are generally more toxic to organisms (Semerci and Cecen 2007). Interestingly, nitrifying microbes exposed *in vitro* to low concentrations of a metal can develop a tolerance to the metal and even resist future exposures of high, normally inhibitory concentrations (Mertoglu et al. 2008). For example, in a stream exposed to mining runoff, long term exposure to copper and lead has been shown to cause changes in metal tolerance levels of the stream microbes and alters the dominant genera (microbial succession) of nitrifying bacteria present (Satchanska et al. 2005), indicating microbial adaptation following metal exposure (Mertoglu et al. 2008).

Copper is an essential trace element in nitrifying bacteria but becomes toxic as concentrations increase to levels that disrupt normal cellular function (Sato et al. 1988). The concentration at which copper becomes toxic depends on bacterial physiology. For example, copper concentrations between 1.27–12.7 mg/L are important for optimal ammonia monooxygenase (AMO) enzyme function (Ensign et al. 1993). However, in both *Nitrosomonas* and *Nitrobacter,* higher copper ion concentrations can decrease nitrification rates (Braam and Klapwijk 1981; Lee et al. 1997; Hu et al. 2004). At higher copper concentrations (> 30 mg/L), ammonia oxidizing bacteria, such as the genus *Nitrosomonas,* show signs of growth delay greater than that of nitrite oxidizers, such as *Nitrobacter* (Lee et al. 1997). In addition, concentrations of only 0.5 mg/L copper can cause significant reduction (50%) in nitrification rates for the genus *Nitrosomonas* (Sato et al. 1988). Copper concentrations in sewer sludge have been reported at 0.10 mg/L (Sato et al. 1988).

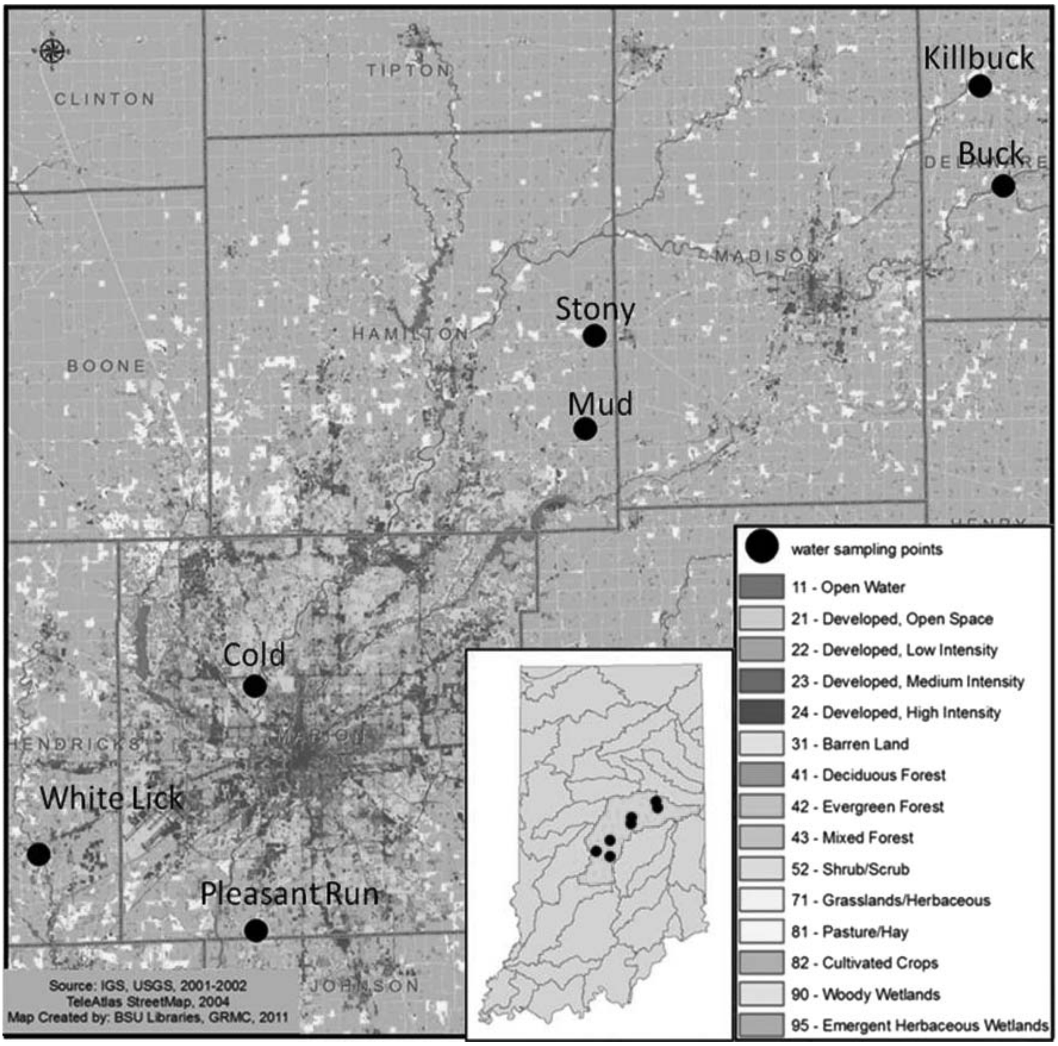
Lead ions are highly toxic to nitrifying microbes and cause greater inhibition relative to copper (Mittal et al. 2004). Lead binds to soil particles and also to living and dead microbial cells (Stucznski et al. 2002; You et al. 2009). Lead also adheres to living cells, and may not enter bacterial cells limiting direct influence on bacterial enzymes (Stucznski et al. 2002; Sato et al. 1988). *In vitro* studies have found that lead has no significant influence on either step of the nitrification process when compared to cadmium and nickel (You et al. 2009). Lead concentrations in sewer sludge have been reported at 0.12 mg/L (Sato et al. 1988).

Similar to copper, iron is also an essential element for nitrifying bacteria. The optimal concentration for nitrification is 6 mg/L for *Nitrosomonas* and *Nitrobacter* (Meiklejohn 1957). The lowest concentration of iron needed for nitrification is 0.1 mg/L for *Nitrosomonas* and 0.3 mg/L for *Nitrobacter* (Meiklejohn 1957). Both *Nitrosomonas* and *Nitrobacter* can tolerate iron concentrations of 112 mg/L, although activity is reduced. Iron concentrations in sewer sludge have been reported at 3.0 mg/L (Sato et al. 1988).

To assess the influence of metal concentrations on sediment microbial nitrification, metal concentrations in central Indiana freshwaters were measured. Further, the influence of metal concentrations on sediment nitrification rates was experimentally quantified. The primary goal of this research was to comparatively quantify the influence of copper, lead, and iron on sediment nitrification rates in the streams of the Upper White River Watershed of central Indiana. It was hypothesized that microbial responses to metals are a function of the history of metal exposure and stream physiochemical characteristics. It was further hypothesized that sediment metal concentrations would affect nitrification rates more than dissolved metal concentrations.

METHODS

Site selection.—Seven sites were selected in the Upper White River Watershed of central Indiana to represent a range of agricultural and urban land use in the surrounding sub-watersheds (Fig. 1). All sites selected were 3rd order streams and topographic maps and aerial photographs obtained from the Indiana University GIS spatial data portal (topographic maps from USGS 1984; and aerial photography from Google Earth 2010) were used to determine stream order according to Cole (1994). The White River Watershed covers an area of 174,830 acres and is located in the



Source: IGS, USGS, 2001-2002
TeleAtlas StreetMap, 2004
Map Created by: BSU Libraries, GRMC, 2011

Figure 1.—Location of study sites in the Upper White River Watershed in central Indiana. Coloring denotes land use within the watershed and sites were selected to represent a gradient of land use from urban to agricultural.

Tipton Till plain of east-central Indiana. It contains mostly sand and gravel from glacial deposits. The predominant soil type has been classified as silt loam and highly erodible.

Streams were sampled in May and August 2010 to encompass stream flow at generally higher (May) and lower levels (August) corresponding to spring runoff and base flow. The May and August sampling times also facilitated incorporation of various seasonal changes in stream physiochemical properties such as changes in water temperature, stream biology, riparian characteristics, and land use.

Sediment and water collection.—At each site and sampling event, sediment and water were collected for laboratory nitrification assays. Specifically, a composite sediment sample was collected from the top 5–10 cm of the benthos at several points along the width of the stream channel. Sediment was placed into an acid-washed bucket with a lid. Additionally, ~2.5 L of stream water (unfiltered) was collected from a well-mixed portion of the stream into acid-washed 1 L Nalgene bottles. An additional 250 mL of stream water was collected and immediately filtered using a syringe fitted with glass fiber filters (Whatman GF/F, 0.7 µm pore size) into a 250 mL Nalgene acid-washed bottle for subsequent analysis of dissolved nutrient and metal concentrations. At each site, stream physiochemical parameters were measured in the stream thalweg including pH, dissolved oxygen concentration, turbidity, total dissolved solids (TDS), and temperature using a Hydrolab minisonde equipped with an LDO oxygen sensor. After collection, samples were immediately placed on ice for transport to the laboratory. Filtered water samples were frozen within 12 h of collection for subsequent analyses of nutrient and metal concentrations. Sediment and unfiltered water were placed at 4°C (< 24 h) until the assay was begun. Filtered water samples were analyzed for anion and cation concentrations including nitrate (NO_3^--N), phosphate (PO_4-P), chloride (Cl^-), sulfate (SO_4^{2-}), bromide (Br^-), ammonium (NH_4^+-N), lithium (Li^+), potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) using ion chromatography (DIONEX, ICS-3000 and 2000).

Laboratory mesocosms.—Laboratory mesocosms were prepared by sieving collected sediment, separately for each site, using a nylon (2.54 mm × 1.25 mm) screen to homogenize the sediment and remove debris. After sediment homogenization, sediment from each site was separately sub-divided for preparation of laboratory mesocosms by filling a graduated cylinder with 40 cm^3 of sediment and placing into 250 mL glass containers. After sediment addition, 56 mL of site-appropriate stream water was added to each mesocosm. Five paired replicate mesocosms ($N = 10$) were prepared for each site and treatment. Prepared laboratory mesocosms from each site were randomly assigned one of 4 treatments including a control (no metal addition), copper addition (127 mg/L), lead addition (127 mg/L), and iron addition (127 mg/L). Metal additions were made using 14 mL of prepared TraceCERT standards for ICP (1000 mg/L Cu, Pb, Fe dissolved in a 2% nitric acid solution) metal stock (Sigma Aldrich) which is 7.62 mg metal per 60 mL total flask content volume. The control mesocosms received 14 mL of deionized water to bring to equal volume relative to treatments. Each treatment had 5 paired replicates prepared for each stream sampled ($N = 280$ total mesocosms).

Nitrification activity.—Nitrification activity was measured using nitrapyrin-inhibition assays on paired replicate mesocosms. Specifically, five replicate mesocosms were treated with nitrapyrin dissolved in dimethly sulfoxide (DMSO) to reach a mesocosm concentration of 10 mg/L nitrapyrin (Kemp and Dodds 2001). The remaining 5 paired replicates were treated with an equal volume of DMSO only. After nitrapyrin and DMSO were added to mesocosms, the mesocosms were gently bubbled with air for ~10 s and covered with a tarp to block light. Mesocosms were incubated for 5 d with all mesocosms briefly uncovered, bubbled with air for ~10 s, and immediately re-covered every 24 h to ensure mesocosms remained oxic.

After incubation, ammonium was extracted from sediment by adding 10 mL of 1 N potassium chloride (KCl), mixing the flasks, then incubating for 10 min, followed by a 30 min sediment settling period. Overlying water was then filtered with glass fiber filters (Whatman GF/F, 0.7 µm pore size) into an acid washed 15 mL Falcon tube and immediately refrigerated (< 24 h) for subsequent analyses of ammonium concentrations using the phenol-hypochlorite technique (Weatherburn 1967). Remaining water was decanted and mesocosms were placed in a 75°C drying oven overnight, followed by measurement of sediment dry mass in each individual mesocosm. Nitrification rates were calculated for each paired replicate mesocosm ($N = 5$ for each treatment) by subtracting the measured ammonium concentration in the nitrapyrin-treated paired replicate from the DMSO-only paired replicate, and then dividing by mesocosm sediment dry mass and total incubation time for expression of the nitrification rate as µg NH_4-N/gdm/d.

Bioavailable sediment metal concentrations.—Biologically available metal concentrations in sediments were quantified according to McKeague (1978). Using the collected homogenized and dried stream sediment from each site, 0.5 g of sediment was placed into a 15 mL acid-washed Falcon tube. For each stream site, two replicates were prepared for a total of two tubes per site, in addition to three water and acid oxalate blanks. Acid oxalate (10 mL) was added to each tube and the tubes were capped. All tubes were then shaken horizontally in the dark for 4 h. After shaking, tubes were centrifuged at 2000 × g for 13 min, and the supernatant decanted and saved for metal analysis on the ICP-OES, Perkin Elmer Optima

2100 DV. Remaining sediment was discarded. Due to high iron concentrations in the sediment, a 1/10 dilution was used to keep the samples within the standard range.

Total sediment metal concentrations.—To determine the total metal concentration in stream sediment, a multi-acid digestion was used, modified from Briggs and Meyer (2002). To perform the assay, 0.2 g of dry homogenized stream sediment was placed into a Teflon vessel with subsequent addition of 3 mL (30% w/v) H_2O_2. After 24 h, 2 mL of concentrated nitric acid (65% w/v) was added to each vessel, followed by 1 mL of concentrated (40% w/v) hydrofluoric acid. The vessels were then capped and heated (~100 °C) overnight, then uncapped and heated until dry. The nitric and hydrofluoric acid steps were repeated 3 times due to undissolved materials. Three mL of H_2O_2 was then added to each vessel and heated until dry to remove remaining organics. The H_2O_2 addition was also repeated 3 times. Due to high iron concentrations in the sediment, iron samples were run with an additional 1/10 dilution.

To determine metal concentrations in the water column, 15 mL acid washed Falcon tubes were used. To each tube, 10 mL of acidified stream water (5 ml of 65% w/v nitric acid per 100 mL of stream water) was added. Three replicates were made for each site for a total of 21 tubes. The samples were then refrigerated until analyzed on the ICP-OES for metal concentration. All transference of liquid was performed using acid washed bottles and pipette tips.

Calculations and statistical analyses.—Differences in control nitrification rates and metal concentrations among streams were compared using one way analysis of variance (ANOVA). Two Sample t-tests were used to compare differences in nitrification rates, sediment and water metal concentrations between the sampling events (May, August). Bonferroni corrected Pearson correlations were used to identify relationships between stream physicochemical parameters, nitrification rates, and metal concentrations (Pearson correlation coefficients, *r*, and probability, *p,* reported). ANOVA and t-tests were performed using MiniTab 16 Software, and correlation statistics were performed using SAS Statistical software using *p-values* ≤ 0.05 to determine significance.

RESULTS

Sediment bioavailable metal concentrations.—Overall, bioavailable copper, lead, and iron concentrations in stream sediment varied among sites (Fig. 2). Sediment concentrations ranged from 654–1,985 mg Fe/kg sediment and from1.00–2.91 mg Cu/kg sediment (Fig. 2). Lead concentrations were below detection limits at all sites except in May at one site (Pleasant Run Creek, 0.47 mg Pb/kg sediment) and in August at one site (Mud Creek, 0.38 mg Pb/kg sediment). Across sites, significant differences in bioavailable metal concentrations were identified between sampling events (May *vs.* August; $p < 0.05$). Specifically, bioavailable iron in Buck Creek was ~25% higher in May (876 mg Fe/kg) relative to August (654 mg Fe/kg; $p = 0.030$, Fig. 2). Similarly, bioavailable iron in Killbuck Creek was ~41% higher in May (1,985 mg Fe/kg) relative to August (1161 mg Fe/kg; $p = 0.01$; Fig. 2). Bioavailable copper concentrations in Killbuck Creek were ~51% higher in May (2.63 mg Cu/kg) relative to August (1.29 mg Cu/kg; p = 0.004).

Dissolved bioavailable metal concentrations.—Copper and iron concentrations in stream water varied among sites and ranged from below detection to 0.10 mg Fe /L, and from 0.01–0.02 mg Cu/L (Fig. 3). Lead concentrations were below detection limits at all sites and sampling events. Overall, bioavailable iron concentrations (mean = 0.026 mg/L) in stream water were greater than copper concentrations (mean = 0.013 mg/L; $p = 0.01$). Across sites, significant differences in bioavailable iron concentrations in the water column were identified between sampling events (May, August; $p < 0.05$) in all streams, except White Lick ($p = 0.904$), with higher dissolved iron concentrations in August relative to May (Fig. 3). Killbuck Creek was the only site with significant differences in dissolved copper concentrations between May and August (0.02 mg/L vs. 0.01 mg/L; $p = 0.037$; Fig. 3).

Control nitrification rates.—Overall, control nitrification rates were ~76% greater in May (mean = 4.31 μg NH_4-N/gdm/d) than August (mean = 1.05 μg NH_4-N/gdm/d; $p = 0.001$; Fig. 4). Across sites, significant differences in control nitrification rates were identified between sampling events (May, August; $p < 0.05$) only in Killbuck Creek (p = 0.001). No other

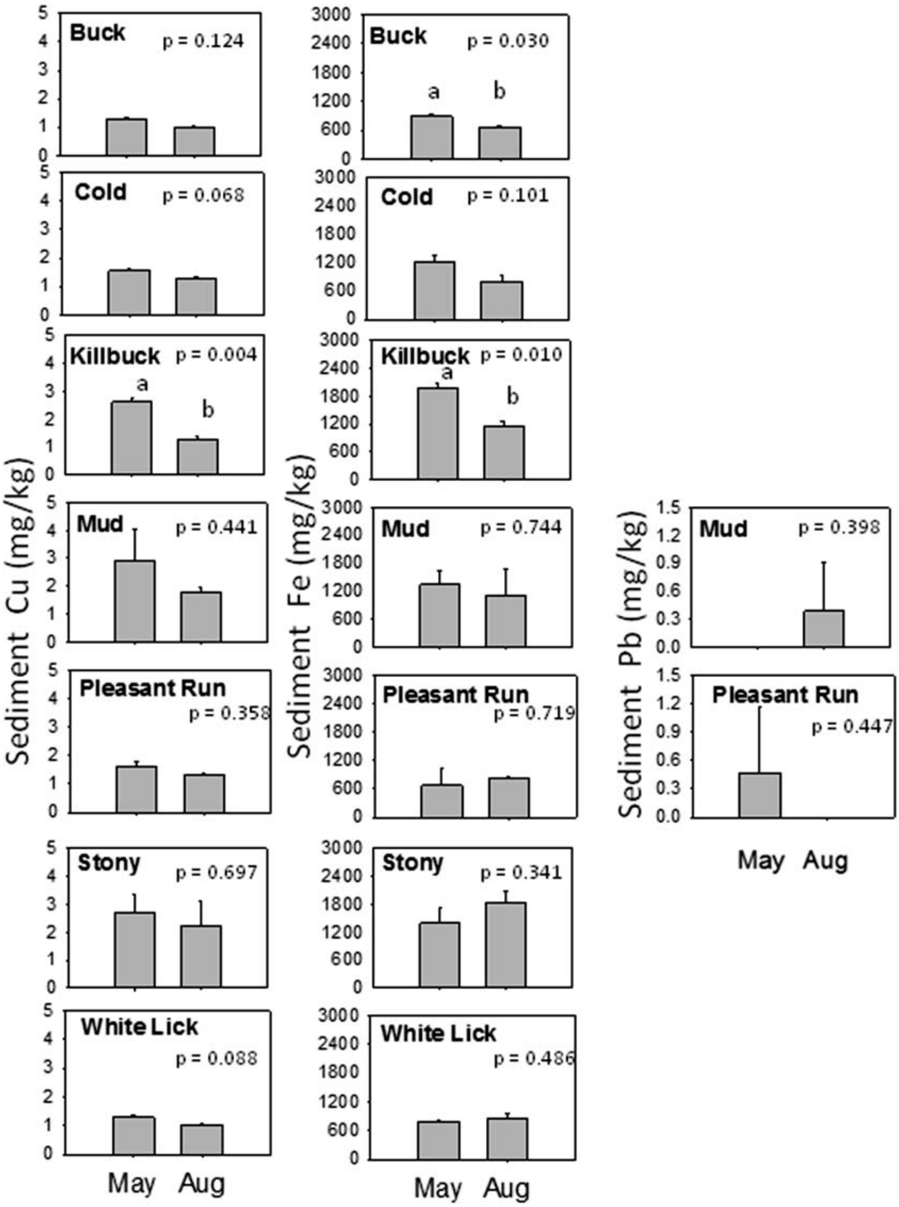


Figure 2.—Mean (N = 5) sediment copper, iron, and lead concentrations in sampled streams during May and August ± 1 standard error (SE). Sediment lead concentrations were below detection except for two sites during one sampling event. Different letters denote significant difference in concentrations between May and August for a given stream. See Figure 1 for stream locations.

stream showed significant differences in nitrification rates with sampling time (Fig. 4).

Nitrification response to metals.—Overall, there was not a consistent nitrification response to metal enrichment across sites (Fig. 5). Nitrification response to metal enrichment did not differ between May and August ($p > 0.05$; *data not shown*). Iron enrichments did reduce nitrification rates compared to the control during August at some sites ($p < 0.05$: Buck Creek - 0.00 vs. 1.72 μg NH_4-N/gdm/d; Mud Creek - 0.00 vs. 8.89 μg NH_4-N/gdm/d; Pleasant Run Creek − 0.02 vs. 1.29 μg NH_4-N/gdm/d; White Lick Creek - 0.03 vs. 0.715 μg

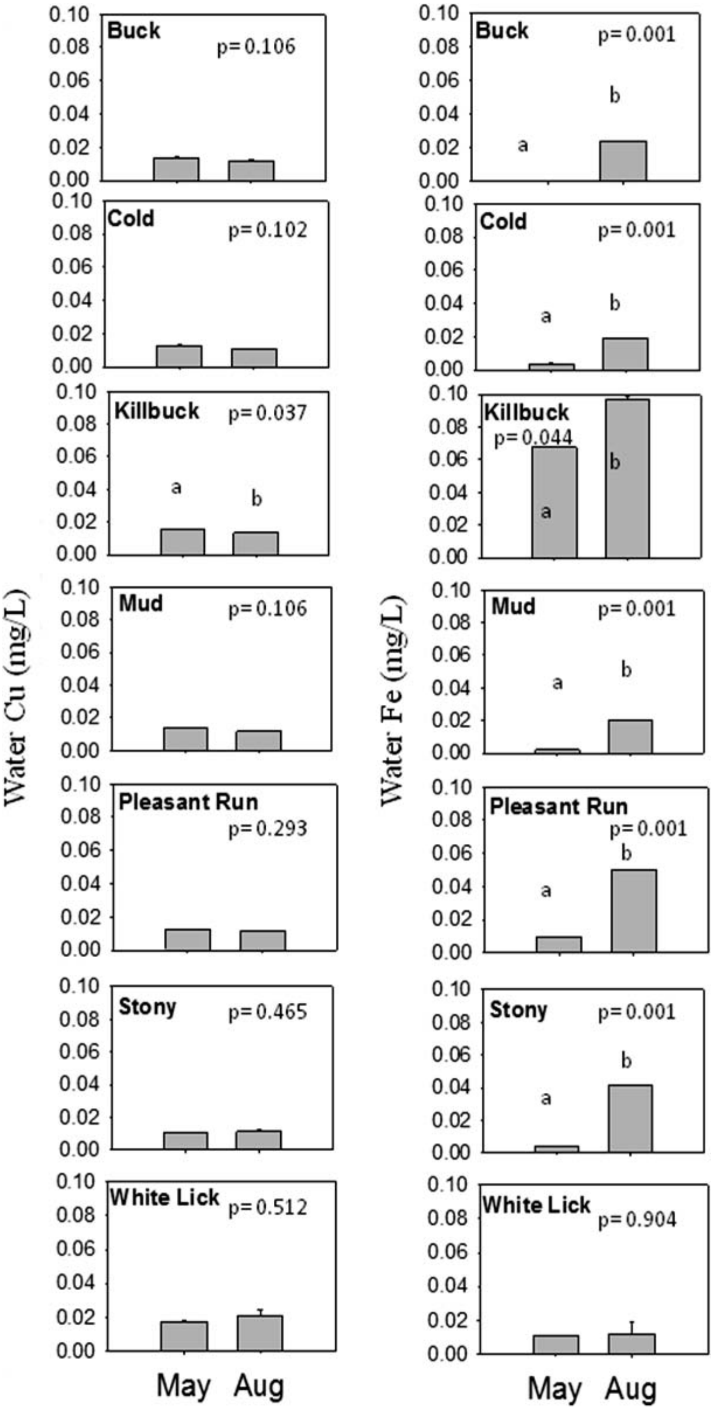


Figure 3.—Mean (N = 5) water column (i.e., dissolved) copper and iron concentrations in sampled streams during May and August ± 1 standard error (SE). Water column lead concentrations were below detection in all samples. Different letters denote significant difference in concentrations between May and August for a given stream. See Figure 1 for stream locations.

NH_4-N/gdm/d; Fig. 5). Copper enrichments decreased nitrification rates in May compared to the control in Killbuck Creek (1.34 vs. 7.58 µg NH_4-N/gdm/d; $p = 0.020$) and Pleasant Run Creek (0.00 vs. 3.86 µg NH_4-N/gdm/d; $p = 0.001$; Fig. 5). Copper enrichments also increased nitrification rates compared to the control during August in Killbuck Creek (1.01 vs. 0.279 µg NH_4-N/gdm/d; $p = 0.024$). Significant decreases in August nitrification rates with copper enrichment compared to the control were found in Buck Creek (0.17 vs. 1.72 µg NH_4-N/gdm/d; $p = 0.032$), Pleasant Run Creek (0.09 vs. 1.29 µg NH_4-N/gdm/d; p = 0.021), and White Lick Creek (0.05 vs. 0.715 µg NH_4-N/gdm/d; $p = 0.045$). Lead enrichments decreased nitrification rates in May compared to the control in Killbuck Creek (0.99 vs. 7.58 µg NH_4-N/gdm/d; $p = 0.001$). Lead enrichments also significantly decreased August nitrification rates compared to the control in Buck Creek (0.13 vs. 1.72 µg NH_4-N/gdm/d; $p = 0.029$), Cold Creek (0.00 vs. 1.14 µg NH_4-N/gdm/d; $p = 0.001$), Pleasant Run Creek (0.00 vs. 1.29 µg NH_4-N/gdm/d; $p = 0.001$), Stony Creek (0.01 vs. 1.13 µg NH_4-N/gdm/d; $p = 0.037$), and White Lick Creek (0.00 vs. 0.715 µg NH_4-N/gdm/d; $p = 0.001$).

Factors influencing metal concentrations and nitrification rates.—Stream pH, temperature, total dissolved solids (TDS), and dissolved oxygen concentrations (DO) were not significantly correlated with sediment iron concentrations ($p > 0.05$; *data not shown*). Dissolved iron concentrations were negatively correlated with stream DO (r = −0.75, $p = 0.003$; Fig. 6). Stream pH, temperature and TDS were not significantly correlated with dissolved iron concentrations ($p > 0.05$; Fig. 6). Sediment copper concentrations were negatively correlated with stream temperature (r = −0.66, $p = 0.010$; *data not shown*). Stream pH, TDS, and DO were not significantly correlated with sediment copper concentrations (p > 0.05; *data not shown*). Stream pH, temperature, TDS, and DO were not significantly correlated with dissolved copper concentrations ($p > 0.05$; *data not shown*).

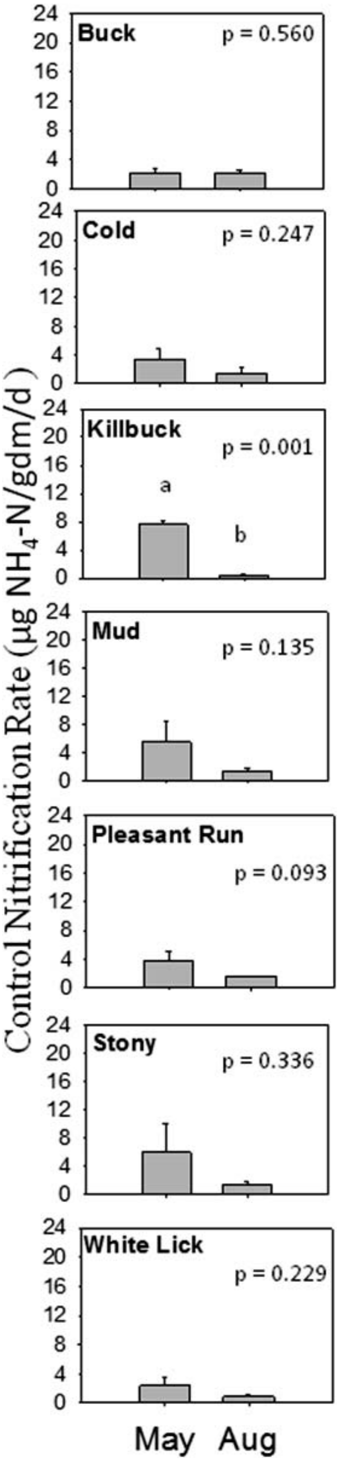


Figure 4.—Mean (N = 5) control sediment nitrification rates in sampled streams during May and August ± 1 standard error (SE). Different letters denote significant difference in nitrification rates between May and August for a given stream. See Figure 1 for stream locations.

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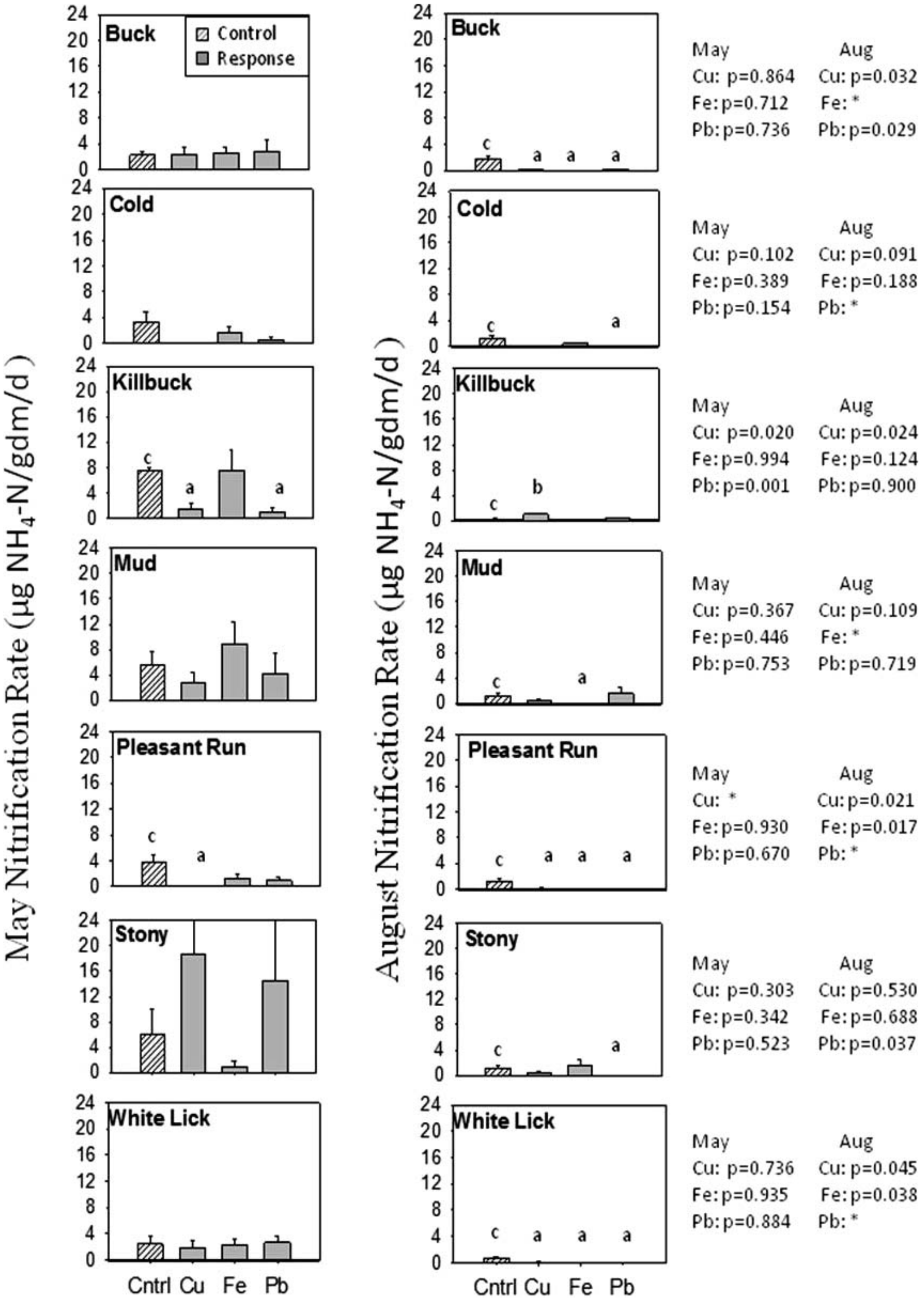

Figure 5.—Mean (N = 5) nitrification rates in metal enriched mesocosms in May and August ± 1 standard error (SE). "a" indicates value is significantly greater than the control. "b" indicates value is significantly less than the control and "c" denotes the control value. P-values are indicated for the comparison of control rates to metal enrichments treatments (a,b,c) (*) indicates no detected nitrification.

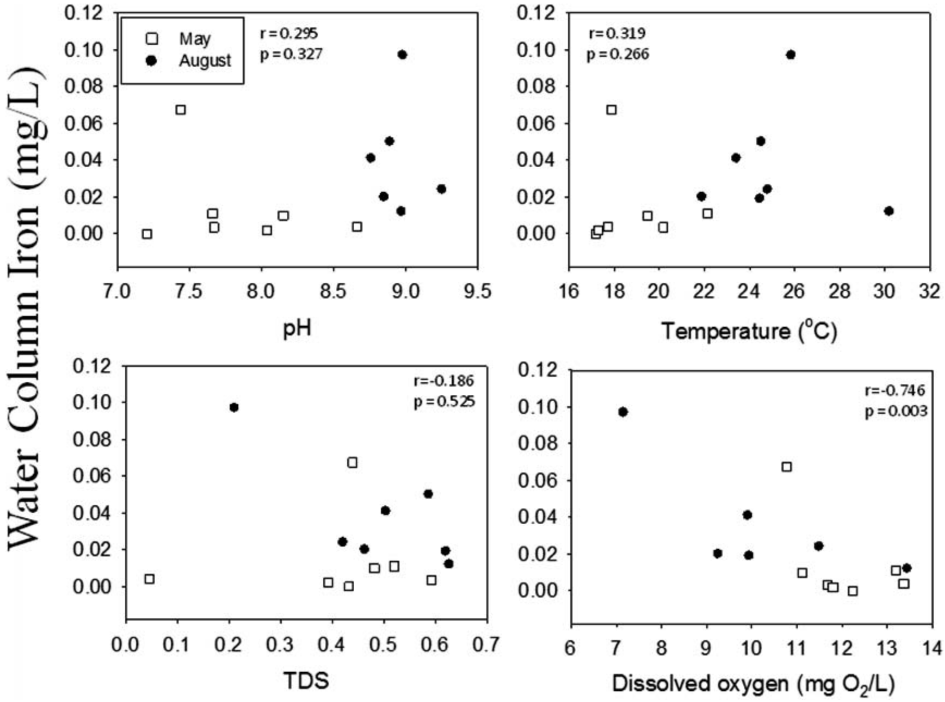

Figure 6.—Correlations between water column (i.e., dissolved) iron concentrations and stream physiochemical parameters: water column pH, total dissolved solids (TDS), dissolved oxygen, and temperature. N = 14.

Control nitrification rates were positively correlated with sediment copper concentrations (r = 0.78, p = 0.001; Fig. 7). There was no significant correlation between control nitrification rates and sediment iron concentrations, water iron concentrations, water copper concentrations, stream pH, or TDS ($p > 0.05$; Fig. 7). Nitrification response to metal enrichment was positively correlated to total iron concentrations (r = 0.61, p = 0.02; Fig. 8) and total copper concentrations (r = 0.742, p = 0.002; Fig. 8).

DISCUSSION

Metal concentrations in central Indiana streams.—These data suggest iron is the most abundant metal in the selected study sites relative to copper, and lead. Copper was the second most abundant and lead was undetectable in all but two samples (N = 14 total). These findings are consistent with previous reports from the Indiana Department of Environmental Management (IDEM) (Holdeman et al. 1999). The higher presence of iron compared to copper and lead may be attributed to less federal and state monitoring of these contaminants. Iron concentrations are not regulated due to minimal adverse effects at environmentally-relevant concentrations (Holdeman et al. 1999). In contrast, copper is toxic to humans and can function as a biocide to aquatic organisms at environmentally-relevant concentrations (Nirel and Pasquini 2010; Moore and Ramamoorthy 1984). Similarly, lead is toxic to both humans and aquatic organisms at environmentally-relevant concentrations (Moore and Ramamoorthy 1984). Higher concentrations of iron in central Indiana streams may also be due to greater natural occurrences of these elements.

Factors controlling metal concentrations in central Indiana streams.—Geochemical processes and sediment dynamics (i.e., sorption) may influence dissolved metal concentrations in streams, with some evidence suggesting metal concentrations may follow diel cycles (Nimick et al. 2003). Urban et al. (1990) found soluble iron concentrations in lakes were positively correlated with dissolved organic carbon (DOC) concentrations and negatively correlated with

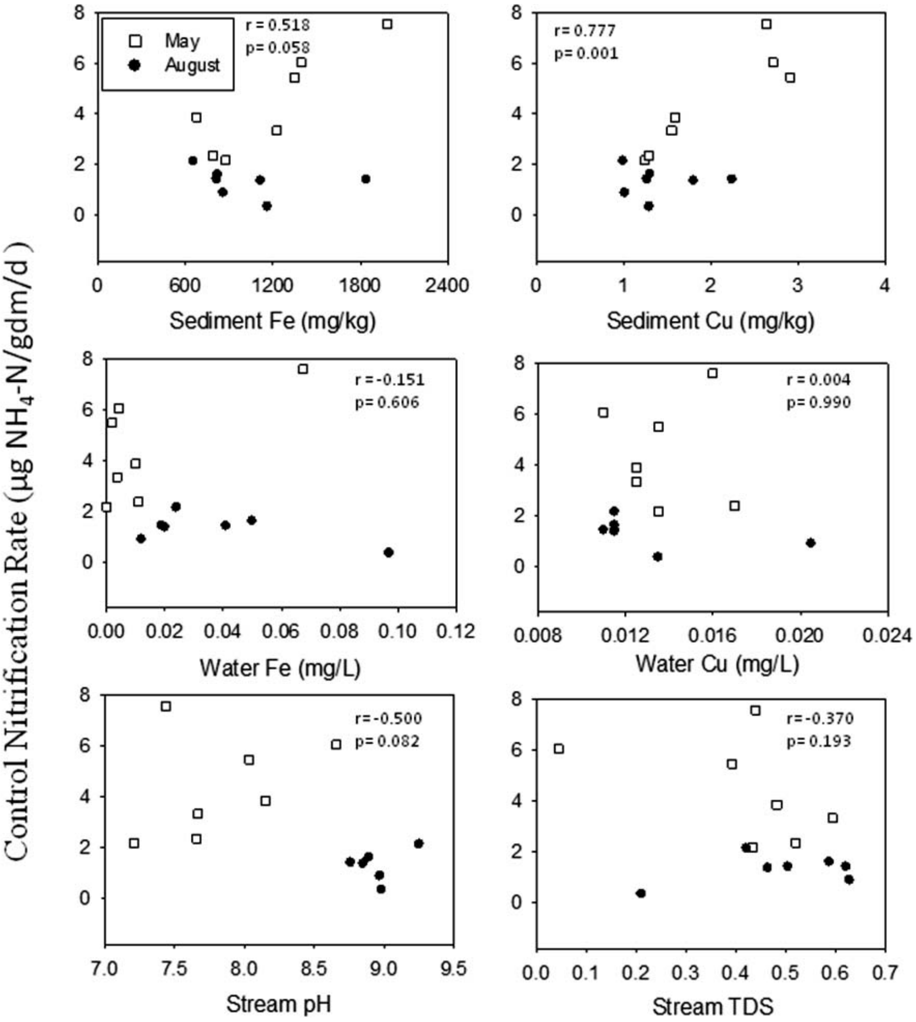

Figure 7.—Correlation between control nitrification rates and stream sediment and water column iron and copper concentrations as well as stream physicochemical parameters (pH and total dissolved solids, TDS). N = 14.

stream pH. Dissolved organic carbon enhances iron mineral phase solubility above pH 5 and buffers dissolved iron content below pH 5 through binding and flocculation processes (Urban et al. 1990). Wen et al. (1998) found pH was positively correlated with copper adsorption rates. Aquatic plants have also been shown to absorb metal from the environment, reducing metal concentrations (Miretzky et al. 2004). The rate at which aquatic plants can remove metals depends on plant species and water conditions such as dissolved oxygen and pH (Miretzky et al. 2004). The relationship between dissolved oxygen and plant metal uptake may be the cause of decreasing dissolved iron concentrations as steam dissolved oxygen increases. Observed relationships between metal concentrations and physiochemical parameters may also be due to biogeochemical processes and factors not measured in this study, such as dissolved organic carbon concentrations and macrophyte abundance.

Factors controlling stream nitrification rates.—Stream sediment nitrification rates measured in this study (1–7 µg NH_4-N/gdm/d) were comparable to rates previously measured in lake sediments (0.4–2.3 µg NH_4-N/gdm/d; Strauss and Dodds 1997). Differences in stream physiochemical factors may have influenced

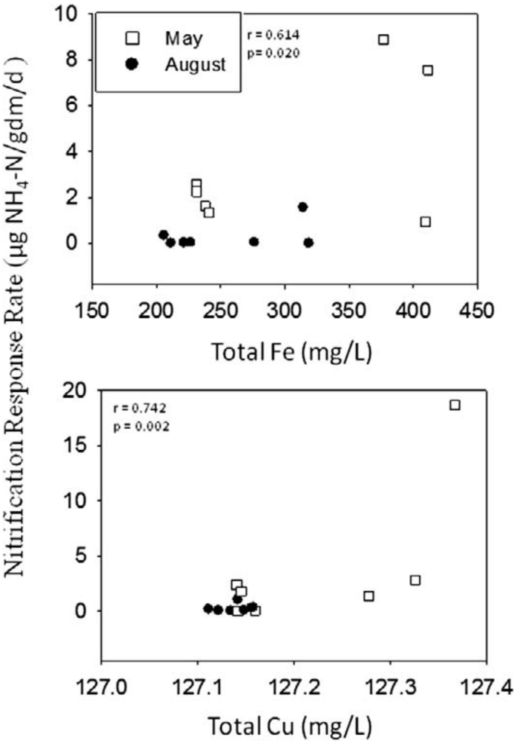

Figure 8.—Correlation between nitrification response and total metal concentration (sum of sediment, dissolved and experimental addition) for iron and copper. N = 14.

nitrification rates measured across sampling events and explain variation between the May and August sampling events. Overall, nitrification rates were greater in the May relative to August. In May, nitrification rate increased with pH around 7.0 to just above 8.5; in contrast, August samples were closely clustered between pH 8.5 and 9. Previous studies (Strauss et al. 2002) show nitrification rate tends to increase with stream pH and optimal conditions of pH 7.5. This is consistent with this study for May samples, but August samples had higher pH (above the optimum of pH 7.5) and lower nitrification rates, potentially due to confounding factors. This also supports the conclusion noted by Strauss et al. (2002) that additional factors other than pH may influence nitrification responses including levels of organic carbon and available ammonium as well as available light. Light can inhibit the growth of nitrifying bacteria (Hagopian and Riley 1998) and even under ideal growth conditions nitrifying bacteria have a relatively slow mean generation time (up to 60 h). Ward et al. (1982) suggested that light levels may strongly influence the location and depth at which nitrifying bacteria are found, with higher nitrification activity occurring below the photic zone of coastal waters. The greater depth of streams in May relative to August (*data not shown*) may have shielded the stream sediment from light, thus allowing for increased activity in May relative to August.

The influence of metals on sediment nitrification rates.—Since nitrifying microbes grow best when bound to a surface and shielded from light (Hagopian and Riley 1998), the stream sediment is the ideal habitat for nitrifying bacteria. Thus, sediment metal concentrations should be more influential than water column metal concentrations. The correlation between nitrification rates and sediment iron and copper concentration suggest that nitrification may be facilitated with increasing concentrations under some conditions. Observations by Dollhopf et al. (2005) indicated an increase in nitrification rate as sediment iron concentrations increased in salt marshes. This increase was attributed to possible protection of ammonium monoxygenase by iron from sulfide. While sulfide was not measured in this study, iron could be performing a similar type of protection which would explain increased nitrification rates as iron concentrations increased. Copper has also been shown to protect nitrifying bacteria against some nitrification inhibition compounds (Campbell and Aleem 1965) which also supports the relationship between increased copper concentrations and increased nitrification rates.

Copper enrichment of Killbuck Creek sediment collected in May decreased nitrification rates relative to controls but increased rates in sediment collected in August when sediment copper concentrations were lower in the ecosystem. Thus, an increase in nitrification rate was observed only when additional copper was added during a period of lower *in situ* copper concentrations in Killbuck Creek. This may have been due to microbial adaption to higher metal concentrations (e.g., Mertoglu et al 2008) or to other factors such as changes in temperature.

The lack of a significant nitrification rate response with iron enrichments in May suggest iron additions of 127 mg/L may not influence microbial nitrification during the May conditions. Sediment iron concentrations were similar in May compared to August with only Killbuck Creek and Buck Creek having lower concentrations in August. Killbuck Creek had the highest dissolved iron concentration and the only evidence of inhibition of nitrification rates with enrichment in August. The lack of a nitrification rate response to iron enrichment may be due to the higher concentrations of iron naturally found in the sampled streams compared to copper. Significant differences were more prevalent for copper treatments including an increase in Killbuck Creek. Nitrifying microbes sampled were from an environment with higher iron concentrations compared to copper. Thus 127 mg/L iron addition did not consistently influence stream nitrification rates. Rather, the total metal concentrations (sum of *in situ* concentration and experimental addition) dictated nitrification response.

Because most samples had lead concentrations below detection and inconsistent responses to lead enrichment, it is difficult to determine with certainty, the influence of lead on microbial nitrification. Observations by You et al. (2009) found that 40 mg/L of lead did not affect nitrification rates in sludge. In this study, the addition of 127 mg/L of lead also did not have a direct influence on nitrification in stream sediment.

Conclusions.—Previous research has indicated that certain metal compounds can influence the physiology of nitrifying microbes although the influence of metals at environmentally-relevant concentrations on stream sediment nitrification rates is not well understood. We found that stream sediment metal concentrations may have a greater influence on nitrification rates relative to dissolved metal concentrations. Nitrification rates in central Indiana streams were comparable to previous nitrification estimates in aquatic ecosystems. Similarly, sediment and dissolved metal concentrations were within previously reported ranges. The nitrification response to metal enrichment in stream ecosystems is likely a function of both physiochemical characteristics of the stream ecosystem and the history of metal exposure. Overall, nitrification rates were lower during August compared to May sampling events regardless of metal enrichment.

The May sampling event was characterized by lower water temperature and pH, but greater dissolved oxygen concentration relative to the August sampling event. Relationships between these physiochemical characteristics and stream sediment nitrification rates suggest that stream physicochemical properties are more influential on microbial nitrification than the 127 mg/L enrichment of copper, lead, and iron. Although a 127 mg/L metal concentration enrichment may reduce stream sediment nitrification rates; stream physiochemical characteristics such as oxygen, pH and light likely dictate the majority of observed microbial responses by affecting biotic activity (i.e., metabolic rates).

ACKNOWLEDGEMENTS

We thank Dr. Carolyn Dowling, Melissa Fear, Rob Osborne, Matt Suter, Angela Gibson, Matt Beer, Joe Meiring, Katrina Van Zant and Leslie O'Neill for laboratory and field assistance and Dr. John McKillip, Dr. Randall Bernot and an anonymous reviewer for helpful edits, comments and discussion.

LITERATURE CITED

Blake, W., R. Walsh, J. Reed, M. Barnsley & J. Smith. 2007. Impacts of landscape remediation on the heavy metal pollution dynamics of a lake surrounded by non-ferrous smelter waste. Environmental Pollution 148:268–280.

Braam, F. & A. Klapwijk. 1981. Effect of copper on nitrification in activated sludge. Water Research 15:1093–1098.

Briggs, P.H. & A.L. Meier. 2002. The determination of forty-two elements in geological materials by inductively coupled plasma- mass spectrometry. Analytical methods for chemical analysis of geologic and other materials, US Geological Survey.

Campbell, N.E.R. & M.I.R. Aleem. 1965. The effect of 2-chloro, 6-(trichlormethyl) pyridine on the chemoautotrophic metabolism of nitrifying bacteria. Antonie Van Leeuwenhoek 31:124–136.

Ciudada, G., R. Gonzalez, C. Bornhardt & C. Antileo. 2007. Modes of operation and pH control as enhancement factors for partial nitrification with oxygen transport limitation. Water Research 41:4621–4629.

Cole, G. 1994. Textbook of Limnology. 4th ed. Waveland. Prospect Heights, Illinois: Heights Press Inc. 61:152.

Dollhopf, S.L., J. Hyunn, A.C. Smith, H.J. Adams, S. O'Brien & J.E. Kostka. 2005. Quantification of ammoni-oxidizing bacteria and factors controlling nitrification in salt marsh sediments. Applied Environmental Microbiology 71:240–246.

Earl, S., H. Valett & J. Webster. 2006. Nitrogen saturation in stream ecosystems. Ecology 87: 3140–3151.

Ensign, S., M. Hyman & D. Arp. 1993. In vitro activation of ammonia monooxygenase from *Nitrosomonas europaea* by copper. Journal of Bacteriology 175:1971–1980.

Galloway, J.N. 1998. The global nitrogen cycle: changes and consequences. Environmental Pollution 102:15–24.

Guinee, J., J. Bergh, J. Boelens, P. Fraange, G. Huppes, P. Kandelaars, T. Lexmond, S. Moolenaar, A. Olsthoorn, H. Haes, E. Verkuijlen & E. Voet. 1999. Evaluation of risks of metal flows and accumulation in economy and environment. Ecological Economics 30:47–65.

Hagopian, D.S. & J.G. Riley. 1998. A closer look at the bacteriology of nitrification. Aquacultural Engineering 18:223–224.

Holdeman, M.A., S.C. Gibson, J.L. McFall, T.J. Beckman, C.C. Christensen & V.A. Erwin. 1999. 1997 Synoptic Sampling Surveys in the Whitewater River Basin. Indiana Department of Environmental Management, Office of Water Management, Assessment Branch, Surveys Section, Indianapolis, Indiana. IDEM 032/02/010/1999.

Hu, Z., K. Chandran, D. Grasso & B. Smets. 2004. Comparison of nitrification inhibition by metals in batch and continuous flow reactors. Water Research 38:3949–3959.

Jones, R.D. & M.A. Hood. 1980. Effects of temperature, pH, salinity, and inorganic nitrogen on the rate of ammonium oxidation by nitrifiers isolated from wetland environments. Microbial Ecology 6:339–347.

Kemp, M.J. & W.K. Dodds. 2001. Centimeter-scale patterns in dissolved oxygen and nitrification rates in a prairie stream. J. N. American Benthological Society 20:347–357.

Lee, Y., S. Ong & C. Sato. 1997. Effects of heavy metals on nitrifying bacteria. Water. Science. Technology 36:69–74.

McKeague, J.A. 1978. Manual on Soil Sampling and Methods of Analysis 2nd ed. Canadian Society of Soil Science. AAFC, Ottawa, Ontario, Canada. 103–104.

Meiklejohn, J. 1957. Iron and the nitrifying bacteria. Journal of General Microbiology 8:58–65.

Mertoglu, B., N. Semerci, N. Guler, B. Calli, F. Cecen & A. Saatci. 2008. Monitoring of population shifts in an enriched nitrifying system under gradually increased cadmium loading. Journal of Hazardous Materials 160:495–501.

Miretzky, P., A. Saralegui & A.F. Ciewlli. 2004. Aquatic macrophytes potential for simultaneous removal of heavy metals (Buenos Aires, Argentina). Chemosphere 57:997–1005.

Mittal, S., S. Goel & A. Sharma. 2004. Metal ion effect on BOD exertion at different temperatures. International Journal of Environmental. Research Public Health 1:132–137.

Moore, J.W. & S. Ramamoorthy. 1984. Heavy metals in natural waters: applied monitoring and impact assessment. Springer Series on Environmental Management. Springer-Verlag, New York, NY. 268p.

Nimick, D.A., C.H. Gammons, T.E. Cleasby, J.P. Madison, D. Skaar & C.M. Brick. 2003. Diel cycles in dissolved metal concentrations in streams: Occurrence and possible causes. Water Resources Research 39:1247.

Nirel, P.M. & F. Pasquini. 2010. Differentiation of copper pollution origin: agricultural and urban sources. Novatech 7p.

Rimmer, D., C. Vizard, T. Pless-Mulloli, I. Singleton, V. Air & Z. Keatinge. 2006. Metal contamination of urban soils in the vicinity of a municipal waste incinerator: Once source among many. Science of the Total Environment 356:207–216.

Satchanska, G., E. Pentcheva, R. Atanasova, V. Groudeva, R. Trifonova & E. Golovinsky. 2005. Microbial diversity in heavy-metal polluted waters. Biotechnology and Biotechnological Equipment 19:61–67.

Sato, C., S. Leung & J. Schnoor. 1988. Toxic response of *Nitrosomonas europaea* to copper in inorganic medium and wastewater. Water Research 22:1117–1127.

Semerci, N. & F. Cecen. 2007. Importance of cadmium speciation in nitrification inhibition. Journal of Hazardous Materials 147:503–512.

Strauss, E.A., N.L. Mitchell & G.A. Lamberti. 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. Canadian Journal of Fisheries and Aquatic Sciences 59:554–563.

Strauss, E.A. & W.K. Dodds. 1997. Influence of protozoa and nutrient availability on nitrification rates in subsurface sediments. Microbial Ecology 34:155–165.

Stucznski, T., G. McCarty & G. Siebielec. 2002. Response of soil microbiological activities to cadmium, lead, and zinc salt amendments. Journal of Environmental Quality 32:1346–1355.

Urban, N.R., E. Gorham, J.K. Underwood, F.B. Martin & G. Ogden. 1990. Geochemical process controlling concentrations of Al, Fe, and Mn in Nova Scotia lakes. Limnology and Oceanography 35:1516–1534.

Villaverde, S., P. Garcia-Encina & F. Fdz-Polanco. 1996. Influence of pH over nitrifying biofilm activity in submerged biofilters. Water Research 31:1180–1186.

Ward, B.B., R.J. Olson & M.J. Perry. 1982. Microbial nitrification rates in the primary nitrite maximum off southern California. Deep Sea Research 29:247–255.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39:971–974.

Wen, X., Q. Du & H. Tang. 1998. Surface complexation model for the heavy metal adsorption on natural sediment. Environmental Science and Technology 32:870–875.

You, J., A. Das, E.M. Dolan & Z. Hu. 2009. Ammonia-oxidizing archea involved in nitrogen removal. Water Research 43:1801–09.

You, S., Y. Tsai & R. Huang. 2009. Effect of heavy metal on nitrification performance in different activated sludge processes. Journal of Hazardous Materials 165:987–994.

Manuscript received 16 October 2013, revised 11 September 2014.

2013. Proceedings of the Indiana Academy of Science 122(2):93–117

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ABSTRACT. Owned by the Red-tail Land Conservancy, Munsee Woods Nature Preserve (MWNP) is an 18.4 ha (~ 45.5 acres) woodland located 9.5 km southeast of downtown Muncie, Indiana, and just west of the northern end of Prairie Creek Reservoir in Delaware County. The inventory of the vascular flora indicates that the site harbors significant plant diversity with 399 taxa representing 252 genera and 85 families. The 10 families containing ~55% of the documented species are Asteraceae (51 spp.), Poaceae (43), Cyperaceae (35), Rosaceae (18), Brassicaceae (15), Lamiaceae (14), Fabaceae (12), Scrophulariaceae (11), Polygonaceae (10), and Liliaceae (10). Of the 399 taxa, 300 [75.3%] are native and 99 [25.8%] are exotics, and 41 represent Delaware County Records. Three species are on the Indiana Watch List, i.e., *Hydrastis canadensis*, *Spiranthes ovalis* var. *erostellata*, and *Viola pubescens*. A physiognomic analysis revealed that the native species consisted of 55 woody species, 182 herbaceous vines or forbs, 56 graminoids, and seven ferns or fern allies. Of the 99 exotics, 14 were woody, 63 were herbaceous vines or forbs, and 22 were grasses. The flora at MWNP is predominately low fidelity (low C-value), i.e., ~ 67% of the taxa have C-values ≤ 3, and only ~5% have C-values ≥ 7. For native species only, the FQI = 55.0 and the mean Coefficient of Conservatism (mean C) is 3.2. For all species FQI = 47.7 and the mean C = 2.4. These numbers indicate that MWNP is a site with high natural quality that is being compromised by exotics. The exotic woody species with the highest visual abundances are *Lonicera maackii*, *Rosa multiflora*, *Euonymus alatus*, *Ligustrum obtusifolium*, and *Ailanthus altissima*. Permanent plot analysis revealed that the five most important species in the overstory [based on relative importance] are *Acer saccharum*, *Quercus alba*, *Celtis occidentalis*, *Prunus serotina*, and *Ulmus americana*. Except for *Q. alba*, the same species dominate the regeneration layers. Tree species composition and diversity at MWNP is similar to other disturbed forests in the region, with an overstory dominated by species with low or intermediate shade tolerance and understory advanced regeneration with greater abundance of shade tolerant species. All these disturbed woods had lower importance of shade tolerant species and higher tree species diversity than an old-growth forest in the region. These observations indicate that Munsee Woods is in an intermediate stage of forest succession after a long history of human disturbance.

Keywords: Delaware County Indiana, Floristic Quality Index (FQI), county records – vascular plants, flora – Indiana, permanent plots, relative importance values

INTRODUCTION

Unlike the majority of nature preserves we have inventoried to date, Munsee Woods Nature Preserve (MWNP) has experienced considerable anthropogenic impact over the past century. In the early 1900s much of the site, especially the northern half, was agricultural, in the 1930s it served as a Civilian Conservation Corp camp, and in 1939 it became a residential Girl Scout camp (Camp Munsee). In the later decades of the 1900s, the camp experienced a steady decline of use and was sold to the Red-tail Land Conservancy in April 2011. The continued intrusion of the site by human activity raises several very interesting questions. For example, during the Camp Munsee period the woodland structure as well as a stream corridor in the southern half of the preserve remained structurally intact. How has camp activity altered the vascular flora of these habitats? How does the overall flora at MWNP compare to analogous sites that have not

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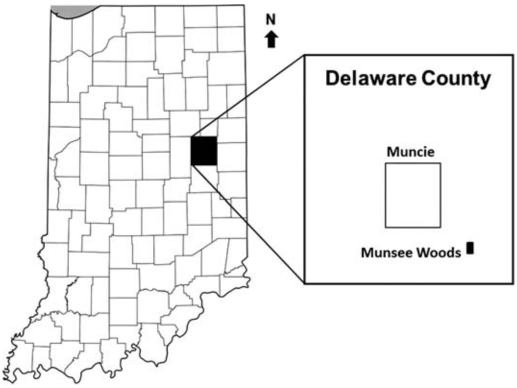

Figure 1.—Maps indicating the location of Munsee Woods Nature Preserve within Delaware County (right) and the location of Delaware County within the state of Indiana (left). The Preserve lies approximately 9.5 km southeast of downtown Muncie.

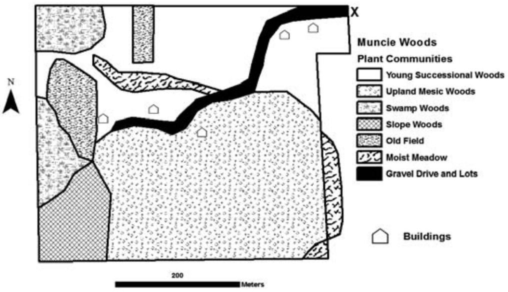

Figure 2.—Diagram illustrating the distribution of the major habitats at Munsee Woods Nature Preserve, Delaware County, Indiana.

experienced similar disturbance? Has the native flora been measurably compromised by a differential loss of sensitive species? Have the number of exotics, both in species number and in number of plants, increased? In part, it was because of questions like these, that this site was included in our larger study pertaining to the flora and floral communities of east-central Indiana. Also, Barry Bank, Executive Director of the Red-tail Land Conservancy, inquired if we could help him realize the scope of plant species on the property, since an inventory of resources is the necessary first step in developing a long-term resource management plan. Additionally, measures of diversity are frequently seen as indicators of the well-being of ecological systems (Magurran 1988). There have been no published studies or informal lists concerning the flora of Camp Munsee or MWNP. Thus, the goals in this study were (1) to inventory the vascular flora, including a visual estimate of the relative abundance and distribution of each species; (2) to determine the floristic quality of the site and to compare MWNP to other sites previously studied in Indiana; (3) to establish permanent plots and analyze the structure and composition of the upland mesic woodland on the southern half of the property; (4) to compare tree species composition and diversity at MWNP to other forests in the region; (5) to identify areas of special concern (e.g., areas with rare or threatened plants, if any, and communities sensitive to disturbance); and (6) to produce a vegetation map illustrating various habitats at the site.

SITE DESCRIPTION

Formerly known as Camp Munsee, Munsee Woods Nature Preserve is a 18.4 ha (~ 45.5 acres) woodland located 9.5 km southeast of downtown Muncie, Indiana, and just west of the northern end of Prairie Creek Reservoir in Delaware County (Fig. 1). The latitude and longitude at the entrance to the lodge are 40°08′24″N 85°18′13″W, with an elevation of 307 m (1007 ft). MWNP was approved as a state nature preserve by the Indiana Natural Resources Commission on January 21, 2014. The property is bordered on the north and west by farm fields, and on the south and east by privately owned woodlands and meadows. Medford Drain, a tributary of the White River, runs north from the southeast corner along the eastern border, then cuts west to the northwest corner of the study site.

Except for a few smaller habitats, the majority of Munsee Woods is covered by woodlands (Fig. 2). The southern half of the property is primarily a relatively flat, upland mesic forest. Most of the former campsites are located in this woodland, as are many of the largest diameter trees. These tree have low, thick branches below the current cover of the overstory and photographs from the 1930s confirm that the woods were more open in the past (Graham, Wapehani Girl Scouts Camp Munsee, Pers. Commun.). Most of the northern half of the property is covered by a young successional woodland dominated by exotic shrubs. Other habitat types include a seasonal pool and open manicured field to the west, a previously farmed old-field to the north that

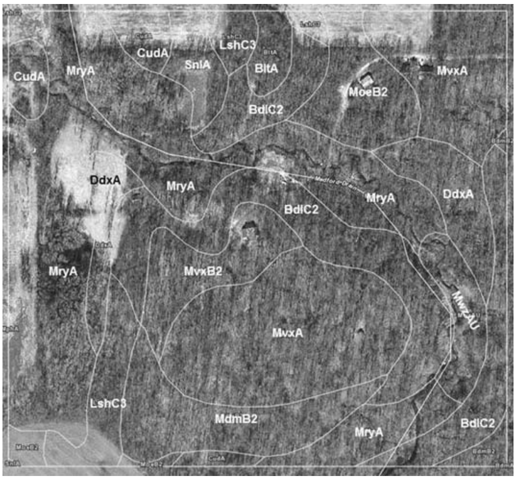

Figure 3.—Soil types and location in Munsee Woods Nature Preserve, Delaware County, Indiana. BdlC2 = Belmore loam, BdmB2 = Belmore silt loam, BltA = Belmore silt loam, CudA = Crosby silt loam, DdxA = Digby Haney silt loam, LshC3 = Losantville clay loam, MoeB2 = Miamian loam, MryA = Millgrove silty clay loam, MvxA = Mountpleasant silt loam, MvxB2 = Mountpleasant silt loam, MwzAU = Muskego muck, and SnlA = Southwest silt loam. (From WSS, 2013).

has been fallow for many years, a small old-field, approximately 10 m across, with a flagpole, and a low moist meadow to the east and southeast, as well as a creek and creek bed, along the Medford Drain. Additionally, there are many woodland edges along roads and paths, and lastly, there are three gravel parking areas.

Munsee Woods lies near the southern border of the Tipton Till Plain Section and the northern border of the New Castle Till Plains and Drainageways Section of the Central Till Plain Region of Indiana (Homoya et al. 1985; Hedge 1997; Clark & Sanders 2005; IUPUI 2007). It occurs in the Upper White River Watershed (USGS Cataloging Unit 05120201, EPA 2009). Medford Drain flows north into the West Fork of the White River. The White River flows southwest into the Wabash River, which in turn, flows south into the Ohio River.

The soil at Munsee Woods is primarily silt and clay loam (Fig. 3). The central upland forest of Munsee Woods consists of both Mountpleasant silt loam, which is characterized as moderately sloping (0–6%), well-drained soil usually associated with kames and till plains, and Belmore silt loam, which is characterized as moderately sloping (1–6%) and well-drained soil usually associated with outwash plains and glacial drainage channels on till plains (WSS 2013). The soils associated with the ephemeral pool and open field along the western border and along most of the Medford Drain are comprised of Milgrove silty clay loam and Digby-Haney silt loams. Both of these soil types are characterized as shallow sloping (0–1%), very to somewhat poorly drained soils usually associated with glacial drainage channels on till plains and outwash plains (WSS 2013). The moist meadow running along the eastern and southeast corner of the site is comprised of Muskego muck soils characterized as shallow sloping (0–1%), very poorly drained soils usually associated with depressions on till plains (WSS 2013). The section of Munsee Woods lying north of Medford Drain comprises several soil types, including the moderately sloping (0–12%) and well to moderately well drained loam soils [i.e., Belmore and Miamian] and the shallow sloping (0–2%) and somewhat poorly to poorly drained silt loam soils [i.e., Blount, Crosby, Mountpleasant, and Southwest] (WSS 2013). Except for the two fields, moist meadows, and gravel parking lots, all the soils at Munsee Woods are covered by woodlands.

BRIEF HISTORY AND LAND USE

The name of the nature preserve, Munsee, came from the Munsee clan of the Delaware Indians. "Munsee" means a place where stones are gathered together (Katherine King, unpublished manuscript entitled "History of Camp Munsee"). The small creek, which runs through the property was named Wolf Run Creek [today know as Medford Drain], and was named after another Delaware clan. These Native Americans inhabited the area in which the preserve is located. A series of fourteen villages was located along the White River ranging from approximately two miles outside of the Muncie city limits to Noblesville (Kemper 1908).

Little is known about the early history of Munsee Woods, other than that its ownership has changed multiple times and its boundaries are unchanged. The information provided below was provided by Bette Graham (Wapehani Girl Scouts Camp Munsee, Pers. Commun.) and Barry Banks (Executive Director Red-tail Land Conservancy, Pers. Commun.).

The land that Munsee Woods currently occupies was originally part of the Whitney Farm. E. Arthur Ball purchased the land and renamed it Green Hills Farm. In 1936 the land was rented to the Girl Scouts and nine Civilian Conservation Corp (CCC) buildings were moved onto the site. In 1939, the Ball family deeded the hilly woodland site, named Camp Munsee, to the Muncie Girl Scouts [Girl Scout Wapehani Council Camp Munsee]. The buildings located on the site were loaned to the Muncie Girl Scouts by the federal government. Camp Munsee operated as a residence camp from 1937 to 1964. In 1965 the residence camp was moved to Camp Windigo in southeastern Grant County, and Camp Munsee was used only as a day camp. In the late 1960s, many of the original buildings were removed including the unit cabins. On December 2, 1970, the Great Lodge burned and was replaced by the current building, Lycoming Lodge. The camp experienced a steady decline of use and was sold to the Red-tail Land Conservancy in April 2011, and the name was changed to Munsee Woods.

Over the years, there has been considerable human impact to the site. There is a large loop-trail through the upland woodlands with numerous campsites. Many of the largest trees at Munsee Woods occur in the campsites. In addition, there are several woodland trails through the upland woodland and the young successional woodland north of Medford Drain. A large gravel parking lot was established south of Medford Drain, just north of the new lodge. Along the west-central section of the property, a large, manicured field was maintained through the years. Off the northwest corner of this field is a deep drainage ditch. Lastly, along the north-central section of the property are two old-fields. One is a small circular field, approximately 10 m across, known as flagpole field because the flagpole was/is located here. To the west of flagpole field is the other old-field. Approximately 0.5 ha, this field was used by the girl scouts to grow garden crops, including pumpkins, and was known as pumpkin patch field.

METHODS

Floristic inventory and analysis.—During the 2011 growing season, one or two forays were made into the study site each week. Forays were random but all areas of the study site were inventoried. Voucher specimens for each species observed were collected and deposited in the Ball State Herbarium (BSUH). Notes on vegetation consisted of a species list with visual estimates of distribution and relative abundance (see Appendix 1). Seasonal changes in the dominant vegetation (based on time of flowering) were noted for the various habitats. Also, the location of potentially sensitive plant communities and rare and endangered species was noted. Nomenclature follows the USDA NRCS Plants Database (USDA 2013).

The floristic quality index (FQI) for Munsee Woods was determined using the program developed by the Conservation Research Institute in conjunction with Rothrock (2004). The program also calculates the mean Coefficient of Conservatism (mean C) and the mean Wetland Indicator Status (mean W). Additionally, it presents a detailed physiognomic analysis of the flora, both native and exotic. For a detailed description of how the FQI is determined and an explanation of C-values, see Swink & Wilhelm (1994); Rothrock (2004); Rothrock & Homoya (2005); Ruch et al. (2010). Briefly, C-values, which range from zero to ten, are 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 conservative the species is to an undisturbed habitat. All exotics are given a C-value of 0. [Exotic species in Indiana was determined by the team of plant taxonomists who determined the C-values for all species (Rothrock 2004). We followed their recommendations.] The FQI is determined by multiplying the mean C for all species present by the square root of the total number of species. [For native FQI and mean C, only the native species are used.] A FQI greater than 35 suggests that a site has remnant natural quality and contains some noteworthy remnants of natural heritage of the region (Rothrock & Homoya 2005; Swink & Wilhelm 1994).

Permanent plot analysis.—Permanent-monitoring plots were established in the mesic upland forest community located in the southern half of Munsee Woods, within which data for woody species were collected. Fourteen 15 m radius circle plots were established, corresponding to an area 0.0706 ha per plot and a total area sampled of 1 ha. The center of each plot was permanently marked with an orange-tipped metal rebar.

Trees in each plot were identified to species and classified into one of three size classes based on diameter at breast height (dbh): 1–4.9 cm dbh, 5–9.9 cm dbh, and $\geq$ 10 cm dbh. Woody stems with a dbh $<$ 10 cm were simply counted. Trees with dbh $\geq$ 10.0 cm were measured for their exact dbh. These data were used to characterize current stand structure and composition and as a basis to predict future successional trends for the site.

Stem density, frequency, and basal area (dominance) were used to characterize woodland structure and composition of the study site. For each woody species a relative importance value was calculated for each of the three size classes. For size classes with dbh $<$ 10 cm, importance values were based on relative density (percentage of all stems in the size class for each species) and relative frequency (percentage of 14 plots where a species was observed). For the dbh $\geq$ 10 cm size class, the importance value was based on relative density, relative frequency, and relative dominance (percentage of total basal area (m^2 ha^{-1}) accounted for by summed basal area of stems for each species. Relative importance values (RIV) were computed as the average of relative density, relative frequency, and relative dominance (for stems with dbh $\geq$ 10 cm). For more detailed description of these calculations see Elzinga et al. (1998) and Ruch et al. (2008a). Chi-squared tests were used to compare species composition among size classes and also to compare the relative abundance of trees in three shade tolerance classes (tolerant, intermediate, intolerant), as defined by Daniel et al. (1979). The results of these comparisons among shade tolerance classes were interpreted with regards to the disturbance history of the site, age of the stand, and the stage of succession.

Relative importance values for tree species in the dbh $\geq$ 10 cm size class were used to compare species composition and species diversity between Munsee Woods and three other forest areas in the vicinity that span a range of disturbance history. Christy Woods in Delaware County and Yuhas Woods in Randolph County were chosen for this comparison because they have a disturbance history similar to that of Munsee Woods. Ginn Woods, in Delaware County, is an undisturbed, late successional forest that represents pre-settlement forests in this region, and represents what second growth forests such as Munsee Woods might approach as they recover from past disturbance. Data for Christy Woods and Ginn Woods were obtained by sampling in permanent plots using methods similar to those used in the analysis of trees with dbh $\geq$ 10 cm at Munsee Woods. Data for Yuhas Woods were obtained using the point-centered-quarter method at 100 systematically located points. Although the sampling methods differed, the data sets for all four woods had similar numbers of stems, with n = 363, 456, 472, and 400 for Munsee, Christy, Ginn, and Yuhas Woods, respectively. Chi-squared analysis was used to compare species composition among the sites, based on relative importance values. Another chi-squared analysis was used to compare the four forests with regards to relative abundance of trees in the three shade tolerance classes. Pairwise two sample t-tests were used to compare Shannon Diversity among the four woods (Zar 1996). A simple Bonferoni adjustment was used to control cumulative Type 1 error rate for these multiple t-tests.

RESULTS

Floristic inventory and analysis.—The vascular flora documented at Munsee Woods Nature Preserve is listed in Appendix 1. The flora consists of 399 taxa representing 252 genera and 85 families. Twenty-nine families (~34%) are represented by only one species and 18 families (~21%) are represented by only two species. The 10 families containing ~55% of the documented species are Asteraceae (51 species), Poaceae (43 species), Cyperaceae (35 species, including 30 *Carex* spp.), Rosaceae (18 species), Brassicaceae (15 species), Lamiaceae (14 species), Fabaceae (12 species), Scrophulariaceae (11 species), Polygonaceae (10 species), and Liliaceae (10 species). The plant community at MWNP is dominated by herbaceous species (Table 1, Appendix 1), especially forbs (241 taxa or ~60.4%). Although grasses and sedges represent fewer total species (78 taxa or ~26%), they are more visually abundant within moist meadows, old fields, and roadside at MWNP.

The MWNP flora is predominately low fidelity (low C-value) native and exotic species (Appendix 1). Only one plant species is classified as having the highest index of fidelity to undisturbed plant communities (C = 10), *Ranunculus hispidus* var. *caricetorum*. Six species, *Aristolochia serpentaria*, *Carex amphibola*,

Table 1.—Physiognomic analysis of the vascular flora documented at Munsee Woods Nature Preserve, Delaware County, Indiana. A = annual, B = biennial, H = herbaceous, P = perennial, W = woody.

	Native species summary		Exotic species summary	
	Number	% of Total	Number	% of Total
# of species	300	75.2%	99	24.8%
Tree	33	8.3%	3	0.8%
Shrub	13	3.3%	10	2.5%
W-Vine	9	2.3%	1	0.3%
H-Vine	4	1.0%	0	0.0%
P-Forbs	131	32.8%	23	5.8%
B-Forbs	11	2.8%	12	3.0%
A-Forbs	36	9.0%	28	7.0%
P-Grass	18	4.5%	13	3.3%
A-Grass	3	0.8%	9	2.3%
P-Sedge	35	8.8%	0	0.0%
A-Sedge	0	0.0%	0	0.0%
Fern	7	1.8%	0	0.0%

Deparia acrostichoides, *Hepatica nobilis* var. *acuta*, *Sedum ternatum*, and *Symplocarpus foetidus*, have a C = 8, and only 12 species have a C = 7. In contrast, 132 species have a C = 0, i.e., 99 exotics and 33 native species, 29 species have a C = 1, 46 species have a C = 2, and 61 species have a C = 3. Thus, 67% of the documented flora at Munsee Woods is categorized with C-values ≤ 3, although only ~5% have C-values ≥ 7. The remaining 28% of the taxa have C-values = 4–6, i.e., 59 species with C = 4, 47 species with C = 5, and six species with C = 6.

Based upon several sources, including the Indiana Natural Heritage Data Center's records for Delaware County, 41 species documented at MWNP are reported for the first time in Delaware County (Appendix 1). In addition, based on the list of endangered, threatened, rare, and extirpated plants of Indiana compiled by the Indiana Department of Natural Resources (IDNR Nature Preserves 2013), the vascular flora of MWNP includes three species on the state watch list, i.e., *Hydrastis canadensis*, *Spiranthes ovalis* var. *erostellata*, and *Viola pubescens*.

Accounting for ~25% of the taxa, exotic species have considerably higher visual abundances than the native species (Table 1, Appendix 1). Most of the native taxa with high visual abundances or widespread distributions are representatives of the low fidelity species categories (C < 4) indicating a notable history of disturbance. Some exotic shrubs, such as *Lonicera maackii* and *Rosa multiflora*, grow so densely that large areas of the site, such as the young successional woodlands north of Medford Drain and the younger slope woodlands in the southwest quadrant, have little or no other shrubs or herbaceous ground cover. Herbaceous exotic species are found in abundance in all disturbed habitats on the property, especially old-fields, manicured fields, lawns around buildings, and the gravel driveways and parking lots (Fig. 2, Appendix 1). Even the less disturbed upland mesic woodland has an excess of exotic shrubs and herbaceous plants. For example, *Alliaria petiolata* lines the many trails cutting through this woodland. For the native species only, the FQI = 55.0 and the mean Coefficient of Conservatism (mean C) is 3.2 (Table 2). When the exotics are included, the FQI drops more than 7 units and the mean C is lowered by 0.8 units (Table 2). Such considerable change in both these matrices suggests that the exotics are having a significant negative impact on the native flora. Although exotics shrubs made up only a small percentage (2.5%,

Table 2.—Floristic Quality summary for the vascular flora documented at Munsee Woods Nature Preserve, Delaware County, Indiana. Mean C = mean Coefficient of Conservatism, FQI = Floristic Quality Index. Total Species is native species plus exotic species.

	Species count	Mean C	FQI
Native species	300	3.2	55.0
Total species	399	2.4	47.7

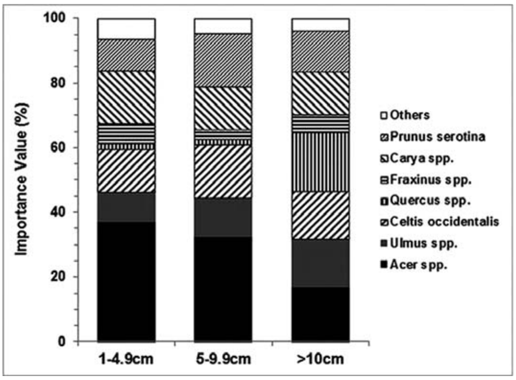

Figure 4.—Relative importance values for tree taxa recorded at Munsee Woods for three size classes defined by diameter at breast height (dbh). Dark solid bars are for tree species classified as shade tolerant. Heavy cross-hatch bars are for species with intermediate shade tolerance. Fine cross-hatch bars are for species classified as shade intolerant.

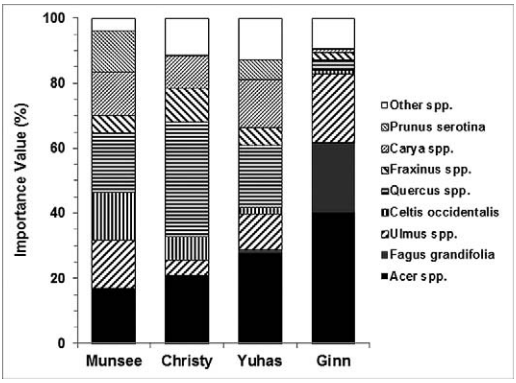

Figure 5.—Relative importance values for tree taxa recorded at three disturbed woods (Munsee, Christy, and Yuhas Woods) and one undisturbed, old-growth forest (Ginn Woods). Description relating bar fill pattern to species shade tolerance same as in Fig. 4.

10 species) of the total number of species (Table 1), they have the highest visual abundances and largest negative impact at the site. The exotic shrub species with the highest visual abundances are *Lonicera maackii*, *Rosa multiflora*, *Euonymus alatus*, and *Ligustrum obtusifolium* (Appendix 1). Lastly, though only infrequent at present, *Ailanthus altissima* is well established in the woodland just north of the manicured field to the west and the woodland just northwest of the eastern meadow.

Permanent plot analysis.—A total of 15 tree species with dbh $\geq$ 10 cm was recorded in the upland mesic woods on the southern half of the property (Prast 2012). The five most important species were *Acer saccharum* (RIV = 16.7), *Quercus alba* (16.0), *Celtis occidentalis* (14.7), *Prunus serotina* (12.9), and *Ulmus americana* (10.2). The high RIV of *Q. alba* is due to a small number of large diameter stems (high relative dominance), whereas the high RIVs of *A. saccharum*, *C. occidentalis*, and *P. serotina* are due to a higher frequency and density of smaller diameter stems.

A total of 20 woody species was recorded in the advanced regeneration layers at Munsee Woods (Prast 2012). Based on RIV, the six most important species in both regeneration layers are *A. saccharum*, *C. occidentalis*, *P. serotina*, *U. americana*, *Carya cordiformis*, and *Lonicera maackii*. Four of these species were also among the five most important in the overstory. *Lonicera maackii* is an invasive shrub that will never attain canopy tree status, but can develop dense populations that shade the understory and interfere with tree regeneration. (Additional raw data for the plots are available in Prast (2012).)

Tree species composition at MWNP differed between the largest size class and the two smaller size classes ($p < 0.001$), but did not differ between the two smaller size classes (Fig. 4). Oaks had the second highest RIV in the overstory but were not found in either of the advanced regeneration layers. The relative abundance of shade tolerant sugar maple in the dbh > 10 cm size class was only half that in the advanced regeneration size classes.

Comparisons of overstory species composition and diversity between MWNP and other mature woods in the region show that MWNP is similar to other disturbed forests, but quite different from an undisturbed old-growth forest (Fig. 5). All three disturbed woods (Munsee, Yuhas, and Christy Woods) are dominated by species that are shade intolerant or have intermediate tolerance, but the old-growth Ginn Woods was over 60% sugar maple and American beech, both highly shade tolerant species. The three disturbed woods had similar Shannon diversity of overstory trees, but all had higher tree species diversity than Ginn Woods ($p < 0.001$, Fig. 6).

DISCUSSION

Floristic inventory and analysis.—The FQI = 55.0 for native species suggests that the site

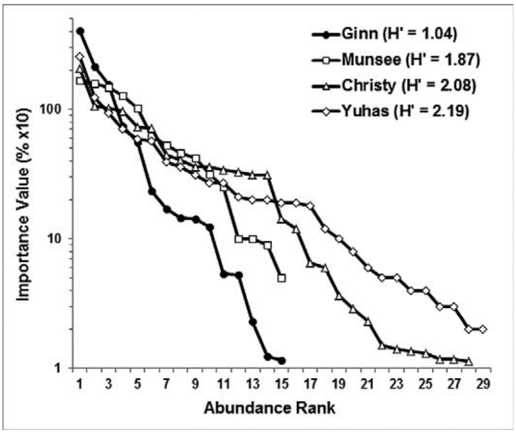

Figure 6.—Rank – abundance curve displaying species richness and species evenness for overstory trees at three disturbed woods (Munsee, Christy, and Yuhas Woods) and one undisturbed, old-growth forest (Ginn Woods). The absolute value of Shannon diversity index for each site is listed in the legend.

contains remnants of the region's natural flora (Swink & Wilhelm 1994). A comparison of the native FQI and mean C-values for various sites in East-Central Indiana is seen in Table 3. Although FQI value for MWNP suggests it is of nature preserve quality (Swink & Wilhelm 1994), it is clearly of lower quality than most other natural areas that have been studied. Rothrock & Homoya (2005) indicated that the best quality central Indiana sites have mean C values ranging from 3.8–4.1. The mean C = 3.2 for MWNP falls shy of this range and is likely due to the excessive, prolonged anthropogenic impact. For example, the majority of the northern half of the site (north of Medford Drain) was agricultural field until about 25–30 years ago, and has since been covered by the young successional woodland (Graham, Wapehani Girl Scouts Camp Munsee, Pers. Commun.). In addition to containing 99 exotic species, the site also includes 169 species of native plants with low C-values ($C \leq 3$). Rothrock & Homoya (2005) noted that central Indiana natural areas a have limited number of species in the highest fidelity categories of C-values, but they gave no specific reason for this observation. Ruch et al. (2008a) suggested the large number of low-fidelity species was due to the anthropogenic impact associated with large amounts of agriculture and construction. As noted above, one of the most noticeable disturbances was the clearing of the northern half of the site for agricultural use when it was still farm property. Additionally, in the upland mesic woodland trees were cleared later for buildings, drive-ways, trails, and campsites associated with the old girl scout camp. The presence of large lower branches among the larger, older stems and the absence of species common in

Table 3.—Comparison of the native Floristic Quality Index and mean Coefficient of Conservatism for various sites in East-Central Indiana. Sites are arranged by descending FQI values. Mean C = native mean Coefficient of Conservatism, FQI = native Floristic Quality Index, * = unpublished data (Ruch), NP = Nature Preserve, FWA = Fish and Wildlife Area, WC = Wetland Complex. (Rothrock & Homoya 2005; Ruch et al. 1998, 2002, 2004, 2007, 2008a, 2008b, 2009, 2012, 2013; Stonehouse et al. 2003; Tungesvick 2011)

Site	# Native spp./ # Total spp.	Native FQI	Mean C	Hectares	County
Mounds State Park	478/584	96.2	4.4	117	Madison
Cabin Creek Raised Bog	400/478	85.6	4.3	7	Randolph
Wilbur Wright FWA	388/536	77.3	3.9	416	Henry
Ginn Woods	364/441	74.1	3.9	61	Delaware
Hayes Arboretum	375/525	72.0	3.7	203	Wayne
Duning Woods NP*	308/380	71.9	4.1	49	Wayne
Lick Creek Summit NP	304/387	68.9	4.0	16	Wayne
Botany Glen	295/357	68.5	4.0	18	Grant
IMI WC	246/287	61.9	3.9	3	Henry
McVey Memorial Woods*	295/378	60.0	3.5	115	Randolph
Munsee Woods NP	300/399	55.0	3.2	18.4	Delaware
Bennett WC	210/264	54.6	3.8	3	Henry
Mississinewa Woods NP	233/311	46.2	3.0	15	Randolph
Dutro Woods NP*	132/239	25.0	2.2	6.6	Delaware

the area, such as *Asarum canadense*, in the southern half of Munsee Woods suggest that the site was once more open and may have been used for grazing when it was part of Whitney Farm or Green Hills Farm.

The FQI for all species is 7.3 units lower and the mean C is 0.8 units lower than the FQI and mean C for native species alone. Rothrock & Homoya (2005) suggested that the quality of a plant community is degraded when including exotic species and lowers the mean C by more than 0.7 units. From these numbers and from visual examination of MWNP, it is obvious that the exotic flora is having a significant negative impact on the native plant community. Sites, such as Fall Creek Gorge in Warren County (Tonkovich & Sargent 1993; Rothrock & Homoya 2005) and Lick Creek Summit Nature Preserve (LCSNP) in Wayne County (Ruch et al. 2008a), have shown a difference in mean C $\geq$ 0.7 units with limited negative impact on the native community. This is due in large part to the highest diversity and concentration of exotic species being located on the margins and only minimally penetrating the interior of these sites. At MWNP, however, many of the exotic species, especially invasive shrubs, were found in the southwestern successional woods and the northern half of the site, and were spreading into the south mesic woods along the Medford Drain and along the many paths. *Lonicera maackii* dominated the northern half of the site, and was growing so thickly in some areas that virtually no other plants could grow. Furthermore, around the gravel drive and parking lot and field adjacent to Medford Drain, herbaceous exotic species dominated. A similar degree of negative impact by exotic species was reported for many areas, especially old-fields and young successional woodlands, at Wilbur Wright Fish and Wildlife Area, where the mean differences between all species and the native species was 1.1 units (Ruch et al. 2002). A study that uses plot analysis to quantify frequency, density, and percent cover of exotic species, especially the shrubs, is recommended to better quantify the abundance and impact of exotic species on the plant community at MWNP.

Permanent plot analysis.—Comparisons among size classes in MWNP and comparisons with other mature forests in the region indicate that Munsee Woods is in early to middle stages of succession from past disturbances. As the large trees in the current overstory die and are replaced by saplings from the understory, the relative importance of sugar maple in the overstory will likely increase. Undisturbed forests in this region, such as Ginn Woods, are dominated by shade tolerant species such as *A. saccharum* and *Fagus grandifolia*. No stems of American beech were recorded in the permanent plots, but sugar maple was abundant in the advanced regeneration of MWNP. Oak species may well disappear from Munsee Woods in the future as the few large trees currently in the overstory die with no oaks in the understory to replace them. However, other tree species with intermediate shade tolerance, such as *P. serotina* and *C. cordiformis*, are relatively abundant in the advanced regeneration size classes and may succeed into the overstory in the future.

Historical disturbance in Munsee, Christy, and Yuhas Woods appears to have resulted in higher tree species diversity compared to the old-growth forest at Ginn Woods. This is consistent with the literature on forest succession, which shows that late successional forests tend to be dominated by a small number of highly competitive, shade tolerant tree species (Christensen 1977). The spatially and temporally complex disturbance history of Indiana woodlots managed by individual landowners created a patchy distribution of niches that could be exploited by tree species with differing life history characteristics, including differences in dispersal mechanism, micro-site requirements, and shade tolerance. If the relative importance of sugar maple in MWNP increases in the future it is likely that tree species diversity will decrease toward a level similar to that documented for the old-growth Ginn Woods. Some research has shown that regular small-scale natural disturbances can maintain overstory tree species diversity (Glitzenstein et al. 1986). In Indiana such disturbances might include strong winds and ice storms that damage or knock down overstory trees, creating large gaps in the canopy. However, there is evidence of regular small-scale blow-down in Ginn Woods, yet the overstory is dominated by beech and maple and has low species diversity. If Munsee Woods is allowed to develop without further human disturbance, it is likely to follow a trajectory toward species composition and diversity similar to Ginn Woods.

ACKNOWLEDGMENTS

We thank the Indiana Academy of Science for financial support through the Winona Welch Award for Botanical Biodiversity Research. We also wish to thank the Department of Biology at Ball State University for use of resources for this research project. We express our appreciation to Barry Banks, Executive Director of the Red-tail Conservancy, for permission to conduct the study. We especially wish to thank Bette Graham, Wapehani Girl Scouts, and her family for providing historical information regarding the site. Lastly, we would like to thank Ball State University student Morgan Roederer for her assistance in the herbarium.

APPENDIX 1

CATALOG OF VACULAR FLORA AT MUNSEE WOODS, DELAWARE COUNTY, INDIANA

(Arranged alphabetically by family)

Listed are voucher specimens for all species documented at Munsee Woods. Nomenclature follows the USDA Plants Database (USDA 2013). Each species report contains the following information: (1) current scientific name based on the USDA Plants Database; (2) current taxonomic synonyms, if appropriate; (3) common name(s), based primarily on Gleason & Cronquist (1991); Swink & Wilhelm (1994); Yatskievych (2000); (4) typical habitat(s) within the study site; (5) a visual estimate of its relative abundance; (6) its Coefficient of Conservatism (C-value) for Indiana (Rothrock 2004); and (7) the Ball State University Herbarium (BSUH) number(s). The relative abundance for species is defined as follows; rare = ≤ 5 sites although a species may be abundant at one site; infrequent = occasional, not widespread throughout its potential habitats, but may be locally abundant at a site; common = frequent throughout its potential habitats and may be locally abundant at one or more sites; and abundant = common and numerous throughout its potential habitats.

All non-native (exotic) species are in capital letters. [Exotics that were planted and have not naturalized are also listed in capital letters.] Potential Delaware County records are indicated by a pound-symbol (#) in parentheses immediately preceding a species. Species were deemed unreported for Delaware County, and hence considered a county record, if they did not appear in Deam (1940), the computer database of Keller et al. (1984); Keller (1986); Overlease & Overlease (2007); Ruch et al. (1998, 2004); or the UDSA Plant Database (UDSA 2013). (The database of plants developed by Keller et al. (1984) contains the same plant list for Delaware County as does the Indiana Natural Heritage Data Center, IDNR.). There are 41 Delaware County records. Lastly, the status of certain species in brackets (e.g, [Watch List]) is from the list compiled by the Indiana Department of Natural Resources (IDNR 2013).

PHYLUM EQUISETOPHYTA

Horsetails and Scouring Rushes

Equisetaceae (Horsetail Family)

Equisetum arvense L.; Common or field horsetail; Eastern meadow and creek bank; Infrequent; C = 1; BSUH 17822.

PHYLUM POLYPODIOPHYTA

Ferns

Aspleniaceae (Spleenwort Family)

Asplenium platyneuron (L.) Britton, Sterns & Poggenb. var. *platyneuron*; Ebony spleenwort; Successional woods north of western old-field; Rare, but locally common; C = 3; BSUH 17798.

Dryopteridaceae (Wood Fern Family)

Cystopteris protrusa (Weath.) Blasdell; Lowland bladder fern; Successional woods along northern creek; Common; C = 4; BSUH 17805.

Deparia acrostichoides (Sw.) M. Kato; SYN: *Athyrium thelypterioides* (Michx.) Desv., *Diplazium acrostichoides* (Sw.) Butters; Silver false spleenwort, silvery glade fern, silvery spleenwort; Upland woods; Rare, one plant; C = 8; Observed but not collected.

Onoclea sensibilis L.; Sensitive fern; Creek bank and meadow in the SE corner of property; Rare, but locally frequent; C = 4; BSUH 17783.

Ophioglossaceae (Adder's Tongue Family)

Botrychium dissectum Spreng.; SYN: *Botrychium dissectum* Spreng. var. *obliquum* (Muhl. ex Willd.)

Clute; Lace-frond or cutleaf grapefern; Upland woods; Rare; C = 3; BSUH 17826.

Botrychium virginianum (L.) Sw.; Rattlesnake fern, Virginia grapefern; Upland woods; Abundant and widespread; C = 4; BSUH 17757.

PHYLUM CONIFEROPHYTA

Gymnosperms or Conifers

Cupressaceae (Cypress Family)

(#) *Juniperus virginiana* L. var. *virginiana*; Eastern red cedar; Young successional woods east of entrance road [Happy Hollow Campsite]; Rare; C = 2; BSUH 17964.

Pinaceae (Pine Family)

PICEA ABIES (L.) Karst.; Norway spruce; West side of gravel lot; One tree, planted not naturalized; C = 0; BSUH 17988.

Pinus strobus L.; Eastern white pine; Entrance road near maintenance building; Rare, one tree, planted not naturalized; C = 5; BSUH 17947.

PHYLUM MAGNOLIOPHYTA

Angiosperms

Acanthaceae (Acanthus Family)

Ruellia strepens L.; Smooth ruellia, Limestone wild petunia; Entrance road, gravel lot and adjacent field; Infrequent; C = 4; BSUH 18059.

Aceraceae (Maple Family)

Acer negundo L. var. *negundo*; Boxelder; Woodlands; Abundant and widespread; C = 1; BSUH 17311.

Acer saccharinum L.; Silver maple; Seasonal pool, woodlands; Common, and locally abundant; C = 1; BSUH 17846.

Acer saccharum Marsh. var. *saccharum*; Sugar maple; Woodlands; Abundant and widespread; C = 4; BSUH 17758.

Alismataceae (Water Plantain Family)

Alisma subcordatum Raf.; SYN: *Alisma plantago-aquatica* L. ssp. *subcordatum* (Raf.) Hultén; Small-flowered or American water-plantain; Northern meadow; Rare; C = 2; BSUH 18022.

(#) *Sagittaria latifolia* Willd.; Common or broadleaf arrowhead; Moist meadow and creek bank in SE corner of property; Rare; C = 3; BSUH 18039.

Amaranthaceae (Amaranth Family)

(#) *AMARANTHUS RETROFLEXUS* L.; Rough green amaranth, redroot; Edge of seasonal pool; Infrequent; C = 0; BSUH 17290.

Anacardiaceae (Cashew Family)

Rhus glabra L.; Smooth sumac; Woodland edge along south end of pumpkin patch old-field; Rare, but locally common; C = 1; BSUH 17940.

Toxicodendron radicans (L.) Kuntze ssp. *negundo* (Greene) Gillis; Common or eastern poison ivy; Woodlands, old-fields; Abundant and widespread; C = 1; BSUH 17738.

Apiaceae (Carrot Family)

Chaerophyllum procumbens (L.) Crantz var. *procumbens*; Wild or spreading chervil; Young successional woods; Abundant and widespread; C = 2; BSUH 17867.

Cicuta maculata L. var. *maculata*; Spotted water hemlock; Northern meadow; Infrequent; C = 6; BSUH 18036.

CONIUM MACULATUM L.; Poison hemlock; Entrance road near maintenance building; Rare; C = 0; BSUH 17745.

Cryptotaenia canadensis (L.) DC.; Canada honewort; Woodlands; Abundant and widespread; C = 3; BSUH 17955.

DAUCUS CAROTA L.; Queen Anne's-lace, wild carrot; Gravel lot and adjacent field; Common; C = 0; BSUH 17972.

Osmorhiza longistylis (Torr.) DC.; Aniseroot, long-style sweetroot; Young successional woods; Abundant and widespread; C = 3; BSUH 17812.

PASTINACA SATIVA L.; Wild parsnip; Edge of gravel lot; Rare; C = 0; BSUH 17742.

Sanicula canadensis L. var. *canadensis*; Canada sanicle, Canada black-snakeroot; Flagpole old-field; Infrequent; C = 2; BSUH 17975.

Sanicula odorata (Raf.) K.M. Pryer & L.R. Philippe; SYN: *Sanicula gregaria* E.P. Bicknell; Clustered black-snakeroot, clustered sanicle; Woodlands; Abundant and widespread; C = 2; BSUH 17794.

Apocynaceae (Dogbane Family)

Apocynum cannabinum L.; Dogbane, American Indian-hemp; Old-fields; Infrequent; C = 2; BSUH 18020.

Araceae (Arum Family)

Arisaema dracontium (L.) Schott; Green dragon; Meadow; Rare, but locally common; C = 5; BSUH 18010.

Arisaema triphyllum (L.) Schott var. *triphyllum*; Jack-in-the-pulpit; Woodlands; Abundant and widespread; C = 4; BSUH 17814.

Symplocarpus foetidus (L.) Salib. ex W.P.C. Barton; Eastern skunk cabbage; Meadows; Common and locally abundant; C = 8; BSUH 17843.

Aristolochiaceae (Birthwort Family)

Aristolochia serpentaria L.; Virginia snakeroot; Successional woods near buildings; Infrequent; C = 8; BSUH 17778.

Asclepiadaceae (Milkweed Family)

Asclepias incarnata L. ssp. *incarnata*; Swamp milkweed; Eastern meadow; Infrequent; C = 4; BSUH 18054.

Asclepias syriaca L.; Common milkweed; Gravel lot and adjacent field; Rare; C = 1; BSUH 18006.

Asteraceae (Aster Family)

Achillea millefolium L.; Common yarrow; Western old-field; Infrequent; C = 0; BSUH 17316.

Ageratina altissima (L.) King & H. Rob. var. *altissima*; SYN: *Eupatorium rugosum* Houtt.; White snakeroot; Woodlands; Abundant and widespread; C = 2; BSUH 17907.

Ambrosia artemisiifolia L. var. *elatior* Descourt.; SYN: *Ambrosia elatior* L.; Common ragweed; Western old-field; Abundant and widespread; C = 0; BSUH 17903.

Ambrosia trifida L. var. *trifida*; Great or giant ragweed; Gravel lot and adjacent field; Common; C = 0; BSUH 17895.

ARCTIUM MINUS (Hill) Bernh.; Common or lesser burdock; Border of western old-field; Infrequent; C = 0; BSUH 18021.

Bidens cernua L.; Nodding bur-marigold, nodding beggar's-ticks; Creek bank north of western old-field; Common; C = 2; BSUH 17292.

Bidens frondosa L.; Common beggar's-ticks, devil's-beggar's-ticks; Successional woods near Happy Hollow camp site; Infrequent but locally common; C = 1; BSUH 17873.

(#) *Bidens tripartita* L; SYN: *Bidens comosa* (A. Gray) Wiegand; Three-parted or three-lobed beggar's-ticks; Northern meadow; Common; C = 2; BSUH 17922.

Bidens vulgata Greene; Tall beggar's-ticks, big devil's-beggar's-ticks; Western old-field; Infrequent but locally abundant; C = 0; BSUH 17904.

CICHORIUM INTYBUS L.; Chicory; Gravel lot and adjacent field; Rare; C = 0; BSUH 17932.

CIRSIUM ARVENSE (L.) Scop.; Canada thistle; Gravel lot and adjacent field; Infrequent; C = 0; BSUH 18012.

Cirsium discolor (Muhl. ex Willd.) Spreng.; Field or pasture thistle; Gravel lot and adjacent field, old-fields; Infrequent but widespread; C = 3; BSUH 17871.

CIRSIUM VULGARE (Savi) Ten.; Bull thistle; Gravel lot and adjacent field; Rare; C = 0; BSUH 18023.

Conyza canadensis (L.) Cronquist var. *canadensis*; SYN: *Erigeron canadensis* L.; Canadian horseweed; Gravel lot and old-fields; Common and widespread; C = 0; BSUH 18034.

Erechtites hieraciifolia (L.) Raf. ex DC. var. *hieraciifolia*; White fireweed, American burnweed; Old-fields; Rare; C = 2; BSUH 17890.

Erigeron annuus (L.) Pers.; Eastern daisy or annual fleabane, whitetop; Old-fields; Abundant and widespread; C = 0; BSUH 17740.

Erigeron philadelphicus L. var. *philadelphicus*; Philadelphia daisy or fleabane; Gravel lot and adjacent field; Infrequent; C = 3; BSUH 17809.

(#) *Eupatorium altissimum* L.; Tall boneset, tall thoroughwort; Gravel lot and adjacent field; Infrequent; C = 1; BSUH 17295.

Eupatorium perfoliatum L. var. *perfoliatum*; Common boneset; Moist meadows; Common and widespread; C = 4; BSUH 18025.

Euthamia graminifolia (L.) Nutt. var. *graminifolia*; SYN: *Solidago graminifolia* (L.) Salisb.; Common flat-topped goldenrod, grass-leaved goldenrod; Old-fields; Infrequent but locally common; C = 3 BSUH 17889.

Eutrochium purpureum (L.) E.E. Lamont var. *purpureum*; SYN: *Eupatorium purpureum* L. var. *purpureum*, *Eupatoriadelphus purpureus* (L.) King & H. Rob.; Purple-node, green-stemmed, or sweet-scented Joe-Pye-weed; Old-fields; Common; C = 5; BSUH 18016.

(#) *GALINSOGA QUADRIRADIATA* Cav.; Common quickweed, Peruvian daisy, shaggy soldier; Edge of gravel lot; Rare; C = 0; BSUH 17288.

Helenium autumnale L. var. *autumnale*; Common sneezeweed; Creek bank near eastern meadow; Rare; C = 3; BSUH 17925.

(#) Helianthus grosseserratus M. Martens; Sawtooth sunflower; Creek bank north of western old-field; Infrequent; C = 3; BSUH 17285.

Helianthus tuberosus L.; Jerusalem-artichoke; Edge of gravel lot, western old-field; Infrequent but locally abundant; C = 2; BSUH 17878.

Heliopsis helianthoides (L.) Sweet var. *helianthoides*; False sunflower, smooth oxeye; Woodlands and creek banks; Common and widespread; C = 4; BSUH 17980.

(#) *HYPOCHAERIS RADICATA* L.; Hairy cat's-ear; Western old-field; Infrequent but locally common; C = 0; BSUH 17779.

Lactuca biennis (Moench) Fernald; Tall blue lettuce; Eastern meadow and woodland edges; Infrequent but widespread; C = 2; BSUH 17882.

Lactuca canadensis L.; Wild or Canada lettuce; Western old-field; Infrequent; C = 2; BSUH 18051.

Lactuca floridana (L.) Gaertn. var. *floridana*; Woodland or blue lettuce; Edge of gravel lot, old-fields, woodlands; Abundant and widespread; C = 5; BSUH 17870.

MATRICARIA DISCOIDEA DC.; SYN: *Matricaria matricarioides* auct. non (Less.) Porter; Pineappleweed, disc mayweed; Gravel lot and adjacent field; Rare; C = 0; BSUH 17324.

Packera glabella (Poir.) C. Jeffrey; SYN: *Senecio glabellus* Poir.; Butterweed, yellowtop; Young successional woods, old-fields; Infrequent but widespread; C = 0; BSUH 17852.

Packera obovata (Muhl. ex Willd.) W.A. Weber & A. Löve; SYN: *Senecio obovatus* Muhl. ex Willd.; Round-leaved golden ragwort; Upland woods; Infrequent but locally common; C = 7; BSUH 17850.

Prenanthes altissima L.; Tall rattlesnake-root, tall white lettuce; Upland woods; Common; C = 5; BSUH 17879.

Ratibida pinnata (Vent.) Barnhart; Gray-headed or pinnate prairie coneflower; Flagpole old-field and path; Rare; C = 5; BSUH 17931.

Rudbeckia hirta L. var. *pulcherrima* Farw.; Black-eyed Susan; Creek bank in SE corner of property; Rare; C = 2; BSUH 17912.

Rudbeckia laciniata L. var. *laciniata*; Cut-leaved coneflower; Creek banks, meadows; Common and widespread; C = 3; BSUH 18035.

(#) *Solidago altissima* L.; SYN: *Solidago canadensis* L. var. *scabra* Torr. & A. Gray; Tall or Canada goldenrod; Old-fields, roadside; Abundant and widespread; C = 0; BSUH 17920.

Solidago caesia L.; Blue-stemmed goldenrod, wreath goldenrod; Upland woods; Infrequent but locally common; C = 7; BSUH 17299.

SONCHUS ASPER (L.) Hill; Spiny sow-thistle; Gravel lot and adjacent field; Rare; C = 0; BSUH 17951.

Symphyotrichum cordifolium (L.) G.L. Nesom; SYN: *Aster cordifolius* L., *Aster sagittifolius* Wedem. ex Willd., *Symphyotrichum sagittifolium* (Wedem. ex. Willd.) G.L. Nesom; Common blue wood aster, blue heart-leaved aster; Field adjacent to gravel lot, woodlands; Common and widespread; C = 5; BSUH 17302.

Symphyotrichum lanceolatum (Willd.) G.L. Nesom ssp. *lanceolatum* var. *lanceolatum*; SYN: *Aster simplex* Willd.; *Aster lanceolatus* Willd. ssp. *simplex* (Willd.) A.G. Jones; White panicle aster; Meadows, creek bank; Common and widespread; C = 3; BSUH 17301.

Symphyotrichum lateriflorum (L.) Á. Löve & D. Löve var. *lateriflorum*; SYN: *Aster lateriflorus* (L.) Britton; Calico, goblet, or side-flowering aster; Gravel lot and adjacent field, woodlands; Abundant and widespread; C = 3; BSUH 17294.

Symphyotrichum novae-angliae (L.) G.L. Nesom; SYN: *Aster novae-angliae* L.; New England aster; Gravel lot and adjacent field; Rare; C = 3; BSUH 17289.

Symphyotrichum pilosum (Willd.) G.L. Nesom var. *pilosum*; SYN: *Aster pilosus* Willd.; Hairy white old-field aster, goodbye meadow; Old-fields, roadside; Common and widespread; C = 0; BSUH 17303.

(#) *Symphyotrichum puniceum* (L.) Á. Löve & D. Löve var. *puniceum*; SYN: *Aster firmus* Nees.; *Symphyotrichum firmum* (Nees) G.L. Nesom; Purple-stem aster; Meadow in SE corner of property; Rare but locally common; C = 4; BSUH 17300.

TARAXACUM OFFICINALE F.H. Wigg. ssp. *OFFICINALE*; Common dandelion; Old-fields, roadside; Common and widespread; C = 0; BSUH 17840.

TRAGOPOGON LAMOTTEI Rouy; SYN: *Tragopogon pratensis* L.; Common goat's-beard, jack-go-to-bed-at-noon; Western old-field; Rare; C = 0; BSUH 18056.

Verbesina alternifolia (L.) Britton ex Kearney; SYN: *Actinomeris alternifolia* (L.) DC.; Wingstem; Creek bank and moist woodlands; Common; C = 3; BSUH 17908.

Vernonia gigantea (Walter) Trel. ssp. *gigantea*; SYN: *Vernonia altissima* Nutt.; Tall or giant ironweed; Western old-field; Common; C = 2; BSUH 17905.

Xanthium strumarium L. var. *glabratum* (DC.) Cronquist; Rough cocklebur; Seasonal pool; Rare but locally abundant; C = 0; BSUH 17917.

Balsaminaceae (Touch-Me-Not Family)

Impatiens capensis Meerb.; SYN: *Impatiens biflora* Walter; Orange jewelweed, spotted touch-me-not; Old-fields, meadows, open woodlands; Abundant and widespread; C = 2; BSUH 17978.

Impatiens pallida Nutt.; Yellow jewelweed, pale touch-me-not; Gravel lot and adjacent field; Rare; C = 4; BSUH 18027.

Berberidaceae (Barberry Family)

(#) *BERBERIS THUNBERGII* DC.; Japanese barberry; Upland woods; Rare; C = 0; BSUH 17785.

Podophyllum peltatum L.; May-apple; Woodlands; Abundant and widespread; C = 3; BSUH 17835.

Bignoniaceae (Trumpet-Creeper Family)

Campsis radicans (L.) Seem. ex Bureau; Trumpet creeper; Woodland along the edge of the western old-field; Locally abundant; C = 1; BSUH 17983.

Catalpa speciosa (Warder) Warder ex Engelm.; Northern catalpa; Gravel lot and successional woods along northern creek; Infrequent but locally common; C = 0; BSUH 17753.

Boraginaceae (Borage Family)

Hackelia virginiana (L.) I.M. Johnst.; Stickseed, beggars-lice; Upland woods; Abundant and widespread; C = 0; BSUH 18042.

Mertensia virginica (L.) Pers. ex Link; Virginia bluebells; Upland woods near RTC Office; Rare but locally abundant; C = 6; BSUH 17839.

Brassicaceae (Mustard Family)

ALLIARIA PETIOLATA (M. Bieb.) Cavara & Grande; SYN: *Alliaria officinalis* Andrz. ex M. Bieb.; Garlic mustard; Woodlands, old-fields; Abundant and widespread; C = 0; BSUH 17823.

Arabis hirsuta (L.) Scop. var. *pycnocarpa* (M. Hopkins) Rollins; SYN: *Arabis hirsuta* (L.) Scop. var. *adpressipilis* (M. Hopkins) Rollins; Hairy rockcress, cream-flower rockcress; Upland woods; Rare but locally common; C = 5; BSUH 17804.

BARBAREA VULGARIS W.T. Aiton; Garden yellow-rocket, bitter winter cress; Edge of gravel lot, old-fields, roadside; Common; C = 0; BSUH 17863.

BRASSICA NIGRA (L.) W.D.J. Koch; Black mustard; Gravel lot and adjacent field; Rare but locally common; C = 0; BSUH 18014.

CAPSELLA BURSA-PASTORIS (L.) Medik.; Shepherd's-purse; Gravel lot and adjacent field, roadside; Infrequent; C = 0; BSUH 17807.

Cardamine bulbosa (Schreb. ex Muhl.) Britton, Sterns & Poggenb.; SYN: *Cardamine rhomboidea* (Pers.) DC.; White spring cress, bulbous bittercress; Northern creek bank and meadow; Infrequent but locally abundant; C = 4; BSUH 17824.

Cardamine concatenata (Michx.) Sw.; SYN: *Dentaria laciniata* Muhl. ex Willd.; Cut-leaved toothwort; Woodlands; Abundant and widespread; C = 4; BSUH 17836.

Cardamine douglassii Britton; Purple spring cress, limestone bittercress; Edge of western old-field, woodlands; Common and widespread; C = 5; BSUH 17845.

(#) *CARDAMINE HIRSUTA* L.; Hairy bittercress; Building lawns; Infrequent but locally abundant; C = 0; BSUH 17837.

Cardamine pensylvanica Muhl. ex Willd.; Pennsylvania bittercress; Young successional woods and meadows; Infrequent; C = 2; BSUH 17851.

(#) *DRABA VERNA* L.; Early whitlow-grass, spring draba; Gravel drive and lawns; Infrequent but locally abundant; C = 0; BSUH 17830.

Lepidium virginicum L. var. *virginicum*; Common peppergrass, poor man's-pepper, Virginia pepperweed; Gravel lot and adjacent field; Infrequent; C = 0; BSUH 17962.

NASTURTIUM OFFICINALE W.T. Aiton; SYN: *Rorippa nasturtium-aquaticum* (L.) Hayek; Watercress; Creek at bridge of entrance road; Rare but locally abundant; C = 0; BSUH 18040.

Rorippa palustris (L.) Besser ssp. *fernaldiana* (Butters & Abbe) Jonsell; SYN: *Rorippa islandica* (Oeder) Borbás var. *fernaldiana* Butters & Abbe; Common or Fernald's yellow cress; Seasonal pool; Rare; C = 2; BSUH 17915.

THLASPI ARVENSE L.; Field pennycress; Gravel lot and adjacent field; Rare but locally frequent; C = 0; BSUH 17767.

Campanulaceae (Bellflower Family)

Campanulastrum americanum (L.) Small; SYN: *Campanula americana* L.; Tall or American bellflower; Edge of woodlands and open woodlands; Abundant and widespread; C = 4; BSUH 18044.

Lobelia inflata L.; Indian-tobacco; Gravel lot and adjacent field, successional woods north of creek; Infrequent; C = 3; BSUH 17874.

Lobelia siphilitica L. var. *siphilitica*; Great blue lobelia; Moist meadows; Infrequent; C = 3; BSUH 17897.

Cannabaceae (Hemp Family)

(#) *Humulus lupulus* L. var. *lupuloides* E. Small; Common or American hops; Edge of western old-field; Rare but locally common; C = 5; BSUH 17775.

Caprifoliaceae (Honeysuckle Family)

LONICERA MAACKII (Rupr.) Herder; Amur bush honeysuckle; Woodlands; Abundant and widespread; C = 0; BSUH 17789.

LONICERA MORROWII A. Gray; Morrow's honeysuckle; Successional woods; Infrequent; C = 0; BSUH 17857.

LONICERA X *BELLA* Zabel; Showy fly honeysuckle; Successional woods; Infrequent; C = 0; BSUH 17859.

Sambucus nigra L. ssp. *canadensis* (L.) R. Bolli; SYN: *Sambucus canadensis* L.; American black elderberry; Moist woodlands, border of eastern meadow; Common; C = 2; BSUH 18005.

Triosteum perfoliatum L.; Common horse-gentian, feverwort; Flagpole old-field; Rare; C = 5; BSUH 17953.

Viburnum lentago L.; Nannyberry, sheepberry; Creek bank in SE corner of property; Rare; C = 5; BSUH 17885.

Viburnum prunifolium L.; Black haw; Upland woods; Common and widespread; C = 4; BSUH 17861.

Caryophyllaceae (Pink Family)

ARENARIA SERPYLLIFOLIA L.; Thyme-leaved sandwort; Building lawns; Rare, one large colony; C = 0; BSUH 17981.

CERASTIUM FONTANUM Baumg. ssp. *VULGARE* (Hartm.) Greuter & Burdet; SYN: *Cerastium vulgatum* L.; Mouse-ear chickweed, big chickweed; Western old-field; Common and widespread in field; C = 0; BSUH 18047.

SILENE LATIFOLIA Poir. ssp. *ALBA* (Mill.) Greuter & Burdet; SYN: *Lychnis alba* Mill.; Evening, white, or bladder campion; Gravel lot and adjacent field; Rare; C = 0; BSUH 17322.

Silene stellata (L.) W.T. Aiton; Starry catchfly, widowsfrill; Young successional slope woods in SW corner; Rare; C = 5; BSUH 17984.

Silene virginica L. var. *virginica*; Fire pink; Upland woods; Rare but locally common; C = 7; BSUH 17319.

STELLARIA MEDIA (L.) Vill.; Common chickweed; Lawns, woodlands; Abundant and widespread; C = 0; BSUH 17827.

Celastraceae (Staff-tree Family)

(#) *CELASTRUS ORBICULATUS* Thunb.; Oriental bittersweet; Shrub thicket between gravel lot and creek; Infrequent but locally common; C = 0; BSUH 17770.

Celastrus scandens L.; American bittersweet; Woodland edge of western old-field; Rare; C = 2; BSUH 17780.

EUONYMUS ALATUS (Thunb.) Siebold var. *ALATUS*; Winged euonymus, winged burning bush; Upland and successional woods; Abundant and widespread; C = 0; BSUH 17808.

EUONYMUS FORTUNEI (Turcz.) Hand.-Maz. var. *RADICANS* (Siebold ex Miq.) Rehder; Winter creeper; Gravel lot and adjacent field; Rare; C = 0; BSUH 17296.

Chenopodiaceae (Goosefoot Family)

CHENOPODIUM ALBUM L. var. *ALBUM*; Lamb's-quarters, pigweed; Gravel lot and adjacent field; Rare; C = 0; BSUH 17921.

Clusiaceae (Mangosteen Family)

Hypericum punctatum Lam.; Spotted St.-John's-wort; Western old-field; Common and widespread in this field; C = 3; BSUH 17987.

Commelinaceae (Spiderwort Family)

COMMELINA COMMUNIS L.; Asiatic or common day-flower; Gravel lot and adjacent field; Rare; C = 0; BSUH 17868.

Tradescantia subaspera Ker Gawl. var. *subaspera*; Zigzag or broad-leaved spiderwort; Edge of western old-field; Infrequent; C = 4; BSUH 17906.

Tradescantia virginiana L.; Virginia spiderwort; Upland woods; Common and widespread; C = 7; BSUH 17801.

Convolvulaceae (Morning-glory Family)

Calystegia sepium (L.) R. Br.; Common hedge bindweed, hedge false bindweed; Gravel lot and adjacent field, edge of old-fields; Abundant; C = 1; BSUH 17743.

IPOMOEA HEDERACEA Jacq.; Ivy-leaved morning-glory; Gravel lot and adjacent field; Rare; C = 0; BSUH 17894.

Ipomoea pandurata (L.) G. Mey.; Wild potato, man-of-the-earth; Western old-field; Infrequent but locally abundant; C = 3; BSUH 18019.

IPOMOEA PURPUREA (L.) Roth; Common or tall morning-glory; Gravel lot and adjacent field; Rare; C = 0; BSUH 18043.

Cornaceae (Dogwood Family)

Cornus drummondii C.A. Mey.; Rough-leaved dogwood; Gravel drive, woodland edges; Common and widespread; C = 2; BSUH 18049.

Cornus florida L.; Flowering dogwood; Woodlands; Abundant and widespread; C = 4; BSUH 17862.

Cornus obliqua Raf.; SYN: *Cornus amomum* Mill. var. *schuetzeana* (C.A. Mey.) Rickett; Silky dogwood, knob-styled dogwood; Northern meadow; Rare; C = 5; BSUH 17332.

Crassulaceae (Stonecrop Family)

Sedum ternatum Michx.; Wild or woodland stonecrop; Campsites in upland woods; Infrequent but locally abundant; C = 8; BSUH 17820.

Cuscutaceae (Dodder Family)

Cuscuta gronovii Willd. ex Schult. var. *gronovii*; Common dodder, scaldweed; Northern meadow; Rare, parasitic on *Lactuca floridana*; C = 2; BSUH 17898.

Cyperaceae (Sedge Family)

(#) *Carex aggregata* Mack.; Smooth clustered sedge, Glomerate Sedge; Fields, especially the western old-field; Abundant and widespread; C = 2; BSUH 17268.

Carex amphibola Steud.; Gray sedge, eastern narrowleaf sedge; Upland woods; Infrequent; C = 8; BSUH 17934.

(#) *Carex annectens* E.P. Bicknell; Large yellow fox sedge, yellow-fruit sedge; Western old-field; Rare but locally common; C = 3; BSUH 17936.

Carex blanda Dewey; Common wood sedge, eastern woodland sedge; Old-fields and woodlands; Abundant and widespread; C = 1; BSUH 17269.

Carex cephalophora Muhl. ex Willd.; Short-headed bracted sedge, oval-leaf sedge; Western old-field; Common; C = 3; BSUH 17271.

Carex conjuncta Boott; Green-headed or soft fox sedge; Small seasonal pool in NW corner of property; Rare but locally abundant; C = 6; BSUH 17270.

Carex cristatella Britton; Crested oval sedge; Eastern meadow; Infrequent but common at this site; C = 3; BSUH 17272.

Carex davisii Schwein. & Torr.; Awned graceful sedge, Davis' sedge; Old-fields and woodlands; Abundant and widespread; C = 3; BSUH 17273.

Carex frankii Kunth; Bristly cattail sedge, Frank's sedge; Gravel lot and adjacent field; Infrequent; C = 2; BSUH 17310.

Carex granularis Muhl. ex Willd.; Pale sedge, limestone meadow sedge; Old-fields; Common; C = 2; BSUH 17274.

Carex grisea Wahlenb.; Wood gray sedge, inflated narrow-leaf sedge; Woodlands; Abundant and widespread; C = 3; BSUH 17275.

Carex hirsutella Mack.; Hairy green sedge, fuzzy-wuzzy sedge; Upland woods; Common and widespread; C = 3; BSUH 17276.

Carex hirtifolia Mack.; Hairy wood sedge, pubescent sedge; Creek bank and field near western old-field; Infrequent; C = 5; BSUH 17277.

(#) *Carex hystericina* Muhl. ex Willd.; Porcupine or bottlebrush sedge; Eastern meadow; Infrequent but locally common; C = 5; BSUH 17278.

Carex jamesii Schwein.; Grass sedge, James' sedge; Young successional woods north of creek; Abundant and widespread; C = 4; BSUH 17813.

Carex laevivaginata (Kük.) Mack.; Smooth-sheathed fox sedge; Northern meadow; Infrequent but locally common; C = 7; BSUH 17279.

Carex laxiculmis Schwein.; Weak-stemmed wood sedge, spreading sedge; Upland woods; Abundant and widespread; C = 7; BSUH 17935.

(#) *Carex lupulina* Muhl. ex Willd.; Common hop sedge; Western old-field near seasonal pool; Rare; C = 4; BSUH 18061.

Carex molesta Mack. ex Bright; Field oval sedge, troublesome sedge; Western old-field; Common; C = 2; BSUH 17966.

Carex normalis Mack.; Spreading oval sedge, greater straw sedge; Old-fields and northern meadow; Common and widespread; C = 3; BSUH 17280.

Carex radiata (Wahlenb.) Small; Straight-styled wood sedge, eastern star sedge; Gravel lot, lawns, woodlands; Abundant and widespread; C = 4; BSUH 17765.

Carex rosea Schkuhr ex Willd.; Curly-styled wood sedge, rosy sedge; Upland woods; Infrequent but locally common; C = 5; BSUH 17933.

Carex shortiana Dewey; Short's sedge; Gravel lot and adjacent field; Infrequent; C = 3; BSUH 17796.

Carex sparganioides Muhl. ex Willd.; Loose-headed bracted sedge, bur-reed sedge; Fields and creek east of the entrance drive; Infrequent; C = 4; BSUH 17281.

Carex stipata Muhl. ex Willd. var. *stipata*; Common fox sedge, awlfruit sedge; Fields and creek east of entrance drive; Common; C = 2; BSUH 17282.

Carex stricta Lam.; Tussock sedge, upright sedge; Northern creek bank and meadow; Common; C = 5; BSUH 18063.

(#) *Carex texensis* (Torr.) L.H. Bailey; Texas bracted sedge; Western old-field; Rare but locally common; C = 0; BSUH 17264. NOTE: a disjunct species north of its normal range.

Carex tribuloides Wahlenb. var. *tribuloides*; Awl-fruited oval sedge, blunt broom sedge; Western old-field, successional woods; Abundant; C = 5; BSUH 17971.

(#) *Carex trichocarpa* Muhl. ex Willd.; Hairy-fruited lake sedge; Northern meadow; Rare but locally common; C = 4; BSUH 17283.

Carex vulpinoidea Michx. var. *vulpinoidea*; Brown fox edge; Gravel lot and adjacent field; Rare but locally abundant; C = 2; BSUH 17749.

Cyperus strigosus L.; Long-scaled or false nut sedge, straw-colored flatsedge; Western old-field; Abundant and widespread in this field; C = 0; BSUH 17884.

Eleocharis erythropoda Steud.; Red-rooted or bald spike rush; Western old-field along seasonal pool; Rare but locally abundant; C = 2; BSUH 17318.

Schoenoplectus tabernaemontani (C.C. Gmel.) Palla; SYN: *Scirpus validus* Vahl; Great or soft-stemmed (softstem) bulrush; Northern meadow; Infrequent but locally common; C = 4; BSUH 17941.

Scirpus atrovirens Willd.; Dark green bulrush; Gravel lot and adjacent field; Infrequent; C = 4; BSUH 18001.

Scirpus pendulus Muhl.; Red or rufous bulrush; Edge of western old-field; Rare but locally common; C = 2; BSUH 17943.

Elaeagnaceae (Oleaster Family)

ELAEAGNUS UMBELLATA Thunb. var. *PARVIFOLIA* (Wall. ex Royle) C.K. Schneid.; Autumn olive; Near creek on entrance road; Rare; C = 0; BSUH 17810.

Euphorbiaceae (Spurge Family)

Acalypha rhomboidea Raf.; SYN: *Acalypha virginica* L. var. *rhomboidea* (Raf.) Cooperr.; Common three-seeded mercury; Woodlands, old-fields; Common and widespread C = 0; BSUH 17896.

Chamaesyce maculata (L.) Small; SYN: *Euphorbia maculata* L.; Milk purslane, spotted or creeping spurge, spotted sandmat; Lawn of the RTC Office, roadside; Infrequent but locally common; C = 0; BSUH 17910.

Chamaesyce nutans (Lag.) Small; SYN: *Euphorbia nutans* Lag.; (Small) Eyebane, nodding spurge; Gravel lot and adjacent field; Infrequent; C = 0; BSUH 17872.

Fabaceae (Pea or Bean Family)

Amphicarpaea bracteata (L.) Fernald; American hog-peanut; Northern meadow and moist path along the creek; Abundant; C = 5; BSUH 17876.

(#) *Apios americana* Medik.; Common groundnut, wild bean; Northern meadow and moist path along creek; Infrequent but locally common; C = 3; BSUH 17877.

Cercis canadensis L. var. *canadensis*; Eastern redbud; Woodlands; Infrequent; C = 3; BSUH 17847.

Desmodium canadense (L.) DC.; Canadian tick-trefoil, showy tick-trefoil; Pumpkin patch old-field; Common in this field; C = 3; BSUH 17297.

Gleditsia triacanthos L.; Honey locust; Western old-field; Infrequent; C = 1; BSUH 17982.

Gymnocladus dioicus (L.) K. Koch; Kentucky coffee-tree; Entrance Road near caretaker's house; Rare; C = 4; BSUH 18013.

MEDICAGO LUPULINA L.; Black medic; Large gravel lot and field along the entrance road; Infrequent; C = 0; BSUH 17790.

MELILOTUS ALBA Medik.; SYN: *Melilotus officinalis* (L.) Pall.; White sweet clover; Entrance Road, gravel lot and adjacent field; Common; C = 0; BSUH 17959.

MELILOTUS OFFICINALIS (L.) Lam.; Yellow sweet clover; Flagpole old-field; Infrequent; C = 0; BSUH 17741.

TRIFOLIUM HYBRIDUM L.; Alsike clover; Large gravel lot and adjacent field; Rare but locally common; C = 0; BSUH 17952.

TRIFOLIUM PRATENSE L.; Red clover; Large gravel lot and adjacent field, roadside; Infrequent; C = 0; BSUH 17787.

TRIFOLIUM REPENS L.; White clover; Large gravel lot and adjacent field, roadside; Infrequent but locally common; C = 0; BSUH 17791.

Fagaceae (Beech Family)

Quercus alba L.; White oak; Parking lot and lawn around the RTC Office, upland woods; Infrequent; C = 5; BSUH 17760.

Quercus imbricaria Michx.; Shingle-oak; Happy Hollow Camp Sites – successional woods; Rare; C = 3; BSUH 18045.

Quercus rubra L.; Northern red oak; Parking lot and lawn around the RTC Office, upland woods; Infrequent; C = 4; BSUH 17747.

Geraniaceae (Geranium Family)

Geranium maculatum L.; Wild geranium, spotted geranium; Upland woods; Common and widespread; C = 4; BSUH 17853.

Grossulariaceae (Currant Family)

Ribes cynosbati L.; Dogberry, eastern prickly gooseberry; Upland woods in the south half of property; Infrequent; C = 4; BSUH 17858.

Hippocastanaceae (Horse-chestnut Family)

Aesculus glabra Willd.; Ohio buckeye; Entrance Road near the caretaker's house; Rare; C = 5; BSUH 18002.

Hydrangeaceae (Hydrangea Family)

PHILADELPHUS INODORUS L.; Scentless mock-orange; RTC Office lawn [planted]; Rare; C = 0; BSUH 17325. NOTE: This plant was removed following this study.

Hydrophyllaceae (Waterleaf Family)

Hydrophyllum macrophyllum Nutt.; Hairy or large-leaf waterleaf; Fields/lawns around buildings, woodlands; Abundant and widespread; C = 7; BSUH 17948.

Phacelia purshii Buckley; Miami mist; Fields and meadows along creek east of entrance road; Infrequent but locally common; C = 3; BSUH 17793.

Iridaceae (Iris Family)

Iris virginica L. var. *shrevei* (Small) E.S. Anderson; SYN: *Iris shrevei* Small; Southern blue flag, Shreve's iris; Northern meadow; Rare; C = 5; BSUH 17957.

Sisyrinchium angustifolium Mill.; Stout or narrowleaf blue-eyed grass; Old-fields; Infrequent but locally common; C = 3; BSUH 17954.

Juglandaceae (Walnut Family)

Carya cordiformis (Wangenh.) K. Koch; Bitternut hickory; Woodlands in the northern half of property; Common and widespread; C = 5; BSUH 17330.

Carya glabra (Mill.) Sweet; Pignut hickory; Upland woods; Common; C = 4; BSUH 17772.

Carya ovata (Mill.) K. Koch; Shagbark hickory; Upland woods; Common; C = 4; BSUH 17773.

Juglans nigra L.; Black walnut; Upland woods; Common; C = 2; BSUH 17774.

Juncaceae (Rush Family)

Juncus dudleyi Wiegand; SYN: *Juncus tenuis* Willd. var. *dudleyi* (Wiegand) F.J. Herm.; Dudley's rush; Large gravel lot and adjacent field; Infrequent; C = 2; BSUH 17939.

Juncus tenuis Willd.; Path or poverty rush; Successional woods in the northern half of the property; Common and widespread; C = 0; BSUH 17938.

(#) *Juncus torreyi* Cov.; Torrey's rush; Pumpkin patch old-field; Rare; C = 3; BSUH 17891.

(#) *Luzula multiflora* (Ehrh.) Lej.; Common wood rush; Upland woods; Infrequent; C = 6; BSUH 17854.

Lamiaceae (Mint Family)

Agastache nepetoides (L.) Kuntze; Catnip or yellow giant hyssop; Path from gravel lot to eastern meadow [open woods]; Rare; C = 4; BSUH 17880.

GLECHOMA HEDERACEA L.; Ground ivy, gill-over-the-ground, creeping Charlie; Old-fields, successional woods, meadows; Abundant and widespread; C = 0; BSUH 17860.

LAMIUM AMPLEXICAULE L.; Henbit, dead nettle; Lawns, upland woods; Infrequent; C = 0; BSUH 17829.

LAMIUM PURPUREUM L. var. *PURPUREUM*; Purple dead nettle; Lawns and upland woods; Infrequent; C = 0; BSUH 17834.

Lycopus americanus Muhl. ex W.P.C. Barton; Common or American water horehound, American bugleweed; Northern meadow; Infrequent; C = 3; BSUH 17900.

Lycopus uniflorus Michx. var. *uniflorus*; Northern water horehound or bugleweed; Seasonal pool, creek banks, meadows; Infrequent; C = 5; BSUH 17919.

Mentha arvensis L.; SYN: *Mentha arvensis* L. var. *villosa* (Benth.) S.R. Stewart, *Mentha arvensis* L. var. *canadensis* (L.) Kuntze; Field or wild mint; Eastern meadow; Rare; C = 4; BSUH 17924.

Monarda fistulosa L. ssp. *fistulosa* var. *mollis* (L.) Benth.; Wild bergamot; Northern meadow and moist field along creek; Common; C = 3; BSUH 18055.

NEPETA CATARIA L.; Catnip; Entrance road, gravel lot and adjacent field; Rare but locally common; C = 0; BSUH 17991.

PRUNELLA VULGARIS L. ssp. *VULGARIS*; Common self heal, lawn prunella, heal-all; Western old-field, lawns; Infrequent; C = 0; BSUH 18000.

Scutellaria incana Biehler var. *incana*; Downy or hoary skullcap; Open woodlands north of eastern meadow, i.e., path between entrance road at the bridge and eastern meadow; Rare but locally common; C = 4; BSUH 17994.

Scutellaria lateriflora L. var. *lateriflora*; Mad-dog skullcap, blue skullcap; Seasonal pool, successional woods north of western old-field; Infrequent; C = 4; BSUH 17883.

Stachys tenuifolia Willd.; SYN: *Stachys tenuifolia* Willd. var. *hispida* (Pursh) Fernald, *Stachys hispida* Pursh; Smooth hedge-nettle; Eastern meadow; Infrequent; C = 4; BSUH 17996.

Teucrium canadense L. var. *canadense*; SYN: *Teucrium canadense* L. var. *virginicum* (L.) Eaton; Canadian (American) germander; Entrance road, gravel lot and adjacent field; Open successional woods; Infrequent but locally common; C = 3; BSUH 17989.

Lauraceae (Laurel Family)

Lindera benzoin (L.) Blume var. *benzoin*; Northern or hairy spice bush; Woodlands; Infrequent but locally common; C = 5; BSUH 17848.

Sassafras albidum (Nutt.) Nees; Sassafras; Woodlands east of entrance road, between Happy Hollow Camp Site and caretaker's house; Rare; C = 1; BSUH 17754.

Lemnaceae (Duckweed Family)

Lemna minor L.; Lesser, small, or common duckweed; Northern meadow; Locally abundant in a small pool; C = 3; BSUH 17803.

Liliaceae (Lily Family)

Allium burdickii (Hanes) A.G. Jones; SYN: *Allium tricoccum* Aiton var. *burdickii* Hanes; Narrow-leaf wild leek; Upland woods; Abundant and widespread; C = 6; BSUH 17782.

Allium canadense L. var. *canadense*; Wild or meadow garlic; Woodland near RTC Office; Infrequent; C = 1; BSUH 17323.

(#) *ASPARAGUS OFFICINALIS* L.; Garden asparagus; Gravel lot and adjacent field; Rare; C = 0; BSUH 17992.

Camassia scilloides (Raf.) Cory; Early-blooming wild hyacinth, Atlantic camas; Upland woods; Rare; C = 5; BSUH 17817.

(#) *HEMEROCALLIS FULVA* (L.) L.; Orange day lily; Entrance Road, gravel lot and adjacent field; Infrequent; C = 0; BSUH 17960.

Maianthemum racemosum (L.) Link ssp. *racemosum*; SYN: *Smilacina racemosa* (L.) Desf.; Feathery false Solomon's seal, feathery Solomon's plume, feathery false lily of the valley; Fields and meadows near creek east of entrance road; Infrequent; C = 4; BSUH 17755.

NARCISSUS PSEUDONARCISSUS L.; Daffodil; Lawn and woodlands around the RTC Office; Rare; C = 0; BSUH 17833.

Polygonatum biflorum (Walter) Elliott var. *biflorum*; Small or smooth Solomon's seal; Slope woods between RTC Office and northern meadow, woodlands; Abundant; C = 4; BSUH 17320.

Polygonatum biflorum (Walter) Elliott var. *commutatum* (Schult. & Schult. f.) Morong; Giant or smooth Solomon's seal; Fields and meadows near creek east of entrance road; Infrequent; C = 4; BSUH 17795.

Trillium sessile L.; Toadshade, sessile trillium, sessile-flowered wake-robin; Lawn around the RTC Office, upland woods; Abundant; C = 4; BSUH 17832.

Limnanthaceae (Meadow-foam Family)

Floerkea proserpinacoides Willd.; False mermaidweed; Upland woods; Infrequent but locally abundant; C = 5; BSUH 17825.

Lythraceae (Loosestrife Family)

Lythrum alatum Pursh var. *alatum*; Winged loosestrife, winged lythrum; Pumpkin patch old-field; Rare; C = 5; BSUH 17977.

Magnoliaceae (Magnolia Family)

Liriodendron tulipifera L.; Tulip popular, tulip tree, yellow popular; Parking lot and lawn around the RTC Office; Rare; C = 4; BSUH 17762.

Malvaceae (Mallow Family)

ABUTILON THEOPHRASTI Medik.; Velvetleaf; Gravel lot and adjacent field; Rare; C = 0; BSUH 17287.

HIBISCUS TRIONUM L.; Flower-of-an-hour; Northern border of western old-field; Rare; C = 0; BSUH 17291.

SIDA SPINOSA L.; Prickly sida, prickly mallow, prickly fan-petals; Northern border of western old-field; Infrequent; C = 0; BSUH 17928.

Menispermaceae (Moonseed Family)

Menispermum canadense L.; Common moonseed; Woodlands; Common; C = 3; BSUH 17806.

Moraceae (Mulberry Family)

MORUS ALBA L.; SYN: *Morus tatarica* L.; White mulberry; Woodlands; Infrequent but widespread; C = 0; BSUH 17761.

Morus rubra L. var. *rubra*; Red mulberry; Upland woods; Infrequent; C = 4; BSUH 17771.

Oleaceae (Olive Family)

Fraxinus americana L.; White ash; Woodlands; Common and widespread; C = 4; BSUH 17756.

Fraxinus pennsylvanica Marsh.; SYN: *Fraxinus pennsylvanica* Marsh. var. *subintegerrima* (Vahl) Fernald, *F. pennsylvanica* Marsh. var. *lanceolata* (Borkh.) Sarg.; Green ash; Woodlands; Common and widespread; C = 1; BSUH 17786.

LIGUSTRUM OBTUSIFOLIUM Siebold & Zucc.; Border privet; Woodlands, especially in the northern half of the property; Abundant and widespread; C = 0; BSUH 17735.

Onagraceae (Evening Primrose Family)

Circaea lutetiana L. ssp. *canadensis* (L.) Asch. & Magnus; Common or broadleaf enchanter's nightshade; Woodlands; Abundant and widespread; C = 2; BSUH 18009.

Epilobium coloratum Biehler; Eastern, cinnamon, or purple-leaf willow-herb; Eastern meadow; Infrequent; C = 3; BSUH 17913.

Gaura biennis L.; Biennial gaura, biennial bee-blossom; Pumpkin patch old-field; Rare; C = 3; BSUH 17888.

Ludwigia palustris (L.) Elliott; Common water purslane, marsh purslane, marsh seedbox; Creek at bridge on entrance road, east side of road; Rare but locally abundant; C = 3; BSUH 17926.

Oenothera biennis L.; Common evening primrose; Gravel lot and adjacent fields; Rare; C = 0; BSUH 18015.

Orchidaceae (Orchid Family)

Aplectrum hyemale (Muhl. ex Willd.) Torr.; Putty-root orchid, Adam and Eve; Successional woods north of the RTC Office; Infrequent; C = 7; BSUH 17818.

(#) *Spiranthes ovalis* Lindl. var. *erostellata* Catling; Lesser ladies tresses, October lady's tresses; Lawn of RTC Office; Rare; C = 3; BSUH 17286. [Watch List]

Oxalidaceae (Wood Sorrel Family)

Oxalis stricta L. Upright yellow wood sorrel, common yellow oxalis; Gravel lot and adjacent field; Common; C = 0; BSUH 17788.

Papaveraceae (Poppy Family)

Sanguinaria canadensis L.; Bloodroot; Lawn and woodlands around the RTC Office; Infrequent; C = 5; BSUH 17838.

Phytolaccaceae (Pokeweed Family)

Phytolacca americana L. var. *americana*; American pokeweed or pokeberry; Entrance Road, gravel lot and adjacent fields, open woodlands; Common and widespread; C = 0; BSUH 17958.

Plantaginaceae (Plantain Family)

PLANTAGO LANCEOLATA L.; English or narrow-leaf plantain, buckhorn; Roadside, parking area and lawns around buildings, gravel lot and adjacent fields; Infrequent but widespread; C = 0; BSUH 17950.

Plantago rugelii Decne. var. *rugelii*; American, purple-stemmed or blackseed plantain; Entrance Road, gravel lot and adjacent fields, open woodlands; Abundant and widespread; C = 0; BSUH 17887.

Platanaceae (Plane-tree Family)

Platanus occidentalis L.; American sycamore, buttonwood; Creek banks near northern meadow and SE corner of property; Infrequent; C = 3; BSUH 18060.

Poaceae (Grass Family)

(#) *AGROSTIS GIGANTEA* Roth; SYN: *Agrostis alba* auct. non L.; Redtop; Pumpkin patch old-field; Common; C = 0; BSUH 17970.

Agrostis perennans (Walter) Tuck.; Autumn or upland bent-grass, thin-grass; Lawn around buildings; Common; C = 2; BSUH 17929.

(#) *Andropogon virginicus* L. var. *virginicus*; Broom-sedge, Virginia bluestem; Western old-field; Infrequent; C = 1; BSUH 17284.

BROMUS COMMUTATUS Schrad.; SYN: *Bromus racemosum* L.; Hairy chess, also hairy, meadow, or bald brome; Pumpkin patch old-field; Infrequent; C = 0; BSUH 17967.

BROMUS INERMIS Leyss.; Smooth or Hungarian brome; Gravel lot and adjacent field, old-fields;

Common and locally abundant; C = 0; BSUH 17942.

Bromus pubescens Muhl. ex Willd.; Hairy woodland brome; Parking area and lawns around the buildings; Infrequent; C = 4; BSUH 17267.

BROMUS TECTORUM L.; Junegrass, cheatgrass, downy chess, or downy brome; Gravel lot and adjacent field; Infrequent; C = 0; BSUH 17750.

Cinna arundinacea L.; Common or sweet woodreed; Northern meadow, woodlands; Abundant and widespread; C = 4; BSUH 18041.

DACTYLIS GLOMERATA L.; Orchard grass; Parking lot and lawns around the RTC Office; Infrequent; C = 0; BSUH 17766.

Danthonia spicata (L.) P. Beauv. ex Roem. & Schult.; Poverty oatgrass; Flagpole path and old-field; Abundant here; C = 3; BSUH 17326.

Dichanthelium acuminatum (Sw.) Gould & C.A. Clark var. *fasciculatum* (Torr.) Freckmann; SYN: *Panicum implicatum* Scribn., *Panicum lanuginosum* Elliot var. *implicatum* (Scribn.) Fernald; Woolly, western, or old-field panic grass; Western old-field; Locally abundant; C = 2; BSUH 17331.

(#) *DIGITARIA CILIARIS* (Retz.) Koeler; SYN: *Digitaria sanguinalis* (L.) Scop. var. *ciliaris* (Retz.) Parl.; Southern crab-grass; Lawn of RTC Office; Rare but locally common; C = 0; BSUH 17886.

DIGITARIA SANGUINALIS (L.) Scop.; Northern or hairy crab-grass; Roadside, gravel lot and adjacent field; Common; C = 0; BSUH 17965.

ECHINOCHLOA CRUS-GALLI (L.) P. Beauv.; Barnyard-grass; Entrance road, gravel lot, and adjacent field; Infrequent; C = 0; BSUH 17266.

Echinochloa muricata (P. Beauv.) Fernald var. *muricata*; Rough-barnyard grass; Seasonal pool adjacent to western old-field; Rare but locally abundant; C = 1; BSUH 17265.

ELEUSINE INDICA (L.) Gaertn.; Yard grass; Indian goose-grass; crowfoot grass; Lawn and drive at RTC Office; Infrequent; C = 0; BSUH 18030.

Elymus hystrix L. var. *hystrix*; SYN: *Hystrix patula* Moench; Eastern bottlebrush grass; Upland woods adjacent to eastern meadow; Rare but locally frequent; C = 5; BSUH 18029.

(#) *Elymus macgregorii* R. Brooks & J.J.N. Campb; Early wild rye; Creek bank near entrance road, north of bridge and east side of road; Infrequent; C = 3; BSUH 17968.

ELYMUS REPENS (L.) Gould; SYN: *Elytrigia repens* (L.) Desv. ex Nevski.; Quack grass; Entrance road, gravel lot and adjacent field; Infrequent; C = 0; BSUH 18057.

Elymus villosus Muhl. ex. Willd.; Downy or hairy wild rye; Woodlands; Abundant and widespread; C = 4; BSUH 17969.

Festuca subverticillata (Pers.) E. Alexeev; SYN: *Festuca obtusa* Biehler; Nodding fescue; Woodlands; Abundant and widespread; C = 4; BSUH 17312.

Glyceria striata (Lam.) Hitchc.; Fowl manna-grass; Meadows and creek banks; Abundant; C = 4; BSUH 17328.

(#) *HOLCUS LANATUS* L.; Common velvet-grass; Western old-field; Abundant; C = 0; BSUH 17327.

HORDEUM JUBATUM L. ssp. *JUBATUM*; Foxtail barley; Gravel lot and adjacent field; Rare; C = 0; BSUH 17746.

Leersia oryzoides (L.) Sw.; Rice cut-grass; Wet meadows, especially the eastern meadow; Infrequent but locally abundant; C = 2; BSUH 17307.

Leersia virginica Willd.; White-grass; Woodlands; Abundant; C = 4; BSUH 17930.

(#) *Muhlenbergia frondosa* (Poir.) Fernald; Common satin-grass, wirestem muhly; Creek bank and meadow in SE corner; Infrequent but locally common; C = 3; BSUH 17911.

Muhlenbergia schreberi J.F. Gmel.; Nimblewill; Gravel lot and adjacent field; Abundant; C = 0; BSUH 18033.

Panicum dichotomiflorum Michx. var. *dichotomiflorum*; Knee grass, fall panic grass; Entrance road, gravel lot and adjacent field; Infrequent; C = 0; BSUH 17306.

Panicum philadelphicum Bernh. ex Trin.; Philadelphia panic grass; Entrance road, gravel lot and adjacent field; Infrequent; C = 4; BSUH 17305.

(#) *PHALARIS ARUNDINACEA* L.; Reed canary grass; Large gravel lot and adjacent fields, creek bank; Common here; C = 0; BSUH 17945.

PHLEUM PRATENSE L.; Timothy; Pumpkin patch old-field; Common; C = 0; BSUH 18058.

(#) *Phragmites australis* (Cav.) Trin. ex Steud.; Common reed; Eastern meadow; Rare but locally common; C = 0; BSUH 17881. NOTE: it is the native genotype.

POA ANNUA L.; Annual bluegrass, speargrass; Lawn and roadside near RTC Office; Common and widespread; C = 0; BSUH 17309.

POA COMPRESSA L.; Canada bluegrass; Flagpole path and old-field, woodlands; Abundant; C = 0; BSUH 17946.

POA PRATENSIS L. ssp. *PRATENSIS*; Kentucky bluegrass; Old-fields, roadside, and open woodlands; Abundant; C = 0; BSUH 17759.

Poa sylvestris A. Gray; Forest or woodland bluegrass; Woodlands; Abundant and widespread; C = 5; BSUH 17797.

POA TRIVIALIS L.; Rough bluegrass; Northern meadow and creek bank; Abundant; C = 0; BSUH 17329.

SCHEDONORUS ARUNDINACEUS (Schreb.) Dumort.; SYN: *Schedonorus phoenix* (Scop.) Holub, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire, *Festuca arundinacea* Schreb., *Festuca elatior* L. var. *arundinacea* (Schreb.) Hook; Tall fescue; Fields and roadsides; Abundant; C = 0; BSUH 17944.

SETARIA FABERI Herrm.; Nodding or giant foxtail-grass, Japanese bristlegrass; Entrance road, gravel lot and adjacent fields; Infrequent; C = 0; BSUH 18024.

SETARIA PUMILA (Poir.) Roem. & Schult. ssp. *PUMILA*; SYN: *Setaria glauca* (L.) P. Beauv.; Yellow foxtail-grass; Gravel lot and adjacent field; Infrequent; C = 0; BSUH 17909.

SORGHUM HALEPENSE (L.) Pers.; Johnson-grass; Gravel lot and adjacent field; Rare; C = 0; BSUH 17986.

Tridens flavus (L.) Hitchc. var. *flavus*; SYN: *Triodia flava* (L.) Smyth; Purpletop, purpletop tridens; Pumpkin patch old-field; Infrequent; C = 1; BSUH 18031.

Polemoniaceae (Phlox Family)

Phlox divaricata L. ssp. *divaricata*; Wild blue or woodland phlox; Young successional woods northern half of property; Abundant and widespread; C = 5; BSUH 17866.

Phlox paniculata L.; Garden, summer, or fall phlox; Creek bank near northern meadow; Rare; C = 3; BSUH 17979.

Polemonium reptans L. var. *reptans*; Greek valerian, spreading Jacob's-ladder; Young successional woods northern half of property; Common and widespread; C = 5; BSUH 17865.

Polygonaceae (Smartweed Family)

Fallopia scandens (L.) Holub; SYN: *Polygonum scandens* L. var. *scandens*; Climbing false buckwheat; Pumpkin patch old-field, woodland edges; Abundant and widespread; C = 0; BSUH 17293.

(#) *PERSICARIA CESPITOSA* (Blume) Nakai, var. *LONGISETA* (Bruijn) C. F. Reed; SYN: *Polygonum cespitosum* Blume var. *longisetum* (Bruijn) A.N. Steward, *Polygonum longisetum* Bruijn, *Persicaria longiseta* (Bruijn) Kitagawa; Creeping smartweed, Oriental lady's thumb; Pumpkin patch old-field, woodlands; Abundant and widespread; C = 0; BSUH 17963.

Persicaria pensylvanica (L.) Small; SYN: *Polygonum pensylvanicum* L.; Pinkweed, Pennsylvania smartweed; Large seasonal pool west side of western old-field; Infrequent but locally abundant; C = 0; BSUH 17916.

PERSICARIA MACULOSA Gray.; SYN: *Persicaria vulgaris* Webb & Moq., *Persicaria maculata* (Raf.) Gray, *Polygonum persicaria* L., *Polygonum dubium* Stein; Spotted lady's-thumb; Entrance road, gravel lot and adjacent field; Infrequent; C = 0; BSUH 18048.

Persicaria punctata (Elliott) Small var. *leptostachya* (Meisn.) Small; SYN: *Polygonum punctatum* Elliott var. *confertiflorum* (Meisn.) Fassett; SYN: Dotted or water smartweed; Creek bank and sandy shoreline north of western old-field; Infrequent, but locally common; C = 3; BSUH 17875.

POLYGONUM AVICULARE L.; SYN: *Polygonum monspeliense* Pers.; Doorweed, common or prostrate knotweed; Lawns around the buildings, roadside; Infrequent; C = 0; BSUH 17923.

RUMEX ACETOSELLA L.; Field sorrel, common sheep sorrel; Western old-field; Rare but locally abundant; C = 0; BSUH 17800.

RUMEX CRISPUS L. ssp. *CRISPUS*; Curly dock, sour dock; Gravel lot and adjacent field; Infrequent; C = 0; BSUH 17752.

RUMEX OBTUSIFOLIUS L.; Bitter dock, blunt-leaved dock; Entrance road, gravel lot and adjacent field; Common; C = 0; BSUH 18062.

Tovara virginiana (L.) Raf.; SYN: *Polygonum virginianum* L., *Persicaria virginiana* (L.) Gaertn.; Jumpseed, Virginia knotweed; Successional woods north of creek; Abundant and widespread; C = 3; BSUH 18018.

Portulacaceae (Purslane Family)

Claytonia virginica L. var. *virginica*; Virginia spring beauty; Woodlands and fields; Abundant; C = 2; BSUH 17831.

Primulaceae (Primrose Family)

Lysimachia ciliata L.; Fringed loosestrife; Field/open woods from entrance road at the bridge to eastern meadow; Common; C = 4; BSUH 17993.

Samolus valerandi L. ssp. *parviflorus* (Raf.) Hultén; SYN: *Samolus floribundus* Kunth, *Samolus parviflorus* Raf.; Seaside brookweed, water pimpernel; Eastern meadow; Rare; C = 5; BSUH 17914.

Ranunculaceae (Buttercup Family)

Anemone virginiana L. var. *virginiana*; Tall anemone or thimbleweed; Pumpkin patch old-field; Rare; C = 4; BSUH 18052.

Caltha palustris L. var. *palustris*; Marsh marigold, cowslip; Meadow in SE corner of property; Rare but locally abundant; C = 7; BSUH 17849.

Hepatica nobilis Schreb. var. *acuta* (Pursh) Steyerm.; SYN: *Hepatica acutiloba* DC., *Anemone acutiloba* (DC.) G. Lawson; Sharp-lobed hepatica; Hillside woods between office and northern meadow; Rare; C = 8; BSUH 17815.

Hydrastis canadensis L.; Goldenseal, yellowroot; Creek bank and meadow in the SE corner of property; Rare but locally common; C = 7; BSUH 17784. [Watch List]

Ranunculus abortivus L.; Kidney-leaved or little-leaf buttercup or crowfoot, small-flowering crowfoot; Lawn and woodlands around the RTC Office, moist woodlands; Common; C = 0; BSUH 17842.

Ranunculus hispidus Michx. var. *caricetorum* (Greene) T. Duncan; SYN: *Ranunculus caricetorum* Greene, *Ranunculus septentrionalis* Poir.

var. *caricetorum* (Greene) Fernald; Bristly or hispid buttercup, swamp buttercup; Creek bank and eastern meadow; Common and locally abundant; C = 10; BSUH 17821.

Thalictrum revolutum DC.; Waxy-leaved or skunk meadow rue; Northern meadow and creek bank; Common; C = 5; BSUH 17737.

Rosaceae (Rose Family)

(#) *Agrimonia gryposepala* Wallr.; Tall hairy or common agrimony; Upland woods; Common and widespread; C = 2; BSUH 17997.

Agrimonia parviflora Aiton; Southern, swamp, or small-flowered agrimony, harvestlice; Border of western old-field; Infrequent; C = 4; BSUH 18037.

Agrimonia pubescens Wallr.; Downy or soft agrimony; Creek bank and succession woods east of entrance road; Infrequent; C = 5; BSUH 18017.

Crataegus mollis Scheele; Downy hawthorn; Successional woods south of the western old-field and seasonal pool; Infrequent; C = 2; BSUH 17777.

Crataegus punctata Jacq.; Dotted hawthorn; Successional woods north of the western old-field; Infrequent; C = 2; BSUH 17776.

(#) *DUCHESNEA INDICA* (Andrews) Focke; Mock or Indian strawberry; Lawns, woodland edges, fields; Common; C = 0; BSUH 17816.

Geum canadense Jacq. var. *canadense*; White avens; Woodlands; Abundant; C = 1; BSUH 18008.

Geum laciniatum Murray; Rough avens; Eastern meadow; Rare; C = 3; BSUH 17892.

Geum vernum (Raf.) Torr. & A. Gray; Spring avens; Woodlands; Abundant; C = 1; BSUH 17864.

Potentilla norvegica L. ssp. *monspeliensis* (L.) Asch. & Graebn. Rough or Norwegian cinquefoil; Pumpkin patch old-field; Rare but locally common; C = 0; BSUH 17976.

POTENTILLA RECTA L.; Sulfur cinquefoil, sulfur five-fingers; Western old-field; Rare; C = 0; BSUH 17739.

Potentilla simplex Michx.; Common or old-field cinquefoil, old-field five-fingers; Upland woods; Rare but locally common; C = 2; BSUH 17802.

Prunus serotina Ehrh. var. *serotina*; Wild black cherry; Woodlands; Abundant and widespread; C = 1; BSUH 17781.

ROSA MULTIFLORA Thunb.; Japanese or multiflora rose; Woodlands; Abundant and widespread; C = 0; BSUH 17949.

Rosa setigera Michx. var. *setigera*; Climbing prairie rose, Illinois rose; Eastern meadow, western old-field; Infrequent; C = 4; BSUH 18011.

Rubus allegheniensis Porter; Common or Allegheny blackberry; Gravel lot and adjacent field, old-fields; Common; C = 2; BSUH 17768.

Rubus occidentalis L.; Black raspberry; Parking lot and yards around the RTC Office, old-fields; Common; C = 1; BSUH 17764.

Rubus pensilvanicus Poir.; SYN: *Rubus abactus* L.H. Bailey; Pennsylvania or Yankee blackberry; Western old-field; Common; C = 5; BSUH 17315.

Rubiaceae (Madder Family)

Cephalanthus occidentalis L.; Common buttonbush; Eastern meadow; Rare; C = 5; BSUH 18004.

Galium aparine L.; Cleavers, annual bedstraw, sticky-willy; Upland woods; Abundant and widespread; C = 1; BSUH 17869.

Galium circaezans Michx. var. *circaezans*; Forest bedstraw, smooth wild licorice, licorice bedstraw; Upland woods around buildings; Abundant in this area; C = 7; BSUH 17313.

Galium concinnum Torr. & A. Gray; Shining bedstraw; Woodlands; Common; C = 5; BSUH 17736.

(#) *Galium tinctorium* (L.) Scop.; Stiff marsh bedstraw; Eastern meadow; Infrequent but locally common; C = 6; BSUH 18026.

Galium triflorum Michx.; Sweet-scented or fragrant bedstraw; Meadows and creek banks, moist woodlands; Abundant and widespread; C = 5; BSUH 17973.

Salicaceae (Willow Family)

Populus deltoides Bartram ex. Marsh. var. *deltoides*; Eastern cottonwood; Successional woods in the northern half of property; Abundant and widespread; C = 1; BSUH 17751.

Salix amygdaloides Andersson; Peach-leaf willow; Western old-field; Rare; C = 4; BSUH 17308.

Salix nigra Marsh.; Black willow; Eastern meadow; Rare; C = 3; BSUH 18003.

Scrophulariaceae (Figwort Family)

Mimulus alatus Aiton; Winged or sharp-wing monkey-flower; Northern meadow and moist path along creek; Common; C = 4; BSUH 18053.

Mimulus ringens L. var. *ringens*; Allegheny monkey-flower; Eastern meadow; Common; C = 4; BSUH 18038.

Penstemon calycosus Small; SYN: *Penstemon laevigatus* Ait. ssp. *calycosus* (Small) Benn.; Smooth beard-tongue, long-sepal beard-tongue; Near screen building in the western old-field; Rare but locally common; C = 4; BSUH 17314.

Scrophularia marilandica L.; Eastern or late figwort, carpenter's-square; Happy Hollow Camp Sites – successional woods; Rare; C = 5; BSUH 18046.

VERBASCUM BLATTARIA L.; Moth mullein; Gravel lot and adjacent field; Rare; C = 0; BSUH 17744.

VERBASCUM THAPSUS L.; Common or woolly mullein; Gravel lot and adjacent field; Rare but locally common; C = 0; BSUH 17961.

Veronica anagallis-aquatica L.; SYN: *Veronica catenata* Pennell; Water speedwell; Eastern meadow; Common; C = 5; BSUH 17321.

VERONICA ARVENSIS L.; Corn speedwell; Old-fields; Abundant; C = 0; BSUH 17855.

(#) *VERONICA PERSICA* Poir.; Birdseye speedwell; Gravel lot and adjacent field; Rare but locally common; C = 0; BSUH 17937.

VERONICA POLITA Fr.; Gray field or wayside speedwell; Lawn and woodlands around the RTC Office; Infrequent but locally common; C = 0; BSUH 17819.

VERONICA SERPYLLIFOLIA L. ssp. *SERPYLLIFOLIA*; Thyme-leaved speedwell; Old-fields; Abundant; C = 0; BSUH 17856.

Simaroubaceae (Quassia Family)

AILANTHUS ALTISSIMA (Mill.) Swingle; Tree-of-heaven; Successional woods in the northern half of property; Infrequent but widespread; C = 0; BSUH 17995.

Smilacaceae (Catbrier Family)

Smilax ecirrhata (Engelm. ex Kunth) S. Watson; Upright carrion flower; Woodlands; Infrequent; C = 5; BSUH 17748.

Smilax tamnoides L.; SYN: *Smilax hispida* Muhl. ex Torr., *Smilax tamnoides* L. var. *hispida* (Muhl. ex Torr.) Fernald; Bristly greenbrier or catbrier; Eastern meadow, woodlands; Common and widespread; C = 3; BSUH 17792.

Solanaceae (Nightshade Family)

Physalis longifolia Nutt. var. *subglabrata* (Mack. & Bush) Cronquist; SYN: *Physalis subglabrata* Mack. & Bush; Long-leaved or smooth ground cherry; Western old-field; Rare; C = 0; BSUH 17927.

Solanum carolinense L. var. *carolinense*; Horse-nettle, Carolina poppy; Pumpkin patch old-field; Common; C = 0; BSUH 18050.

Solanum ptycanthum Dunal; SYN: *Solanum nigrum* auct. non L., *Solanum americanum* auct. non Mill.; Eastern black nightshade, West Indian nightshade; Seasonal pool north of the western old-field; Rare; C = 0; BSUH 17918.

Typhaceae (Cattail Family)

TYPHA ANGUSTIFOLIA L.; Narrow-leaved cattail; Meadows; Rare; C = 0; BSUH 18028.

Typha latifolia L.; Common or broad-leaved cattail; Meadows; Rare but locally common; C = 1; BSUH 17893.

Ulmaceae (Elm Family)

Celtis occidentalis L.; Northern or common hackberry; Woodlands; Abundant; C = 3; BSUH 17317.

Ulmus americana L.; White or American elm; Woodlands, Abundant; C = 3; BSUH 17799.

Ulmus rubra Muhl.; Red or slippery elm; Woodlands; Common; C = 3; BSUH 17956.

Urticaceae (Nettle Family)

Boehmeria cylindrica (L.) Sw.; Small-spike false nettle; Meadows; Infrequent but locally common; C = 3; BSUH 17899.

Laportea canadensis (L.) Weddell; SYN: *Urtica canadensis* L.; Canadian wood nettle; Moist woodlands; Abundant; C = 2; BSUH 17901.

Parietaria pensylvanica Muhl. ex Willd.; Pennsylvania pellitory; Western old-field; Infrequent but locally abundant; C = 1; BSUH 17902.

(#) *Pilea fontana* (Lunell) Rydb.; Bog or lesser clearweed; Northern meadow; Rare; C = 5; BSUH 17298.

Pilea pumila (L.) A. Gray var. *pumila*; Canadian clearweed; Creek bank, moist old-fields, woodlands; Abundant and widespread; C = 2; BSUH 17304.

Urtica dioica L. ssp. *gracilis* (Aiton) Seland.; SYN: *Urtica dioica* L. var. *procera* (Muhl. ex Willd.) Weddell, *Urtica procera* Muhl. ex Willd.; Tall, California, or stinging nettle; Creek banks and meadows; Infrequent but locally common; C = 1; BSUH 17974.

Valerianaceae (Valerian Family)

Valerianella umbilicata (Sull.) Alph. Wood; Navel or navel-fruited cornsalad; Young successional woods north of the creek; Abundant; C = 5; BSUH 17811.

Verbenaceae (Vervain Family)

Phryma leptostachya L.; American lopseed; Woodland; Common; C = 4; BSUH 18007.

Phyla lanceolata (Michx.) Greene; SYN: *Lippia lanceolata* Michx.; Lance-leaf fogfruit; West old-field bordering large seasonal pool; Rare but locally common; C = 2; BSUH 17999.

Verbena hastata L. var. *hastata*; Common or blue vervain, swamp verbena; Entrance road, gravel lot and adjacent field; Rare but locally common; C = 3; BSUH 17990.

Verbena urticifolia L. var. *urticifolia*; White vervain; Creek bank and meadow in SE corner of property; Infrequent; C = 3; BSUH 17998.

Violaceae (Violet Family)

Viola pubescens Aiton; Forest yellow violet; Lawn and woodlands; Abundant and widespread; C = 5; BSUH 17828. [Watch List]

Viola sororia Willd.; Common blue violet, dooryard violet; Lawn and woodlands; Abundant and widespread; C = 1; BSUH 17844.

Viola striata Aiton; Striped cream violet, cream violet, white violet; Lawn and woodlands; Abundant and widespread; C = 4; BSUH 17841.

Vitaceae (Grape Family)

Parthenocissus quinquefolia (L.) Planch.; Virginia creeper, woodbine; Woodlands; Abundant; C = 2; BSUH 17763.

Vitis cinerea (Engelm.) Engelm. ex Millard var. *cinerea*; Graybark or winter grape; On shrubs between the eastern meadow and creek; Rare but locally abundant; C = 4; BSUH 17985.

Vitis riparia Michx.; Riverbank grape; Roadside, gravel lot and adjacent field; Rare; C = 1; BSUH 18032.

Vitis vulpina L.; Frost or fox grape; Woodlands; Common and widespread; C = 3; BSUH 17769.

LITERATURE CITED

Christensen, N.L. 1977. Changes in structure, pattern and diversity associated with climax forest maturation in Piedmont, North Carolina. American Midland Naturalist 97:176–188.

Clark, R. & S.R. Sanders. 2005. Wild and Scenic Indiana. Brown Trout Publishers, Inc., San Francisco, California. 168 pp.

Daniel, T.W., J.A. Helms & F.S. Baker. 1979. Principles of Silviculture, 2nd ed. McGraw-Hill Publishing Co., New York. 500 pp.

Deam, C. 1940. Flora of Indiana. Department of Conservation, Wm. B. Burford Printing Co., Indianapolis, Indiana. 1236 pp.

EPA (U.S. Environmental Protection Agency). 2009. Upper White River Watershed. Available at http://cfpub.epa.gov/surf/huc.cfm?huc_code=05120201.

Elzinga, C.L., D.W. Salzer & J.W. Willoughby. 1998. Measuring & Monitoring Plant Populations. BLM Technical Reference 1730-1. U.S. Department of the Interior, Bureau of Land Management, Denver, Colorado. 478 pp.

Gleason, H.A. & A. Cronquist. 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. New York Botanical Garden, Bronx, New York. 910 pp.

Glitzenstein, J.S., P.A. Harcombe & D.R. Streng. 1986. Disturbance, succession, and maintenance of species diversity in an east Texas forest. Ecological Monographs 56:243–258.

Hedge, R.L. 1997. Forested swell and swale: the Central Till Plain natural region. Pp. 195–199. *In* The Natural Heritage of Indiana (M.T. Jackson, Ed.). Indiana University Press, Bloomington, Indiana.

Homoya, M.A., D.B. Abrell, J.F. Aldrich & T.W. Post. 1985. The natural regions of Indiana. Proceedings of the Indiana Academy of Science 94:245–268.

IDNR Nature Preserves. 2013. Endangered, Threatened, Rare, and Extirpated Plants of Indiana. Available at http://www.in.gov/dnr/naturepreserve/files/np-etrplants042513.pdf. Accessed December 21, 2013.

IUPUI. 2007. Indiana Geology. What are the features of central Indiana? Available at http://www.iupui.edu/~g135/g135/05_topography/explore05.html.

Keller, C. 1986. The computerization of regional floristic data. Proceedings of the Indiana Academy of Science 95:412.

Keller, C., T. Crovello & K. Guild. 1984. Floristic database program (See C. Keller 1986. The computerization of regional floristic data. Proceedings of the Indiana Academy of Science 95:412).

Kemper, G.W.H. (ed.). 1908. A Twentieth Century History of Delaware County, Indiana, Volume II. The Lewis Publishing Company, Chicago, Illinois. 1085 pp.

Overlease, W. & E. Overlease. 2007. 100 Years of Change in the Distribution of Common Indiana Weeds. Purdue University Press, West Lafayette, Indiana. 270 pp.

Prast, Z.B. 2012. The Vascular Flora and Vegetative Communities of Munsee Woods in Delaware County, Indiana. Master of Science Thesis. Cardinal Scholar, University Libraries, Ball State University, Muncie, Indiana. Available at https://cardinalscholar.bsu.edu/handle/123456789/196151.

Rothrock, P.E. 2004. Floristic quality assessment in Indiana: The concept, use and development of coefficients of conservatism. Final Report for ARN A305-4-53, EPA Wetland Program Development Grant CD975586-01. 96 p. Available at http://www.in.gov/idem/water/planbr/401/publications.html.

Rothrock, P.E. & M.A. Homoya. 2005. An evaluation of Indiana's Floristic Quality Assessment. Proceedings of the Indiana Academy of Science 114:9–18.

Ruch, D.G., A. Schoultz & K.S. Badger. 1998. The flora and vegetation of Ginn Woods, Ball State University, Delaware County, Indiana. Proceedings of the Indiana Academy of Science 107:17–60.

Ruch, D.G., B.G. Torke, C.R. Reidy, K.S. Badger & P.E. Rothrock. 2002. The flora and vegetational communities of Wilbur Wright Fish and Wildlife Area, Henry County, Indiana. Proceedings of the Indiana Academy of Science 111(2):147–176.

Ruch, D.G., C.R. Reidy, B.G. Torke, K.S. Badger & P.E. Rothrock. 2004. Additions to the flora of Ginn Woods, Delaware County, Indiana. Proceedings of the Indiana Academy of Science 113:1–6.

Ruch, D.G., B.G. Torke, K.S. Badger, C.R. Reidy, P.E. Rothrock, R. Waltz, E.G. Urly, J.L. Chance & L. Click. 2007. The vascular flora and vegetational communities of Hayes Arboretum in Wayne County, Indiana. Proceedings of the Indiana Academy of Science 116:11–41.

Ruch, D.G., B.G. Torke, K.S. Badger, B.R. Hess, B.N. Christian & P.E. Rothrock. 2008a. The vascular flora and vegetational communities of Lick Creek Summit Nature Preserve in Wayne County, Indiana. Proceedings of the Indiana Academy of Science 117:29–54.

Ruch, D.G., B.G. Torke, B.R. Hess, K.S. Badger & P.E. Rothrock. 2008b. The vascular flora and vegetational communities of the wetland complex on the IMI Property in Henry County near Luray, Indiana. Proceedings of the Indiana Academy of Science 117:142–158.

Ruch, D.G., B.G. Torke, B.R. Hess, K.S. Badger & P.E. Rothrock. 2009. The vascular flora and plant communities of the Bennett wetland complex in Henry County, Indiana. Proceedings of the Indiana Academy of Science 118:39–54.

Ruch, D.G., B.G. Torke, B.R. Hess, K.S. Badger & P.E. Rothrock. 2010. The vascular flora in three prairie cemeteries in Henry County, Indiana. Proceedings of the Indiana Academy of Science 119:35–51.

Ruch, D.G., K.S. Badger, B.C. Daugherty, B.G. Torke & P.E. Rothrock. 2012. The vascular flora and vegetational communities of Mississinewa Woods in Randolph County, Indiana. Proceedings of the Indiana Academy of Science 121:23–44.

Ruch, D.G., B.G. Torke, K.S. Badger, J.E. Taylor, B.R. Hess & P.E. Rothrock. 2013. The vascular flora and vegetational communities of Cabin Creek Raised Bog, Randolph County, Indiana. Castanea 78:290–311.

Stonehouse, A.L., K.S. Badger, D.G. Ruch & P.E. Rothrock. 2003. A floristic inventory and description of the structure and composition of the plant communities of Botany Glen, Grant County, Indiana. Proceedings of the Indiana Academy of Science 112:135–159.

Swink, F. & G. Wilhelm. 1994. Plants of the Chicago Region, 4th ed. Indiana Academy of Science, Indianapolis, Indiana. 921 pp.

Tonkovich, G.S. & M.L. Sargent. 1993. The vascular plants of Fall Creek Gorge Nature Preserve, Warren County, Indiana. Proceedings of the Indiana Academy of Science 102:9–45.

Tungesvick, K., D.G. Ruch, B.G. Torke, K.S. Badger & P.E. Rothrock. 2011. Additions to the flora of Mounds State Park and Preserve, Madison County, Indiana. Proceedings of the Indiana Academy of Science 120:1–11.

USDA. 2013. Natural Resources Conservation Services Plants National Database. Available at http://plants.usda.gov/index.html. Accessed on October 28, 2013.

WSS. 2013. Web Soil Survey, Natural Resources Conservation Service. Available at http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm. Accessed on December 22, 2013.

Yatskievych, K. 2000. Field Guide to Indiana Wildflowers. Indiana University Press, Bloomington, Indiana. 357 pp.

Zar, J.H. 1996. Biostatistical Analysis, 3rd ed. Prentice Hall, Upper Saddle River, New Jersey. 121 pp.

Manuscript received 3 November 2013, revised 31 March 2014.

2013. Proceedings of the Indiana Academy of Science 122(2):118–125

STRUCTURAL VIBRATIONS: NORMAL MODES IN A HAND-BELL

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ABSTRACT. The vibrational modes contributing most significantly to the radiated sound of hand-bells, are the bending waves travelling along the bell surface with periodic boundary conditions in the angular direction, nearly clamped boundaries near the crown, and free boundary conditions at the mouth of the bell. They are usually identified by two indices (m,n), where m counts the number of nodal lines crossing the crown, and n the number of circumferential nodal lines. The (m,0) modes, for values of m higher than a critical value, are missing. They are replaced by a mode for which the first circumferential nodal line lies very close to the mouth of the bell. These modes are designated as (m,$1^{\#}$) modes. Using Finite Element Analysis (FEA) a hand-bell is modeled with increasing complexity to show that both positive and negative curvatures are required in the bell wall to account for the presence of the (m,$1^{\#}$) modes.

Keywords: Normal modes, nodal lines, hand-bell, boundary condition, Finite Element Analysis (FEA)

INTRODUCTION

The partial differential equation in space and time associated with a local disturbance in an infinite medium, generally is a wave equation with solutions representing traveling waves. Imposing boundary conditions usually limits the possible solutions to a discrete set of standing waves identified with normal modes of vibration. The shape of a hand-bell naturally forms a free boundary at the mouth of the bell, whereas the crown essentially clamps the bell wall, imposing a condition of no translational or rotational motion at the top of the bell.

Experimental studies on hand-bells by T. D. Rossing, R. Perrin, H. J. Sathoff, and R. W. Peterson[1] include holographic interference patterns shown in figure 1.

Systematic grouping of those images by T. D. Rossing and R. Perrin[2] gives a periodic table-like representation shown in figure 2.

Figure 3[1] shows a systematic relationship between the frequency and the first mode index m, identifying the number of nodal lines crossing the crown, on logarithmic scales.

From all of these illustrations it becomes apparent that the (m,$1^{\#}$) modes naturally fall into the sequence of (m,0) modes. This paper uses FEA modeling to investigate which bell characteristics are responsible for the transition from (m,0) to (m,$1^{\#}$) modes. FEA solutions also include torsional and extensive modes, which will not be considered in this paper. This paper is limited to the study of transverse bending waves.

As a starting point, reference is made to the solution of a one dimensional wave equation for an elastic string under tension, where hinged boundary conditions at the ends limit the solutions to standing waves in the form of integral numbers of loops. Weinreich[3] shows, that relaxing the hinged condition at one end to make it either more spring-like or more mass-like, results in either an open end, or a fish-tail-like configuration respectively (figure 4).

It is not entirely unreasonable to expect a two dimensional extension of that model for a clamped top with hinged boundaries at the side and a free bottom, to result in an effective relaxing of the boundary, yielding an (m,$1^{\#}$) mode. This suggests that for sufficiently narrow plates, a strictly free boundary is not compatible with the hinged boundary on the sides, resulting in a fish-tail-like configuration at the open end in the two dimensional extension of the Weinreich one-dimensional model, corresponding to the (m,$1^{\#}$) modes for a sufficiently narrow mode section.

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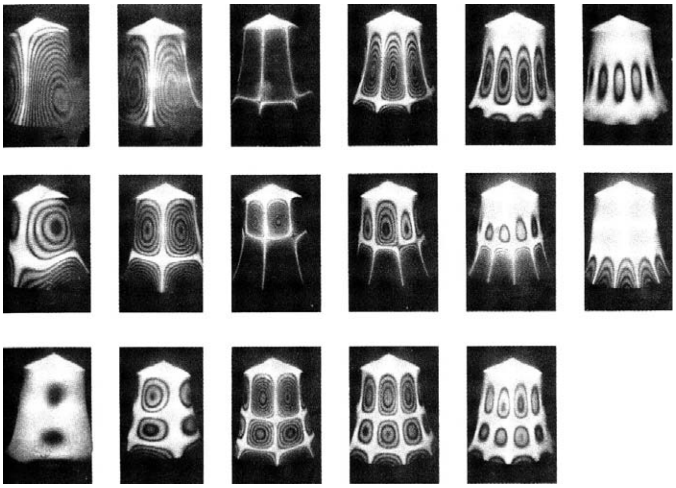

Figure 1.—Vibrational modes of a hand-bell imaged with holographic interferometry (from Rossing, Perrin, Sathoff & Peterson[1]).

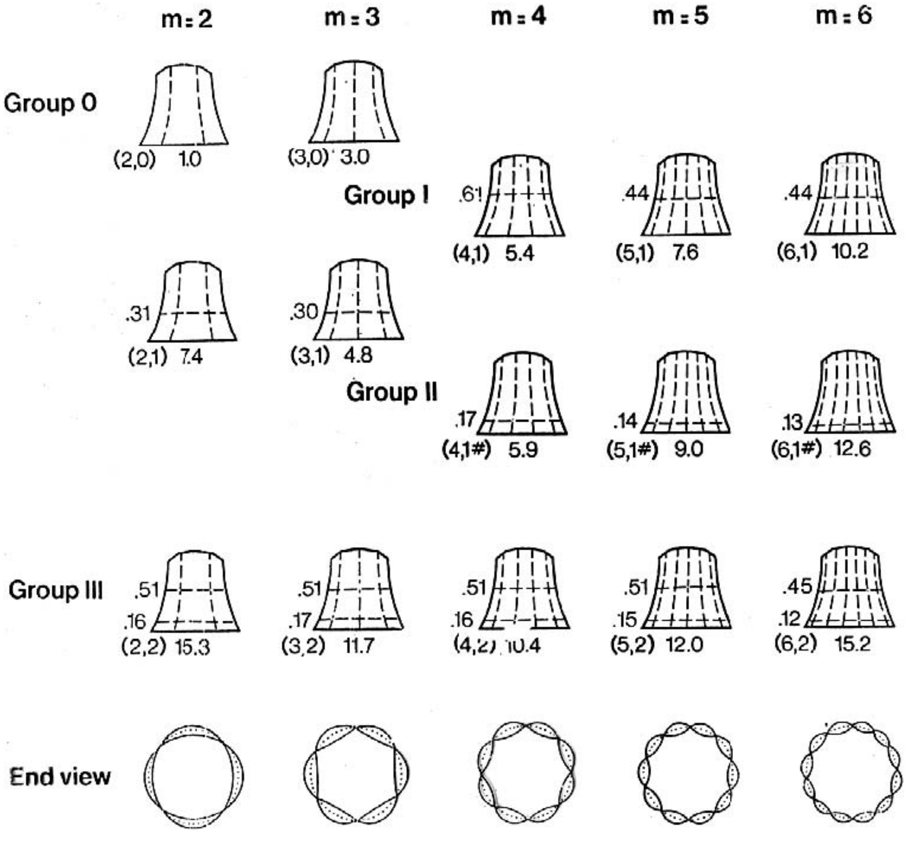

Figure 2.—Systematic grouping of modes of vibration in a hand-bell (from Rossing & Perrin[2]).

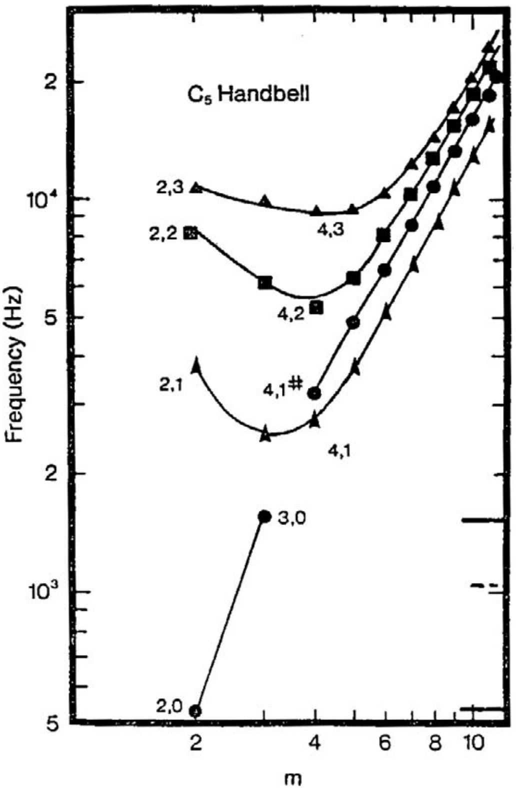

Figure 3.—Relationship of frequency and first mod index of a C$_5$ Hand-bell (from Rossing, Perrin, Sathoff & Peterson[1]).

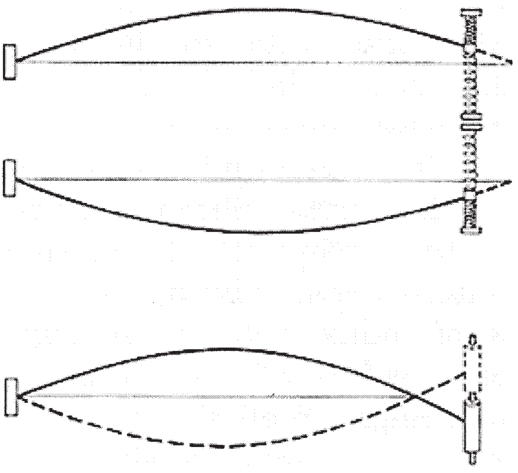

Figure 4.—Relaxing boundary conditions at one end of a vibrating string under tension (from Weinreich[3]).

METHOD

In order to investigate the origin of the $(m,1^{\#})$ modes, models of increasingly complex geometry were studied using Finite Element Analysis (FEA). FEA requires the introduction of geometric and structural parameters including boundary conditions. The former involves imposing a finite number of grid points on the shape of the structure, and the latter requires specifying mechanical and elastic parameters. Values representative of Bronze alloys used for hand-bells were chosen from standard tables. Limits on translational and rotational freedoms for certain parts of the structure are imposed as boundary conditions. For the crudest approximation in the (m,0) mode, a wall section between nodal lines is approximated as a flat rectangular plate of constant wall thickness. The boundary conditions imposed are: clamped at the top, hinged at the sides, and free at the bottom. (figure 5a)

ANSYS^{R}-ED^{TM} [4] an FEA program, commercially available at treasonable cost to students and faculty of educational institutions, provides a large enough number of degrees of freedom to accommodate all increasingly complex approaches to the normal mode hand-bell problem considered in this work. Figure 5a shows the geometry and the mesh chosen for the lowest order of approximation. Figures 5b-d show modes for increasing values of m at increasing frequencies. Clearly the approximation is too crude to illustrate the appearance of the $(m,1^{\#})$ mode. An additional check is made by reducing the plate width. Figures 6a–c again show the inadequacy of the flat rectangular plate model, $(m,1^{\#})$ modes do not appear.

The next approximation takes the rectangle to a trapezoid, reflecting the realization that the circumference of the bell at the mouth is larger than at the crown. Figures 7a–b clearly illustrate that this approximation is still inadequate.

In the next approximation the flat plate is replaced by a curved section of the bell, and again the inadequacy of the model is evidenced in figures 8.

A crown is added for the fourth level of approximation, with the clamped boundary condition imposed near the center of the crown. This modification is still inadequate as shown by figures 9.

For the fifth approximation, an additional curvature, opposite to the curvature added in the third approximation, is introduced.

This approximates the actual bell cross-section more closely. Now the $(m,1^{\#})$ mode appears (fig 10). Imposing a hinged boundary condition at the location of expected nodal lines is difficult for this double curvature configuration, consequently the entire bell is modeled with a fixed thickness contour, and the clamped boundary condition is imposed on the center hole of the crown. Mode (2,1) is shown in fig 11 in gray scale representation. For this configuration modes (2,0), (2,1), (3,1), (4,1) and $(3,1^{\#})$ are shown in figures 12, 13, 14, 15, and 16 respectively. These figures show the extreme displacements of the modes as viewed from the top of the bell.

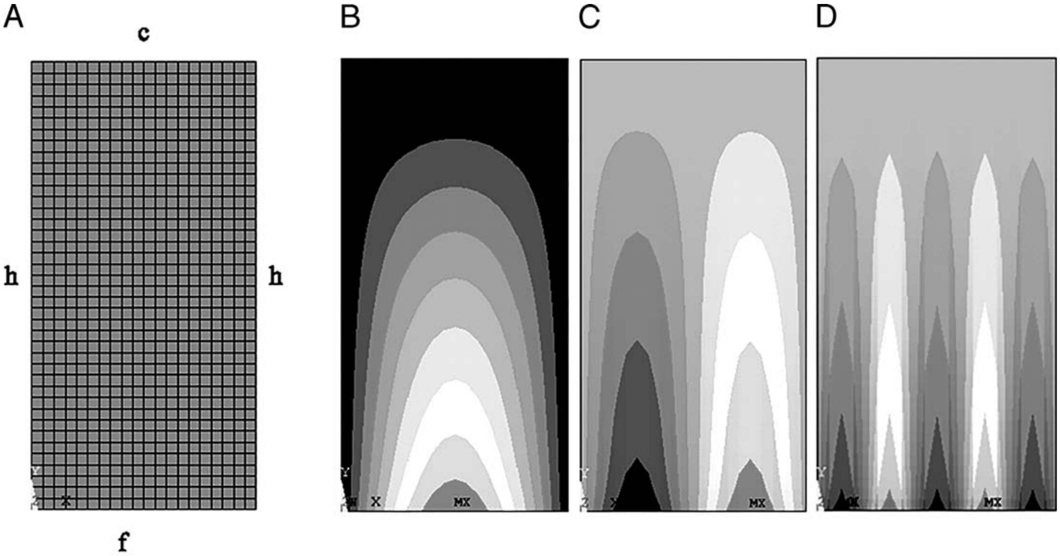

Figure 5.—A. Flat plate with FEA grid and boundary conditions. c—clamped, h—hinged, f—free. B. Mode (0,0). C. Mode (1,0). D. Mode (4,0).

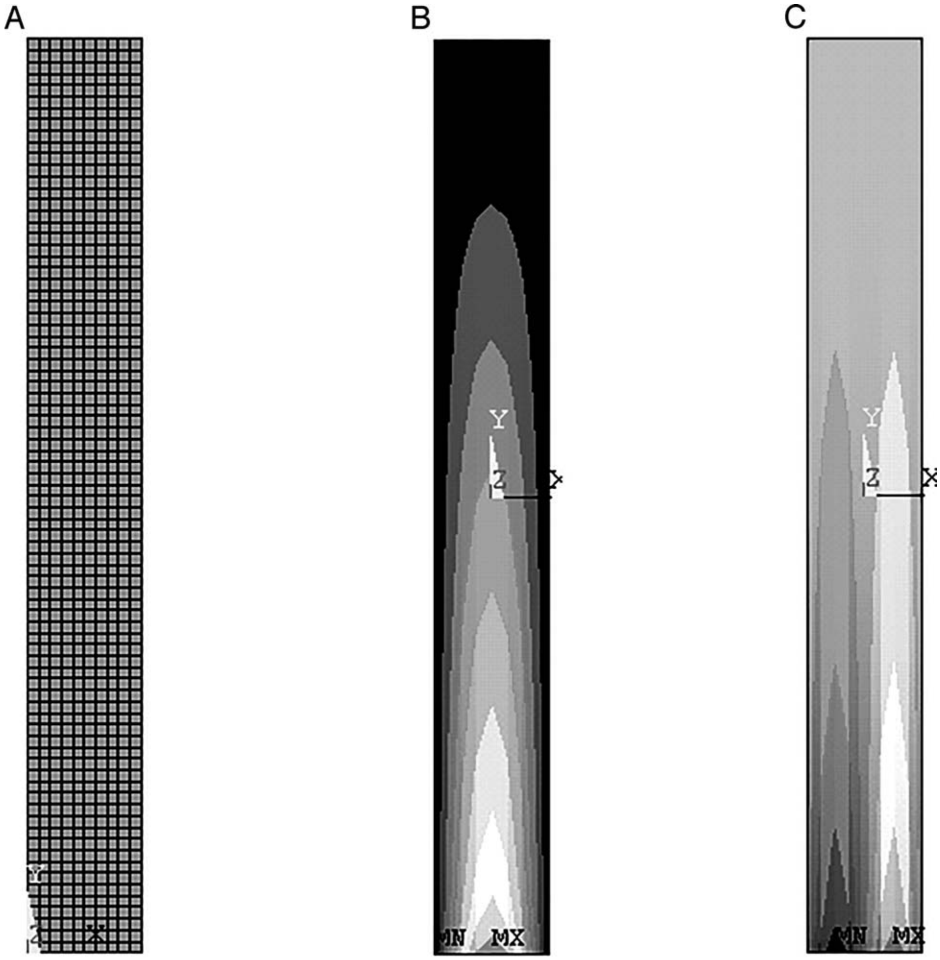

Figure 6.—A. Narrow rectangular plate, same boundary conditions as Figure 5. B. Mode (0,0). C. Mode (1,0).

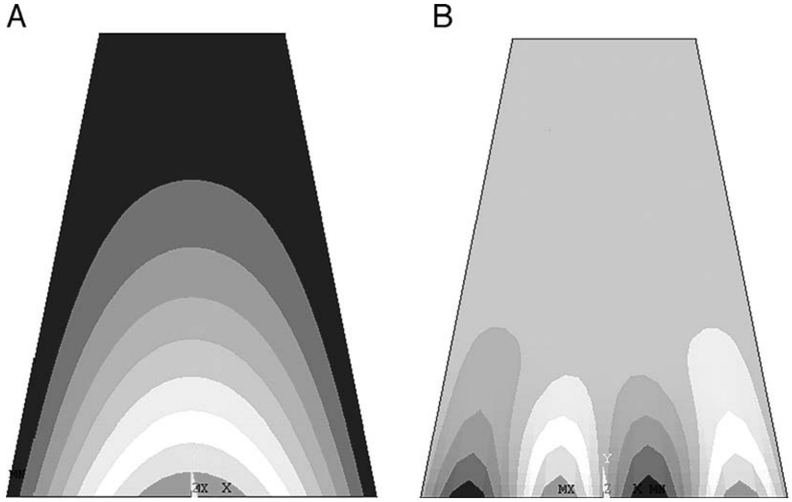

Figure 7.—Flat trapezoidal plate. A. Mode (0,0). B. Mode (3,0).

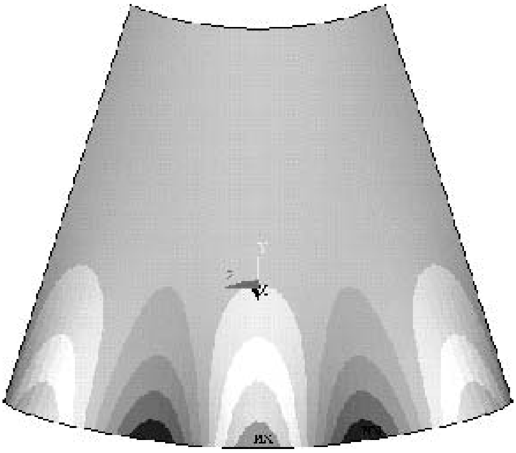

Figure 8.—Conical plate section. Mode (4,0).

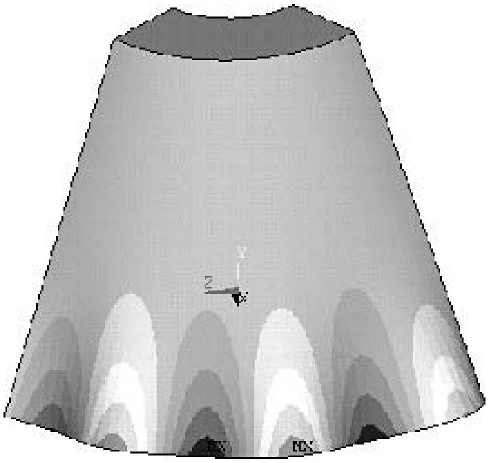

Figure 9.—Conical plate section with crown. Mode (5,0).

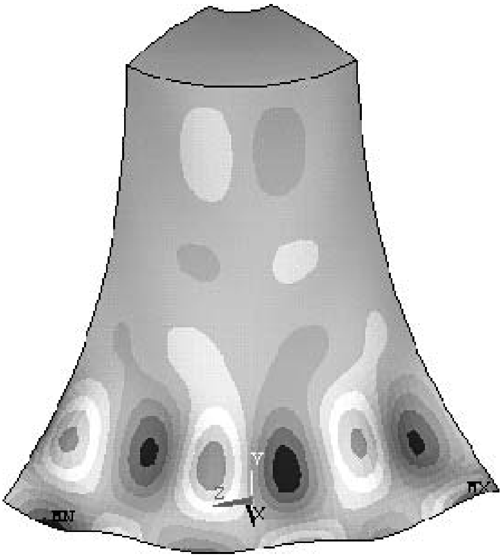

Figure 10.—Bell section with double curvature. Mode (5,1).

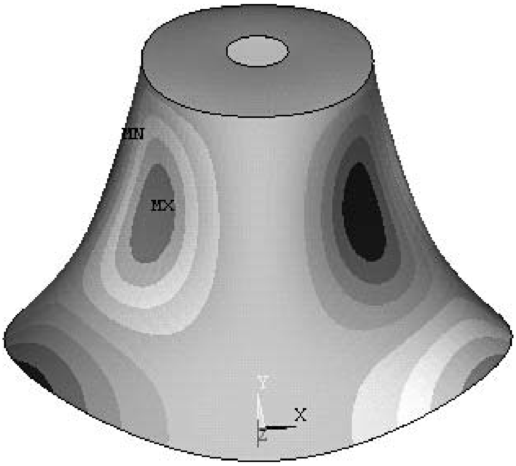

Figure 11.—Bell model. Mode (2,1).

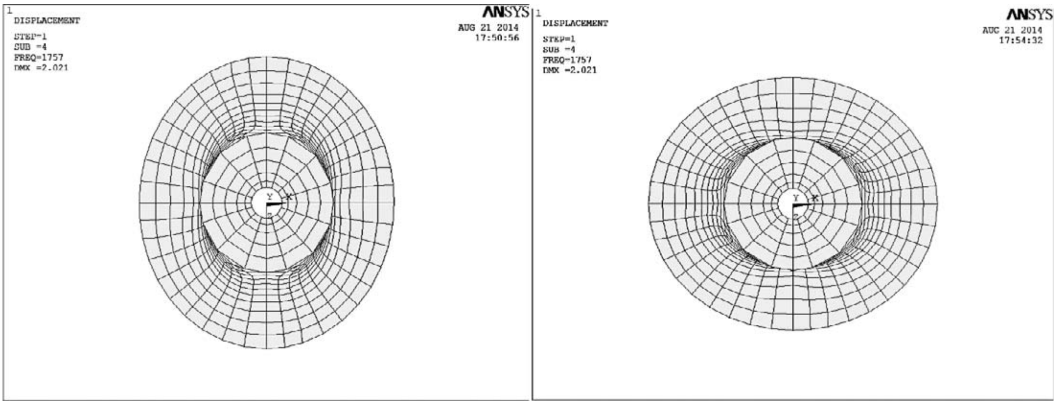

Figure 12.—Two FEA frames of opposite extreme displacement for the (2,0) mode.

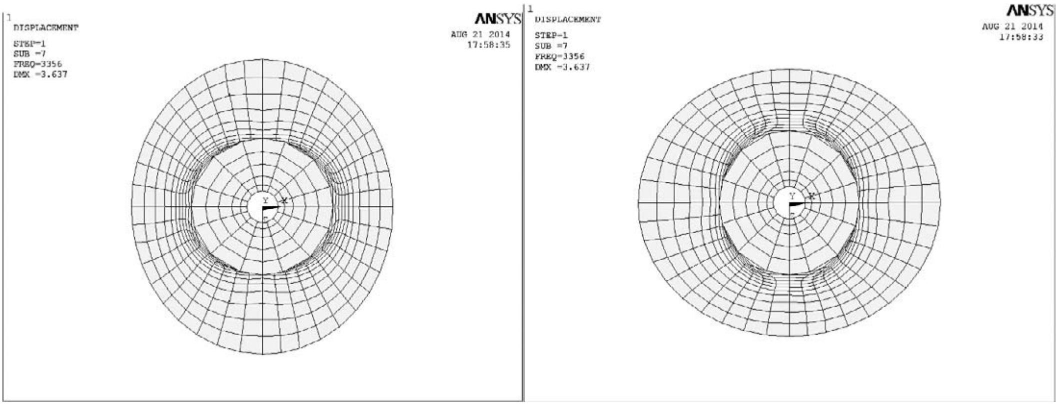

Figure 13.—Two FEA frames of opposite extreme displacement for the (2,1) mode. The nodal line lies approximately between the third and the fourth circumferential grid line.

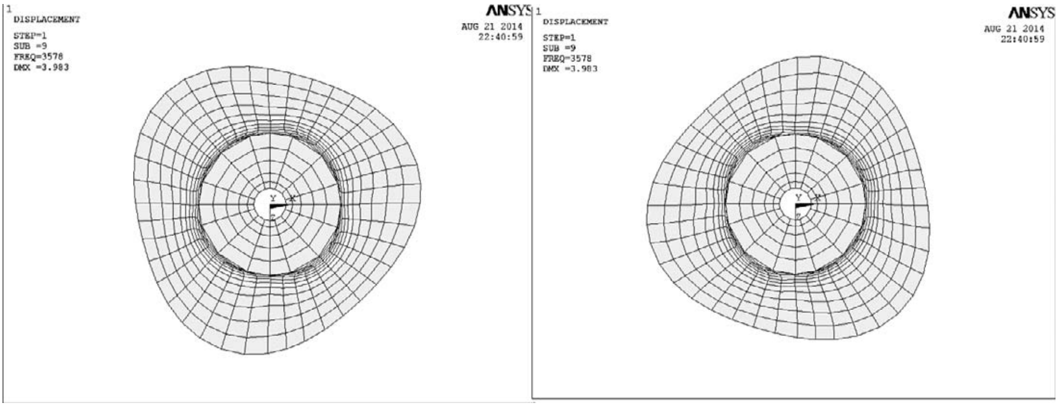

Figure 14.—Two FEA frames of opposite extreme displacement for the (3,1) mode. The nodal line lies approximately between the fourth and the fifth circumferential grid line.

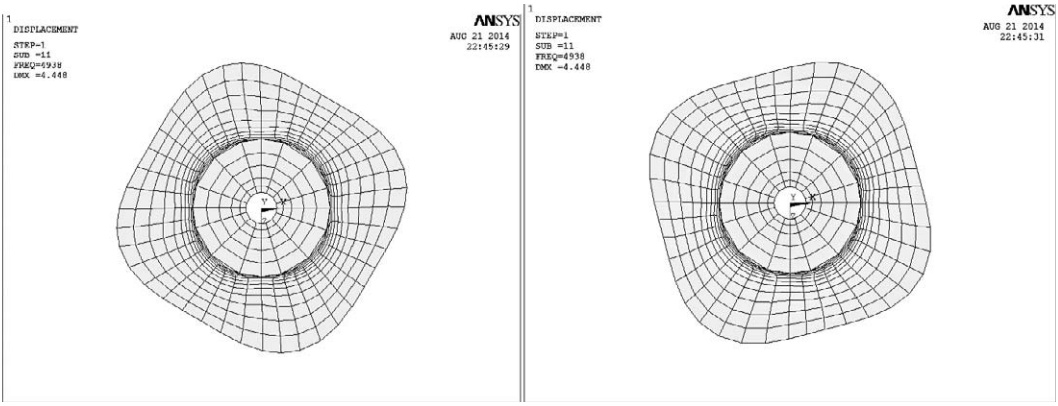

Figure 15.—Two FEA frames of opposite extreme displacement for the (4,1) mode. The nodal line lies approximately between the fourth and the fifth circumferential grid line.

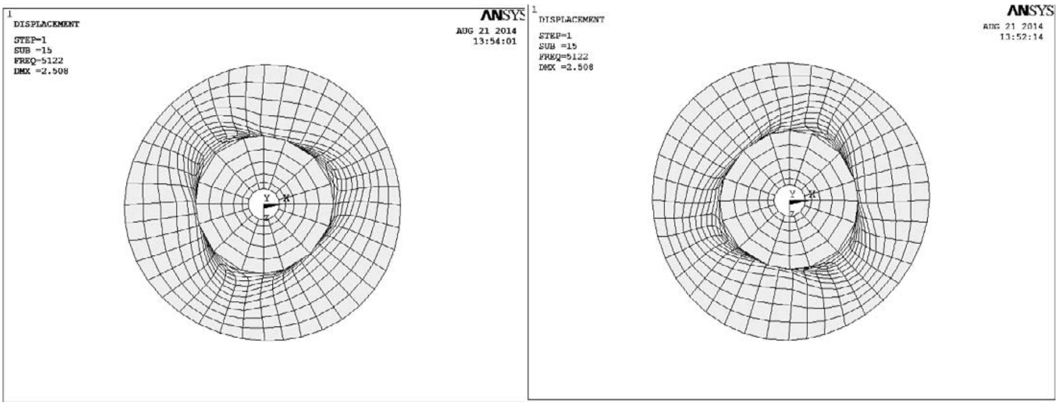

Figure 16.—Two FEA frames of opposite extreme displacement for the (3,1) mode. The nodal line is located between the rim and the first circumferential grid line.

CONCLUSION

From this FEA study of hand-bell models of increasing complexity it appears that the saddle-like double curvature in the side wall of the bell is essential for the formation of the (m,$1^{\#}$) modes. Frequency comparisons with an actual bell are not possible since the cross-section of a real bell would require a full three dimensional FEA model for which the number of degrees of freedom exceed the capability of this program. That study is left for future work with access to a full ANSYS FEA program.

REFERENCES

Rossing, T.D., R. Perrin, H. Sathoff & R.W. Peterson. 1984. Vibrational modes of a tuned handbell. *J.Acoust. Soc. Amer.* 76:Nr. 4, 1263–1267.

Rossing, T.D. & R. Perrin. 1987. Vibrations of Bells. *Applied Acoustics* 20:41–70.

Weinreich, G. Jan 1979. The Coupled Motions of Piano Strings, *Scientific American,* 124.

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Manuscript received 2 October 2013, revised 6 August 2014.

2013. Proceedings of the Indiana Academy of Science 122(2):126–129

TRAP ELEVATION AND BIOTIC FACTORS INFLUENCING CAPTURE FREQUENCIES OF WESTERN HARVEST MICE (*REITHRODONTOMYS MEGALOTIS*) IN PRAIRIE GRASSES OF INDIANA

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ABSTRACT. Western harvest mice, *Reithrodontomys megalotis*, are used as a prairie indicator species but low capture probabilities in Indiana may make them unreliable for this purpose at this eastern edge of their range. To increase capture probabilities, researchers have experimented with vertical trap elevation with varying degrees of success. Our objective was to investigate if elevated traps increased captures of *R. megalotis* and to determine if competition for traps with meadow voles, *Microtus pennsylvanicus,* contributes to this pattern. To investigate these questions a 5×6 grid of trapping stations with 15 meter spacing was established in the Purdue Wildlife Area. Three Sherman traps; ground, semi-elevated, and elevated, were placed at each station. Independence of frequency of capture in the vertical strata was compared between these two species using a G-test. We rejected the null hypothesis of independence between trap vertical strata and small mammal species, providing support for the role of competition for traps as contributing to differences in captures of R. megalotis across the vertical stratum. Post hoc tests were then conducted to determine significance in trap comparisons. Significance was found in ground vs. elevated and semi-elevated vs. elevated traps. Surprisingly, 24 captures of *M. pennsylvanicus* were recorded in the higher stratum traps, despite no previous records of captures of this species above the ground. These results suggest when using *R. megalotis* as an indicator species of prairie health in Indiana, investigators should elevate traps.

Keywords: Elevated traps, Indiana, *Microtus pennsylvanicus*, *Reithrodontomys megalotis*, semi-elevated

Western harvest mice (*Reithrodontomys megalotis*) are at the eastern periphery of their range in northwestern Indiana and little research has been conducted on them in Indiana. Ford (1977) hypothesized that *R. megalotis* began its eastward expansion into northwestern Indiana around the 1950s, but the first recorded captures occurred in 1969 near Morocco, Indiana (Whitaker & Sly 1970). Ford (1977) conducted research on the range, distribution, and habitat of *R. megalotis* in Indiana. Leibacher and Whitaker (1998) demonstrated that twenty years later the range of this species in Indiana continued to expand. Whitaker and Mumford (1972) documented reproduction, parasites, and food preferences of *R. megalotis* in Indiana.

Native prairie once extended into northwest and west-central Indiana (Transeau 1935), but land conversion associated with European settlement eliminated prairies from 99.9% of their former range (Samson & Knopf 1994). As of 1994, government agencies had placed less than 0.01% of the remaining prairie under protection (Samson & Knopf 1994). Subsequently, efforts to restore prairies to their native range have increased. To monitor the success of these restorations, biologists look for the presence of indicator species. Western harvest mice are one such indicator species that are associated with prairie-like habitats (Ford 1977). However, low capture success in Indiana make it difficult to estimate abundance and survival rates for *R. megalotis* (Ford 1977), impacting the suitability of this species as an indicator of prairie restoration.

Researchers in Europe and North America have experimented with vertical trap stratification and have demonstrated species specific variation in the use of the vertical vegetative stratum. In a vertical trap experiment, Jensen et al. (2001) observed no captures of *Microtus agrestis* in elevated traps, while three other rodent species exploited the upper vegetation stratum extensively, and two additional rodent species exploited that stratum to a lesser degree. Cummins and Slade (2007) reported higher

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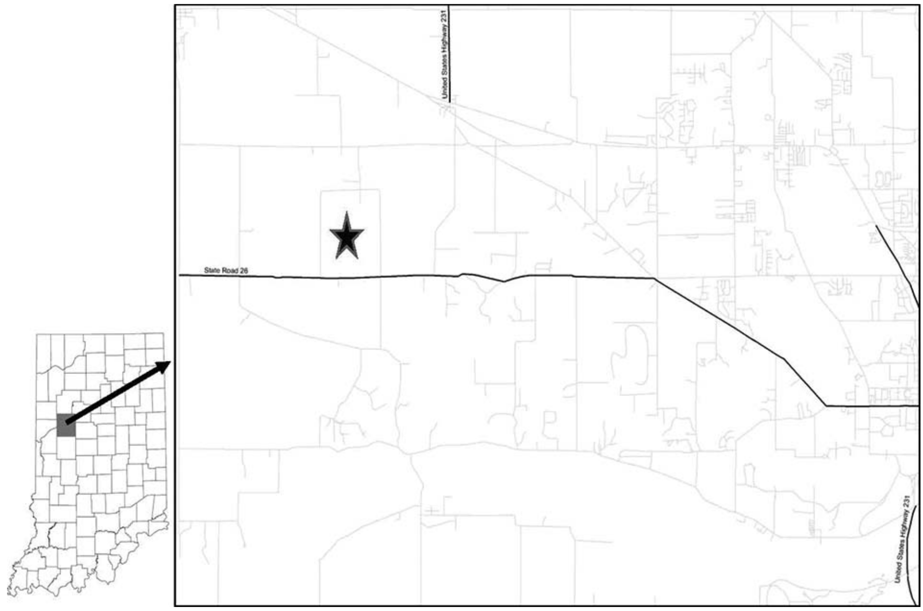

Figure 1.—The star indicates the location of the study site in relation to the closest major city in Tippecanoe county, Lafayette/West Lafayette.

captures of *R. megalotis* in elevated Sherman traps. Their research demonstrated that traps on platforms had higher success rates when compared to traps on the ground (Cummins & Slade 2007). Johnson and Gaines (1988) introduced vertical traps into their experiment to increase captures of *R. megalotis* with mixed success. Increasing probability of capture is important if *R. megalotis* is to be used as a reliable indicator species and if we are to learn more about its ecology in Indiana.

One proposed explanation for increased trapping success in elevated traps is *M. pennsylvanicus* outcompetes *R. megalotis* for traps on the ground (Meserve 1977; Johnson & Gaines 1988; Jekanoski & Kaufman 1995). In this context competition for traps means that *M. pennsylvanicus* are being captured in traps on the ground and precluding *R. megalotis* from access to this trapping stratum. An alternative explanation is that *R. megalotis* spends so much in the grass canopy that they do not often encounter traps on the ground. Our primary objective is to determine if competition for traps between these two species is influencing capture success of *R. megalotis* in elevated traps. To investigate this we will test for independence of captures of these two species across three vertical strata because we could find no records of captures of *M. pennsylvanicus* in elevated traps. We predict that if competition for traps is influencing this phenomenon then we should statistically reject a null hypothesis of frequency of captures at each strata as independent of species.

METHODS

Study site.—Our study was conducted at the Purdue Wildlife Area (PWA), a research property located in the Central Till Plain of Tippecanoe County, Indiana (Fig. 1). In 2003, a prairie restoration project converted portions of the Purdue Wildlife Area from invasive brush and agricultural land to native tall grass prairie and savannah (Benage 2007). The portion of the property where this research occurred is characterized by native prairie on a 6–7 year burn regime. This property was chosen because of its proximity to campus and a history of capturing more than two western harvest mice per year for three years prior to our experiment.

Data collection.—A 5×6 grid of trapping stations was established in the northern portion of PWA. Trapping stations had 15 meter spacing. Three Sherman traps were placed at each station. A vertical trap was placed on a wooden platform elevated off of the ground by a 0.5m wooden stake and level with the surrounding prairie grass. The semi-elevated

Figure 2.—A typical trapping station set up with three Sherman traps in different positions, ground, semi-elevated, and elevated.

trap was placed against the stake at a 45° angle with the door opening upwards. The third trap was placed on the ground at the base of the wooden stake (Fig. 2).

Traps were checked each morning and evening. For each captured animal the species, age, sex, weight, and trap position of capture were recorded. Each animal was given a uniquely numbered ear tag and released. Following identification recaptured animals had the aforementioned characteristics re-measured and were released. All trapping and handling of small mammals was consistent with American Society of Mammalogists guidelines (Gannon & Sikes 2007) and described in Purdue Animal Care and Use Protocol (07-032).

Statistical analysis.—To determine if the pattern of captures of *R. megalotis* was independent of trap position (elevated, semi-elevated, ground) we conducted a G-test to compare observed frequencies of *R. megalotis* captures in each trap position with a null expectation of even distribution of captures across all three trap positions. We then conducted post hoc tests on the three pairwise comparisons (Table 1) using Gardner and MacDonald's Bonferroni-corrected pairwise technique (2000), substituting their Chi-square test for Fisher's exact test, given our small sample size.

Table 1.—Frequency of captures for *R. megalotis* and *M. pennsylvanicus* with the resulting P-value for each pairwise comparison. G = Ground, SE = Semi-elevated, E = Elevated, M.pen = *M. pennsylvanicus* R.meg = *R. megalotis.*

	M. pen	R. meg	P-value
G	34	5	0.6739
SE	13	1	
G	34	5	2.7285E-05
E	1	8	
SE	13	1	1.5541E-04
E	1	8	

RESULTS

We recorded 14 captures of *R. megalotis*, 7 individuals, and 48 captures of *M. pennsylvanicus*, 22 individuals, in 900 trap nights. With a high degree of statistical significance (G = 42.7533, df = 2, P-value = 5.2028E-10) we rejected the null hypothesis that the frequencies of captures of individuals across these three strata was independent of species. Of the three pairwise comparisons ground vs. elevated and semi-elevated vs. elevated were significant, (P-value = 2.7285E-05, P-value = 1.5541E-04), respectively (Table 1).

DISCUSSION

Our primary objective was to determine if competition for traps with *M. pennsylvanicus* influences capture success of *R. megalotis* in elevated traps. Our findings are consistent with the observations of Cummins and Slade (2007) that elevating traps increase captures of *R. megalotis*. Our data suggests that the underlying factors may be a combination of competition with *M. pennsylvanicus* and the foraging behavior of *R. megalotis*. Despite these results our observations were based upon a small population size.

Previous researchers have shown that the introduction of vertical traps have had success in increased capture success of *R. megalotis* (Slade & Cummins 2007; Johnson & Gaines 1988). Our data has found support for these claims. After conducting the post hoc test on ground vs. elevated traps significance was found. This supported our initial assumption of selection of elevated traps by *R. megalotis*.

Our experimental design assumed that *M. pennsylvanicus* would have no access to semi-elevated and elevated traps. We based this assumption upon previous research (Pagels & Wright 1977; Manson & Ostfeld 1996; Jensen et al. 2001) which found *Microtus spp.* exhibits the ability to climb sturdy vegetation and man-made wiring but no evidence of capture in elevated traps. Surprisingly, we recorded 23 captures of *M. pennsylvanicus* in semi-elevated traps and one capture in an elevated trap. We believe that *M. pennsylvanicus* utilized the trap and compacted vegetation to gain access to semi-elevated traps and the wooden stake to access the elevated trap. Johnson and Gaines (1988) hypothesized that *R. megalotis* utilizes the vertical stratum to avoid voles which results in trap avoidance in the lower stratum. We recorded one capture of *R. megalotis* in semi-elevated traps, which leads us to believe that the presence of *M. pennsylvanicus* in this stratum may have affected capture probabilities of *R. megalotis*. This assumption is supported by the Fisher's exact test comparing the semi-elevated and elevated trap positions which show selection by *M. pennsylvanicus* is influencing avoidance by *R.* megalotis in this stratum.

In conclusion, we have found that *R. megalotis* populations in Indiana exhibit similar behavior as other populations within its range. Our experiment was consistent with our hypothesis of competition for traps with *M.* pennsylvanicus as a mechanism contributing to disproportionate captures of *R. megalotis* in elevated traps. Additionally, our results reinforce observations made by Cummins and Slade (2007) that elevating traps increases capture success of *R. megalotis*. Such increases in capture success can have profound implications for abundance estimates, (Jensen et al. 2001) and therefore practical implications for how best to utilize *R. megalotis* as indicator species of prairie restorations.

ACKNOWLEDGEMENTS

We would like to thank Jessica Rodkey, Christina Bienz, and Cole Bleke for their assistance in the field. Without their help this research would have been a daunting task. We would like to thank Casey Day for his advice and assistance with statistical analysis. We are also grateful for Timothy Boszor for his assistance in making the elevated traps. His advice and expertise was a welcome addition to the project. We would also like to extend our gratitude to Purdue University for allowing us to use their research property.

LITERATURE CITED

Benage, M.M. 2007. Ecological Effects of Restoration: an Assessment for Small Mammals and Birds. M.S. thesis, Purdue University, West Lafayette, Indiana.

Cummins, T. & N.A. Slade. 2007. Summer Captures of *Reithrodontomys megalotis* in Elevated Traps. The Southwestern Naturalist 52:79–82.

Ford, S.D. 1977. Range, Distribution and Habitat of the Western Harvest Mouse, *Reithrodontomys megalotis* in Indiana. American Midland Naturalist 98:422–432.

Gannon, W.L. & R.S. Sikes. 2007. Guidelines of the American Society of Mammalogists For the Use of Wild Mammals in Research. Journal of Mammalogy 83:809–823.

Gardner, R.C. & P.L. MacDonald. 2000. Type I Error Rate Comparisons of Post Hoc Procedures for I j Chi-Square Tables. Educational and Psychological Measurement 60:735–754.

Jekanoski, R.D. & D.W. Kaufman. 1995. Use of Simulated Herbaceous Canopy by Foraging Rodents. American Midland Naturalist 133:304–311.

Jensen, T.S., K. Nordvig & J. Reddersen. 2001. Small Mammal Exploitation of Upper Vegetation Strata in Non-forest, Mixed Farmland Habitats. Mammalian Biology 66:129–134.

Johnson, M.L. & M.S. Gaines. 1988. Demography of the Western Harvest Mouse, *Reithrodontomys megalotis*, in Eastern Kansas. Oecologia 75:405–411.

Leibacher, B. & J.O. Whitaker, Jr. 1998. Distribution of the Western Harvest Mouse *Reithrodontomys megalotis* in Indiana. Proceedings of the Indiana Academy of Science 107:167–170.

Manson, R.H. & R.S. Ostfeld. 1996. Long-Distance Homing in Meadow Voles, *Microtus pennsylvanicus*. Journal of Mammalogy 77:870–873.

Meserve, P.L. 1977. Three-Dimensional Home Ranges of Cricetid Rodents. Journal of Mammalogy 58:549–558.

Pagels, J.F. & D.E. Wright. 1977. Climbing Activity in Hispid Cotton Rat, *Sigmodon hispidus*, and the Easter Meadow Vole, *Microtus pennsylvanicus*. Chesapeake Science 18:87–89.

Samson, F.B. & F.L. Knopf. 1994. Prairie Conservation in North America. BioScience 44:418–421.

Transeau, E.N. 1935. The Prairie Peninsula. Ecology 16:423–437.

Whitaker, J.O. Jr. & R.E. Mumford. 1972. Ecological Studies on *Reithrodontomys megalotis* in Indiana. Journal of Mammalogy 53:850–860.

Whitaker, Jr., J.O. & G.R. Sly. 1970. First Record of *Reithrodontomys megalotis* in Indiana. Journal of Mammalogy 51:381.

Manuscript received 23 October 2013, revised 3 July 2014.

2013. Proceedings of the Indiana Academy of Science 122(2):130–139

DAVE'S POND: HISTORY OF A SIGNIFICANT INDIANA HERPETOLOGICAL SITE

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ABSTRACT. Dave's Pond, 1 ¼ miles NE of Sandcut in northeastern Vigo County, Indiana, is compared to "Sand Hill", now part of Deming Park on the eastern edge of Terre Haute, Indiana, which was a favored collecting site of the naturalist W. S. Blatchley in the 1890s. Both sites lie at the juncture of Illinoian uplands and Wisconsinan lowlands, have sandy soils, and include ponds, woods, and meadows. At Sand Hill, Blatchley collected 35 species of amphibians and reptiles; some have not been found elsewhere in Vigo County and some are probably no longer extant in the county. In comparison, Dave's Pond yielded 21 species of amphibians and reptiles including the state endangered crawfish frog (*Lithobates areolatus*); the spadefoot toad (*Scaphiopus holbrookii*), which had previously been known in Indiana no closer than 65 miles to the southeast; the newt (*Notophthalmus viridescens*), which has not been found recently at any other locality in Vigo County or the surrounding counties in Indiana and Illinois; and Kirtland's snake (*Clonophis kirtlandii*), another species of special concern. The Dave's Pond population of crawfish frogs is the only known extant population in Vigo County and may be the most northern extant population in the state. Most species of pond breeding amphibians found in Vigo County were found at both Sand Hill and Dave's Pond. Sand Hill has been considerably altered and no longer has the habitat or the herpetofauna that existed in the 1890s. Dave's Pond, while degraded to some extent, remains a significant herpetological site.

Keywords: Dave's Pond, amphibians and reptiles, Vigo County, Sand Hill

BACKGROUND

Over a century ago, in the 1880s and '90s, the famous Naturalist/Biologist/Geologist Willard S. Blatchley roamed Indiana, spending a good deal of time in Vigo County. He taught for many years at Wiley High School in Terre Haute while studying the biota and geology of the state. He was State Geologist of Indiana but was most famous for his works in entomology. He also published his work on plants, birds, mammals, reptiles and amphibians.

In two papers on the amphibians and reptiles of Vigo County, Blatchley (1891, 1899) recorded 52 species. Some of his favorite collecting areas were "the bluffs overlooking five mile pond, Hecklund Prairie, and Sand Hill," all near Terre Haute. A small portion of the bluffs overlooking Five Mile Pond has now been preserved as Little Bluestem Prairie. Hecklund Prairie was along the railroad tracks just SW of the Rio Grande School but was lost to farming operations in the 1980s. Sand Hill was on the south edge of current Deming Park, at the east end of Terre Haute where Poplar Street passes over a knoll. Blatchley described Sand Hill as a 40 acre woodland pasture with thick underbrush and several shallow ponds. The soil, he said, was a loose, sandy, black loam. At Sand Hill, Blatchley (1891, 1899) collected 35 species of amphibians and reptiles, several of which are currently either rare or no longer extant in Vigo County (ex. *Hemidactylium scutatum*, *Notophthalmus viridescens*, *Ambystoma jeffersonianum*); he felt the area was so productive because here "the upland met the lowland."

Blatchley's "lowland" is actually the old floor of the glacial Wabash River, and the "upland" is its shore. During the Illinoian glaciation, Vigo County was completely covered by glacial drift. This material, still present in the "upland," produced a clayey loam soil which Shannon (1911) referred to as "Knox silt

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loam." Later, during the Wisconsinan glaciation, when the Wabash River served as a glacial spillway, the Wisconsinan melt waters washed away the clayey loam in the river valley and laid down stratified deposits with sand lying on top of gravel. The soil of these deposits is a black sandy loam which Shannon (1911) called "Sioux sandy loam." The present Wabash River is incised in these stratified deposits.

Blatchley's Sand Hill is now a much disturbed and degraded site, and has been for many years. The environment in which Blatchley collected is gone, as are most of the amphibians and reptiles. But knowledge of a comparable site in Vigo County emerged in the 1960s, and, while degraded to some extent, this site is still significant for its herpetological diversity. The site, Dave's Pond, is the subject of this paper.

DAVE'S POND

Dave's Pond is farther north than Sand Hill but is also along the shore of the glacial Wabash River. It is located on farmland about 5 miles NE of Terre Haute and about 1 ¼ miles E of Sandcut on the Rio Grande Road (Rosedale Quadrangle, T 14 N, R 8 W, in the south-central portion of section 16). There are actually three ponds: the northern pond (Dave's Pond) is about ¼ mile north of the road, the middle pond (Scaphiopus Pond) is at the edge of the road to the north, and the third pond (Areolatus Pond) is at the edge of the road to the south. They lie amidst meadows and a small woods. Dave's Pond retains water all year round, while the two ponds along the road are temporary.

The Dave's Pond site, as it existed in the mid-1960s, was described by Rubin (1965a) as follows: "The pond near Sandcut covers one eighth of an acre. It nearly dries in late summer after partially filling with aquatic vegetation, particularly arrowleaf (*Sagittaria* sp.). South of the pond is a meadow which floods with the rains of March and April but dries in the beginning of June. Southeast of the pond is an open woods which harbors some flood pools after heavy rains."

Since the 1960s, there has been some degradation of the habitat, but much of the original conditions and the original herpetofauna remain. Perhaps the most serious degradation occurred in the late 1960s and early 1970s. The woods was significantly thinned and much of its undergrowth removed, and a residence was built in it. During much of the year, the woods may have served as the reservoir for the eft stage of the newt, for some of the mole salamanders (*Ambystoma* spp.), and for species such as the wood frog (*Lithobates sylvaticus*). Indeed, the newt (*Notophthalmus viridescens*) and the spotted salamander (*Ambystoma maculatum*) have not recently been found at the site and may very well be extirpated here. Regardless, Dave's Pond remains a significant herpetological site.

"Dave" is Dave Rubin, the junior author of this paper. Dave was a graduate student at Indiana State University (ISU) from 1963 to 1969, and the first Ph.D. student of senior author John O. Whitaker, Jr., who started his career on the faculty at ISU in 1962. Dave's master's thesis (Rubin 1965a) was a survey of the amphibians and reptiles of Vigo County. Dave was working two sites near Burnett, Indiana, in northeastern Vigo County that had several species of frogs calling from temporary pools. One evening in late March 1964, while travelling to the Burnett sites, he heard a strange frog call in a flooded meadow along the Rio Grande Road, about 1 ¼ miles E of Sandcut. It was the crawfish frog, *Lithobates areolatus*, uncommon then, and now an Indiana state endangered species. That was the first of many visits to this locality. On April 6 of the same year, at this site, Earl Zimmerman, an undergraduate student of Whitaker, and later Chairman of the Biological Sciences Department at North Texas State University, collected a single crawfish frog. Rubin returned to the site the same evening, along with another student, Phil Evers, and found a single spadefoot toad, *Scaphiopus holbrookii*, in the flooded meadow. The authors found breeding choruses there two weeks later. At that time, the closest known Indiana locality for spadefoots was 65 miles to the southeast. The spadefoot range extension was reported by Rubin (1965b). Over the next few years, the site was often visited and many species were added to the list of those found there. In total, 21 herpetofaunal species–15 amphibians (including most of the pond-breeding species known from Vigo County) and 6 reptiles–were found at the site. Besides crawfish frogs and spadefoot toads, they included the newt (*Notophthalmus viridescens*), which had not been reported for Vigo County (or any adjacent counties) since Blatchley's 1890s Sand Hill record, and Kirtland's snake (*Clonophis kirtlandii*), another species of special concern in Indiana.

Table 1.—Comparison of the species of amphibians and reptiles found at Sand Hill (Blatchley 1891; 1899) and at Dave's Pond. X = certain; P = probable.

Species	Common name	Sand Hill	Dave's Pond
Ambystoma jeffersonianum	Jefferson salamander	X	
Ambystoma maculatum	spotted salamander	X	X
Ambystoma opacum	marbled salamander	X	
Ambystoma texanum	small-mouthed salamander	X	X
Ambystoma tigrinum	eastern tiger salamander	X	X
Notophthalmus viridescens	eastern newt	X	X
Hemidactylium scutatum	four-toed salamander	X	
Plethodon cinereus	red-backed salamander	X	
Plethodon glutinosus	northern slimy salamander	X	
Scaphiopus holbrookii	eastern spadefoot toad		X
Anaxyrus fowleri	Fowler's toad	P	X
Acris crepitans	eastern cricket frog	X	X
Hyla versicolor	gray treefrog	X	X
Pseudacris crucifer	spring peeper	X	X
Pseudacris triseriata	western chorus frog	X	X
Lithobates areolatus	crawfish frog		X
Lithobates catesbeianus	American bullfrog	P	X
Lithobates clamitans	green frog	P	X
Lithobates sphenocephalus	southern leopard frog	P	X
Lithobates sylvaticus	wood frog	P	X
Chelydra serpentina	snapping turtle	P	X
Chrysemys picta	painted turtle	P	X
Terrapene carolina	eastern box turtle	P	X
Carphophis amoenus	common worm snake	X	
Clonophis kirtlandii	Kirtland's snake	X	X
Heterodon platirhinos	eastern hognose snake		X
Lampropeltis calligaster	prairie kingsnake	X	
Nerodia sipedon	common water snake	P	X

* Besides the 25 species listed above as certain or probable for Sand Hill, Blatchley saw an additional 10 species there. Possibilities for these additional 10 species include one frog (*Lithobates palustris*), one turtle (*Apalone spinifer*), one lizard (*Plestiodon* sp.), and eleven snakes (including *Heterodon platirhinos* listed in the table above). See the text for further discussion. Common and scientific names follow Crother et al. 2012.

Rubin in 1973 published the material from his master's thesis. Earlier, Rubin (1968) reported on amphibian breeding dates in Vigo County based largely on data from the site. Some of these records were cited by Minton (1972). Whitaker et al. (1977) compared the food habits of four species of spadefoot toads; the specimens of *Scaphiopus holbrookii* used in this study were all from Vigo County and largely from the Dave's Pond site.

From 1963 to 1969, Whitaker (1971) and students in his herpetology classes conducted research on the western chorus frog (*Pseudacris triseriata*) in Vigo County. Most data were collected from a pond NE of Terre Haute that was referred to as the "Pseudacris Pond," but considerable data from Dave's Pond were included. In this work, the locality NE of Sandcut was first referred to, in print, as "Dave's Pond."

Subsequent researchers, and herpetology class students, have often visited Dave's Pond. The most recent references to it are included in reports on the status of the crawfish frog in Indiana (Engbrecht and Lannoo 2010; Engbrecht et al. 2012). Engbrecht et al. (2012) indicated that Dave's Pond contains the only known remaining population of this species in Vigo County, and perhaps the northernmost remaining population in the state.

HERPETOFAUNA OF DAVE'S POND

The 21 herpetofaunal species recorded from the Dave's Pond site from 1964 through 1966 included four salamanders, eleven anurans, three turtles, and three snakes. These are listed in Table 1, along with a comparison to the list of species most likely found by Blatchley (1891, 1899) at Sand Hill. After repeated visits in the mid-1960s, the Dave's Pond area was frequently visited by Whitaker and his students in herpetology classes from the late 1960s into the early 2000s. Changes in ownership of the property subsequently affected the ability to access the site.

Rubin (1968) summarized amphibian pond breeding in Vigo County based largely on observations at Dave's Pond and another site about two miles away: The first spring breeder is *Ambystoma texanum* which may breed during warm spells in January and February and peaks in early March. *Ambystoma maculatum* and *A. tigrinum* breed through March with the peak in mid-March. The first frogs to call are *Pseudacris triseriata* and *Pseudacris crucifer*, beginning at the end of February, with *Lithobates pipiens*, *L. sylvaticus*, and *L. areolatus* joining in that order during March. Breeding in April is dominated by *Hyla versicolor*, *Anaxyrus woodhousii*, and *Acris crepitans*. *Lithobates clamitans* breeds in May and June and *L. catesbeianus* breeds later in the summer.

Comments on the individual species found at Dave's Pond follow:

Salamanders

Ambystoma maculatum (spotted salamander).—Several spotted salamanders were collected during intensive work at Dave's Pond in April 1964 (ISU 20, 22) and March and April 1965 (ISU 863, 874, 882, 883, 938), but this species was quite scarce then compared to the tiger salamander, and especially to the small-mouthed salamander. The spotted salamander has not been seen there since 1965 and is likely extirpated from that site, perhaps as a consequence of degradation of the woods adjacent to the main pond. Until recently, Dave's Pond and Sand Hill were the only known Vigo County localities for the spotted salamander, which is now likely gone from both sites. However, another Vigo County locality for the spotted salamander has subsequently been found. A site in Seelyville, with several small ponds, yielded this species in 2002, 2003, 2004, and 2006 (Foster, pers. comm. 2013). One collected there on March 9, 2002, is in the ISU Vertebrate Collection (ISU 4091). The Seelyville site is 1.25 km N and 1 km E of the intersection of US 40 and North Road, and approximately 4.75 miles from Dave's Pond.

A. texanum (small-mouthed salamander).—Of the three species of *Ambystoma* taken at Dave's Pond, the small-mouthed salamander was by far the most numerous. This species is widely distributed in Vigo County, and often seen in large numbers during breeding season. It is also the earliest ambystomid to migrate to the breeding ponds with peak breeding activity in February and early March. Many voucher specimens from Dave's Pond are in the ISU Vertebrate Collection. Use of minnow traps at Dave's Pond resulted in 64 captures from January 31 through March 9, 1974, and 161 captures from February 13 through March 7, 1976. Use of minnow traps at Scaphiopus Pond resulted in 123 captures from February 13 through March 7, 1976; 20 captures from February 20 through March 27, 1986; and 69 captures from February 16 through April 20, 1992.

A. tigrinum (Eastern tiger salamander).—The tiger salamander is reasonably common at Dave's Pond, but is not as numerous as the small-mouthed salamander. Voucher specimens of adults were taken at Dave's Pond in 1964 (ISU 24), 1965 (ISU862, 868, 909, 923), 1972 (ISU 2609), 1974 (ISU 2836-37, 3163-65, 3185-90), and 1975 (ISU 3337, 3339). All were taken between late February and early April. Larvae collected by seining on June 30, 1964, were also preserved (ISU 24, 26, 28). Use of minnow traps at Dave's Pond by Whitaker's herpetology class from February 24 through April 4, 2000 resulted in 51 captures of tiger salamanders, of which 50 were males and one was a female, although some may have been recaptures. The first capture was on February 24 and the last was on March 24. Nearly half (23 of 51) of the captures occurred during the last five days of February.

Data collected by Whitaker and his herpetology class in 1976 gives good insight into the relative abundance of small-mouthed and tiger salamanders at Dave's Pond. Using minnow traps, the class worked at both Dave's Pond proper and Scaphiopus Pond. Traps were first set on February 14 and were removed on March 17. At Dave's Pond, total captures were 161 individuals of *A. texanum* and 27 of *A. tigrinum*. At Scaphiopus Pond, total captures were 123 individuals of *A. texanum* and 13 of *A. tigrinum*. For *A. texanum*, approximately 90% of captures were between February 17 and February 22. Captures of *A. tigrinum* were more evenly spread over the time period.

Notophthalmus viridescens (Eastern newt).—A larval newt (ISU 58) was taken from Dave's Pond on March 25, 1964. This was the first record of the newt for Vigo County since Blatchley recorded it for Sand Hill. This was also significant in view of the lack of recent records for adjacent areas in Indiana and Illinois (Minton, 1972, 2001; Smith, 1961). Three adults (ISU 870) were taken from the pond on March 4, 1965, and another on April 5, 1965. Additional newts were taken in February and March 1966; March 1967; March and April 1972; and January, February, and March 1974. Most of these specimens are in the ISU Vertebrate Collection (ISU 1440, 1442, 1448, 1834, 2610-13, 2802, 2825, 2835, 3166, 3184). Those collected in 1972 included 24 individuals that were caught using minnow traps. No specimens of this species have been taken at Dave's Pond since 1974. Modification of the adjacent woods, eliminating appropriate habitat for the eft stage, may have doomed this species at Dave's Pond.

Anurans

Foster (pers. comm. 2013) also informed us that Nathan Engbrecht heard spadefoots calling at 10 locations in Vigo and Parke counties in 2011 or 2012. Specimens in the ISU Vertebrate Collection from Dave's Pond are from April, May, and June 1964 (ISU 325-32, 2970); May and July 1965 (ISU 1030, 1065, 1220-21); April and May 1966 (ISU 1499, 1502-03, 1507, 1512).

Scaphiopus holbrookii (eastern spadefoot).—The spadefoot is listed as a species of "Special Concern" in Indiana. When first discovered at Dave's Pond in April 1964, the new Vigo County record represented an extension of the known range of the species by about 65 miles to the northwest of the closest localities in south-central Indiana (Rubin 1965b). Since its discovery at Dave's Pond, the spadefoot has been found at a number of other Vigo County localities and at least one locality (Parke County 750 W road between Lyford and Clinton) in Parke County to the north (Foster); and May 1967 (ISU 1859).

Most of the species in this genus inhabit the arid western U.S, spend most of their lives underground, and breed only in temporary pools created by heavy rains. While not living under arid conditions, the eastern spadefoot follows the same pattern–breeding seems tied to rainfall more than to season, temporary pools are used almost exclusively, and both breeding and the larval period are very much compressed. Rubin (1968) reported spadefoot breeding at the Dave's Pond site from April 19 to 21, 1964; on July 9, 1965; and on April 27-28, 1966. No breeding activity was observed in 1967, even though the site was repeatedly visited. The flooded pasture (Scaphiopus Pond) was utilized in 1964 and 1965; in 1966, the breeding congregation was found in flood pools in the woods. Rubin (1965b, 1968) reported finding fully transformed toadlets in May 1964 only 37 days after the first known breeding at the site.

Anaxyrus fowleri (Fowler's toad).—Fowler's toad is common at Dave's Pond. Voucher specimens in the ISU Vertebrate Collection are from 1964, 1965, and 1972 with dates from mid-April into July. Rubin (1965a) first heard this species calling at Dave's Pond in 1964 on April 20; it was still present in large numbers on July 7. ISU 3246 includes 35 adults collected on April 19, 1972, indicating that this species was breeding at Dave's Pond on that date.

Acris crepitans (Eastern cricket frog).—The eastern cricket frog is the least abundant hylid found at Dave's Pond. Rubin (1965a) noted that this species was widespread in Vigo County, but was (most) often seen along creeks during autumn. Rubin first observed this species in 1964 at Dave's Pond on May 14. The herpetology class heard approximately 15 calling at Scaphiopus Pond on May 3, 1972, and 4 to 8 calling two weeks later on May 17. Voucher specimens are from May 14, 1964 (ISU 233); April 15, 1969 (ISU 2817); April 21, 1972 (ISU 3260); and May 18, 1972 (ISU 3180, 3236).

Hyla chrysoscelis (Gray treefrog).—The gray treefrog breeds later than the chorus frog and spring peeper, but earlier than the cricket frog. It is common at Dave's Pond, but far less abundant than the chorus frog and spring peeper. Voucher specimens in the ISU Vertebrate Collection are from 1964, 1965, 1966, 1969, 1972, 1974, and 1980. All were collected between April 15 and July 7. Rubin (1965a) reported this species first calling at Dave's Pond in 1964 on April 19, and he also heard it on July 7, the last day he visited the site that year. Rubin (1968) also reported that this species often called from "on top of arrowleaf leaves." Whitaker's herpetology class reported gray treefrogs calling at Scaphiopus Pond on May 16 and May 17.

Pseudacris crucifer (spring peeper).—The spring peeper is abundant at Dave's Pond. Voucher specimens are from 1965, 1969, 1972, 1974, and 2002. Rubin (1965a) reported first hearing the peeper call at Dave's Pond in 1964 on March 15, and hearing it for the last time that year on April 27. The 1972 herpetology class reported hearing this species at Scaphiopus Pond on many days between March 4 and May 8, with "numerous" individuals present on March 4, 8, and 28. The 1974 herpetology class reported this species calling at Scaphiopus Pond on February 18, March 3, and from April 3 to 21. Estimated numbers on April 10, 14, 17, and 21 were 200, 300, 200, and 150, respectively. In 2002, Whitaker heard 2 peepers calling at Scaphiopus Pond on February 13. On March 7, he heard about 25 calling at Dave's Pond and numerous individuals calling at Scaphiopus and Areolatus Ponds. He heard full choruses of peepers at Scaphiopus Pond on April 1 and at Scaphiopus and Areolatus Ponds on April 5.

P. triseriata (western chorus frog).—The chorus frog is abundant at Dave's Pond. Voucher specimens are from 1964, 1965, 1966, 1967, 1969, 1972, 1974, 1979, and 1980. With the exception of a recently transformed individual collected on May 26, 1964 (ISU 291), all voucher specimens were taken in March and April. Rubin (1965a) reported this species first calling at Dave's Pond in 1964 on March 15 and last calling on April 27. For 1965, he reported it first calling there on March 2. On March 4, 1965, Dave's Pond was half covered with ice; water temperature was 1 C and air temperature was 20 to 25 F. Seining that day yielded 1 chorus frog (ISU 871) along with 12 *Ambystoma texanum*, 5 *A. tigrinum*, 1 *A. maculatum*, 3 *Notophthalmus viridescens*, and 3 *Lithobates sphenocephalus*. In 1972, Whitaker's herpetology class found chorus frogs calling in large numbers, estimated several times at 100 to 200, at Scaphiopus Pond from March 15 to April 14. In 1974, the herpetology class recorded chorus frogs at Scaphiopus and Areolatus Ponds from February 22 to April 1; the first full chorus was on March 3 and the last on March 28. In 2002, Whitaker found a few chorus frogs each calling at Dave's, Scaphiopus, and Areolatus Ponds. On April 1, 2002, there was a full chorus at Scaphiopus Pond; on April 5, only 1 individual was heard.

Lithobates areolatus (crawfish frog).—In 1964, crawfish frogs were first heard calling at Dave's Pond on March 24 and last heard on April 26 (Rubin 1965a). Eggs were collected in the field on April 2 (ISU 402) and a captive female deposited eggs on March 24 (ISU 403). An individual (ISU 397), completely transformed except for a small tail stub, was taken on June 24, although tadpoles (ISU 398) were still present on June 30. In 1965, the crawfish frog was first heard at Dave's Pond on April 5 (ISU 937). In 1972, Whitaker's herpetology class heard crawfish frogs on March 21 and found individuals at Scaphiopus Pond on April 14, 17, and 21. In 1974, the herpetology class heard crawfish frogs calling at Scaphiopus and Areolatus Ponds from March 4 through April 1. In 2002, Whitaker heard crawfish frogs calling at Scaphiopus Pond on April 1 and estimated the number at 30. On April 5 of that year, he heard a full chorus at Scaphiopus and Areolatus Ponds, and estimated the number at 60.

Voucher specimens of the crawfish frog in the ISU Vertebrate Collection are listed by Engbrecht and Lannoo (2010). These include individuals collected in 1964, 1965, 1969, 1972, and 1974. Most were taken in March and April, but two are from June 1964. Engbrecht and Lannoo (2010) reported the crawfish frog as still being present at Dave's Pond in 2008. Engbrecht et al. (2012) reported seeing it at Dave's Pond in 2009, 2010, and 2011, but estimated the population at only 24 individuals.

Engbrecht and Lannoo (2010) described the status of the crawfish in Indiana, reviewing known records for the frog, including Vigo County. Noting that it had once been described as plentiful, declines had led to its being listed as a state endangered species in 1988. Two years later, an update by Engbrecht et al. (2012) concluded that the crawfish frog had been extirpated north of Vigo County and that Dave's Pond had the only remaining county population and the northernmost extant population in the state.

L. catesbeianus (American bullfrog).—The bullfrog is common at Dave's Pond and widespread in Vigo County. It is the latest of the true frogs to breed, with breeding activity not occurring until summer. Rubin (1965a) noted that he did not hear the bullfrog calling at Dave's Pond in 1964, and July 7 was the last date that year for visitation to the site. Whitaker's herpetology classes regularly visited the site during late winter and early spring of 1972 and 1974 but never recorded bullfrog vocalizations. Tadpoles, however, have been collected there in late winter and early spring, indicating that they overwinter in that stage. A number of adults from Dave's Pond are in the ISU Vertebrate Collection with collection dates ranging from March through May.

L. clamitans (green frog).—The green frog is also common at Dave's Pond and is the next to the latest of the true frogs to breed there. Rubin (1968) noted that the green frog, in Vigo County, calls from late April through June. In 1964, Rubin (1965a) first heard the green frog at Dave's Pond on May 14 and also heard it on July 7, the last day of that year that he visited the site. In 1972, the herpetology class reported calling by the green frog at Scaphiopus Pond on May 16 and 17. Tadpoles taken on March 4, 1965, had either hind legs or developing limb buds, indicating that they had overwintered as tadpoles. Voucher specimens in the ISU Vertebrate Collection from Dave's Pond were collected from March through May.

L. sphenocephalus (southern leopard frog).—The leopard frog is common at Dave's Pond and widespread in Vigo County. Voucher specimens for Dave's Pond in the ISU Vertebrate Collection are from 1964, 1965, 1969, 1972, and 1974. Most of the voucher specimens were collected in March or April. Leopard frogs and wood frogs are the earliest breeding ranids at Dave's Pond, followed by the crawfish frog, with green frog and bullfrog breeding significantly later. In 1964, first and last dates for observed leopard frog breeding activity at Dave's Pond were March 25 and April 26 respectively (Rubin, 1965a). The earliest date in 1965 was March 2. In 1972, Whitaker's herpetology class heard leopard frogs calling at Scaphiopus Pond from April 10 to April 21. In 1974, the herpetology class heard this species calling from March 3 to April 10. In 2002, at Scaphiopus Pond, Whitaker heard a chorus of about 20 individuals on April 1 and a chorus of about 30 individuals on April 5.

L. sylvaticus (wood frog).—In the mid-1960s, the wood frog was the scarcest of the ranids present at Dave's Pond, although it was common at some sites not too distant. In 1964, the wood frog was heard calling at Dave's Pond on April 2 (ISU 489) and eggs (ISU 493) were collected the same day (Rubin, 1965a). Nearby, it had been heard calling as early as March 16. On March 7, 2002, Whitaker heard 2 wood frogs calling just south of Dave's Pond, a few at Scaphiopus Pond, and a chorus of about 20 wood frogs at Areolatus Pond.

Turtles

Chelydra serpentina (snapping turtle).—The snapping turtle is a common county resident, and a usual inhabitant of ponds. Several have been seen in Dave's Pond, usually picked up in seine nets. Voucher specimens from Dave's Pond were taken in April 1964 (ISU 778), March 1965 (ISU 1447), and February 1976 (ISU 3498). An individual found DOR on the Rio Grande Road on April 17, 1972 was not saved.

Chrysemys picta (painted turtle).—Like the snapping turtle, painted turtles frequent county ponds and have been captured in seines at Dave's Pond. Voucher specimens were collected in April, May, and June 1964 (ISU 544, 543, 547); March 1966 (ISU 3499); and April 1974 (ISU 3313). An individual found DOR on the Rio Grande Road (along with the snapping turtle mentioned above) on April 17, 1972 was not saved.

Terrapene carolina (eastern box turtle).—A single specimen was found DOR on the Rio Grande Road in spring 1966. It was not saved.

Snakes

Clonophis kirtlandii (Kirtland's snake).—Kirtland's snake is a species of "Special Concern" in Indiana. This species is known at Dave's Pond from only one specimen taken on 5 May 1966 (ISU 1513) on the Rio Grande Road, between Scaphiopus Pond and Areolatus Pond. The flooded meadows at the Dave's Pond site, along with numerous crayfish burrows with which Kirtland's snake is often associated, provide appropriate habitat for this species. Besides Dave's Pond and Sand Hill, this species is also known in Vigo County from a DOR specimen found in West Terre Haute (Foster, pers. comm. 2004a) and from the Terre Haute Airport where two individuals were taken in late May 2004 (Foster, pers. comm. 2004b).

Nerodia sipedon (common water snake).—The common water snake appears to be found in nearly every stream and pond in Vigo County, and Dave's Pond is no exception. Voucher specimens from Dave's Pond are from April, May, and June 1964 (ISU 707, 719, 726); April 1967 (ISU 1842); and April and May 1972 (ISU 3237, 3418).

Heterodon platirhinos (eastern hog-nosed snake).—A single specimen was found DOR on the Rio Grande Road in spring 1966. It was not saved. Given the sandy soil and the large populations of anurans upon which this species primarily feeds, it was not surprising to find this snake there.

HERPETOLOGICAL COMPARISON OF DAVE'S POND AND SAND HILL

Blatchley (1899) stated that he found 35 species of amphibians and reptiles at Sand Hill. We know many, but not all, of these species. Blatchley (1891, 1899) specifically mentioned spotted salamander, marbled salamander, tiger salamander, newt, slimy salamander, gray treefrog, worm snake (*Carphophis amoenus*), and prairie kingsnake (*Lampropeltis calligaster*) as being taken at Sand Hill. Specimens of others species are still extant in the Museum of Comparative Zoology at Harvard University. We have examined Blatchley's specimens of Jefferson's salamander (MCZ 7067, 7068) and Kirtland's snake (MCZ 14812, 14813) from Sand Hill. Finally, some species can be deduced from Blatchley's comments, from knowledge of the typical habitat in which they are found, and from their abundance in Vigo County.

Blatchley (1899), writing of the salamanders and tree frogs from Sand Hill, stated that "out of 11 species of the former and four of the latter all but two have been seen here, and nine of the 15 nowhere else in the county." To consider only 11 species of salamanders, Blatchley must have omitted the completely aquatic forms, *Necturus* and *Siren*. Blatchley (1891) did record the mudpuppy as common in the Wabash River, and later (Blatchley 1899) recorded a siren from a lowland pond in southern Vigo County. Of the 11 species of salamanders, Blatchley's descriptions preclude his having found the two-lined salamander (*Eurycea cirrigera*) and the long-tailed salamander (*E. longicauda*) at Sand Hill. Blatchley took the two-lined salamander from the Coal Creek area and from a "worn out field, where the soil was wholly clay" and the long-tailed salamander from "beneath rocks in the bed of a branch." Thus, it may be assumed that Blatchley found the following nine salamanders and four tree frogs at Sand Hill: *Ambystoma jeffersonianum*, *A. maculatum*, *A. opacum*, *A. texanum*, *A. tigrinum*, *Notophthalmus viridescens*, *Plethodon cinereus*, *P. glutinosus*, *Hemidactylium scutatum*, *Acris crepitans*, *Hyla chrysoscelis*, *Pseudacris crucifer*, and *P. triseriata*.

Blatchley (1899) stated that the American toad, *Anaxyrus americanus*, was the only toad he found in Vigo County and that it was common. Rubin (1965a), on the other hand, found Fowler's toad (*Anaxyrus fowleri*) at 22 county localities but did not record a single American toad (the American toad has since been recorded for Vigo County, but it is not common). No Vigo County American toads collected by Blatchley are at Harvard, but MCZ 7242, 7243, 7245, and 7247 are Vigo County specimens of Fowler's toad that Blatchley collected. It is thus clear that Blatchley misidentified the toads and that Fowler's toad was common in Vigo County in the 1890s, and it is probable that Blatchley saw this species at Sand Hill.

Six species of true frogs (family Ranidae) were known to occur in Vigo County (seven now as *L. blairi* was found in Vigo County in 2008 (Enbrecht, et al. 2009). We know that Blatchley did not collect *L. areolatus* at Sand Hill. However, he did report *L. areolatus* from Vigo county (Blatchley, 1899): three specimens were collected from two localities–two by C. Stewart in "the south part of the city of Terre Haute" and one by H. McIlroy "three miles west from where the others were secured." Four of the other ranid species (*Lithobates catesbeianus, L. clamitans, L. sphenocephalus*, and *L. sylvaticus*) were reported for Vigo County by both Blatchley (1899) and Rubin (1965a). All four are common and widely distributed in Vigo County, and it is probable that Blatchley saw them at the ponds at Sand Hill. The seventh Vigo County ranid is *Lithobates palustris*, the pickerel frog, which is not common. It is impossible to say whether Blatchley saw this frog at Sand Hill or elsewhere; the same can be said for L. blairi which was not described until 1973.

Based on the comments above, it is likely that Blatchley saw 18 or perhaps 19 species of amphibians at Sand Hill. The remaining 16 or 17 species that he saw at Sand Hill were reptiles. Compared to the amphibians, it is much more difficult to deduce which reptilian species he actually saw there.

We can be fairly certain about seven species of reptiles. From his papers, we know that Blatchley took the worm snake and the prairie kingsnake there, and two of his Sand Hill specimens of Kirtland's snake are at Harvard. He must also have seen the common water snake, a species ubiquitous in county ponds and streams. He probably also saw snapping turtles and painted turtles; he found both to be common in the county and both are commonly found in small ponds. And he probably found the box turtle at Sand Hill; he said this species was common in "sandy upland woods" such as existed there.

For the remaining 9 or 10 reptiles, of 21 possibilities, it is easier to say what was not found at Sand Hill than what was found. There are five species of turtles that Blatchley definitely did not find at Sand Hill. He found only one specimen of the smooth softshell, in the Wabash River. He reported the map turtle from the Wabash and its larger tributaries. He reported a single specimen of the false map turtle that he found on a road about a half mile from water (presumably the Wabash or some other large stream); two of his specimens of this species at Harvard (MCZ 16470 and 16471) bear the locality data "Wabash River." The only two red-eared turtles he found were at the margin of a pond 5 miles NE of Terre Haute. And he found a single "mud turtle" (presumably a musk turtle – see Rubin 1965a) at a large pond in the southern part of the county. That leaves only one additional species of turtle, the spiny softshell, which he might have found at Sand Hill.

Blatchley did not specify Sand Hill as a locality for skinks but he may very well have seen some there. Blatchley recorded only the five-lined skink (*Plestiodon fasciatus*) for the county, but his descriptions of red-headed adults and a female 10 ½ inches long make it likely that he saw the broad-headed skink (*Plestiodon laticeps*) as well. Both species are possibilities for Sand Hill. With regard to snakes, we know that he did not see the queen snake or the diamond-backed water snake there. The former he found in "rocky branches" and the latter at ponds in the southern part of the county. That leaves, as possibilities, the following snake species: red-bellied (*Storeria occipitomaculata*), DeKay's (*Storeria dekayi*), eastern ribbon (*Thamnophis sauritus*), western ribbon (*T. proximus*), garter (*Thamnophis sirtalis*), black rat (*Elaphe obsolete*), rough green (*Opheodrys aestivus*), racer (*Coluber constrictor*), ringneck (*Diadophis punctatus*), common king (*Lampropeltis getula*), milk, (*L. triangulum*) and hognose (*Heterodon platirhinos*).

Elimination of five species of turtles and two snakes leaves 14 possibilities for the remaining 9 or 10 reptiles. In what is clearly conjecture, but based on Blatchley's comments about abundance and habitat and what we know of distributions in the county, we believe that the remaining 9 or 10 included, in part, DeKay's, garter, black rat, racer, common king, hognose, and at least one of the ribbon snakes, and the skink.

Using this information, Table 1 compares the known herpetofauna of Dave's Pond with the known and probable herpetofauna of Sand Hill. Blatchley's Sand Hill and Dave's Pond both were at the juncture of Illinoian uplands and Wisconsinan lowlands. Both harbor(ed) an extremely large number and variety of species of amphibians and reptiles–35 species at Sand Hill and 21 species at Dave's Pond. At least 18 species, and possibly more, were found at both sites. Most pond-breeding amphibian species were found at both sites. Snakes provide the greatest faunal difference between the two sites.

Both Blatchley and Rubin attributed the herpetofaunal diversity to the fact that the sites lay at upland-lowland junctures, with the mix of upland and lowland conditions providing proper habitat for a wider range of species. Permanent and temporary ponds, along with woods and open fields, exist(ed) at both sites. The sandy soil is ideal for burrowing amphibians, such as the mole salamanders and spadefoot toads, and is preferred substrate for reptiles such as the hog-nosed snake which feed largely on amphibians.

As noted above, Blatchley found 14 more species at Sand Hill than the present authors have found at Dave's Pond. The aquatic and semi-aquatic herpetofauna of the two sites, however, are very similar. The real difference in number of species is due to more terrestrial forms, with Sand Hill having more terrestrial salamanders, such as red-backed and slimy, and many more snakes. This may be due, in part, to the fact that Sand Hill, at 40 acres, was significantly larger than the Dave's Pond site, and probably had much more woodlands. It should also be remembered that it is much easier to overlook animals such as snakes and terrestrial salamanders than it is to overlook species that form large breeding congregations during the appropriate season. The great majority of visits to the Dave's Pond site took place at night in late winter and early spring when it was much more likely to find amphibians than reptiles. Additional reptiles likely exist in the vicinity of Dave's Pond. For example, because of their abundance in the county and because of the habitat around Dave's Pond, garter snake, racer, and black rat snake would be expected.

STATUS OF DAVE'S POND

Sand Hill, as it was known to Blatchley, is gone; it is too late to save what might have been the most herpetologically diverse site in Indiana. But Dave's Pond remains, and it is not too late to save it. It is still a site of significant herpetological diversity and has added significance because of the populations of crawfish frogs and spadefoot toads that exist there. Several attempts by the authors to have state or private conservation agencies purchase the site have been unsuccessful. Since most of the property changed hands in 2005, researchers have been denied access to Dave's Pond and Scaphiopus Pond, on the north side of the Rio Grande Road. Areolatus Pond, on the south side of the road, continues to be accessible.

ACKNOWLEDGMENTS

We thank Jagger Foster for helping in the field and supplying information, especially on the spotted salamander. Laura Bakken helped in the editing and submitting of the final draft of the manuscript. We also thank the many friends, colleagues, and students who assisted with collecting specimens and recording data.

LITERATURE CITED

Blatchley, W.S. 1891. Notes on the batrachians and reptiles of Vigo County, Indiana. Journal of the Cincinnati Society of Natural History 14:22–35.

Blatchley, W.S. 1899. Notes on the batrachians and reptiles of Vigo County, Indiana – II. Annual Report of the Indiana Department of Geology and Natural Resources 24:537–552.

Crother, B.I., et al. 2012. Scientific and Standard English Names of Amphibians and Reptiles of North America, North of Mexico, with Comments Regarding Confidence in our Understanding. Seventh Edition. Publications of the Society for the Study of Amphibians and Reptiles No. 39. 92 pp.

Engbrecht, N.J., J.A. Crawford & M.J. Lannoo. 2009. Geographic Distribution: Lithobates (=Rana) blairi. Herptological Review 40(4):446.

Engbrecht, N.J. & M.J. Lannoo. 2010. A review of the status and distribution of Crawfish Frogs (*Lithobates areolatus*) in Indiana. Proceedings of the Indiana Academy of Science 119(1):64–73.

Engbrecht, N.J., M. Lannoo, P.J. Williams, J.R. Robb, T.A. Gerardot, D.R. Karns & M.J. Lodato. 2012. Is there hope for the Hoosier frog? An update on the status of Crawfish Frogs (*Lithobates areolatus*) in Indiana, with recommendations for their conservation. Proceedings of the Indiana Academy of Science 121(2):147–157.

Minton, S.A. Jr. Amphibians and Reptiles of Indiana. Indiana Academy of Science Monograph 3. Indianapolis, Indiana. 346 pp.

Minton, S.A. Jr. Amphibians and Reptiles of Indiana. Second edition. Indiana Academy of Science, Indianapolis, Indiana. 404 pp.

Rubin, D. 1965a. Amphibians and Reptiles of Vigo County, Indiana. M.A. Thesis. Indiana State University, Terre Haute.

Rubin, D. 1965b. Occurrence of the Spadefoot Toad, *Scaphiopus holbrooki* (Harlan), in Vigo County, Indiana. Herpetologica 21(2):153–154.

Rubin, D. 1968. Amphibian breeding dates in Vigo County, Indiana. Proceedings of the Indiana Academy of Science 77:442–444.

Rubin, D. 1973. Distributional summary of the amphibians and reptiles of Vigo County, Indiana. Proceedings of the Indiana Academy of Science 82:465–468.

Shannon, C.W. 1911. Soil survey of Clay, Knox, Sullivan, and Vigo Counties, Indiana. Annual Report of the Indiana Department of Geology and Natural Resources 36:135–280.

Smith, P.W. 1961. The Amphibians and Reptiles of Illinois. Illinois Natural History Survey Bulletin 28(1):1–298.

Whitaker, J.O. Jr. A study of the Western Chorus Frog, *Pseudacris triseriata*, in Vigo County, Indiana. Journal of Herpetology 5(3–4):127–150.

Whitaker, J.O. Jr., D. Rubin & J. Munsee. 1977. Observations on food habits of four species of Spadefoot Toads, genus *Scaphiopus*. Herpetologica 33(4):468–475.

Manuscript received 14 October 2013, revised 30 March 2014.

2013. Proceedings of the Indiana Academy of Science 122(2):140–151

RELATIONSHIPS BETWEEN MULTI-SCALE ENVIRONMENTAL AND LAND-USE FACTORS AND SUMMER DEMOGRAPHICS OF THE NORTHERN CLEARWATER CRAYFISH, *ORCONECTES PROPINQUUS* (DECAPODA: CAMBARIDAE)

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ABSTRACT. Crayfish are structurally important in streams as a main component in the food chain and as decomposers of organic material. They exhibit wide sensitivities to environmental disturbance and serve as response indicators of habitat degradation and anthropogenic effects. Thirty stream reaches in central Indiana were sampled to determine relationships between relative abundance, size, age, sex, and habitat associations of the Northern Clearwater Crayfish, *Orconectes propinquus*. Females were significantly more abundant than males ($P = 0.08303$). The frequency of crayfish in gravel substrate was significantly higher than that of cobble substrate ($P < 0.0001$). The size of crayfish in cobble substrate were significantly larger ($P < 0.001$) than individuals found in gravel substrates, while females were significantly larger ($P = 0.013$) than males in gravel substrates. Watershed variables were not significantly related to crayfish abundance. The only reach scale variable that proved to be significant ($P = 0.084$) was a boulder substrate score. Microhabitat variables showed a significant increase between catch-per-unit-effort (CPUE) and cobble ($P = 0.083$) and gravel ($P = 0.099$) substrates.

Keywords: watershed-scale, reach-scale, microhabitat, age, cumulative frequency distribution

INTRODUCTION

Tertiary burrowing crayfish play a vital role in the structure and function of stream ecosystems (Momot 1995; Butler et al. 2003). Tertiary burrowing crayfish are decapods that live the majority of life in open bodies of water and depend on weakly constructed burrows during drought conditions for survival (Hobbs 1981). They are structurally important in a stream as ecosystem engineers and are a key component of the food chain (Taylor et al. 1996; Creed & Reed 2004). Fish, turtles, salamanders, birds, and mammals all utilize crayfish as a primary source of food (Bovbjerg 1952; Rabeni 1992). Crayfish can be a keystone species in many stream ecosystems by affecting species throughout many trophic levels in aquatic food webs (Parkyn et al. 1997; Flinders & Magoulick 2005). Crayfish are also decomposers of organic material within a stream and contribute to overall stream health and energy transformation between trophic levels (Butler et al. 2003; Montemarano et al 2007; Stewart et al 2010). Crayfish are sensitive indicators of habitat degradation and respond to anthropogenic effects in streams. Species composition and relative abundance reflects anthropogenic response to water quality, habitat, land use change, and stressors (Butler et al. 2003; Simon & Morris 2009).

The Northern Clearwater Crayfish, *O. propinquus* (Girard 1852) is a tertiary burrowing crayfish (Hobbs Jr. 1989). The species is common in areas of North America ranging from southern Ontario and Quebec, as far south as southern Illinois, Indiana, Ohio, Pennsylvania, and New York, and as far west as Iowa and Minnesota (Hobbs Jr. 1989; Crocker & Barr 1968). The species is most common in Midwestern United States headwater streams (Simon 2001). *O. propinquus* is found in both stream and lake ecosystems (Page 1985; Hobbs III 1988). The species' habitat is typically rocky riffle habitat in

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streams (Crocker & Barr 1968; Page 1985; Taylor & Schuster 2004; Momot 1966), but they prefer coarse habitat that can provide cover from predators. They function as omnivores and feed on plant material and various invertebrate larvae in streams (Page 1985).

Watershed and reach-scale land use can influence chemical and physical factors associated with a stream (Allan 2004). On a reach-scale, the channel morphology can be largely influenced by bank material, riparian vegetation, and the slope at which water and other inputs enter the stream (Allan 2004; Wang et al. 1997). The resulting channel morphology and substrate can determine the types of species that will likely inhabit that particular reach. Watershed-scale land uses are known to have negative impacts on stream ecosystems (Burskey & Simon 2010). For example, agricultural practices increase sediment inputs and nutrients into streams and may negatively affect water quality, habitat, and biological assemblages (Allan 2004; Nerbonne & Vondracek 2001). Excessive sediment loads in streams can negatively affect macroinvertebrates, such as crayfish, by reducing food sources and filling in interstitial pore spaces in preferred habitats (Nerbonne & Vondracek 2001). Urban land use has also been found to reduce stream habitat quality by the addition of chemical contaminants (Wang et al. 1997). Alternatively, many types of land use can improve stream quality. Forest land use has been found to correlate with high quality habitat and also for bank stability and instream cover (Wang et al. 1997)

Factors known to influence crayfish distribution at the stream reach-scale include presence of predators, amount and stability of instream cover, age and body size, food sources, and competition among other crayfish (Stewart et al. 2010; Rabeni 1985). Larger crayfish are best able to defend themselves (Stein 1977) and are more capable of obtaining preferred cover through competition (Stewart et al. 2010).

The four primary objectives of this study were to: (1) determine overall CPUE patterns with watershed-, reach-, and microhabitat-scale associations for *O. propinquus* based on gender and habitat; (2) determine variation in size among *O. propinquus* based on gender and habitat associations; (3) examine the age range that exist in headwater streams based on gender and habitat associations; and (4) determine those factors that affect the CPUE of *O. propinquus* at watershed-, reach-, and microhabitat-scales.

METHODS

Study area.—The study area was within the Interior Plateau Level III ecoregion of Indiana (Woods et al. 2011). This ecoregion is characterized by rolling and heavily dissected, rugged terrain (Woods et al. 2011). The underlying soil is composed of sandstone, siltstone, shale, and limestone (Woods et al. 2011). The ecoregion consists of high hills and knobs, and low and narrow valleys. The streams of this region are medium to high gradient (Woods et al. 2011).

A total of 30 sites were sampled in the counties of Brown, Monroe, Morgan and Lawrence in south central Indiana (Figure 1). Sites are located in the East Fork White River watershed, which is dominated by karst topography and limestone quarries (Rapid Watershed Assessment 2011). Land use is mainly agricultural cropping and livestock pasturing, but includes several forest types. Forested areas were the most common land use buffering the focus streams, composing 57% of the total land use.

Study design.—Site selection was chosen based on a random probability study design. Sites were classified by Strahler stream order (Strahler 1957) and selected without replacement from the universe of wadeable first through third order streams in the four county areas (Stevens & Olsen 2004). These sites were previously sampled as part of the U.S. Environmental Protection Agency (USEPA) Regional Monitoring and Assessment Program (REMAP) in the Eastern Corn Belt Plain study. Stream site conditions ranged from the highest quality streams in south central Indiana to those of lower quality due to poor land management practices (Simon & Dufour 1998).

The following research questions and *a priori* hypotheses were tested to evaluate the association between habitat, relative abundance, and CPUE of Northern Clearwater Crayfish based on gender, age, and landscape scale factors.

Questions regarding relative abundance included whether there is an equal distribution of males and females based on gender ratios, and whether male to female gender ratios are equal in cobble and gravel substrates. We evaluated the percentage of cobble and gravel substrates at each site using a qualitative habitat proce-

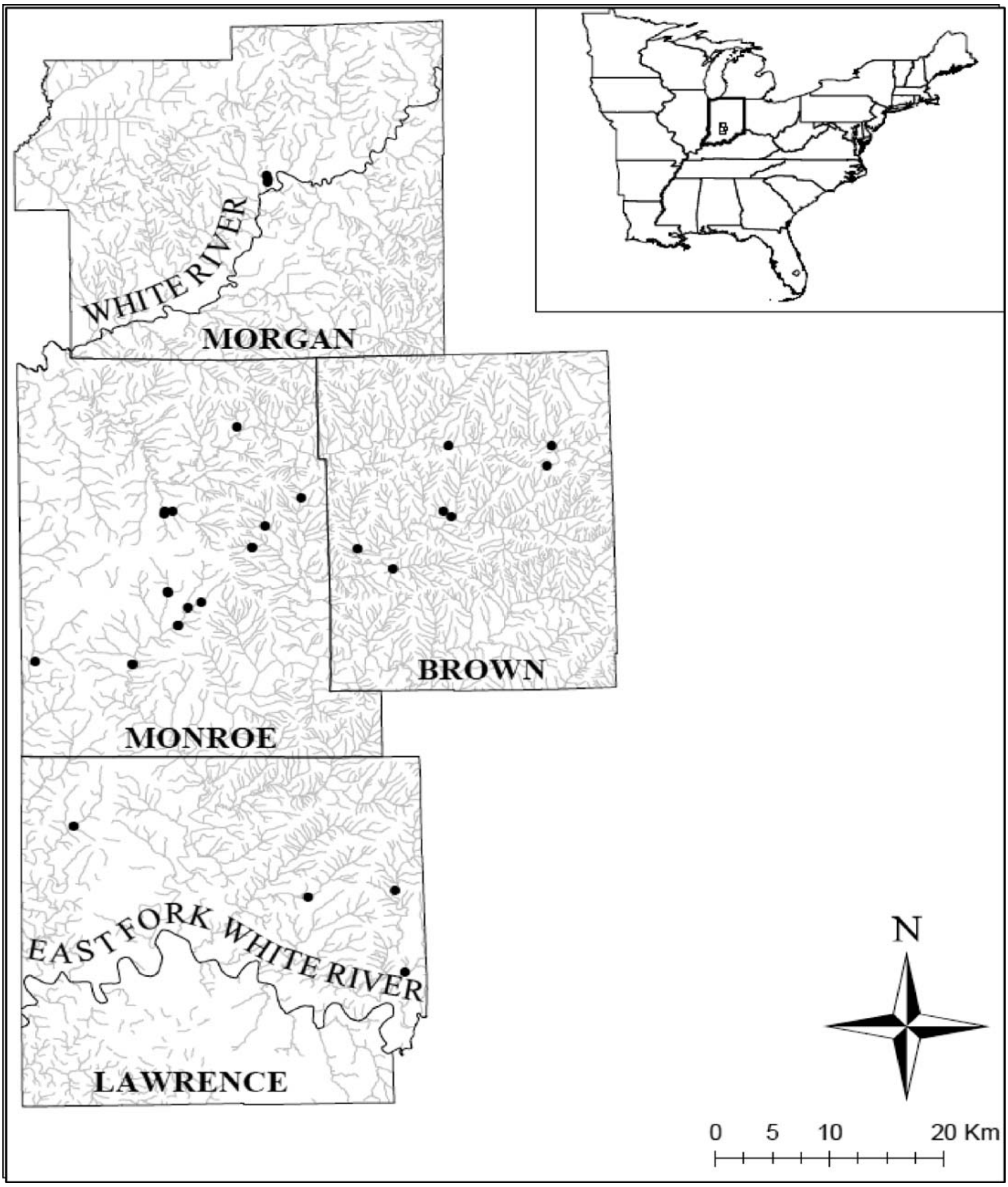

Figure 1.—Study sites (black dots) sampled during an investigation of *O. propinquus* habitat associations in southcentral Indiana, USA, headwater streams.

dure (Rankin 1989). Within similar relative abundance categories, we evaluated whether there would be a greater CPUE of *O. propinquus* in cobble substrate compared to gravel substrates

We explored two size-related questions, including whether *O. propinquus* is larger in size in large coarse substrates compared to smaller coarse substrates, and whether males are larger than females.

Scale and habitat associated questions were placed into three categories, including watershed-, reach-, and microhabitat-scales. For the watershed-scale associations, we evaluated whether the CPUE of crayfish changes with land use, whereas for reach scale we evaluated whether increasing reach scale habitat heterogeneity led to greater CPUE. For microhabitat scale associations we explored whether the CPUE increases with increasing size of substrate. Finally, age was evaluated to determine if ontogenetic differences existed in age class use of large-coarse compared to moderate-coarse substrates.

Field sampling.—The stream reach length sampled was 15 times the wetted width (Simon 2004). The study stream reaches ranged from a minimum distance of 50 meters (m) to a maximum distance of 250 m. Sampling proceeded in an upstream manner beginning at the downstream end of the stream reach, thereby reducing disturbance to upstream crayfish.

The sampling events occurred between June 17 and July 18, 2010, and generally followed the method used by Simon (2004). A one-man minnow seine (1 m × 1 m) with 3.1 mm standard mesh netting was used to collect crayfish by kick-seining a 1 m^2 area of substrate directly upstream of the seine (Mather & Stein 1993). Crayfish were sampled from twenty 1 m^2 plots of habitat randomly distributed in the stream reach, which represent the coarse substrate habitat portions of each stream reach (Barbour et al. 1999). The CPUE is defined by the number of crayfish collected within a m^2. Captured individuals of *O. propinquus* and all other crayfish species were counted, sexed, and released after the completion of the sampling event.

A total of twenty 1 m^2 seine samples were completed at each site; ten samples each were randomly located in both gravel-dominated and cobble-dominated substrates. Substrate size was classified following USEPA physical habitat procedures (Kaufmann et al. 1999), and seine sample locations were classified as either cobble or gravel based on the dominate substrate ($>$ 50%).

Stream width measurements at each site included wetted and bankfull widths (Kaufmann et al. 1999). The wetted width is the perpendicular measurement from shoreline to shoreline. Bankfull width measures the lateral extent of water that fills the channel to the top of each bank during periods of high flow.

Laboratory methods.—Individual *O. propinquus* that were too small to sex or measure in the field were taken to the laboratory where carapace length (CL) and postorbital carapace length (POCL; Hobbs Jr. 1981) and sex were recorded (Figure 2). Crayfish specimens were deposited in the Astacology collection at the Aquatic Research Center of the Indiana Biological Survey, Bloomington, Indiana.

Watershed-scale variables.—ArcMap 10.0 was used to overlay the watershed boundary with stream hydrology and 2006 land cover site information. The stream and land cover data were obtained from IndianaMap.org (Indiana map 2011). The stream layer included the 2008 National Hydrology Dataset (NHD) and was derived at 1:100,000 scale. The land cover layer included the 2006 USGS 30-meter resolution National Land Cover Data (NLCD). The percentage of each land use type was determined from the land use layer for each individual watershed.

Watershed-scale variables included 15 land cover types (Watershed Delineation Model 2013), which represent the number of acres contained within the area upstream of the most downstream margin of the sampled reach (listed in Table 5) and three additional variables (i.e., latitude, longitude, drainage area). The watershed boundaries and land cover types were delineated using the Watershed Delineation Model (2013), which utilizes the digital elevation associated with specific latitudes and longitudes. The drainage area for each of the 30 sites sampled were obtained from US Geological Survey sources (Hoggatt 1975).

Reach-scale variables.—Reach-scale variables were derived from qualitative habitat measures defined in the Qualitative Habitat Evaluation Index (QHEI; Rankin 1989). The habitat variables include a variety of habitat qualities within the wetted stream width and the riparian area of the stream. The qualitative habitat variables include the following categories: substrate types, instream cover, channel morphology, riparian quality/bank erosion, pool/glide and riffle/run quality, and local stream gradient. Each qualitative habitat category is ranked by a series of categories representing varying states of stream habitat condition. The total reach habitat score is the sum of each of the category scores, which provides a cumulative score for the entire stream reach. Each qualitative categorical score and the total reach habitat score was regressed against crayfish relative abundance to determine any significant relationships between the habitat category and crayfish relative abundance.

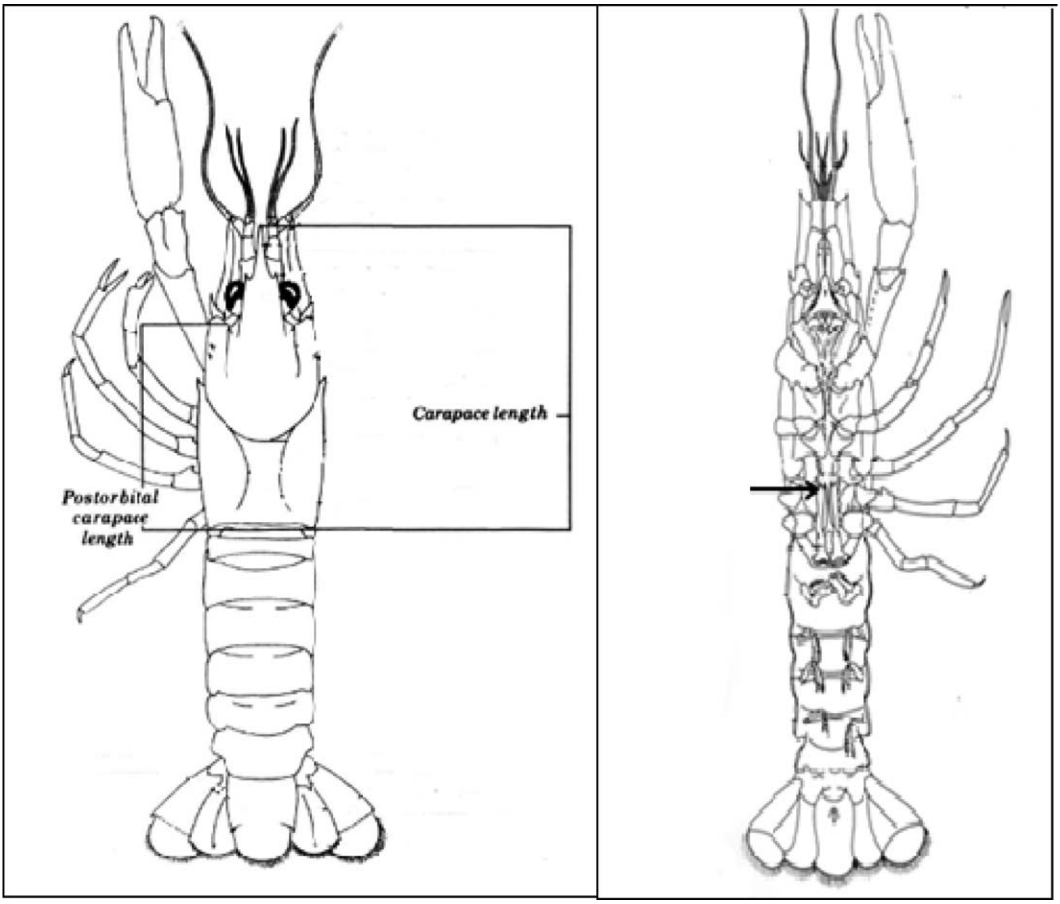

Figure 2.—Dorsal and ventral view of crayfish showing various measurements. a) CL and POCL are shown, and b) ventral view of male crayfish showing the location of sexual reproductive organs (adapted from Page 1985).

Individual substrate particle size categories for each stream reach were compared to crayfish CPUE to determine if any significant relationships existed. The substrate types observed included boulder, cobble, gravel, sand, bedrock, detritus/muck, and artificial. Each specific substrate size class was determined for each reach site and was used for the comparison based on the percent of the sample area occupied by each substrate type.

Several other physical reach-scale factors were evaluated including the total percentage of pool, run, and riffle habitat, and the wetted and bankfull width measurements for each reach. Each variable was compared to the CPUE of crayfish at each site.

Microhabitat-scale variables.—Two microhabitat-scale variables examined include the two primary coarse substrate types (cobble-dominated substrate and gravel-dominated substrate). At each site 10 random m^2 samples in each of the two substrate types were sampled using a kick seine method to collect individual crayfish. A CPUE was calculated based on the 10 seine samples in each substrate size class and compared to evaluate associations between gender, size, and CPUE with each of the microhabitat substrate types.

Statistical methods.—A variety of statistical methods were used to analyze each category of questions that were examined (Sokal & Rolf 1995). Basic statistics using Statistica (StatSoft Inc. 2012) were used for all analyses. Each statistical analysis conducted used a significance value of $\alpha = 0.10$ for field evaluation and a Tukey HSD post-hoc test. Analysis of

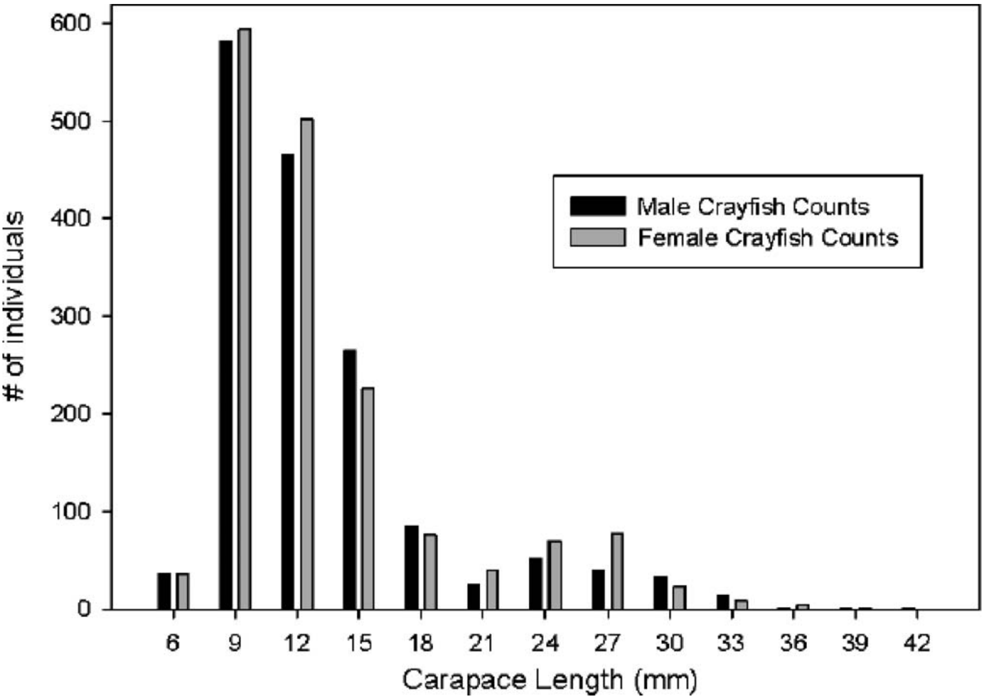

Figure 3.—Length frequency distribution showing the number of *O. propinquus* by sex.

biological community effects at landscape scales commonly utilize an $\alpha = 0.10$ since sample sizes are small ($n=30$) and the landscale units are often at large units (Burskey & Simon 2010; Stewart et al. 2010). Differences between relative abundance and CPUE of male and female *O. propinquus* among cobble and gravel habitats were determined using a Z-test. Differences between populations in crayfish length were assessed using a one-way Analysis of Variance (ANOVA). A length-frequency distribution was developed to evaluate differences in age structure. A simple univariate linear regression was used to analyze each category of the habitat-scale factor questions. The regressions compared a specific watershed, reach, or microhabitat variable with the CPUE of crayfish at each site.

RESULTS

Relative abundance and CPUE.—A total of 2,648 *O. propinquus* were collected from 29 of the 30 sites that were sampled during this study. *O. propinquus* individual CPUE effort ranged from 0 to 19.1 individuals/ m^2 at each stream reach. The number of males compared to females was consistent by site with males comprising 990 individuals and females 1,048 individuals (Figure 3). The sex ratio was 1:1.05 males to females. A total of 610 juveniles (range: 4.4 mm to 9.8 mm CL) were captured. The crayfish were classified as juvenile if the individuals were too small to determine the sex. In crayfish early development the primordial gonads of both genders have an androgenic gland, which develops further in males while disappearing in females. The number of crayfish captured in cobble-dominated substrates was 989, while 1,049 were collected from gravel-dominated substrates (Figure 4).

The predicted outcome was an equal CPUE of males and females for all sites, and an equal CPUE of males and females in both cobble- and gravel-dominated substrates. Females were significantly more abundant than males in the stream reaches (Z-statistic $= -1.733$, $P = 0.083$). For the comparison of crayfish occupying cobble-dominated compared to gravel-dominated substrate, the CPUE was significantly ($P < 0.001$) different in gravel-dominated substrates (Table 1).

Length frequency distribution and age range.—The mean CL for all of the crayfish collected was 12.7 mm. *O. propinquus* ranged in CL from

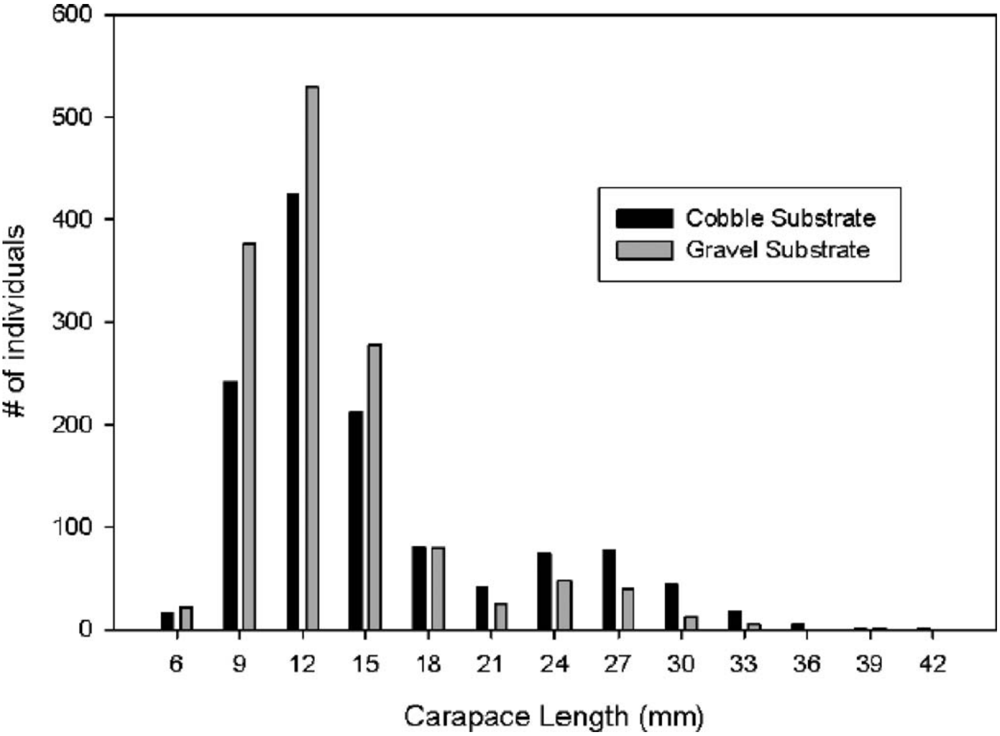

Figure 4.—Length frequency distribution of *O. propinquus* partitioned by substrate size class (cobble-dominated, gravel-dominated) in which they were collected. Large substrates include boulder and cobble, while small substrates include large and fine gravel.

4.4 mm to 39.8 mm. Mean CL was significantly larger for crayfish found in cobble-dominated substrates ($P < 0.001$) compared to gravel-dominated substrates (Figure 4). Females collected in gravel-dominated substrates had significantly larger CLs than males ($P = 0.013$; Table 2).

Three age classes were observed in this study (Table 3). Both male and female *O. propinquus* individuals attained similar size at each age. Age 0 individuals were 3–18 mm CL; age 1 individuals were 18–33 mm CL; and age 2 individuals were 33–42 mm CL. The length-frequency distribution showed the greatest number of individuals occurred at age 0 (cobble = 973, gravel = 1285; and males = 1433, females = 1434). The number of individuals decreased with increasing age group. Only eight individuals were found in the 2-year age group, and no individuals reached age 3 (Tables 3 and 4). Large individuals (>18 mm CL) had a greater occurrence in cobble substrates compared to smaller individuals (3–18 mm CL), which were more common with gravel substrates (Table 4).

Habitat scale factors.—None of the 18 watershed-scale variables showed a significant relationship with *O. propinquus* CPUE (Table 5).

Table 1.—Z-test statistical values for CPUE (individuals/m^2) comparisons between *O. propinquus* gender and substrate size (α=0.10).

Relative Abundance	Z	P -two-tail
Male vs. Female: all sites	−1.733	0.083
Male vs. Female Cobble	<.001	0.999
Male vs. Female Gravel	<.001	0.999
Cobble vs. Gravel	−4.340	<0.0001

Table 2.—F-test P-values (α=0.10) for *Orconectes propinquus* CPUE (individuals/m^2) comparisons between size, gender, and substrate type.

Carapace Length	F	P-value
Cobble vs. Gravel	70.643	< 0.001
Male vs Female: All Sites	0.547	0.460
Male Cobble vs. Female Cobble	0.601	0.438
Male Gravel vs. Female Gravel	6.140	0.013

Table 3.—Age class frequency distribution for male and female by age class. CL= carapace length.

Sex/Size class CL (mm)	N	Age
Male		
3–18	1433	0
18–33	164	1
33–42	3	2
Female		
3–18	1434	0
18–33	219	1
33–42	5	2

Only a single reach-scale variable, boulder substrate, was significantly correlated with crayfish CPUE (Table 6). An increasing amount of boulder habitat was associated with a decrease in crayfish relative abundance. Both microhabitat-scale factors, cobble- (P = 0.083) and gravel-dominated (P = 0.099) substrates, were positively associated with *O. propinquus* CPUE (Table 7).

DISCUSSION

The relative abundance of crayfish is dependent on available stream substrate types (Stewart et al. 2010; Burskey & Simon 2010; Rabeni 1985). The five lowest crayfish CPUE, i.e., 0, 2, 5, 10, and 15 individuals/m^2, occurred at sites with reduced reach-scale habitat. Guthrie Creek, Bean Blossom Creek, Griffey Creek, and two reaches at Sycamore Creek included variables that influenced the crayfish population. For example, Bean Blossom Creek (n = 2 individuals/m^2) was a stagnant stream with muck substrate, whereas Griffey Creek (n=5 individuals/m^2) was heavily impounded with an embedded substrate. These substrate factors are considered responsible for declining crayfish relative abundance due to reduced amounts of preferred substrate and instream cover.

Table 4.—Age class frequency distribution by size and coarse substrate type.

Substrate/Size class, CL (mm)	N	Age
Cobble		
3–18	973	0
18–33	254	1
33–42	7	2
Gravel		
3–18	1285	0
18–33	129	1
33–42	1	2

Table 5.—Simple linear regression (R^2, F-test, Significant F, and P-value, α=0.10) relationships between watershed-scale land use variables and *O.s propinquus* CPUE from headwater streams in south central Indiana.

Watershed Variables	R^2	*F*	P-value
Open water	0.0002	0.005	0.945
Developed open spaces	0.003	0.080	0.779
Developed low intensity residential	0.001	0.038	0.846
Developed medium intensity residential	< 0.0001	0.0009	0.976
Developed high intensity residential	0.0006	0.017	0.896
Deciduous forest	0.002	0.044	0.835
Evergreen forest	0.009	0.247	0.623
Mixed forest	0.042	1.213	0.280
Shrub/Scrub	0.021	0.607	0.443
Grasslands/Herbaceous	0.088	2.716	0.111
Pasture/Hay	0.002	0.047	0.830
Cultivated crop	0.072	2.113	0.157
Barren land	0.034	0.990	0.328
Woody wetland	0.005	0.153	0.699
Emergent herbaceous wetland	0.003	0.094	0.761
Latitude	0.050	1.461	0.236
Longitude	< 0.0001	0.0005	0.982
Drainage area	0.014	0.405	0.530

Rabeni's (1985) study, based on two *Orconectes* species, demonstrated that larger individual crayfish correlated with larger substrates particles. Crayfish substrate preference is typically associated with areas that offer the most overall cover and protection from predators (Stein & Magnuson 1976). Larger crayfish select larger substrates that provide the most cover. The larger substrates will provide more overall interstitial spaces, which provide more areal coverage for protection from predators (Stein & Magnuson 1976). CL was significantly correlated with large substrate sizes compared to small substrates; however, since the study area was not glaciated during the latest Wisconsin glaciation event the dominant particle size in the study area is cobble. Individual *O. propinquus* were associated with cobble substrates that exhibited the highest CPUE (Table 4), so that our study found that as substrate particle size increases so does the CPUE and size of individual crayfish.

Large individuals were associated with large substrate particle size and when mature adults

Table 6.—Simple linear regression (R^2, F-test, Significant F, and P-value, α=0.10) relationships between reach-scale variables and *O. propinquus* relative abundance from headwater streams in south central Indiana.

Reach-Scale Variable	R^2	*F*	P-value
Stream width			
Wetted Width (m)	0.0004	0.010	0.921
Active Width (m)	0.0018	0.050	0.825
Bank Full (m)	0.0300	0.866	0.360
Habitat			
Substrate	0.0150	0.426	0.519
Instream Cover	0.0043	0.122	0.729
Channel Morphology	0.0072	0.204	0.655
Bank Erosion and Riparian Zone	0.0480	1.412	0.245
Pool/ Current	0.0043	0.122	0.729
Riffle/Run	0.0213	0.608	0.442
Gradient	0.0016	0.046	0.831
QHEI Total Score	0.0006	0.017	0.897
Substrate			
Boulder	0.1026	3.202	0.084
Cobble	0.0544	1.611	0.214
Gravel	0.0222	0.637	0.431
Sand	0.0226	0.649	0.427
Bedrock	0.0026	0.074	0.787
Detritus/Muck	0.0698	2.102	0.158
Artificial	0.0287	0.828	0.371
Morphology			
% Pool	0.0374	1.090	0.305
% Run	0.0394	1.148	0.293
% Riffle	0.0003	0.008	0.930

Table 7.—Simple linear regression (R^2, F-test, Significant F, and P-value, α=0.10) relationships between microhabitat-scale substrate type and *O. propinquus* CPUE (number individuals/ m^2) from headwater streams in south central Indiana.

Microhabitat Variables	R^2	*F*	P-value
CPUE Cobble	0.103	3.232	0.083
CPUE Gravel	0.094	2.904	0.099

were present, smaller individual crayfish typically were associated with small, gravel substrates (Stewart et al. 2010; Rabeni 1985). *O. propinquus* individuals were more abundant in gravel substrates than in cobble substrates; however, this was based on the association between CPUE and high number of age 0 individuals. Overall, Age 0 crayfish comprised the largest proportion of individual crayfish at all sites (n = 2258; 85.3%). A niche shift from small substrates to large substrate occurs at lengths greater than 18 mm CL. This niche shift demonstrates that individual crayfish select increasing substrate particle size proportional to increasing body size. Likewise, small age 1 individuals showed similar response as age 0 individuals with increasing CPUE in the less preferred gravel substrates.

Rabeni (1985) demonstrated that often the primary factor that determines crayfish dominance is size. Other studies have also shown that the dominance of many freshwater crayfish is based on size (Stewart et al. 2010; Pavey & Fielder 1996). The study area male to female sex ratio is 1:1.05. Male CPUE was expected to be greater than the CPUE for females, which was based on another assumption that males would be significantly larger than females. However, both of these expected outcomes did not prove to be true. Females were not larger than males in general; however, females were significantly larger than males in gravel substrates. This suggests that females could have a slight numerical advantage over males during the early stages of their lives or be forced into smaller substrate particle sizes due to dominance and territoriality. This would provide one explanation to females being significantly more abundant than males in the streams sampled. However, females would be exposed to increased predation pressure affecting female CPUE with increasing age class. Another possible explanation would be that females grow at a slightly slower pace than males, which could possibly skew the defined age classes. More research into the growth rates and size classes based on sex could better determine the causes.

Linear regression models showed little significance between scale variables tested at watershed- and reach-scales. This was a similar result observed by Burskey & Simon (2010) and Stewart et al. (2010). All study area watersheds comprised relatively small drainage area sizes (range: 9.1 to 49,166 acres). We selected headwater streams to isolate potential impacts and increase the percentage of catchment forested land use. Forested areas provided a large amount of coarse particulate organic matter (CPOM), which are positive factors for stream ecosystems (Englan & Rosemond 2004). Forests provide large amounts of organic material and detritus, which are very important for crayfish survival (Saffran & Barton 1993).

The high percentage of forested areas (mean: 57% for all sites) in these watersheds represent a least impacted condition for crayfish populations. A large amount of forested area is considered to be the most important factor for explaining low significance in watershed-scale analyses, since the forested landscape represented by our study area is larger than usual for most other watersheds. Forested landscapes represent the best case scenario and the high relative abundance attained in this study represents the maximum attainable condition.

Watershed land cover effects were not found to affect crayfish populations, whereas other studies linked various land use types to low crayfish abundance (Stewart et al. 2010; Simon & Morris 2009; Burskey & Simon 2010; Hrodey et al. 2009). Row-crop agriculture, urban, and developed areas have been shown to negatively impact aquatic habitats and fish and macroinvertebrate communities (Simon & Morris 2009); however, agricultural land use was not a predominant component in the study streams.

Reach scale stream variables scores showed increasing levels in the study area (cumulative habitat score range: 37.5 to 91.0, mean = 72.9). These relatively high reach scale habitat values show that streams represented relatively high overall ecological integrity. The only correlated variable with crayfish abundance included reach scale habitat substrate boulder proportion. Boulder presence showed a negative correlation with individual crayfish CPUE. This result seems contradictory; however, boulder substrate provide large interstitial spaces affording cover and habitat for predators. The univariate microhabitat-scale regression models showed a significant relationships between CPUE and both cobble and gravel substrates. This suggests that increasing amounts of coarse substrates correlates with increases in *O. propinquus* CPUE and may be differentially important for various life stages.

ACKNOWLEDGEMENTS

We thank the Indiana Biological Survey Aquatic Research Center, and Indiana University School of Public and Environmental Affairs, for professional courtesies and support of this project. Vehicles and equipment were provided by the Aquatic Research Center. Special thanks to Wade Kimmon, Richard Barendt, and Alex Jackson for field assistance.

LITERATURE CITED

Allan, J. 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics 35:257–284.

Barbour, M.T., J. Gerritsen, B.D. Snyder & J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Bovbjerg, R.V. 1952. Comparative ecology and physiology of the crayfish, *Orconectes propinquus* and *Cambarus fodiens*. Physiological Zoology 25:34–56.

Burskey, J.L. & T.P. Simon. 2010. Reach- and watershed-scale associations of crayfish within an area of varying agriculture impact in west-central Indiana. Southeastern Naturalist 9:199–216.

Butler, R.S., R.J. DiStefano & G.A. Schuster. 2003. Crayfish: An overlooked fauna. Endangered Species Bulletin 27:10–11.

Creed, R.P. & J.M. Reed. 2004. Ecosystem engineering by crayfish in a headwater stream community. Journal of the North American Benthological Society 23:224–236.

Crocker, D.W. & D.W. Barr. 1968. Handbook of the Crayfishes of Ontario. Royal Ontario Museum, University of Toronto Press, Toronto (CA).

England, L.E. & A.D. Rosemond. 2004. Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater streams. Freshwater Biology 49:721–734.

Flinders, C.A. & D.D. Magoulick. 2005. Distribution, habitat use and life history of stream-dwelling crayfish in the Spring River drainage of Arkansas and Missouri with a focus on the imperiled Mammoth Spring crayfish (*Orconectes marchandi*). American Midland Naturalist 154: 358–374.

Girard, C. 1852. A revision of the North American Astaci, with observations on their habits and geographical distribution. Proceedings of the Academy of Natural Sciences, Philadelphia 6.

Hobbs, Jr., H.H. 1981. Crayfishes of Georgia. Smithsonian Contributions in Zoology Number 318.

Hobbs, Jr., H.H. 1989. Illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). Smithsonian Contributions in Zoology Number 480.

Hobbs, III, H.H. & J.P. Jaas. 1988. The crayfishes and shrimp of Wisconsin. Milwaukee Public Museum, Milwaukee, WI.

Hoggatt, R.E. 1975. Drainage areas of Indiana. U.S. Department of the Interior, Geological Survey, Water Resources Division, Indianapolis, IN.

Hrodey, P.J., T.M. Sutton, E.A. Frimpong & T.P. Simon. 2009. Land-use impacts on watershed health and integrity in Indiana warmwater streams. American Midland Naturalist 161:76–95.

Indiana Map. 2011. Indiana Geological Survey. Retrieved January 21, 2011, from http://inmap.indiana.edu/download.html.

Kaufmann, P.R., P. Levine, E.G. Robison, C. Seeliger & D.V. Peck. 1999. Quantifying physical habitat in wadeable streams. Environmental Protection Agency (US), EPA/620/R-99/003, Corvallis, OR.

Mather, M.E. & R.A. Stein. 1993. Direct and indirect effects of fish predation on the replacement of a native crayfish by an invading congener. Canadian Journal of Fisheries and Aquatic Sciences 50:1279–1288.

Momot, W.T. 1966. Upstream movement of crayfish in an intermittent Oklahoma stream. American Midland Naturalist 75:150–159.

Momot, W. T. 1995. Redefining the role of crayfish in aquatic ecosystems. Review of Fisheries Science 3:33–63.

Montemarano, J.J., M.W. Kershner & L.G. Leff. 2007. Crayfish effects on fine particulate organic matter quality and quantity. Fundamentals of Applied Limnology./Archive für Hydrobiologie 168(3):223–229.

Nerbonne, B.A. & B. Vondracek. 2001. Effects of local land use on physical habitat, benthic macroinvertebrates, and fish in the Whitewater River, Minnesota, USA. Environmental Management 28:87–99.

Page, L.M. 1985. The crayfishes and shrimps (Decapoda) of Illinois. Illinois Natural History Survey Bulletin 33:406–412.

Parkyn, S.M., C.F. Rabeni & K.J. Collier. 1997. Effects of crayfish (*Paranephrops planifrons*: Parastacidae) on in-stream processes and benthic faunas: a density manipulation experiment. New Zealand Journal of Marine and Freshwater Research 31:685–692.

Pavey, C.R. & D.R. Fielder. 1996. The influence of size differential on agonistic behaviour in the freshwater crayfish, *Cherax cuspidatus* (Decapoda: Parastacidae). Journal of Zoology 238:445–457.

Rabeni, C.F. 1985. Resource partitioning by stream-dwelling crayfish: The Influence of body size. American Midland Naturalist 113:20–29.

Rabeni, C.F. 1992. Trophic linkage between stream centrarchids and their crayfish prey. Canadian Journal of Fisheries and Aquatic Sciences 49(8): 1714–1721.

Rankin, E.T. 1989. The Qualitative Habitat Evaluation Index (QHEI): Rationale, Methods, and Application. Ohio Environmental Protection Agency, Columbus, OH.

Rapid Watershed Assessment Lower East Fork White Watershed. 2011. US Department of Agriculture. Retrieved April 20, 2011, from http://www.in.nrcs.usda.gov/technical/RWA/Lower%20East%20Fork%20White/Lower%20East%20Fork%20White.pdf.

Saffran, K.A. & D.R. Barton. 1993. Trophic Ecology of *Orconectes propinquus* (Girard) in Georgian Bay (Ontario, Canada). Freshwater Crayfish 9:350–358.

Simon, T.P. 2001. Checklist of the crayfish and freshwater shrimp (Decapoda) of Indiana. Proceedings of the Indiana Academy of Science 110:104–110.

Simon, T.P. 2004. Standard Operating Procedures for the collection and study of burrowing crayfish in Indiana. I. Methods for the collection of burrowing crayfish in streams and terrestrial habitats. Miscellaneous Papers of the Indiana Biological SurveyAquatic Research Center 2:1–14.

Simon, T.P. & R. Dufour. 1998. Development of index of biotic integrity expectations for the ecoregions of Indiana: V. Eastern Corn Belt Plain. Environmental Protection Agency (US), Region 5, EPA 905-R-96-004, Chicago, IL.

Simon, T.P. & C.C. Morris. 2009. Biological response signature of oil brine threats, sediment contaminants, and crayfish assemblages in an Indiana watershed, USA. Archives Environmental Contamination and Toxicology 56:96–110.

Sokal, R.R. & F.J. Rolf. 1995. The Principles and Practices of Statistics in Biological Research. 3rd Edition. Freeman and Company, New York, NY.

StatSoft Inc. 2012. Electronic Statistics Textbook. Tulsa, OK: StatSoft. http://www.statsoft.com/textbook/.

Stein, R.A. 1977. Selective predation, optimal foraging, and the predator-prey interaction between fish and crayfish. Ecology 58:1237–1253.

Stein, R.A. & J.J. Magnuson. 1976. Behavioral response of crayfish to a fish predator. Ecology 57:751–761.

Stevens, D. & A.R. Olsen. 2004. Spatially-balanced sampling of natural resources. Journal of the American Statistical Association 99:262–278.

Stewart, P.M., M.M. Miller, W.H. Heath & T.P. Simon. 2010. Macrohabitat partitioning of crayfish assemblages in wadeable streams in the Coastal Plains of southeastern Alabama. Southeastern Naturalist 9:245–256.

Strahler, A.N. 1957. Quantitative analysis of watershed geomorphology. Transactions of the American Geophysical Union 38:913–920.

Taylor, A.T., M.L. Warren, J.F. Fitzpatrick, H.H. Hobbs III, R.F. Jezerinac, W.L. Pflieger & H.W. Robinson. 1996. Conservation status of crayfishes of the United States and Canada. Fisheries 21:25–38.

Taylor, C.A. & G.A. Schuster. 2004. Crayfishes of Kentucky. Illinois Natural History Survey Special Publication 28, Champaign, IL.

Wang, L., J. Lyons, P. Kanehl & R. Gatti. 1997. Influences of watershed land use on habitat quality and biotic integrity in Wisconsin streams. Fisheries. 22:6–12.

Watershed Delineation Model. (2013), Watershed delineation software, retrieved June 27, 2013, from https://engineering.purdue.edu/~lthia/.

Woods, A.J., J.M. Omernik, C.S. Brockman, T.D. Gerber, W.D. Hosteter & S.H. Azevedo. 2011. Ecoregions of Indiana and Ohio [map]. U.S. Environmental Protection Agency (US), Corvallis, OR. Retrieved December 4, 2012, from http://www.epa.gov/wed/pages/ecoregions/ohin_eco.htm.

Manuscript received 17 September 2013, revised 14 March 2014.

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