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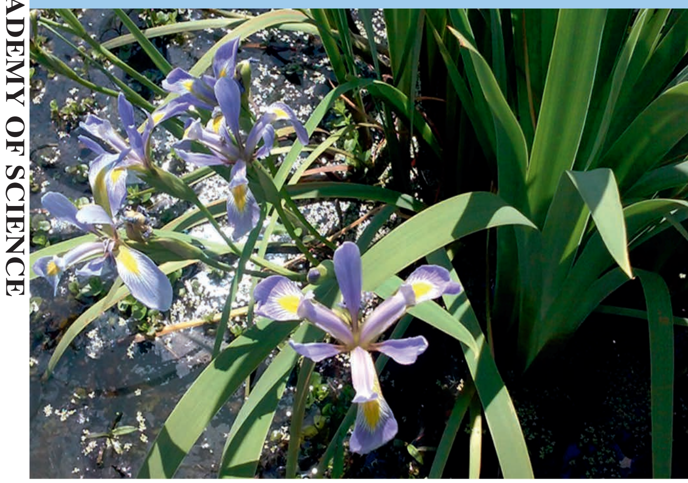

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Cover: Some of the species observed during the 2014 Eagle Marsh Biodiversity Survey or Bioblitz, Allen County, Indiana. (See the article summarizing the results of the bioblitz in this issue of the Proceedings). Upper left: *Iris virginica* var. *shrevei* (Iridaceae), Southern Blue Flag. This is the most common species of wetland iris found in Indiana. (Photo by Paul McMurray) Bottom left: *Xylobolus frustulatus* (Stereaceae), Ceramic Parchment fungus. This lignicolous (wood rotting) mushroom is common and widespread in Indiana. The fruit body (basidioma) forms small, hard, flat crust-like aggregations that resemble broken pieces of ceramic tile. (Photo by Steve Russell) Top right: *Apalone spinifera* (Trionychidae), Spiny Softshell Turtle. This species gets its name from the spiny, cone-like projections on the leading edge of its upper shell (carapace). It is one of the largest freshwater turtle species in North America. (Photo by Bob Brodman) Lower right: *Etheostoma nigrum* (Percidae), Johnny Darter, female. This small, slender fish, averaging 51 mm in length, is native to shallow waters throughout North America east of the Rocky Mountains. (Photo by Brant Fisher)

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

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MONITORING *IN VITRO* RESPONSE OF SELENIUM-TREATED HUMAN PROSTATE CELLS BY ^{1}H NMR SPECTROSCOPY

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ABSTRACT. NMR metabolomics provides a potent method for monitoring alterations in the metabolic signature within tissues and biofluids. In this study, NMR analysis was utilized to determine variation in metabolite levels of human DU145 prostate cancer and non-tumorigenic PNT1A prostate epithelial cells after treatment with selenomethionine (SeM) and Se-methylselenocysteine (SeMSC). Currently, these are the first ^{1}H NMR spectroscopic data on selenium-treated prostate cell lines. Fluorescence microscopy of SeM-incubated PNT1A cells revealed morphological features characteristic of apoptosis. SeMSC-treated PNT1A and DU145 prostate cells indicated greater changes in cellular morphology and in metabolite levels than SeM-treated cells. NMR of prostate cells treated with selenium showed a decreasing trend in metabolite levels with the largest change exhibited by creatine. This is mainly due to disrupted energy metabolism, and probably due to loss of structural integrity combined with dissipation of metabolites. Lactate, choline-containing compounds, and glycine levels increased depending on the type of selenium and cell line used. No clear pattern of variation in metabolite concentration levels from ^{1}H NMR spectroscopy to distinguish apoptotic versus non-apoptotic pathway was observed. Factor analysis (FA) indicated the change in the concentration levels of twelve metabolites was able to distinguish DU145 cells from PNT1A cells when treated using SeM. This study indicated that NMR of intact cells treated with selenium can provide information on the biochemical processes of tissues; thus metabolic fingerprints for compromised cells can be acquired.

Keywords: NMR spectroscopy, metabolomics, selenomethionine, Se-methylselenocysteine, prostate cancer, apoptosis

INTRODUCTION

Selenium is an essential nonmetallic trace element necessary for normal metabolic functions in humans and animals. It serves as a cofactor for the antioxidant enzymes glutathione peroxidase and thioredoxin reductase. Its role in cancer prevention and treatment has gained attention in recent years based on cell culture and animal studies, epidemiological evidence, and human intervention findings demonstrating the anticarcinogenic potential of selenium (Rayman 2005; Jackson & Combs 2008; Zeng 2009; Sanmartin et al. 2012; Chen et al. 2013). The effectiveness of selenium as an anticancer agent has been refuted due to dismal findings from Selenium and Vitamin E Cancer Prevention Trial (SELECT), a phase III randomized placebo-controlled human trial investigating the chemopreventive effects of selenium, vitamin E, or combination of these dietary supplements against prostate cancer. However, post-SELECT studies suggested that the primary reason for its failure was the inadequate dose or formulation of selenium administered to produce optimum effect (Dunn et al. 2010; Ledesma et al. 2011; Hurst et al. 2012; Kristal et al. 2014). A recent report on a series of observational studies conducted on more than 1,100,000 volunteers showed lower cancer incidence and cancer mortality associated with higher selenium exposure (Vinceti et al. 2015). In addition, conflicting results including inverse, null, and direct associations have been reported for certain types of cancer. Therefore, the collection of more preclinical data on selenium is necessary for the scientific community to better understand its mechanism of action. Additional-

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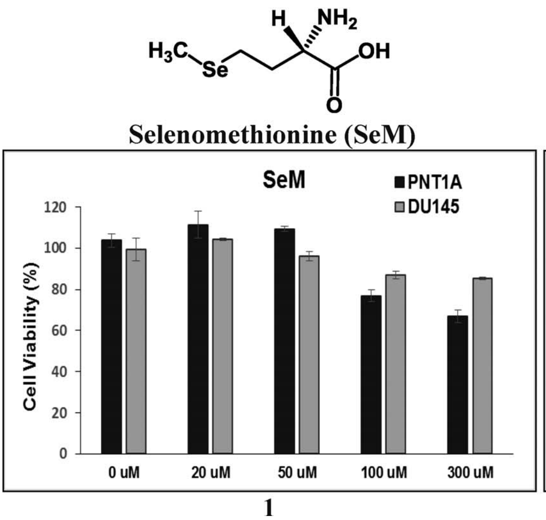

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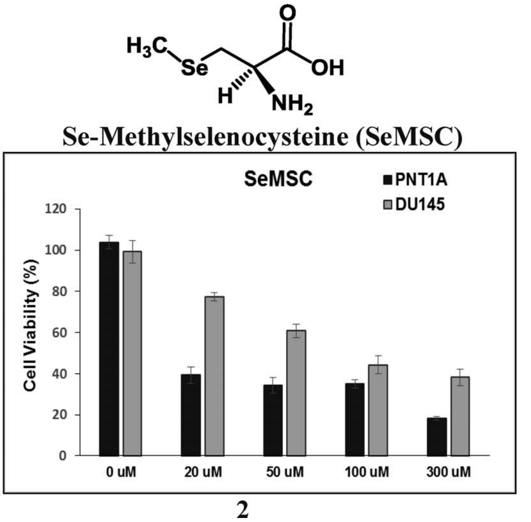

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Figures 1–2.—Bar plots of cell survival assay of non-tumorigenic PNT1A and tumorigenic DU145 prostate cells upon incubation with 20-300 μM solutions of SeM (1) and SeMSC (2). The data represent the average corrected absorbance of eight wells carried out in triplicate independent experiments (96 wells/experiment) and based on untreated cells as controls; *bars*: ±SE.

ly, numerous existing investigations supporting the anti-proliferative effects of selenium against prostate cancer sufficiently justify further studies in this direction (Menter et al. 2000; Wang et al. 2002; Zhong & Oberley 2002; Meuillet et al. 2004; Reagan-Shaw et al. 2008; Richie et al. 2014; Singh et al. 2014), especially since prostate cancer (PCa) is the second leading cause of cancer deaths in US men as of 2011 (Siegel et al. 2015).

Selenomethionine (SeM) or Se-methylselenocysteine (SeMSC), two naturally prevalent organoselenium amino acids (Fig. 1) shown to have potential anticancer activity, are utilized in most experimental as well as clinical studies. SeM was the major component in dietary Se-supplementation in the completed SELECT trial in which participants were randomized to receive either 200 μg or 800 μg of selenized yeast or matched placebo per day. SeMSC is a naturally-occurring amino acid found in Brazil nuts, garlic, onion, and broccoli. Nutritional levels of selenium supplementation provide antioxidant protection against reactive oxygen damage, while supra-nutritional levels of 200 μg/day, about fourfold more than the recommended daily allowance (RDA), may initiate anticancer activity (Seo et al. 2002; Reid et al. 2004). According to the National Institutes of Health (NIH) dietary supplement fact sheet, the RDA of selenium for adults is 55–70 μg/day (NIH 2015). Previous experimental findings reported that SeM activates p53 tumor suppression protein (Seo et al. 2002), stimulates DNA repair response in human fibroblasts *in vitro* (Seo et al. 2002), induces cell-cycle arrest in human colon cancer cells (Goulet et al. 2005; Nelson et al. 2005), and apoptosis in lung (Yamamoto et al. 2003) and prostate cancer cells (Zhao et al. 2006). SeMSC, on the other hand, affects tumor growth by targeting androgen receptor signaling in prostate cancer cells (Lee et al. 2006), and stimulates apoptosis through caspase activation in human promyelocytic leukemia HL-60 cells (Kim et al. 2001) and in SKOV-3 ovarian cancer cells (Yeo et al. 2002). Different forms of selenium compounds were demonstrated to target different mechanisms to inhibit cell growth and proliferation of human DU145, PC-3, and LNCaP prostate cancer cell lines (Pinto et al. 2007; Li et al. 2008; Abdulah et al. 2011). However, investigations of the metabolomic profile response of prostate cancer cells as a result of selenium treatment are not yet available.

Metabolomics (Beger 2013; Vermeersch & Styczynski 2013), which involves simultaneous measurement of all the possible metabolites produced by living cells or tissues, has been successfully applied to prostate cancer research by utilizing nuclear magnetic resonance (NMR) spectroscopy (Jordan & Cheng 2007; Roberts et al. 2014; Lin & Chung 2014;). NMR analysis, which is a non-invasive technique to study biological systems undergoing physiological transformation and involves inexpensive minimal sample preparation, has been widely used as a metabolic profiling tool to generate quantitative data of low-molecular weight metabolites produced by living cells (Palmnas & Vogel 2013; Shao et al. 2014). To date, the literature abounds with numerous NMR-based platforms to study PCa cells *in vitro* (Cornel et al. 1995; Ackerstaff et al. 2001; Milkevitch et al. 2005; Podo et al. 2011; Teahan et al. 2011; MacKinnon et al. 2012), *ex vivo* (Serkova et al. 2008; Levin et al. 2009; DeFeo & Chen 2010; DeFeo et al. 2011; Stenman et al. 2010, 2011; Giskeødegård et al. 2013), and *in vivo* (Zakian et al. 2008; Thomas et al. 2013). In spite of this, NMR-based metabolomic investigation into the alterations of the metabolic profile of PCa cells after selenium treatment has not been explored.

The objectives of this study were to monitor changes in metabolite levels upon selenium treatment of human DU145 prostate cancer cells and to compare with a non-tumorigenic PNT1A prostate cell line. Two organoselenium compounds, selenomethionine (SeM) and Se-methylselenocysteine (SeMSC), known to have anticancer properties (Zeng 2009; Sanmartin et al. 2012), were used for treatment. NMR spectroscopy detected reduced metabolite concentrations dependent upon the organoselenium used and the cell type. However, no clear pattern of metabolite change distinguished apoptotic versus non-apoptotic pathways.

METHODS

Cell line cultivation and sub-culturing.—Androgen-insensitive human prostate adenocarcinoma DU145 was purchased from the American Type Culture Collection (ATCC HTB-81). Normal PNT1A prostate epithelial cell line was obtained from Sigma (Cat No. 95012614). DU145 cells were cultured in EMEM (ATCC), while PNT1A cells were grown in RPMI 1640 medium (MediaTech, Inc.). Both media were supplemented with 10% fetal bovine serum (FBS, MediaTech, Inc.) and with 1% solution containing 10,000 IU/mL penicillin and 10,000 μg/mL streptomycin (MediaTech, Inc.). Cells were grown to 80–90% confluence ($\sim 2 \times 10^7$ cells) in 75-cm^2 culture flasks (Corning) for about a week (6–7 days) in a humidified incubator (Fisher Scientific Isotemp) with 5% CO_2 at 37° C. During the incubation period, growth media was changed once with fresh pre-warmed medium (pH 7.2). To harvest the cells, old growth media was aspirated and 5 mL 0.25% trypsin solution (Thermo Sci Hyclone) were added. The cells were incubated for an additional 10 min or until they visibly detached from the flask walls. Clumped cells were broken up gently and 1 mL of the trypsinized cell homogenate suspension was transferred into a new T75 cell culture flask containing pre-warmed media (20 mL) for further culturing.

Cell survival assay.—Cells were grown to confluence in a 96-well plate (2×10^3 cells/well) and incubated for 24 hrs with SeM or SeMSC (20, 50, 100 and 300 μM) in growth media from a stock solution of 10 mM in PBS (phosphate buffered saline, Fisher). Incubation period of 24 hrs for SeM and SeMSC was based on previous studies conducted by others to stimulate apoptosis using selenium compounds (Kim et al. 2001; Wang et al. 2002). The following day, cells were washed with pre-warmed PBS. MTT (3-[4,5-dimethyl-thiazol-2-yl]-2.5-diphenyltetrazolium bromide, Sigma, 0.5 mg/mL) in PBS was added to each well. Samples were allowed to incubate for an additional 2 hrs, after which dark blue crystals formed. DMSO was added to each well and allowed to stand at room temperature for 1 hr to dissolve the purplish-blue formazan crystals. Plates were shaken for 2 min and thereafter the absorbance values at 490 nm were measured on a microplate reader (BioRad 550). Absorbance readings were calculated based on the absorbance of the cells alone (as control) and were directly proportional to the number of viable cells in culture. Results were reported as average of triplicate measurements.

Fluorescence microscopy.—Cells (1 mL aliquots) obtained from a diluted cell suspension were seeded into each well (1.7 cm^2, $\sim$1000 cells/well) of a 4-well culture slide (BD Biosciences) and grown to confluence in 5% CO_2 at 37° C for 6–7 days for attachment to the substratum. After aspirating the old growth media, 1 mL of SeM or SeMSC (20, 50, 100 and 300 μM) in fresh pre-warmed media at 37° C was added to each well. After incubation for 24 hrs, cells were washed twice with 1 mL fresh growth media. Cells were stained either with Hoechst 33258 (Molecular Probes) or trimethylrhodamine methylester perchlorate (TMRM, Setareh Biotech) or crystal violet (CV, Fisher). Cells were stained in the dark with 1 mL of 0.1 mg/mL Hoechst 33258 or with 2 μM TMRM in pre-warmed media for 10 min at 37° C, washed twice with 1 mL filtered PBS, then fixed with 1 mL filtered pre-warmed 4% paraformaldehyde for 15 min in the incubator. For CV staining, cells were fixed first with methanol (5 min), and then stained with 0.05% crystal violet dissolved in methanol for 30 min after aspirating out methanol. After thorough liquid aspiration, the wells were removed and allowed to air dry in the dark for 1 hr. Slides were protected with coverslips, whose edges were sealed using a clear fast-drying nail polish and allowed to dry at room temperature in the dark for 30 min. Images were recorded by means of fluorescence microscopy (DAPI for Hoechst 350–390 nm excitation and 460–490 nm emission filters; Texas Red for TMRM 550–560 nm excitation and a 570–650 nm emission filters; and, phase contrast for CV) using an upright fluorescence microscope with Retiga imaging 2000R (Nikon Optiphot-2, 20× and 40×) and an image processing Nikon NIS-Elements V4.0 Qimaging software.

Treatment and preparation of cells for NMR spectroscopy.—Cells were grown to 80% confluence in Petri dishes (50 mm in diameter) in 4.5% CO_2 at 37° C for 5–6 days for full attachment to the substratum. After aspirating the old growth media, 3 mL of SeM or SeMSC (300 μM) in fresh pre-warmed media at 37° C was added to each dish. After incubation for 24 hrs, cells were washed twice with 0.5 mL PBS in D_2O (Cambridge Isotope Laboratories). D_2O (0.7 mL) was again added to each petri dish and cells were gently scraped from the surface with a sterile cell scraper. A volume of 0.7 mL of the harvested cell suspension was transferred into a 5 mm NMR tube and 10 μL of 100 mM TMSP in D_2O was added to each tube. Prior to NMR analysis, tubes were vortexed to ensure samples were in suspension. Control experiments using untreated cells were conducted in parallel with treated cells with the same incubation protocols and sample preparation. The protocol followed for the *in vitro* NMR analysis in this study was based on published procedure (Bezabeh et al. 2001; Al-Saffar et al. 2002; Rainaldi et al. 2008; Opstad et al. 2009) in which cells (control and treated) were analyzed on intact cell samples and not on an extract (i.e., perchloric acid extract), thereby eliminating alteration of metabolite levels caused by extraction (Whitehead & Kieber-Emmons 2005).

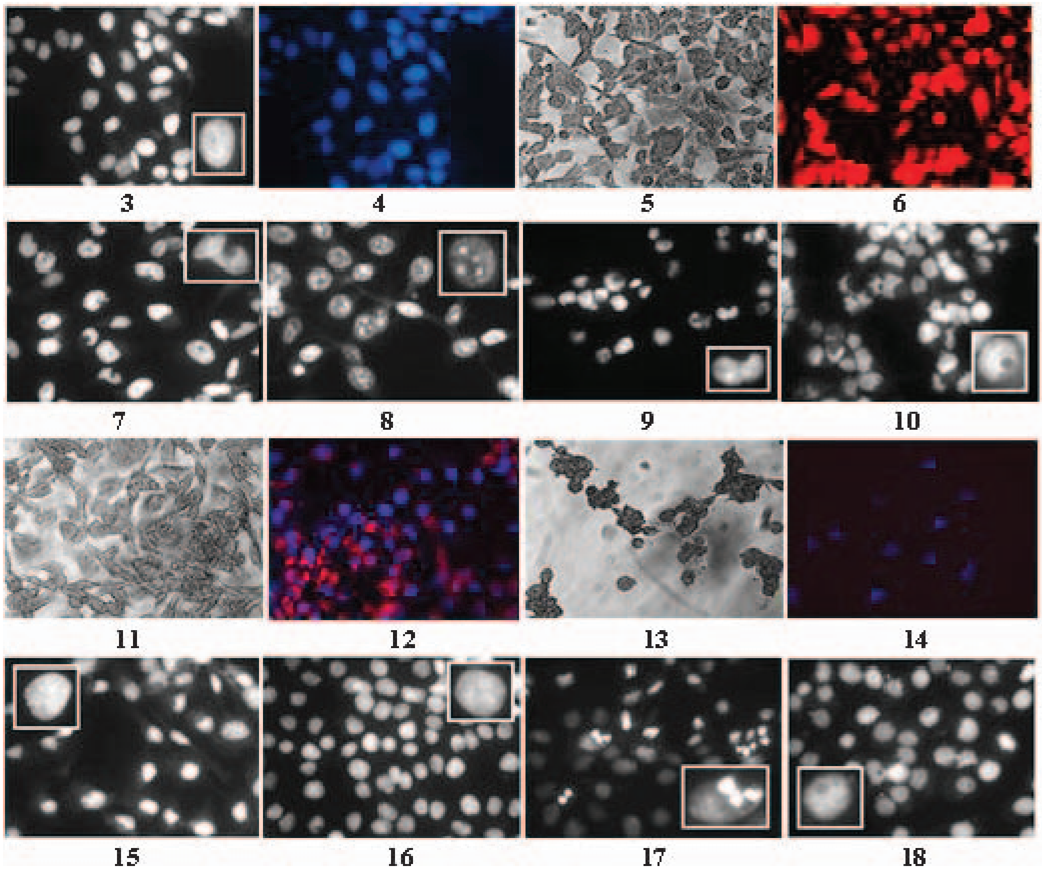
^{1}H NMR spectroscopy.—^{1}H NMR spectra were obtained on a Bruker AV-III-800 NMR spectrometer operating at 800.1337665 MHz and equipped with 5-mm TX1 probe. Gradient shimming was utilized prior to spectral acquisition. Chemical shifts (δ) are given in ppm relative to TMSP: $(CH_3)_3Si(CD_2)_2CO_2Na$, sodium 3-trimethylsilyl-propionate-2,2,3,3-d_4, set at 0 ppm. One-dimensional, one pulse ^{1}H NMR spectra were acquired using the zg one-pulse sequence available in the Bruker pulse sequence library. FIDs for the 1D spectra were collected at 298K by employing a relaxation delay of 2.0 s, a spectral width of 11160 Hz, a data size of 32 K, a pulse width of 90° (8.5 μs), and 256 scans. The FIDs were apodized with an exponential multiplication function using a line-broadening factor of 1.0 Hz prior to Fourier transformation. Water suppression for ^{1}H was carried out using the zgpr pulse sequence from the Bruker pulse sequence library.

Spectral analysis.—Each NMR sample consisted of approximately 10^6 cells and concentration of the metabolites was calculated using the formula:

$$[\text{Metabolite}] = (PA_M/PA_{TMSP}) \times (\text{Number of H's})_M / (\text{Number of H's})_{TMSP} \times [\text{TMSP}]$$

where [Metabolite] and [TMSP] are metabolite concentrations in mM of a metabolite and reference, respectively; and, PA refers to peak integration from NMR signal of the particular metabolite M or TMSP. Percent relative change after selenium treatment compared to untreated control cells was calculated by: % Relative Change = $(PA_{treated}\text{-}PA_{untreated}/PA_{untreated}) \times 100$.

Statistical analysis.—Library psych (1.4.3 library in R, W. Revelle (revelle@northwestern.edu, accessed March 2014) in statistical package R, version 3.0.3 (http://www.r-project.org/, accessed March 2014), with integrated development environment RStudio, version 0.98.932 (http://www.rstudio.com/, accessed March 2014), was used to perform factor analyses (FAs) of the average percent change of 12 metabolites in both normal epithelial PNT1A and prostate



Figures 3–18.—Fluorescence microscopy images of fixed human prostate cells. Grayscale and blue fluorescence images of non-tumorigenic PNT1A cells alone stained with Hoechst (3 and 4, respectively), crystal violet (5), and TMRM (6). Cell morphology after 24 h incubation with SeM at 100 and 300 μM concentrations (7 and 8, respectively), and SeMSC at 100 and 300 μM concentrations (9 and 10, respectively). CV staining of cells with 300 μM SEM- and SeMSC-treated cells (11 and 13, respectively). TMRM/Hoechst double staining after treatment with 300 μM SeM (12) and SeMSC (14). DU145 PCa cells alone (15) and after 24 h incubation with SeM at 100 μM (16) and 300 μM (17), and with SeMSC at 300 μM (18) concentrations. Apoptotic cells are evident in 300 μM SeM-treated cells (8 & 17). Inserts indicate enlarged view of a single cell.

DU145 cancer cells incubated with SeM and SeMSC, replicated three times: PNT1A SeM trials 1, 2, and 3; PNT1A SeMSC trials 1, 2, and 3; DU145 SeM trials 1, 2, and 3; and, DU145 SeMSC trials 1, 2, and 3 (Kabacoff 2011).

RESULTS

Cell survival and morphology.—Cells were incubated with SeM and SeMSC at concentrations of 20–300 μM for 24 hrs. Cell survival assay (Figs. 1 & 2) indicated that SeMSC reduced the growth of PNT1A cells more than DU145 PCa cells by about 10–40%. SeM at the highest concentration caused a slight growth inhibition of PNT1A (69% viability) and DU145 (85% viability). SeMSC decreased cell proliferation of PNT1A and DU145 more than SeM by approximately 30%.

Hoechst nuclear staining was used to monitor the morphological changes that occurred after PNT1A cells and DU145 PCa cells were incubated with SeM or SeMSC (20–300 μM). Untreated normal prostate cells (without exposure to selenium) served as control samples (Figs. 3–6). After 24 hr incubation with SeM or SeMSC, PNT1A cells exhibited greater morphological changes than DU145 PCa cells with increasing concentrations of SeM and SeMSC. No change was apparent at the lowest concentration of 20 μM of SeM-incubated normal prostate cells. At 100 μM SeM, PNT1A cells (Fig. 7) appeared slightly shrunken into an angular or irregular shape. At the highest concentration of SeM (300 μM), grayscale fluorescence image of stained PNT1A nuclei revealed chromatin condensation, nuclear fragmentation, and margination characteristics of apoptosis and possibly necrosis (Fig. 8) (Sinha & El-Bayoumy 2004; Elmore 2007; Bai & Wang 2014; Walsh 2014). PNT1A cells treated with SeMSC showed a different pattern of cell morphology not characteristic of apoptosis. Neither nuclear fragmentation nor margination was observed but cell rounding or shrinking and formation of few cytoplasmic vacuoles could be discerned (Figs. 9 &10).

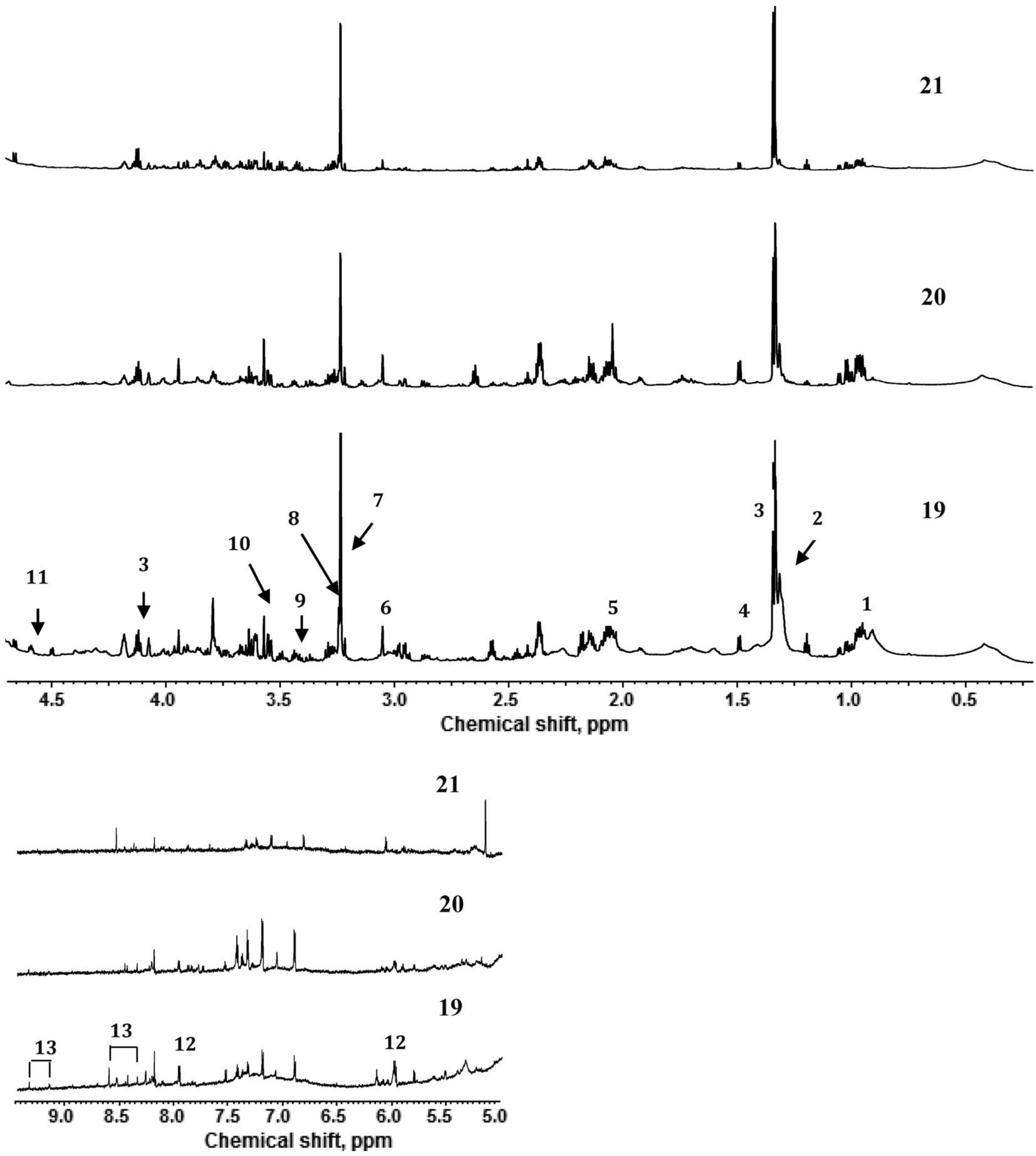
Loss of mitochondrial membrane potential, an indicator of cellular apoptosis, was checked using tetramethylrhodamine methyl ester (TMRM), a cell permeant cationic red fluorescent dye (Griffin et al. 2011; Joshi & Bakowska 2011). SeM or SeMSC treatment of PNT1A at 300 μM concentrations produced partial loss of TMRM puncta, indicating collapse of mitochondrial membrane potential (Figs. 12 & 14). In addition, crystal violet staining (Ito 1984; Lane 2001) of the cells indicated loss of cellular membrane and reduced cell growth in PNT1A cells exposed to 300 μM SeMSC (Fig. 13) but not in SeM-incubated cells (Fig. 11). Compared to the control (Fig. 15), fluorescence microscopy images of fixed SeM- (Figs. 16 & 17) and SeMSC-treated (Fig. 18) DU145 PCa cells indicated no significant morphological changes except for a small amount of apoptotic cells caused by SeM (300 μM, Fig. 17), showing that this PCa cell line was less affected by the inhibitory effects of either SeM or SeMSC.

^{1}H NMR spectroscopy of selenium-treated human prostate cells.—Acquired ^{1}H NMR spectra of non-tumorigenic PNT1A prostate cells and DU145 PCa cells are shown in Figs. 19–24. Control NMR data are cell samples without selenium compounds (Figs. 19 & 22). Peaks were calibrated and corrected based on a TMSP standard (0 ppm), and were assigned according to literature values (Govindaraju et al. 2000). Metabolite concentrations for an average of $\sim 10^6$ cells were calculated based on an internal TMSP standard (1.4 mM) and were corrected depending on the number of protons under the peak. Only those specific metabolite protons that were observed distinctly with relatively minimum overlap in the ^{1}H NMR data were chosen for calculations. It should be noted that the NMR spectra collected only represented intact cells at the end of post-treatment period and metabolites released during the incubation period including extracellular SeM and SeMSC were removed by washing or rinsing with PBS in D_2O prior to NMR experiment. Table 1 shows the percent relative change (positive values for increase or negative values for decrease) in peak area of the metabolites after treatment with selenium compounds. More specifically, the four trellis plots in Fig. 25 show percent changes in all metabolites in all three trials were negative for PNT1A cells except glycine—where positive percent change occurred in all three trials for SeM-treated cells. Percent changes in metabolites were both positive and negative for DU145 cells, particularly, lactate in SeMSC-treated cells. SeMSC produced greater percent changes, either negative or positive. The overall pattern of all twelve metabolites in the four plots appears to be different from one another implying that it is possible not only to distinguish normal cells from cancerous cells but also whether SeM or SeMSC was used.

Statistical analysis.—Factor analysis (FA) is a statistical procedure that reduces a large number of factors to a smaller more interpretable number of factors in a dataset. The FA method was applied to the acquired NMR data to determine which changes in the concentration levels of the metabolites would indicate prostate cells were normal or cancerous and the type of treatment performed. Results from the two factor FA analysis in Fig. 26 &27 showed a separation of cancerous cells from normal cells: trials 1, 2 and 3 of DU145 SeM (upper left part of plot) are separate from trials 1, 2 and 3 of PNT1A SeM (lower right part of plot). Oblique components RC1 (standardized loadings 0.87, 0.98, 0.72, 0.36, –0.01, –0.20 for three trials of normal and cancerous cells, respectively) and RC2 (standardized loadings 0.14, –0.09, 0.03, 0.76, 0.76, 0.80 for three trials of normal and cancerous cells, respectively) had 0.41 correlation and communality values $h^2 = 0.88$, 0.89, 0.54, 0.93, 0.57 and 0.54, respectively, described 41% and 31% respectively of the variation in the loadings, a total of 72% of the total variation in all twelve variables. However, trials 1, 2 and 3 of DU145 SeMSC are not

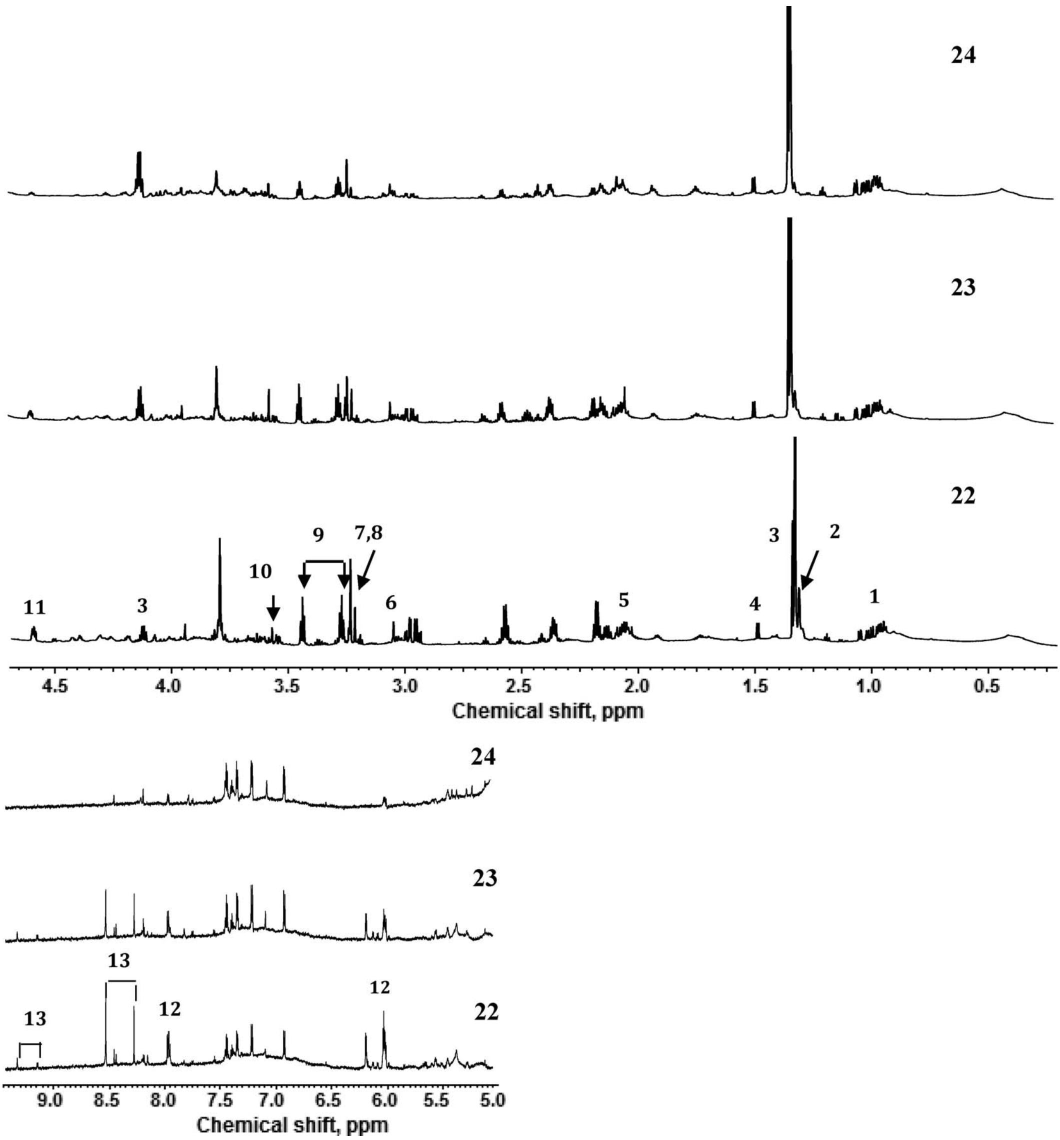
Table 1.—Percent relative change of ^{1}H NMR detectable metabolite levels of SeM- and SeMSC-treated PNT1A non-tumorigenic prostate cells and DU145 PCa cells. Change in metabolite concentrations were measured relative to untreated cell samples (control). Results are average of three independent cell preparations. Abbreviations used: Ala, alanine; Cr, creatine; Cho, choline; PC, phosphocholine; GPC, glycerophosphocholine; Tau, taurine; Gly, glycine; Lac, lactate; β-Glc, β-glucose; UDP, uridine 2′-diphosphate; NADP, nicotine adenine dinucleotide phosphate.

			Conc mM per ~10^6 untreated control		PNT1A cells		DU145 PCa cells	
Metabolites	δ ^{1}H ppm	Number of H's	PNT1A cells	DU145 cells	SeM	SeMSC	SeM	SeMSC
Lipids	1.31	2	0.448 ± 0.057	0.240 ± 0.053	−56.1 ± 12.6	−75.4 ± 3.64	−28.3 ± 7.9	−54.9 ± 1.4
Ala	1.50	3	0.120 ± 0.001	0.098 ± 0.023	−23.3 ± 5.7	−55.8 ± 1.1	−35.8 ± 8.8	−12.3 ± 25.9
Cr	3.05	3	0.135 ± 0.008	0.050 ± 0.010	−56.1 ± 10.4	−80.7 ± 1.2	−25.0 ± 14.4	−35.4 ± 17.1
Cho	3.21	9	0.017 ± 0.003	0.013 ± 0.003	−45.8 ± 16.4	−70.3 ± 6.6	10.0 ± 5.1	−39.7 ± 15.5
PC	3.23	9	0.113 ± 0.007	0.026 ± 0.008	−59.6 ± 2.2	−54.4 ± 3.1	−34.2 ± 7.2	−5.7 ± 7.8
GPC	3.24	9	0.024 ± 0.000	0.011 ± 0.003	−33.3 ± 17.1	−58.3 ± 5.5	5.4 ± 16.5	−35.2 ± 7.7
Tau	3.42	2	0.068 ± 0.010	0.166 ± 0.038	−10.9 ± 1.6	−19.2 ± 5.8	−14.8 ± 9.3	−29.0 ± 8.6
Gly	3.55	2	0.084 ± 0.011	0.061 ± 0.021	25.4 ± 12.9	−57.5 ± 3.8	−4.2 ± 12.3	−17.5 ± 2.5
Lac	4.09	1	0.523 ± 0.015	0.364 ± 0.057	−25.9 ± 7.2	−31.9 ± 3.7	11.4 ± 33.4	65.6 ± 50.4
β-Glc	4.63	1	0.322 ± 0.057	0.431 ± 0.152	−60.8 ± 21.3	−64.0 ± 14.2	−37.7 ± 4.1	−28.9 ± 20.5
UDP	7.98	1	0.084 ± 0.000	0.061 ± 0.027	−33.3 ± 9.6	−61.1 ± 11.1	0 ± 0.0	−35.2 ± 17.7
NADP	8.53	1	0.070 ± 0.000	0.047 ± 0.015	−40.0 ± 0.0	−30.0 ± 5.7	0 ± 0.0	−75.0 ± 25.0



Figures 19–21.—^{1}H NMR spectra (0.25-8.7 ppm) of non-tumorigenic PNT1A prostate cells (19), and after treatment with 300 μM SeM (20) and SeMSC (21). 1: Lipids CH_3, branched chain amino acids; 2: Lipids CH_2; 3: Lactate (Lac); 4: Alanine (Ala); 5: Glutamine (Gln), Glutamic acid (Glu); 6: Creatine (Cr); 7: Choline (Cho), Phosphocholine (PC); 8: Glycerophosphocholine (GPC); 9: Taurine (Tau); 10: Glycine (Gly); 11: β-Glucose (β-Glc); 12: Uridine 5′-diphospho-*N*- acetylglucosamine or Uridine 5′-diphospho-*N*-acetylgalactosamine (UDP); 13: Nicotinamide adenine dinucleotide 2′- diphosphate ($NADP^+$).

grouped separately from trials 1, 2 and 3 of PNT1A SeMSC (Fig. 27). These two factor analyses indicate, in spite of the small sample size of 12 trials and possible dangers of spurious correlations due to the use of percent change data, the measurements of all twelve metabolites reacting to SeM (but not SeMSC) are able to distinguish between normal (PNT1A) and cancerous (DU145) cells, although any one particular metabolite alone was not able to do so. This implies that the lists of metabolite percent reductions given in Table 1



Figures 22–24.—^{1}H NMR spectra (0.25-8.7 ppm) of DU145 prostate cancer cells (22), and after treatment with 300 μM SeM (23) and SeMSC (24). 1: Lipids CH_3, branched chain amino acids; 2: Lipids CH_2; 3: Lactate (Lac); 4: Alanine (Ala); 5: Glutamine (Gln), Glutamic acid (Glu); 6: Creatine (Cr); 7: Choline (Cho), Phosphocholine (PC); 8: Glycerophosphocholine (GPC); 9: Taurine (Tau); 10: Glycine (Gly); 11: β-Glucose (β-Glc); 12: Uridine 5′-diphospho-*N*- acetylglucosamine or Uridine 5′-diphospho-*N*-acetylgalactosamine (UDP); 13: Nicotinamide adenine dinucleotide 2′- diphosphate ($NADP^+$).

(or Fig. 25) could be used as profiles to identify whether cells were cancerous or not: if, for example, the 12 metabolites of cells incubated with SeM had the same percent reduction as given in the second-to-last column of Table 1, this would indicate these cells were characteristic of DU145 PCa cells.

DISCUSSION

Metabolic alterations of prostate cells responding to selenium treatment are not currently available. In this *in vitro* study, we have conducted metabolic profiling of prostate cells after treatment with SeM and SeMSC. The anticarcinogenic properties of SeM and SeMSC are related to the

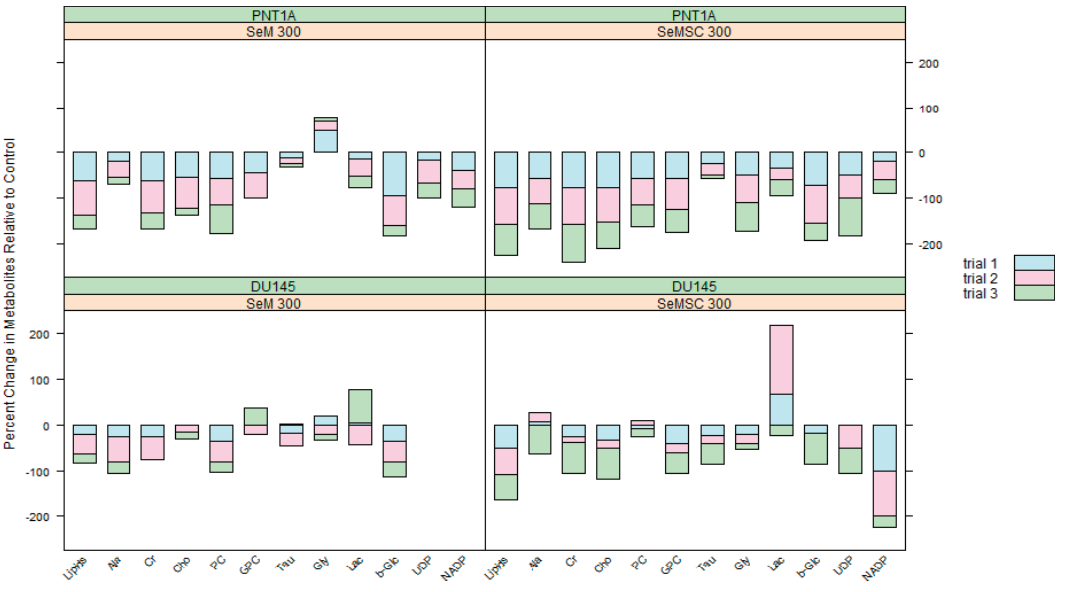
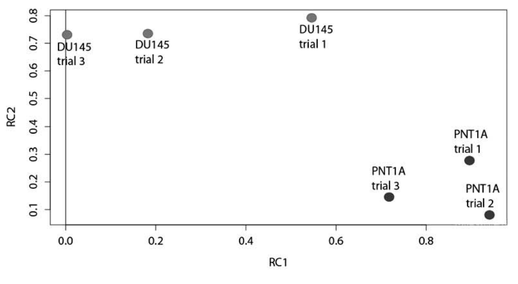


Figure 25.—Bivariate trellis plots of percent changes in three trials of all metabolites relative to control, grouped by PNT1A and DU145 cells and by SeM (300 µM) and SeMSC (300 µM) compounds. Abbreviations used: Ala, alanine; Cr, creatine; Cho, choline; PC, phosphocholine,; GPC, glycerophosphocholine; Tau, taurine; Gly, glycine; Lac, lactate; β-Glc, β-glucose; UDP, uridine 2′-diphosphate; NADP, nicotine adenine dinucleotide phosphate. Note: both UDP and NADP had zero percent changes from control in SeM-treated DU145.

reactive oxygen species (ROS) produced by redox cycling, modification of protein thiols, and induction of DNA strand breaks (Zeng 2009; Sanmartin et al. 2012). Results from NMR data showed perturbations in metabolite levels that correlate with the type of organoselenium and the prostate cells used.

Lactate (2-hydroxypropanoic acid) is the end product of anaerobic glycolysis and is observed to be elevated in metastatic prostate (Moestue et al. 2011), cervical (Walenta et al. 2000), and squamous head and neck (McFate et al. 2008) cancers. Increased glycolysis occurs in tumor cells wherein lactate is produced directly from glucose in the presence of oxygen, known as the Warburg effect (Vander Heiden et al. 2009). The methyl and methine groups of lactate occur as a doublet at 1.31 ppm and a quartet at 4.09 ppm, respectively. After SeM and SeMSC treatment, peak intensity for lactate, based on methine quartet, decreased in PNT1A cells, but increased in DU145 cells (Table 1 and Fig. 25). Results suggest that decreased



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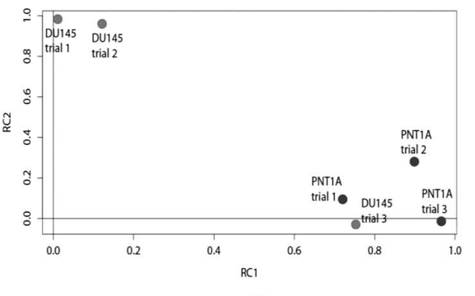

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Figures 26–27.—Factor analysis diagram of oblimin rotation of two factors based on 6 metabolite percent change data, grouped into (a) PNT1A and DU145 SeM all trials, and (b) PNT1A and DU145 SeMSC all trials.

production of lactate can be attributed to increased lactate metabolism as a result of cell stress and disordered energy metabolism during selenium treatment in PNT1A cells. Increased lactate production in Se-treated DU145 cells reflects the typical hallmark of tumor cell metabolism in which glycolysis is upregulated by converting glucose to lactate in an energy inefficient manner. Lactate is also responsible for the consistently acidic environment of tumor tissues enhancing tumor invasion *in vitro* and increased metastasis *in vivo* (Walenta et al. 2000; McFate et al. 2008).

Alanine contains methyl and methine groups similar to lactate with a doublet at 1.50 ppm and a quartet at 3.77 ppm. In our study, a decrease in alanine concentration was detected in both prostate cell lines after selenium treatment. A decrease in alanine concentration was identified in apoptosis-induced HT-29 cells following an initial increase (Lutz et al. 2003).

Glycine is a catabolic product of choline through the choline biosynthetic pathway via irreversible oxidation to betaine producing sarcosine (*N*-methylglycine) as an intermediate product, which is eventually metabolized to glycine (Locasale 2013). High levels of glycine, as a result of increased choline concentration in cancerous tissues, have been proposed as a biomarker for malignancy, and glycine concentration has been shown to decrease in response to drug therapy (Davies et al. 2008, 2010; Mirbahai et al. 2011). In SeM-treated PNT1A prostate cells in this study, the amount of cellular glycine increased by about 25% (Table 1), but decreased in SeMSC-treated cells by 58%. In contrast, glycine only decreased by approximately 4–18% in DU145 PCa Se-treated cells.

Phosphocholine is a metabolic product from phosphatidylcholine that forms the typical cellular membrane bilayer structure with phosphorylethanolamine and other neutral lipids controlling membrane integrity. Elevated levels of phosphocholine and choline-containing compounds have been established as characteristics of cancers of the breast and prostate, as well as other types of solid tumors (Ackerstaff et al. 2003). High levels of choline and choline-containing metabolites have been postulated to correlate with induced membrane transport and upregulation of phospholipases and choline kinase activity taking place in the Kennedy pathway during carcinogenesis (Oakman et al. 2011; Podo et al. 2011). Choline-containing metabolites in malignant human prostatic epithelial cell line (LNCaP, DU145, TSU and PC-3) have been quantified using ^{1}H NMR spectroscopy and results demonstrated that the amount of choline compounds (or total choline) calculated to be 0.4–4.5 mM per 100–250 cells is significantly higher than non-tumoral epithelial cells determined to be ~0.02–0.2 mM (Ackerstaff et al. 2001). Compared to untreated cells in our study, there is a general reduction in the peak area for choline (3.21 ppm) and phosphocholine (3.23 ppm) for both Se-treated normal and PCa cells (Table 1), demonstrating the significance of phospholipid precursors as biochemical indicators of cells responding to selenium treatment. Decrease in total choline in DU145 SeMSC-treated PCa cells is in agreement with published data in which a marked decrease in choline-containing metabolites was observed in doxorubicin-induced or ionizing radiation-induced apoptotic HL60 leukemia cells (Rainaldi et al. 2008) as well as in *in vivo* rat liver cell apoptosis induced by high dose sodium selenite (Cao et al. 2008).

Creatine is a high-energy metabolite linked with choline metabolism. In our study, SeMSC treatment of PNT1A prostate cells caused a significant decrease in the amount of cellular creatine (81%, Table 1). Creatine/creatine kinase system plays an important role in cellular energy regulation and transport, especially in cells with high and disrupted energy metabolism (Wang et al. 2013; Onda et al. 2006). A reduced amount of creatine has been identified in thymidine kinase-ganciclovir gene therapy treated rat glioma cells (Lehtimäki et al. 2003; Valonen et al. 2005).

Taurine (2-aminomethanesulfonic acid) is an amino acid that functions in maintaining cell osmoregulation (Schaffer et al. 2000). In our study, the quantity of taurine in DU145 cells is approximately three times more than the amount detected in PNT1A cells. Others observed that an elevated taurine concentration is associated with increased malignancy in human cerebral neoplasms (Peeling & Sutherland 1992; Kovanlikaya et al. 2005). Studies conducted on apoptotic rat brain glioma cells and in HL60 leukemia cells showed a substantial decline in taurine signal (Friis et al. 2005; Rainaldi et al. 2008), which coincides with results of the present study. Peak area of taurine resonances for SeM- or SeMSC-treated PNT1A and DU145 cells compared to untreated control (Figs. 19–24) was calculated to decrease by 11–29% (Table 1). In contrast, other investigations reported an increase in taurine concentration during apoptosis in a variety of cell types such as cerebellar neurons (Moreno-Torres et al. 2004), 3T3 fibroblasts (Friis et al. 2005), and glioblastomas (Opstad et al. 2009; Opstad 2012). The study conducted on 3T3 fibroblasts showed that protein kinase activation results in cysteine protease caspase-3 stimulation before an efflux of potassium and taurine from the cell, enhancing DNA fragmentation and cell shrinkage characteristic of apoptosis (Friis et al. 2005). However, accumulation of taurine was also observed in necrotic HL60 leukemia cells (Rainaldi et al. 2008).

Glucose is an essential source of cellular energy and serves as a precursor for various biochemical compounds. Peak intensity for β-glucose at 4.63 ppm exhibited a decline of approximately 29–64% for both prostate cell lines after selenium treatment (Figs. 19–24). Accurate peak integration of α-glucose at 5.22 ppm is complicated by the proximity of the proton signal with water resonance during water suppression experiment; therefore, result for α-glucose was omitted.

UDPs, which correspond to both uridine 5′-diphospho-*N*-acetylglucosamine (UDP-GlcNAc) and uridine 5′-diphospho-*N*-acetylgalactosamine (UDP-GalNAc), are nucleotide sugar precursor molecules in lipid glycosylation and in polypeptide biosynthetic pathways. Mediated by the hexosamine pathway, glucose is converted to uridine 5′-diphospho-*N*-acetylhexosamine (UDP-GNAc), which is subsequently synthesized to glycoproteins, proteoglycans, and glycolipids (Lau & Dennis 2008). Some glycosylation metabolic pathways are identified to be involved in tumor progression (Grande et al. 2011). The presence of UDP may imply increase synthesis of nucleotides necessary for replication of proliferative cells. Based on uracil (U_6) signal at 7.98 ppm, UDP showed no change or a 33–61% decline in peak intensity upon selenium treatment of prostatic cells relative to untreated cells (Table 1). Previous studies on human glioblastoma cells indicate that cell death does not cause an increase in UDP-GlcNAc or UDP-GalNAc levels (Opstad 2012), while cisplatin-treated rat brain cells showed elevated amounts of UDP-GlcNAc and UDP-GalNAc as a result of reduced activity of UDP-*N*-acetylglucosamine-polypeptide-*N*-acetylglucosaminyl transferase during cell stress (Pan et al. 2011).

Strong lipid signals originating from saturated methyl at 0.9 ppm and methylene at 1.3 ppm are evident as shown in Figs. 19–24. A lipid proton peak at 1.31 ppm, which is resolved from lactate resonance, indicated a significant decrease in intensity ranging from 28–76% for both normal and tumorigenic prostate cells upon incubation with SeM or SeMSC. This result does not agree with other *in vitro* studies reporting lipid accumulation of drug-induced apoptosis and necrosis in human prostate (Milkevitch et al. 2005), cervical (Bezabeh et al. 2001), leukemia (Locasale 2013), Jurkat T-cell lymphoblasts (Al-Saffar et al. 2002), and rat gliomas (Lehtimäki et al. 2003). Another study revealed that saturated lipid peaks are present in damaged tumor cells irrespective of cell death mechanism (Kauppinen 2002).

Citrate levels are usually high in normal prostatic tissue and prostatic fluid at a concentration of about 100 mM, but are reduced in malignant prostate cancer cells (Teahan et al. 2011). Citrate signals as doublets at 2.55–2.65 ppm were neither observed in the NMR spectra of DU145 PCa cells nor in PNT1A cells (Figs. 19–24). This clearly implies that normal prostate function marked by high citrate levels is not retained in PNT1A cells. Therefore, generating normal physiological phenotypes in immortalized cell models poses a challenging task. A similar study conducted on a non-malignant prostate epithelial cell line RWPE-1 also indicated absence of citrate peaks (Teahan et al. 2011).

Nicotine adenine dinucleotide (NAD) or nicotine adenine dinucleotide phosphate (NADP) has a complex coupling pattern, and contributions from ribose resonating upfield from the water signal are small and overlap with other metabolites. Proton resonances for the two ribose moieties at 4.26–6.15 ppm occur in the most crowded region of the spectrum and are not clearly discernible. Aromatic pyridine (at 8.31–9.44 ppm) and adenine-ring (at 8.42–8.52 ppm) protons are pH-dependent and are sometimes not distinctly apparent due to slow exchange with water (Govindaraju et al. 2000). Selenium-treated prostate cells showed no change or a decrease in NADP level by 30–75% based on pyridine proton at 8.53 ppm (Table 1). Relatively small peak intensities associated with NADP protons caused large variations in the calculated values.

In summary, results from NMR data indicated that greater alterations in metabolite levels were induced by SeMSC than by SeM treatment. Fluorescence microscopy images showed more morphological changes for SeMSC-incubated prostate cells than SeM-treated cells. The weaker anticancer effect of SeM observed in this study is in agreement with previous studies indicating that SeM anticancer activity can be enhanced enzymatically by methioninase which catalyzes the formation of superoxide and other SeM metabolites responsible for its anticancer activity (Zhao et al. 2006). SeMSC does not need to be induced enzymatically for it to cause an anticancer effect. Greater cellular changes were also observed in non-tumorigenic PNT1A than DU145 PCa cells suggesting that PCa cells may have a mechanism of protection triggered by the oxidizing property of selenium compounds, and SeM and SeMSC may be producing an undesirable proliferative effect on PCa cells rather than a destructive effect. Recent studies on dietary antioxidants suggest these compounds initiate biochemical pathways that stimulate cancer cell proliferation and neoplastic transformations (Chandel & Tuveson 2014; Sayin et al. 2014). The NMR data showed a higher taurine content in DU145 PCa cells compared to the non-tumorigenic PNT1A cells due mainly to the origin of the metastasis. DU145 is an androgen-independent and poorly-differentiated human prostate cancer cell line of moderate metastatic potential derived from brain metastasis. Taurine, found to be abundant in the human brain, functions as a neuromodulator, neurotransmitter, and an osmolyte.

Reduced concentrations of metabolites observed with selenium treatment suggested catastrophic collapse of viable cells with the greatest change demonstrated by creatine, a high-energy metabolite. However, lactate, choline-containing compounds (Cho and GPC), and glycine levels increased depending on the type of selenium used and the cell type. No clear pattern of variation in metabolite concentration levels to distinguish apoptotic versus non-apoptotic pathway was observed probably due to heterogeneity of the harvested cells utilized during NMR acquisition, and the possibility that more than one cell death pathway (Chaabane et al. 2013; Su et al. 2013) was induced simultaneously by SeM and SeMSC.

Statistical analysis by FA method performed in this study, based on the change in the concentration levels of twelve metabolites determined by ^{1}H NMR spectroscopy, was able to distinguish between normal (PNT1A) and cancerous (DU145) cells for SeM-treated cells, although any one particular metabolite alone was not able to do so as shown in Figs. 26 &27.

NMR spectroscopy is a valuable tool in monitoring cellular response upon drug treatment. In this study, ^{1}H NMR was exploited to assess the effect of organoselenium treatment of non-tumorigenic and tumorigenic prostate cell lines. Although the exact cell death mechanism stimulated by the selenium compounds used was not established spectrometrically, NMR spectral data confirmed microscopy results that SeMSC triggered more cell stress response in the prostate cell lines used. The results in this study represent one of the first sets of NMR spectroscopic data in metabolic profiling of selenium-treated prostate cells coupled with pattern recognition techniques. Obtaining more complete information of biochemical processes occurring during treatment is an important step towards a better understanding of cellular processes that can have future applications in the clinical setting to monitor treatment response and to improve management of anticancer therapy.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

COMPLIANCE WITH ETHICAL REQUIREMENTS

This article does not contain any studies with human or animal subjects.

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CROSSROADS OF ART, EDUCATION, AND GEOLOGY IN NEW HARMONY, INDIANA: A NEW EXHIBIT AT THE WORKING MEN'S INSTITUTE

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ABSTRACT. The Working Men's Institute (WMI) in New Harmony is the oldest continuously operating public library in Indiana. WMI was established in 1838 by William Maclure, "Father of American Geology", to establish a common place for people to further their knowledge and education. The concept of a combined library and museum evolved from Maclure's emphasis on education, and in particular, the Pestalozzian method. A new exhibit at the WMI entitled "New Harmony: Crossroads of Geology" was completed in August 2014. The exhibit displays a reproduction of the 1818 geologic map of the eastern United States compiled by William Maclure. Panels in the exhibit also highlight the evolution of the geologic time scale, localities near New Harmony significant to early scientific studies, and contributions of David Dale Owen, Richard Owen, and Edward Cox to westward expansion of the United States in the early 19th century. Moreover, panels in the exhibit highlight modern studies in southern Indiana, such as seismic monitoring of the Wabash Valley Fault Zone and flooding hazards of the Wabash River. In addition to the exhibit, fossil and mineral kits for use by K–12 teachers are available from the WMI. Activities planned with the kits include: sketching, building models, conducting hands-on experiments, and identifying fossil and mineral specimens. These applied approaches are aligned with teaching methods championed by Maclure. Furthermore, the new exhibit follows the educational tradition of the WMI established by Maclure in 1838.

Keywords: education, geology, New Harmony, sketching, William Maclure, Indiana

INTRODUCTION

The historic town of New Harmony, located along the Wabash River in Posey County, Indiana, was an early 19th century hub for natural scientists, especially geologists (Fig. 1). Notable geologists that lived in New Harmony in the 19th century included: William Maclure, David Dale Owen, Richard Owen, Gerard Troost, Edward Cox, Joseph Norwood, and James Sampson (Pitzer 1989, 1998; Straw & Doss 2008). Other naturalists that worked in New Harmony included Charles Alexandre Lesueur and Thomas Say, and the town was also visited by Charles Lyell, James Hall, and Maximilian, Prince of Wied (Thomas & Hannibal 2008).

The Working Men's Institute (WMI) in New Harmony was established in 1838 by William Maclure, "Father of American Geology", as a place for citizens to further their knowledge and education (Williams 1950; Douglas 1991; Lowe & Stone 2003; Warren 2009). WMI pioneered the combination of library with museum, a concept that evolved from Maclure's emphasis on education, and in particular, the Pestalozzian method. Today WMI is the oldest continuously operating public library in Indiana (Lowe & Stone 2003).

The scientific accomplishments of William Maclure, David Dale Owen, and other early geologists were highlighted in a comprehensive exhibit in the Keppler House of Historic New Harmony until 2007. At this time, the Keppler House was renovated and the geology displays were dismantled and placed into storage. In preparation for the New Harmony Bicentennial in August 2014, a new exhibit was planned and completed at the WMI from September 2012 to July 2014. The new exhibit highlights the geologic contributions of William Maclure, David Dale Owen, Richard Owen, and Edward Travers Cox, and includes items from the Keppler House, fossil and mineral specimens from WMI, and other items from the geology collections at the University of Southern Indiana. In addition to the new exhibit, fossil and mineral kits were assembled to provide hands-on activities at WMI and to serve the needs of teachers in the Tri–State region (Indiana, Illinois, and Kentucky). Moreover, instructional guides were prepared to outline

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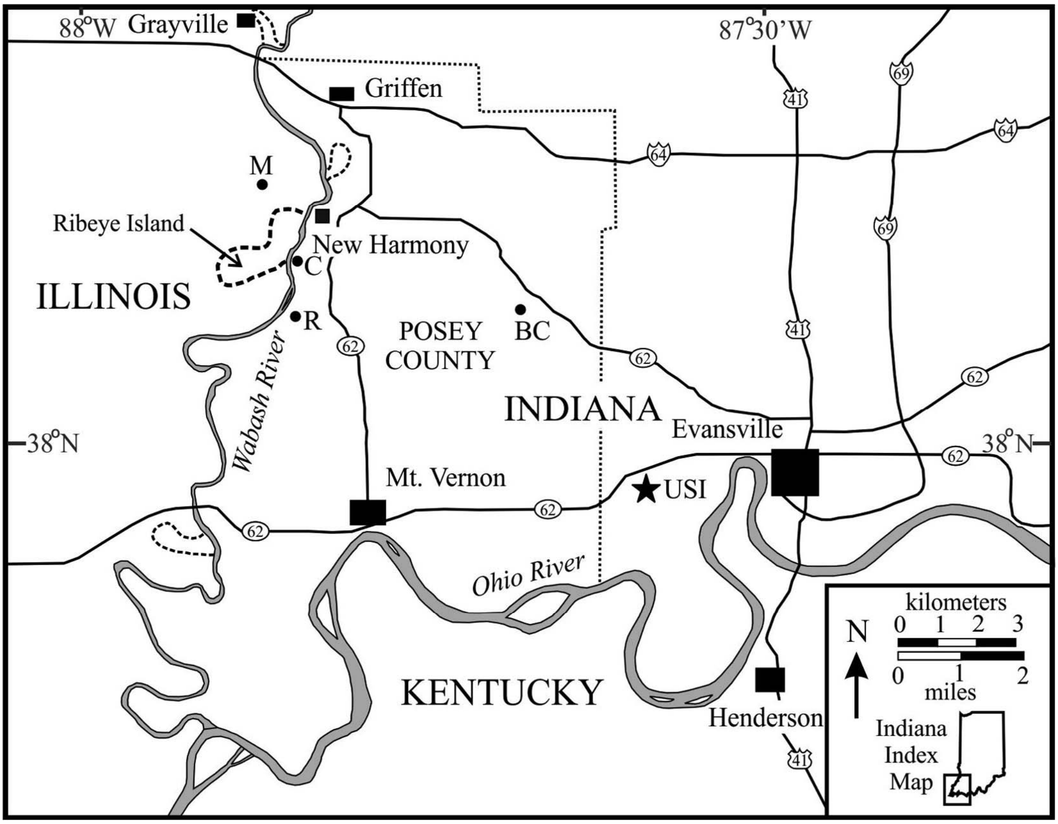


Figure 1.—Map showing the location of New Harmony and several historic geologic localities: BC – Big Creek, C – Wabash Cutoff, M – Mumford Hills, R – Rush Creek, and USI – University of Southern Indiana.

lesson plans for engaged learning activities. These activities include, but are not limited to: (1) maintaining a science notebook; (2) sketching fossils and minerals; (3) recording field observations; and (4) using field guides to identify fossil and mineral specimens.

The objectives of this paper are to: (1) summarize the unique characteristics of the educational enterprise in relation to the natural sciences in the early 19th century of New Harmony, Indiana; (2) highlight the components of a new geology exhibit and related outreach activities at the WMI in New Harmony, Indiana; and (3) focus on the contemporary implications of engaged learning and the integration of arts with natural sciences in achieving modern educational goals.

ART, EDUCATION, AND GEOLOGY IN 19TH CENTURY NEW HARMONY

Robert Owen (1771–1858) was born in Newton, Wales, and married Caroline Dale in 1799 in Scotland, the daughter of the New Lanark cotton mill's proprietor David Dale (1739–1806). Robert Owen's success with mill operations made him a rich industrialist by the early 19th century. In his industrial pursuits, Robert Owen evolved into a social reformer interested in developing a balanced and educated society. To expand his ideas on social reform, Robert Owen purchased the town of New Harmony, Indiana from the Harmonists in 1824 to establish a Utopian society of artists, scientists, and social reformers (Carmony & Elliott 1980).

William Maclure (1763–1840) is known as the "Father of American Geology". Notably, he completed the first geologic map of the eastern United States in 1809, published in the *Transactions of the American Philosophical Society* (Maclure 1809; Merrill 1924, p. 31–32; Winchester 2013, p. 82). In 1812, Maclure was a founding member of the Academy of Natural Sciences of Philadelphia, and later became president in 1817. He also presented a revised geologic map of the eastern United States to the American Philosophical Society in 1817, with printed and hand-colored copies distributed with the *Transactions of the American Philosophical Society* in 1818 (Maclure 1818; Merrill 1924, p. 34).

Maclure became intrigued with the Pestalozzian teaching method in 1805 while living in Switzerland. Specifically, the Pestalozzian method focuses on learning through hands-on, concrete experiences such as sketching and individualized curricula (Pitzer 1998). Maclure realized the importance of this new approach and established a Pestalozzian school in Philadelphia led by Joseph Neef (1770–1854) in 1809. Through his professional activities Maclure established friendships with many of the prominent scientists and educators of his time, such as Charles Alexandre Lesueur and Thomas Say. Thus, it was no surprise that Robert Owen collaborated with William Maclure to conduct an experiment in social and educational reform in New Harmony, Indiana (Burgess 1998; Pitzer 1998; Warren 2009; Winchester 2013, p. 85).

The partnership between Robert Owen and William Maclure resulted in artists, educational reformers, and natural scientists joining the New Harmony social experiment. Many started their journey to New Harmony in Pittsburgh, Pennsylvania on Thursday, December 8, 1825 (Pitzer 1998). The passengers and crew departed on a keel boat named "Philanthropist", often referred to as the "Boatload of Knowledge". They arrived safely in Mount Vernon, Indiana on January 23, 1826 (Fig. 1) and the following day were transported by wagons to New Harmony (Pitzer 1998).

The passengers on the Philanthropist included Thomas Say (1789–1834), an American naturalist, entomologist, and conchologist, and Charles Alexandre Lesueur (1778–1846), a French naturalist, zoologist, ichthyologist, artist, and teacher (Pitzer 1998). Additionally, prominent educators of the time made the journey on the Philanthropist. Madame Marie Louise Duclos Fretageot (1783–1833), French educator who established a Pestalozzian school in Philadelphia (1821–1825), joined the social movement in New Harmony. Likewise, William S. Phiquepal d'Arusmont, a French educator, became engaged with the School of Industry in New Harmony shortly after their arrival in 1826. Pestalozzian teacher Joseph Neef, although not on the Philanthropist, joined the New Harmony community March 20, 1826 after encouragement by Maclure (Pitzer 1998).

In 1827, Maclure arranged for a printing press to be delivered to New Harmony from New Orleans to aid in production of educational materials (MacPhail & Sutton 1998). Maclure realized the importance of printing books and, most significantly, reproducing sketches and drawings. The following passage from an essay entitled "Education" first printed on 13 February 1828 in the *The Disseminator of Useful Knowledge* highlights Maclure's emphasis on the importance of art to learning: "The art of drawing or delineation, which has been placed (because its utility was not well understood) amongst the fine arts, must be ranked amongst those which are useful, as it is probably the most expeditious, correct, easy and pleasant mode, of giving ideas both to children and adults" (*The Disseminator of Useful Knowledge* 13 February 1828 in Maclure 1831, p. 48).

Maclure also recognized the importance of sketching to natural history and visualizing concepts that would simply be too difficult to convey through words: "Representation is the only defined language, and is perhaps equal in value and utility to all the languages together; without it, we can have no correct idea of mechanics or natural history; when the objects themselves are absent, descriptions, from the undefined nature of words, must be equally vague and uncertain" (*The Disseminator of Useful Knowledge* 13 February 1828 in Maclure 1831, p. 48–49).

The following passage is from an essay entitled "Industrial System of Education – Obstacles to Reform in Education" first printed on 26 April 1828 in the *The Disseminator of Useful Knowledge*: "The drawing from the object itself therefore probably is the most useful mode [of learning]. Substituting a long tedious process of copying, in place of a short expeditious mode by the copperplate press, would be losing the benefit of that admirable invention" (*The Disseminator of Useful Knowledge* 26 April 1828 in Maclure 1831, p. 67). In this passage, Maclure suggests that sketching from an original object is much more conducive to learning than observing images produced by copper plate etching, wood block engraving, or lithography.

As part of the social experiment, Maclure recruited scientists with artistic skills to live in New Harmony to teach their approach to scientific observation and documentation, as well as conduct studies of the natural environment. These included Charles Alexandre Lesueur

(1778–1846), who lived in New Harmony from 1825 to 1837, where he filled sketchbooks of drawings of flora and fauna. Likewise, Thomas Say (1787–1834) studied shells and insects, leading to the publication (1830–1834) of *American Conchology* in three volumes. The sketches of shells in Say's *Conchology* volumes were printed and hand colored in New Harmony at the School Press (Banta 1948; Pitzer 1998; Thomas & Hannibal 2008). Ultimately, the significance of art to documenting scientific observations was most likely taught to David Dale Owen (1807–1860) and Richard Owen (1810–1890) by Lesueur and Say.

David Dale Owen, son of Robert Owen, became one of the most prominent American geologists in early 19th century (Hendrickson 1940; Johnson 1977; Thomas & Hannibal 2008). Although David Dale Owen and William Maclure only overlapped in New Harmony in 1828, this interaction may have had a lasting impact on David Dale's passion for geology (Straw & Doss 2008).

In 1836, David Dale Owen was involved with a geological survey of Tennessee conducted under the supervision of Gerard Troost (1776–1850). After completing a medical degree in 1837 from the Medical College of Ohio, David Dale Owen was appointed by the Governor to conduct a geological survey of Indiana, which was published in 1839 (Hendrickson 1943; Kimberling 1996; Johansen 1997). In addition, he was later appointed by the federal government to survey the mineral lands north and west of Indiana, which was paramount to the economic development of the Midwest (Hendrickson 1940, 1943; Johnson 1977; Kimberling 1996). In the case of these early geological reports, sketches and drawings were used to illustrate important geographic landmarks, geological features, and fossils.

Richard Owen, fourth son of Robert Owen, worked under the guidance of his older brother David Dale in 1849 to conduct a geological survey of northern Minnesota and the shores of Lake Superior. Richard, like his brother, was a proficient artist and included many sketches of geologic features in his reports. Following David Dale's death in 1860, Richard Owen continued the geologic enterprise in New Harmony (Johansen 1997; Thomas & Hannibal 2008). In 1864, he became Professor of Natural Science at Indiana University, where he taught for 15 years. He also served as the first president of Purdue University briefly in 1872.

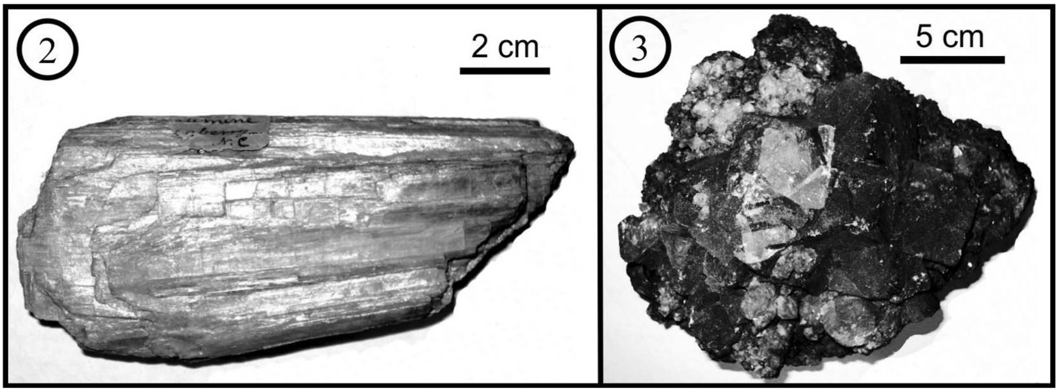
Leo Lesquereux, the "Father of American Paleobotany", worked with David Dale Owen, and later with Richard Owen, on the Pennsylvanian paleofloras of Indiana (Canright 1957). This work entailed documenting various plant fossils from Indiana, with sites in close proximity to New Harmony, such as Rush Creek and the Wabash Cutoff (Fig. 1; Canright 1957; Petzold et al. 1987). Documentation involved detailed drawings of fossil plants that were replicated in many 19th century geologic reports of Indiana (Lesquereux 1862, 1884). Illustrations have always been, and remain, key components of the paleontological sciences (Davidson 2008, p. 182–184).

Edward Travers Cox (1821–1907) served as an assistant to David Dale Owen in conducting early geological surveys of Kentucky. In New Harmony, Cox and James Sampson (1806–1890), who was a saddler, storekeeper and Maclure agent, collected and studied plant fossils from Rush Creek and the Wabash Cutoff (Fig. 1; Collett 1884; Petzold et al. 1987). From 1862 to 1868, little geological work was conducted in Indiana because of the Civil War. In 1869, the Indiana General Assembly passed an Act establishing the Indiana Geological Survey and the position of State Geologist. In accordance with this Act, Governor Baker appointed Edward Travers Cox as Indiana's first official State Geologist (Blatchley 1916). The Working Men's Institute is home to collections of Edward Travers Cox and James Sampson, with many of their geological specimens on display (Figs. 2–6).

Art, education, and geology in 19th century New Harmony established a legacy of significant natural studies. In some cases, the work of these scientists is viewed as fine art, including the work of Charles Alexandre Lesueur, David Dale Owen, and Thomas Say. The lessons learned from this synergetic educational approach may be applicable to inspiring the next generation of scientists and contribute to improving science education in the 21st century.

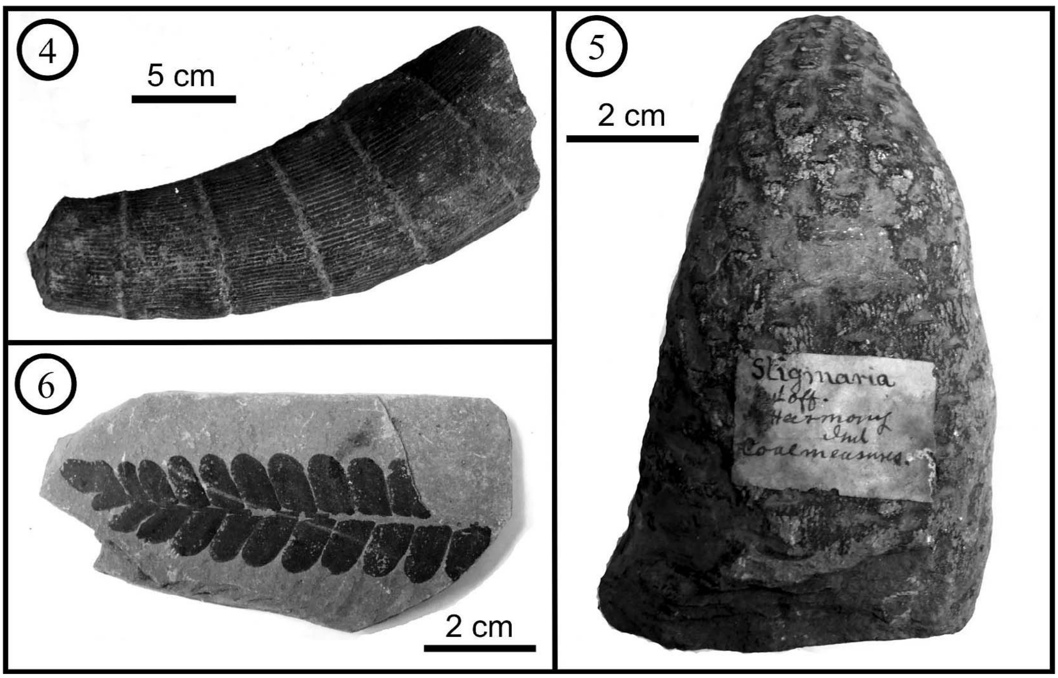
HISTORIC GEOLOGIC EXHIBITS IN NEW HARMONY

From 1834 until 1894, the WMI was located in the old Harmonist Church. With the generous support of Dr. Edward Murphy (1813–1900), who was befriended by both Robert Owen and William Maclure, a new Victorian Romanesque Revival building was constructed in 1894 and



Figures 2 & 3.—Mineral specimens from the Edward Travers Cox Collection at the Working Men's Institute in New Harmony, Indiana. 2. Specimen of kyanite from North Carolina (WMI 304.674). 3. Fluorite with quartz, unknown locality (WMI 304.671).

became the new home for the WMI (Williams, 1950). The museum portion of the WMI building contains several exhibits, including: (1) natural history collections of minerals, fossils, rocks, shells of fresh water mussels, and preserved insects, fish, amphibians, reptiles, birds, and mammals; (2) copies of 19th century Italian art masterpieces purchased by Dr. Edward Murphy to be displayed in the museum; (3) folk art of Jacob Maental (1778–1863), who emigrated from Germany to America in 1805 and lived his last years in New Harmony; and (4) Harmonist



Figures 4–6.—Plant fossil specimens from the James Sampson Collection at the Working Men's Institute in New Harmony, Indiana. 4. Specimen of *Calamites* (WMI 300.228). 5. *Stigmaria* specimen collected from the cutoff locality near New Harmony, Indiana. Please note the original label also indicating "coal measures" (=Upper Carboniferous) (WMI 300.159). 6. Specimen of *Neuropteris* collected from the Rush Creek locality near New Harmony, Indiana (WMI 300.18).

artifacts donated to the WMI by the Harmony Society in 1914.

The natural history collections at WMI were arranged in a "discovery" style consistent with Maclure's emphasis on learning through observation. In particular, mineral and fossil specimens were displayed with minimal labeling and grouped by donor, such as the Sampson collection or the Cox collection. Unfortunately, these exhibits provided no historical background on the importance of New Harmony to the science of geology, and minimal details were provided about the works of William Maclure, David Dale Owen, Richard Owen, and Edward Travers Cox, all prominent geologists who lived and worked in New Harmony, Indiana.

In 1979, an exhibit opened in the Keppler House highlighting the geologic studies conducted from the 19th century New Harmony, focusing on the work of David Dale Owen. In 2007, the exterior siding needed to be replaced on the Keppler House. As the old siding was removed, it was discovered that the roots of a tree growing near the building had comprised the foundation. To make repairs, the building had to be lifted from its foundation and rotted boards replaced, which required the closure of the Keppler House exhibit. During the dismantling of this exhibit, seven fossil specimens and nine printed fossil plates were transferred to the Atheneum and all other remaining items moved to storage.

With the closure of the Keppler House exhibit, plans were initiated to create a new display at WMI to highlight the historic significance of New Harmony to early 19th century geologic studies of the Midwest. This new exhibit needed to combine the original "discovery" feel of the original WMI displays, but provide narrative panels that would guide a visitor through geologic studies conducted by William Maclure, David Dale Owen, Richard Owen, and Edward Cox, as well as summarize basic geologic principles and provide relevance of modern geologic studies in the region.

NEW GEOLOGY EXHIBIT AT THE WORKING MEN'S INSTITUTE

With the loss of the exhibit in the Keppler House in 2007, a new exhibit was proposed to highlight the geologic contributions of William Maclure, David Dale Owen, Richard Owen, and Edward Travers Cox at the WMI. The design for the exhibit at WMI was developed from September 2012 to May 2014 with the assistance and support of stakeholders in New Harmony, including the Director and Board Members of the WMI, Director of Historic New Harmony, and the Collections Manager of the New Harmony State Historic Site.

The installation of the new exhibit at WMI involved the curation and temporary removal of 125 mineral and 89 fossil specimens from seven wood trimmed glass display cases; dimensions of each of these display cases are provided in Table 1. The display cases are original to the WMI building completed in 1894, and efforts were made to preserve these cases with minor alteration (Figs. 7 & 8). All samples removed from the cases were photographed and curated prior to placing them in temporary storage in the Lilly Archive at WMI. A digital catalog was prepared, including the accession number, photograph, and a brief description of each specimen.

Using published literature and resources at the WMI, narrative panels were prepared to summarize the historic significance of New Harmony to early 19th century geologic studies. In particular, a reproduction of an 1818 geologic map of the United States compiled by William Maclure is included in the new exhibit. An original copy of this map is stored in the Lilly Archive at WMI. Additionally, a specimen of *Maclurites*, a planispiral fossil gastropod, is on display, named in honor of William Maclure by Charles Alexandre Lesueur in 1818. Additional panels in the new exhibit highlight the evolution of the geologic time scale, localities near New Harmony significant to early scientific studies, and contributions of David Dale Owen, Richard Owen, and Edward Cox to resource exploration of the Midwest United States in the early 19th century. Moreover, narratives in the exhibit highlight modern studies in southern Indiana, such as seismic monitoring of the Wabash Valley Fault Zone and flooding hazards of the Wabash River.

The new exhibit at WMI includes items once in the Keppler House and items from Historic New Harmony. Mineral and fossil samples in the new exhibit were arranged by themes: (1) mineral identification and properties; (2) crystallography of minerals; (3) ore minerals and their economic importance; (4) fluorite and associated minerals from southern Illinois and northern Kentucky; (5) plant fossils; and (6) invertebrate fossils (Table 1). Selected geologic specimens from the WMI collections were used to supplement narrative panels (Fig. 8). Eight specimens, including granite, rhyolite, blueschist, septarian concretions, and a triolibite, were loaned to WMI from

Table 1.—Dimensions and content of display cases with the new geology exhibit at the Working Men's Institute in New Harmony, Indiana.

Case	Width (cm)	Height (cm)	Depth (cm)	Content of display cases
Mineral Identification & Properties	160	81	32	Mineral properties, including hardness, color, luster, cleavage, and crystal form. Specimens displayed include apatite, calcite, corundum, fluorite, gypsum, jadeite, kyanite, microcline, muscovite, quartz, sulfur, topaz, and tourmaline.
Crystallography of Minerals	228	244	32	Early geologic studies including a reprint of Maclure's 1818 geologic map of the United States, Werner's proposed geologic history, Hutton's concept of Plutonism. Brief history of the Harmonist society, the Utopian society, and Working Men's Institute. Bottom of the case displays mineral specimens and highlights the focus on crystallography in the 18th and 19th centuries.
Ore Minerals & Economic Importance	160	81	32	Ore minerals and their economic importance to recovery of copper, iron, lead, and zinc. Specimens on display include: azurite, calcite, fluorite, galena, hematite, magnetite, malachite, marcasite, native copper, and sphalerite.
Fluorite Minerals from Illinois & Kentucky	226	244	62	Geologic contributions of David Dale Owen, Richard Owen, and Edward Travers Cox. Five specimens from the Cox Collection at WMI are used in this display case. Panels also highlight significant geologic localities near New Harmony, Indiana. Constructed pedestals display seven fluorite specimens probably collected from the fluorspar district of southern Illinois and northern Kentucky.
Plant Fossils	158	81	32	Eleven plant fossils from the James Sampson Collection at WMI displayed with plates of the hand drawn sketches from David Dale Owen's geologic reports.
Invertebrate Fossils: Brachiopods, Bryozoans, & Trilobites	224	244	32	James Sampson and his natural history collection, including specimens in the Smithsonian Institution. Focus on various geologic laboratories used in 19th century New Harmony. Emphasis on the Indiana Geological Survey and the Geology program at University of Southern Indiana. Discussion of geologic hazards in southern Indiana, such as seismic risk along the Wabash Valley Fault Zone and flooding along the Wabash River. Bottom of the case displays invertebrate fossils from WMI collections.
Invertebrate Fossils: Corals & Mollusks	158	81	32	Invertebrate fossils from the WMI Collections displayed with plates of the hand drawn sketches from David Dale Owen's geologic reports. Includes specimen of *Maclurites*, named in honor of William Maclure by Charles Alexandre Lesueur in 1818.

Figures 7 & 8.—Before and after photographs of one of the large display cases on the second floor of the Working Men's Institute in New Harmony, Indiana. 7. Case before construction of new exhibit with unlabeled mineral specimens. Note that the shelving is not original to the display case. 8. Display case after installation of the new exhibit with specimens integrated with the narrative panels (arrows).

the geology collections at the University of Southern Indiana. The exhibit was completed in August 2014 for the New Harmony bicentennial celebration.

In addition to the new exhibit at WMI, two hands-on teaching kits were prepared for use by K–12 teachers and visitors to WMI. One kit focuses on mineral identification, and the other on fossil identification (Figs. 9 & 10). The mineral kit contains: (1) 12 cases of 12 minerals each; (2) 20 magnets; (3) 20 streak plates; (4) 20 glass plates; (5) 20 steel nails; and (6) 20 hand lenses (10×). The fossil kit contains: (1) 12 cases of 12 fossils each; (2) educational panels showing important features of each fossil group; and (3) 20 hand lenses (10×). Teaching guides accompany the kits to provide teachers with background information, identification keys, and suggestions for hands-on activities. The kits and activities are aligned with the specimens on display in the new exhibit and focus upon students conducting hands-on activities that involve sketching. Each kit is packaged in a plastic tub that may be easily transported. These kits are available for check–out from the WMI in New Harmony, Indiana.

IMPORTANCE OF SKETCHING TO LEARNING SCIENCE

Illustrations were paramount to scientific discovery and documentation in the early 19th century, especially prior to portable photography (Rudwick 1976; Davidson 2008, p. 45–46; Johnson 2008). In the 20th century, photographs became pervasive in introductory science textbooks and 35 mm slide shows were commonplace in the introductory science classroom. In the 21st century, software and audiovisual projectors have resulted in pervasive expansion of digital slide presentations in the introductory science classroom.

In the past decade, many studies have focused on active learning and increasing student interest in science, as well as improving performance in the classroom (Lorenzo et al. 2006; Haak et al. 2011; Henderson et al. 2011; Freeman et al. 2014). Sketching is one active tool that improves

Figures 9 & 10.—Teaching kits available at Working Men's Institute. 9. Fossil kit with sample sets and instructional panels. 10. Mineral kit with sample sets and mineral testing materials. Both kits are accompanied with lesson plans and teaching guides.

cognitive retention of scientific principles (Lane et al. 2009; Ainsworth et al. 2011). Ainsworth et al. (2011) outlined five reasons learning by drawing is a key component to science education: (1) enhances engagement; (2) offers representations of scientific processes; (3) promotes reasoning in science; (4) affords a unique learning strategy; and (5) encourages communication. Likewise, concept-sketches are used in textbooks and by instructors to assist students in learning earth science processes, such as plate tectonics, relative dating, and paleontology (Johnson & Reynolds 2005; Yacobucci 2012).

Interestingly, the synergetic approach to education proposed by Robert Owen and William Maclure in the early 19th century aligns with the goals of active learning and the importance of sketching to science comprehension by students. The unique visual language that emerged in the early 19th century led to significant progress in the natural sciences, especially geology (Rudwick 1976). In the modern world, Arnheim (1969) concluded that this graphicacy, visual and graphic skills, remains as important to education as literacy and numeracy. Geology provides a unique opportunity for students to learn visual and graphic skills, and the discipline has a rich history of using illustrations and maps in scientific publications (Rudwick 1976). Furthermore, sketching by students has been an important component to teaching invertebrate paleontology in the 19th and 20th centuries, and continues to be an important pedagogical method.

Recent studies have emphasized the importance of sketching and visual-spatial skills in education for the purpose of increasing comprehension of basic scientific principles (Mathewson 1999, 2005; Jee et al. 2014), and to geology specifically (Johnson & Reynolds 2005; Martínez–Peña & Gil–Quílez 2014). Thus, it is vital to encourage students to sketch in the science classroom in order to gain important visual-spatial skills and to demonstrate understanding of scientific concepts gained through focused observation (Mogk & Goodwin 2012). As part of the new geology exhibit at the WMI, fossil and mineral kits provide students with an opportunity to conduct hands-on activities that require sketching and engage students with visual-spatial exercises (e.g., building models).

In addition to sketching, Mueller & Oppenheimer (2014) have concluded students that take handwritten notes are more likely to retain information than those students relying on technology, such as laptops, cameras, and/or smartphones. This suggests that students should be encouraged to take handwritten notes in the science classroom, while also providing an opportunity for students to draw sketches. In geology, notetaking and sketching are especially important to field notebooks and recording field observations.

The progress toward incorporating more sketching in the science classroom begins with engaging K–12 teachers with active learning. Almquist et al. (2011) developed an integrated field–based approach to enhance geoscience skill

Figure 11.—Participants in the USI day camp in June 2015 involved with activities using the mineral kits prepared for the Working Men's Institute.

sets of teachers. Specifically, this field experience was designed to enhance spatial visualization through detailed study of stratigraphic sequences. They practice three visual skills that are particularly important to geologists (Titus & Horsman 2009): (1) spatial relations; (2) spatial manipulation; and (3) visual penetrative ability. Thus, active learning activities that incorporate these spatial visualizations are important to the engagement of teachers and the transfer of more visual learning to their science classrooms.

As part of the new exhibit at the WMI in New Harmony, the fossil and mineral kits provide K–12 teachers an opportunity to incorporate active learning into their science classrooms. Through lesson plans and guides provided with the kits, teachers are encouraged to require students to maintain a field notebook, build models, and sketch for the purpose of understanding key scientific processes and concepts. Additionally, day camps and visitors to the WMI may use these kits for educational activities in New Harmony (Fig. 11). Active learning approaches such as these have been found to increase student interest in pursuing science degree programs and careers (Watkins & Mazur 2013; Xu 2013; Freeman et al. 2014; Maltese et al. 2014).

SUMMARY

The new geology exhibit at WMI and the related hands-on teaching kits provide regional K–12 teachers with an important resource. The new exhibit and teaching kits follow the tradition of the Pestalozzian method and align with the original goals established for the WMI by Maclure in 1838. Furthermore, the development and installation of the new geology exhibit at WMI provides a unique outreach activity for faculty members and undergraduate students at the University of Southern Indiana. The new exhibit, a fitting replacement for its predecessor in the Keppler House, provides a vital addition to New Harmony. Visitors can learn more about the connections of New Harmony to the 19th century geologists whose studies in the Middle West America shaped that science. Finally, community outreach activities and new Museum exhibits are vital to expanding the visibility of and creating public interest in the natural sciences, especially in geology and the earth sciences.

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WIND POWER FEASIBILITY STUDY FOR BALL STATE UNIVERSITY

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ABSTRACT. Based on two years of site-specific wind speed measurements and actual power curve performance estimates of five commercial wind turbines, a feasibility study of wind-power potential near Ball State University has been conducted. The measured wind speed data from the study site allow a more accurate estimate of the expected energy produced per year from a given turbine than would be obtained by just scaling the estimates based on the rated output power of the turbines. Results show that four out of the five selected turbines could be expected to achieve payoff of combined lifetime costs well within the turbines' estimated lifetimes. Expected savings on the cost of electrical energy range from $2 million to $4 million for a 25-year lifetime. Based on the predicted monetary savings from energy produced by a turbine over its expected lifetime, coupled with trends of decreasing costs and increasing turbine performance, the option of installing a wind turbine to supplement the electrical energy needs of Ball State University appears economically feasible. A renewable energy source, such as a wind turbine, also provides an opportunity for the University to profit from the sale of renewable energy credits (RECs).

Keywords: Wind power, economic feasibility, Muncie, Indiana

INTRODUCTION

Alternative and sustainable energy options have been prioritized at Ball State University (BSU), as exemplified by the Ball State geothermal energy system, the nation's largest ground-source, closed loop system (BSU 2015). Another sustainable energy option for north-central Indiana is wind power, which is expected to grow in production over the next decade (Swiatek 2015).

Currently, installed wind power facilities in Indiana have a combined capacity of nearly 1800 MW, mostly in larger utility wind farms, contributing 2.7% of Indiana's total electricity production (IOED 2015). In order to assess the wind power potential in various regions of the State, long-term records of wind speed data are preferred. One such study recorded wind speeds for a period of one year (2004–2005) on fixed towers at five locations throughout Indiana (Indiana Energy Group 2005). The tower sites were widely distributed throughout the State, with the closest one to BSU, or Muncie, IN, located approximately 35 miles to the southwest. Since wind speeds can vary substantially across geographical regions of this size, a local long-term wind speed study is necessary for assessing the feasibility of wind power for BSU.

Average annual wind speed maps are available for Indiana, and can be used to gain perspective on which regions of the State may produce the most wind energy. For example, regions northwest of Indianapolis show greater potential than regions directly south of Indianapolis (U.S. DOE 2015). The annual average wind speed in the Muncie area (at 80 m above ground level) is estimated at approximately 7.0 m/s. However, reliance on these model-derived estimates is not advised for preparing an economic feasibility study of installing a wind turbine in a given location. The practical reasons requiring a local long-term (non-averaged) study of wind speed in order to develop a wind power feasibility study are described in the data and analysis section.

Ball State University owns a rural property known as Cooper Farm located about 4 miles northwest of the main campus in Muncie, IN (BSU FSEEC 2015). In order to conduct a feasibility study of the wind power potential near the BSU campus, in June 2012, an anemometer was installed 15 m above ground level on an existing tower on the property. Wind speed data have been continuously recorded since then and are archived on a secure digital card and on a web site that receives the data via transmission from

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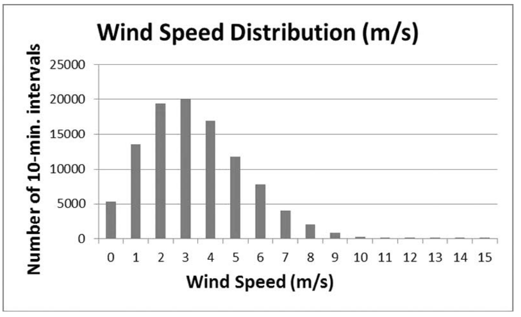

Figure 1.—Wind speed distribution plot in m/s for a two-year period at Ball State's Cooper Farm location, for a measurement height of 15 m.

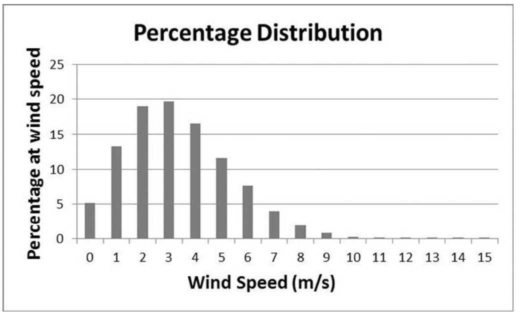

Figure 2.—Percentage wind speed distribution (giving the percentage of time that the wind blew at each speed) for a two-year period at Ball State's Cooper Farm (15 m height).

the wind data logger to a wireless internet site on the property.

DATA AND ANALYSIS

The wind speed data analyzed for this feasibility study covered a two-year period from 26 February 2013 through 25 February 2015. For accurate annual average wind power estimates, it is important that the data window cover an integral number of years, and not include a fraction of an integral number of years. The reason for this is that wind speeds vary significantly for different seasons of the year, and analyzing a fractional year would yield skewed annual wind power estimates. The raw wind data from the anemometer at Cooper Farm are archived in the form of a number equal to the number of rotations, N_r, of the anemometer during a 10-min interval, yielding 144 data points per day. Over the two-year data window, a total of 102,126 wind speed data points were available, corresponding to 97.2% coverage. The slight variation from 100% coverage is due to intermittent loss of the wi-fi connection between the on-site data logger and the BSU wireless internet connection in the residential classroom facility at Cooper Farm.

To conduct the data analysis, raw data were downloaded into a spreadsheet where N_r was converted into a wind speed in m/s, according to the manufacturer's conversion formula for the anemometer. The unit installed at Cooper Farm is a solar-powered, self-contained wind data logger unit from APRS World (APRS6063) with anemometer #40R (current cost of about $900). The number of rotations of the anemometer is typically many thousands during the 10 min sampling interval. For example, a wind speed of 15.0 mph (6.7 m/s) corresponds to $N_r = 10{,}000$. In order to make sense of the large number of data points over the two-year period, a distribution plot of the wind speed data is produced, in which the wind speed is binned in increments of 1 m/s (Fig. 1). The wind speed distribution exhibits a typical Weibull distribution, which is sometimes used to approximate wind speeds when actual data are unavailable (Wizelius 2007).

From the wind speed distribution data, one may obtain the number of hours that the wind blew at a given speed by dividing the distribution data by six intervals per hour. Then, the percentage of time that the wind blew at a given speed is obtained by dividing this result by the number of hours in a two-year period (corrected for 97.2% coverage). The result for the percentage wind speed distribution in m/s is shown in Fig. 2.

The power available from the wind is proportional to the wind speed cubed (v^3), meaning that a disproportionate fraction of the total power available from the wind occurs when wind speeds are highest. For example, the power output of a turbine for a wind speed of 2 m/s is eight times higher compared to the power output for a wind speed of 1 m/s. The cubic dependence of wind power on wind speed is one of the underlying reasons why actual wind speed distributions are necessary for accurate power estimates at a given site, rather than relying on an estimation of average wind speeds.

Another factor that must be taken into account when determining the power output expected from a given wind turbine is the hub height of the turbine. Wind speed typically increases with distance above the ground (or water) level according to a standard formula, depending only

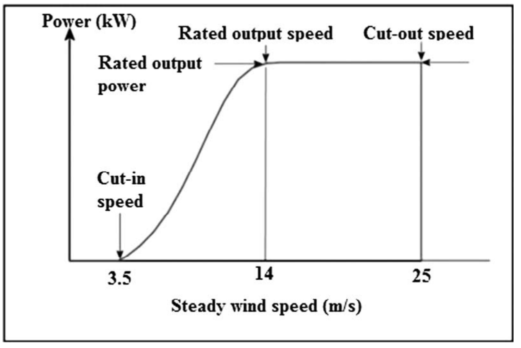

Figure 3.—A typical power curve for a wind turbine, showing the power produced as a function of wind speed (graph adapted from Wind Power Program 2015).

upon the height and the type of surrounding terrain. The wind gradient is greatest when the surrounding region has tall obstacles (such as a city or a forest), and it is least when the surrounding terrain is relatively flat and smooth (such as open farmland or water). To estimate the wind speed, v, at a height h compared to the wind speed v_0 at height h_0, the following formula is used (Wizelius 2007),

$$v = v_0 \left(\frac{h}{h_0}\right)^{\alpha}. \quad (1)$$

The parameter α is determined by the roughness category of the surrounding terrain. For the Cooper Farm location, the terrain is open farmland along with some trees and a couple of one-level structures, for which $\alpha = 0.25$. Using Eq. (1) with this factor of α indicates that the wind speed at, for example, 100 m is 1.6 times greater than it is at 15 m. For the given hub height of a selected commercial wind turbine, Eq. (1) will be used to adjust the raw wind speed data shown in Figs. 1 & 2. Employing an estimated correction for height, instead of measuring the wind speeds at the actual turbine hub heights, is a source of uncertainty in the analysis. Nonetheless, with the small value of the exponent α, errors in the height correction for the wind speed should be less than 10%. But since the wind power varies as the wind speed cubed, inaccuracies in the estimated power could be as much as 30%. More accurate data and results would require installing anemometers on a tower and collecting long term wind speed data at increments of about 20 m (from 80 m to 140 m height). The main expense for collecting data at heights relevant for commercial wind turbines would be the installation of the tower itself, but compared to the cost of a commercial turbine, it would be a reasonable initial investment.

COMMERCIAL WIND TURBINES

In order to transform the wind speed distribution data into potential electrical power produced, wind turbine specifications need to be obtained for various commercially available models. The two most important specifications are the turbine hub height (to be used in Eq. 1) and the *power curve* of the turbine. The turbine's power curve is determined by the manufacturer and shows how much power the turbine will actually produce at different wind speeds. At higher wind speeds, there is a cut-out speed above which the turbine will shut down in order to avoid possible mechanical damage. A typical power curve is shown in Fig. 3. The power produced increases with wind speed up to a rated maximum. Below a threshold wind speed, the turbine will not turn and produces no power output.

New wind turbine models are continually being developed by manufacturers. For this study, wind turbine power curve data were obtained for turbines from five different manufacturers (The Wind Power 2016): Vestas, General Electric (GE), Nordex, Repower, and Gamesa. At this step in the wind power analysis, the procedure begins with the data of Fig. 2 and first calculates the adjusted wind speed at the hub height of the particular turbine, using Eq. (1). This result yields the percentage of time that the wind blows at a given speed at the specified turbine hub height. Next, using the power curve data for a particular turbine, the percentage fraction at each wind speed is multiplied by the turbine output power at that wind speed, and then multiplied by the number of hours in a year to obtain the output energy per year at each wind speed. These results are shown in Fig. 4 for each of the selected wind turbines.

The total estimated output energy per year for each of the five commercial wind turbines is found by summing data points shown in Fig. 4 across the total range of wind speeds (0–25 m/s) (Table 1).

ECONOMIC ANALYSIS OF WIND TURBINE POTENTIAL

On the basis of total energy production (Table 1) the Vestas 126, 3.3 MW turbine would be the best pick; however, other factors, collectively known as Total Cost of Ownership (TCO), factor

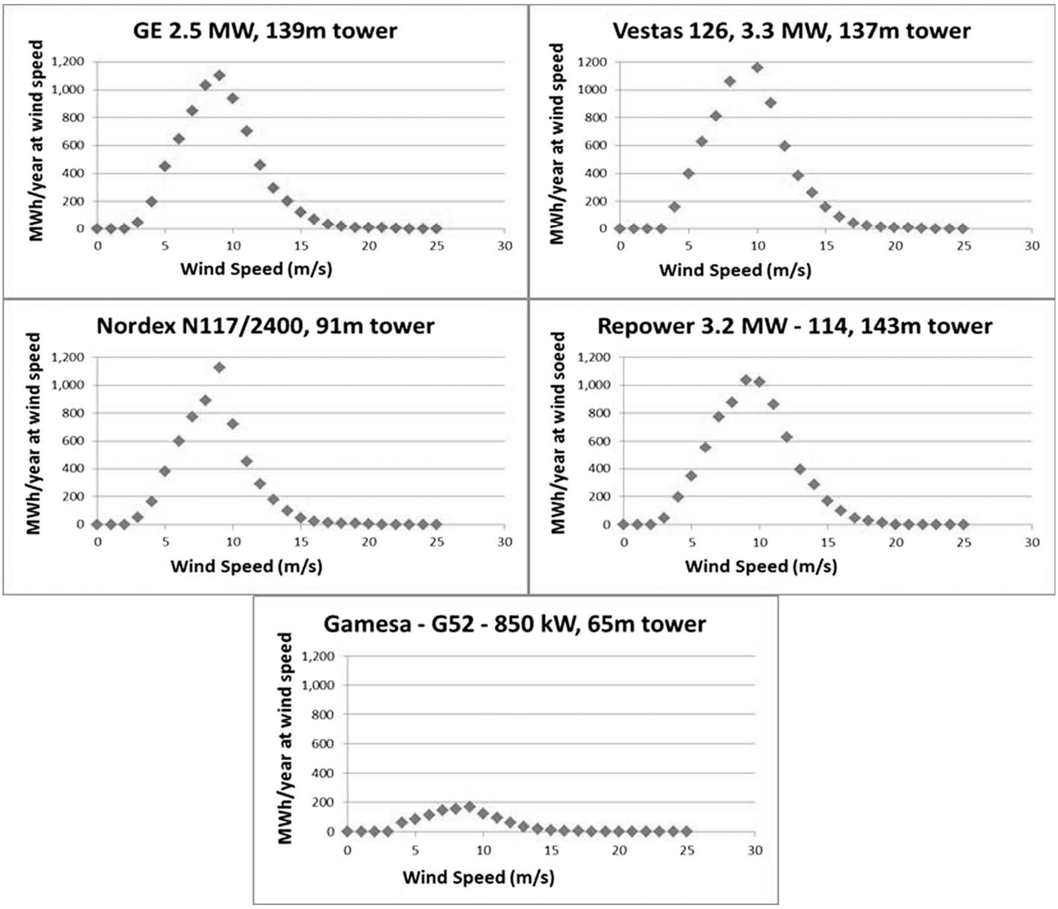

Figure 4.—Estimated wind turbine output energy per year (in megawatt-hours per year) versus wind speed at the specified hub heights for five commercial turbines.

into a turbine's overall economic feasibility. TCO includes the initial cost of the turbine, the on-site installation cost of the turbine, and its lifetime maintenance cost. Commercial-grade wind turbine pricing is available on an average dollars-per-kilowatt basis, and has shown significant variation over the last 10 years (IRENA 2012). Department of Energy data give an average *installed* capital turbine cost of \$1940/kW for 2012 (AWEA 2016). Installed costs include, in addition to the turbine itself, the tower foundation, grid connection, site preparation, consulting, and permits, totaling an average of \$700/kW, increasing the total capital cost of a turbine from about \$1240/kW to \$1940/kW. The estimated capital cost of wind turbines is expected to decline over the next several decades, with costs in the year 2020 estimated at 85% of 2011 costs, and the cost in 2040 estimated at 72% of 2011 costs (IRENA 2012). Reducing the 2012 capital costs for a wind turbine by 15% yields an estimated capital cost in 2020 of approximately \$1054/kW. Possible reductions in the installation costs of a turbine are expected to be modest over the decade from 2011 to 2020 and are not considered here. For our calculations, we will use the average installation cost of \$700/kW, as specified above.

Operation and maintenance (O&M) costs for installed commercial-grade wind turbines must also be factored into an estimate of the economic feasibility of a wind turbine facility. O&M costs have decreased significantly from 1980 and now average \$10/MWh for newer projects (IRENA 2012). Another method of estimating O&M costs utilizes a percentage of the capital cost of the turbine: "For modern machines the estimated maintenance costs are in the range of 1.5% to 2% of the original investment per annum" (WMI

Table 1.—Estimated energy production per year by each of the five selected commercial wind turbines using wind data from the Cooper Farm site.

No.	Wind turbine type	Energy/year (MWh/yr)
1	GE 2.5 MW, 139 m tower	7190
2	Vestas 126 - 3.3 MW, 137 m tower	7920
3	Nordex N117/2400, 91 m tower	5830
4	Repower 3.2 MW – 114, 143 m tower	7370
5	GAMESA - G52 - 850 kW, 65 m tower	1070

2016). For the estimated O&M costs presented in Table 2, a value of 2% of the capital cost per year is used. Projections of O&M costs suggest a declining trend over the next couple of decades, with approximately 7% lower costs in 2020 compared to 2011, according to a study of wind turbines in the United Kingdom (IRENA 2012).

A summary of the economic feasibility of the five sample wind turbines used in this study is given in Tables 2a & 2b. While the capital, installation, and maintenance costs scale directly with the rated output power of the various turbines, the energy produced per year does not, being dependent upon how each turbine responds to the spectrum of wind speeds at the chosen site. In general, turbines with higher hub heights produce more energy for a given output rating, since average wind speeds increase with height above the ground.

The calculated results of Table 2b indicate that of the five commercial wind turbines included in the study, the GE 2.5 MW turbine (#1 in the table) with a 139 m hub height is projected to yield the best estimated performance, based on the measured wind speed data at the Cooper Farm site. The installed cost of this turbine could be paid off in just under 11 years, and factoring in O&M costs for 20 years yields a payoff time of 13.5 years. Each year the wind turbine is used after payoff results in savings of approximately $400,000, based on the current cost of electricity paid by Ball State (Lowe, Associate Vice President for Facilities Planning and Management at Ball State University, Pers. Comm. 2016). The total savings for a 20-year lifetime is projected to yield $2.6 million in avoided costs, and for a 25-year lifetime the savings is projected to yield nearly $4.4 million in avoided costs. The mathematical formulae used to obtain these results are spelled out in the Appendix. Obviously, the magnitude of the estimated savings depends strongly upon the actual lifetime of the turbines. A recent comprehensive study in the UK has indicated a positive trend in wind turbine lifetimes, stating that newly installed turbines should operate effectively for up to 25 years (Myers 2014). Allowing for a modest decrease in turbine output power with age could reduce the estimated lifetime savings by approximately 20–25%.

An additional consideration affecting the economic viability of a renewable energy source such as a wind turbine is the possibility of selling renewable energy certificates (RECs). Each REC validates an amount of renewable or "green" energy equivalent to 1 MWh (Lau & Aga 2008). The green energy market is growing, with the sale of RECs allowing customers to purchase green energy even if local sources of green energy are not actually available. The price of RECs varies with location and time, and commercial vendors are often used to establish specific transaction pricing. Nationally-sourced RECs sold at approximately $0.50/MWh in September 2015 (U.S. DOE 2016). With an average turbine output of 7200 MWh/yr.

Table 2a.—For the 5 wind turbines listed in Table 1, estimates of the capital and installation costs, the lifetime maintenance costs, and the overall 20-year lifetime cost for each turbine are given. In addition, the estimated energy produced by each turbine for the Cooper Farm site is shown (referenced from Table 1). Calculations used in obtaining the numerical values presented in Tables 2a & 2b are detailed in the Appendix.

Turbine	MW rating of turbine	Cost of turbine (2020)	Installation cost	Maintenance cost of 20-yr life-time	Total 20-yr lifetime cost of turbine	Energy produced by turbine in a year (kWh/yr)
1	2.5	$2,635,000	$1,750,000	$1,054,000	$5,439,000	7,192,548
2	3.3	$3,478,200	$2,310,000	$1,391,280	$7,179,480	7,917,526
3	2.4	$2,529,600	$1,680,000	$1,011,840	$5,221,440	5,827,897
4	3.2	$3,372,800	$2,240,000	$1,349,120	$6,961,920	7,374,531
5	0.85	$895,900	$595,000	$358,360	$1,849,260	1,074,734

Table 2b.—Total Cost of Ownership (TCO). Continuing from Table 2a, estimates of the number of years for each turbine to achieve payoff of installed costs and total lifetime costs are shown (including O&M costs for 20 years and 25 years). The financial savings to the University per year after payoff are also estimated for both 20 year and 25 year turbine lifetimes.

Turbine	Years to payoff installed cost	Average maintenance cost per year	Years to pay off 20-yr lifetime cost	Money saved/yr by using turbine (after payoff)	Money saved over 20-yr lifetime	Years to pay off 25-yr lifetime cost	Money saved over 25-yr lifetime
1	10.9	$52,700	13.5	$402,783	$2,616,654	14.2	$4,367,067
2	13.1	$69,564	16.2	$443,381	$1,688,149	17.0	$3,557,236
3	12.9	$50,592	16.0	$326,362	$1,305,805	16.8	$2,684,656
4	13.6	$67,456	16.9	$412,974	$1,297,555	17.7	$3,025,144
5	24.8	$17,918	30.7	$60,185	-$645,558	32.2	-$434,223

(referring to Table 2a), this could provide annual income from the sale of RECs averaging $3600/yr.

With climate change and the prospect of global warming due to increasing atmospheric CO_2 concentrations being a continuing concern, the possibility of future carbon taxes may also enhance the economic viability of investing in wind power as a sustainable alternative energy source. “Economists and international organizations have long advocated carbon taxes, because they can achieve the same emissions reduction target at lower costs than conventional command-and-control regulations” (Zhang & Baranzini 2003). Experts also suggest that once instituted, the carbon tax rate would increase over time, further increasing the desirability of investing in a viable alternative energy source, such as wind power.

Ball State University is a member of the Association for the Advancement of Sustainability in Higher Education (AASHE) and is a charter and ongoing participant in the Sustainability Tracking, Assessment & Rating System (STARS). In 2012, Ball State’s campus sustainability efforts earned the University a STARS Gold rating, and it has continued to be awarded this high ranking for sustainability performance (BSU 2012; STARS 2016). A major BSU initiative towards sustainability is its comprehensive geothermal energy system (BSU 2015). An investment in wind energy would help to further reduce BSU’s carbon footprint and enhance its reputation as a global leader in sustainability efforts in higher education.

Based on the offset in the cost of purchased electricity, the cumulative savings from the installation of a commercial wind turbine at the Cooper Farm site are expected to range from $2 million to $4 million for a 25-year lifetime. Natural variability in wind speeds from one year to the next is a potential source of uncertainty in the results of this study. Other factors that may affect the estimated economic savings of an installed wind turbine relate to actual costs for turbines at the time of purchase, as established by the manufacturer. This study focused on estimating the expected wind power produced from commercially available turbines, and provided an estimate of economic viability using the payback method. A more comprehensive economic viability analysis would need to incorporate adjustments for expected increases in the utility rate for electrical energy, future inflation, and a net present value calculation of the proposed project with appropriate effective annual discount rates. Installing two or more turbines at the site may result in favorable economies of scale related to the capital and installment costs of the turbines. In addition to the economic and environmental benefits of a wind turbine installation, the facility would provide significant educational opportunities for multiple students to become involved in longitudinal research studies of the turbine’s energy performance and economic impact over its operational lifetime. In summary, based on the predicted monetary savings from energy produced by a turbine over its expected lifetime, coupled with trends of decreasing costs and increasing turbine performance, the option of installing a wind turbine to supplement the electrical energy needs of Ball State University appears economically feasible.

APPENDIX

The mathematical formulae used to obtain the numerical values in Tables 2a & 2b are presented below.

$$\textbf{Cost of turbine (2020)} = (\text{MW of turbine}) \times (1000\ \text{MW/kW}) \times (\$1054/\text{kW})$$

$$\textbf{Installation cost} = (\text{MW of turbine}) \times (1000\ \text{MW/kW}) \times (\$700/\text{kW})$$

Note: "Installed cost" = (Cost of turbine) + (Installation cost)

$$\textbf{Operation \& Maintenance cost for 20 years} = (\text{Cost of turbine}) \times (2.0\%/\text{yr}) \times (20\ \text{yr})$$

$$\textbf{Total 20-year cost of turbine} = (\text{Cost of turbine}) + (\text{Installation cost}) + (\text{O \& M cost for 20 yr})$$

$$\textbf{Years to pay off installed cost} = \frac{(\text{Installed cost})}{(\$0.056/\text{kWh})(\text{kWh/yr produced by turbine})}$$

Note: The current utility price that Ball State pays for electricity is \$0.056/kWh.

$$\textbf{Average maintenance cost per year} = (\text{Cost of turbine}) \times (2\%/\text{yr})$$

$$\textbf{Years to pay off 20-year lifetime cost} = \frac{(\text{Total 20-yr cost of turbine})}{(\$0.056/\text{kWh})(\text{kWh/yr produced by turbine})}$$

$$\textbf{Money saved per year after payoff} = (\text{kWh/yr produced by turbine}) \times (\$0.056/\text{kwh})$$

$$\textbf{Money saved after 20-year lifetime} = (20\ \text{yr} - \text{years to payoff 20-yr lifetime}) \times (\text{Money saved per yr after payoff})$$

The last two columns of Table 2b (for a 25-year lifetime) are calculated in the same manner as for the corresponding columns for a 20-year lifetime.

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RESULTS OF THE 2014 EAGLE MARSH BIODIVERSITY SURVEY, ALLEN COUNTY, INDIANA

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ABSTRACT. Eagle Marsh, a 289.8 ha (716-acre) wetland nature preserve located on the southwest border of Fort Wayne, Indiana, is one of the largest wetland restorations ever undertaken in Indiana. The Little River Wetlands Project (LRWP) began acquisition, planning, and restoration in 2005 to 2007. The first biodiversity survey (also known as a bioblitz) of Eagle Marsh was conducted on 31 May and 1 June 2014. Over 125 scientists, naturalists, students, and other volunteers on thirteen different taxonomic teams observed and reported 728 taxa during the event. The thirteen taxonomic teams included aquatic macroinvertebrates, beetles, birds, butterflies, dragonflies and damselflies, fish, freshwater mussels, herpetofauna, small mammals, mushrooms/fungi, singing insects, snail-killing flies, and vascular plants. This manuscript presents both a brief history of Eagle Marsh and a summary overview of the results gathered by the thirteen taxonomic teams.

Keywords: Bioblitz, biodiversity survey, Eagle Marsh, state-endangered, county records

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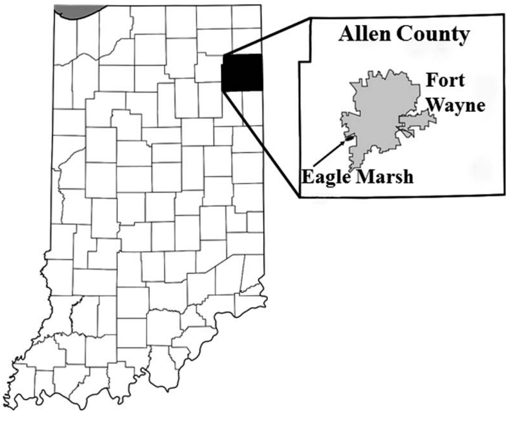


Figure 1.—Map of Indiana showing the location of Allen County (left) and Eagle Marsh within Allen County (right).

INTRODUCTION

Eagle Marsh, a 716-acre wetland nature preserve, is located on the southwest border of Fort Wayne, Indiana (Fig. 1). The wetland preserve has over 11 miles of trails allowing hikers access to the preserve's variety of habitats which include permanent ponds, ephemeral open bodies of water, marsh, sedge meadow, wet to mesic prairie, shrubland, and a mature swamp woodland. Eagle Marsh is one of the largest wetland restorations ever undertaken in Indiana. With the support of the federal Wetlands Reserve Program, the Indiana Heritage Trust of the Indiana Department of Natural Resources, The Nature Conservancy of Indiana, various foundations, and others, Little River Wetlands Project (LRWP) acquired Eagle Marsh (then 676 acres) in 2005. The restoration included digging shallow areas deeper, breaking drain tiles, and removing pumps to increase the volume of water on the land, thus restoring some elements of wetland hydrology. Over 500 acres were seeded with native rushes, grasses, and wildflowers and more than 45,000 native trees and shrubs planted. Between 2007 and 2010, the 40-acre mature swamp woodland was added to the preserve, thus bringing the total acreage to the present day 716. In addition to expanding floral diversity, the swamp woodland provided important habitat for many birds and other fauna that required large trees, sandy soil, or leaf litter to complete their life cycles (LRWP 2016a). Furthermore, the acquisition of this woodland allows for future protection of this important site.

The first biodiversity survey (known as a bioblitz) of Eagle Marsh was conducted on 31 May and 1 June 2014. The information gathered during the Eagle Marsh BioBlitz significantly increased our understanding of the vast biological resources at the site. Additionally, it allowed members of LRWP to evaluate the success of their efforts to restore the biodiversity of the site and it provided valuable information to determine future management strategies.

This paper presents a brief history of Eagle Marsh, results, and a summary overview of the information gathered by the thirteen taxonomic teams. The history of Eagle Marsh was provided by Judy Nelsen and Tony Fleming.

A BRIEF HISTORY OF EAGLE MARSH

Around 15,000 years ago, a huge lake formed between the melting Wisconsin glacier and a recently-deposited moraine, which acted like a dam to impound the lake. About 14,000 years ago, the lake overtopped the moraine near what is now downtown Fort Wayne and eroded a large outlet, unleashing a flood of unimaginable proportions, commonly called the "Maumee Torrent". In a matter of days or weeks, billions of gallons of water scoured out an existing river channel into a valley up to two miles wide. Related geologic events later changed the course of nearby waterways and location of a continental divide. The valley, stretching between present-day Huntington and Fort Wayne, developed into a fertile 25,000-acre wetland where wildlife thrived and Native Americans hunted.

When settlers arrived, this vast wetland became known as the "Great Marsh," and one of the streams traversing it was called the Little River. This river was of great strategic value because, in all but the driest times, one could canoe up it from the Wabash River to a place near present-day Fort Wayne where a shorter or longer portage (depending on the season) allowed passage to the St. Mary's River and Lake Erie. A "golden gateway" between the Mississippi River and the Great Lakes had been found. The continental divide near the portage later became the high point along the Wabash & Erie Canal.

By the 1870's, Fort Wayne had grown from a small outpost to a town of 18,000. Farmers and others began pushing for the Great Marsh to be drained. After several attempts, by the late 1880's the job was largely done, mostly by creating three main drainage ditches and many minor ones. One of the larger efforts was the Graham-McCulloch

Ditch, which crosses what is now Eagle Marsh. Agriculture quickly expanded across the fertile soil of the old marsh, at first with crops such as onions, lettuce, and celery; then, after World War II, with the grain crop rotation of corn-soybeans-wheat. However, even extensive systems of closely spaced drainage tile, ditch laterals, and large pumps running 24 hours a day were sometimes not enough to prevent crops from being flooded out.

In 1990, Little River Wetlands Project (LRWP) was formed with the mission of restoring and protecting some of the lost wetlands of the Little River valley (later watershed). Between 2000 and 2004 the organization had acquired two restored wetlands a few miles southwest of Fort Wayne. The owner of the land that was to become Eagle Marsh had enrolled it in the Wetlands Reserve Program, a federal program that paid farmers to let the government restore wet agricultural land back to wetland and place it under a conservation easement in perpetuity. The original 676 acres consisting of farmed portions, a wooded fringe, barn and parking area were sold to LRWP in 2005. The Indiana Heritage Trust, The Nature Conservancy in Indiana, local foundations and generous donors helped fund the purchase, with the Indiana Department of Natural Resources (of which the Indiana Heritage Trust is part) retaining partial ownership.

After extensive planning by the Wetlands Reserve Program and LRWP, one of the largest contiguous wetland restorations ever undertaken in Indiana began at Eagle Marsh in late 2006. It was designed to emulate the original bottomland communities of the marsh, while adapting to a condition of relative water scarcity because hydrologic changes in the valley have limited the flow of water to the area. In addition to breaking tiles, plugging ditch laterals, and removing pumps to hold water on the land, earth was moved to enhance or recreate some of the old meander scrolls. These basins retain water that might otherwise run off and serve as crucial habitat for wildlife ranging from the tiniest insect larva to large shorebirds. The restoration incorporated extensive prairie areas along with what could be considered true marsh. A highly diverse mix of plants was used to increase the probability of success given the land's variable hydrology. Virtually all the property was seeded with a combination of native grasses, sedges, and wildflowers and approximately 45,000 native trees and shrubs were planted, with the initial restoration completed in 2009.

Over time the new native vegetation has become established to create a varied habitat of shallow water wetlands, wet and drier prairie, a sedge meadow, and areas of newly planted native trees. Forty more acres of mature forested wetland were added to the preserve between 2007 and 2010, bringing it to its present size. The mature wet woods provide important habitat for many birds and remnant populations of native amphibians, reptiles, and insects. Since the restoration, LRWP staff has sought to deal with invasive plant species that appeared. Prescribed burns, mowing, hand-pulling, and use of herbicides are part of the annual invasive management effort.

Today more than 11 miles of nature trails allow the preserve's many visitors to enjoy its varied habitats and the extensive free nature programming offered there. A total of 227 kinds of birds and numerous other wild creatures, many of them endangered or of special concern in Indiana, have been seen at Eagle Marsh. Bald eagles have nested recently just outside the preserve boundary, and are often seen there with their young. With the adjacent Fox Island County Park and other privately owned natural properties, Eagle Marsh creates almost two square miles of natural habitat for native wildlife. (For further information see "Geology of the Little River Valley" (LRWP 2014), "From a Peaceful Stream to a Power Pawn" (LRWP 2016b), and "Results from the 2014 Eagle Marsh Biodiversity Survey, Allen County, Indiana", pages 10–20 (Ruch 2014).

SUMMARY OF RESULTS AND METHODS

The Eagle Marsh BioBlitz, conducted 31 May and 1 June 2014, attracted over 125 scientists, naturalists, students, and others volunteering their time and expertise to make the event an overwhelming success. Thirteen taxonomic teams and their leaders reported 728 taxa (Table 1). To obtain a complete picture of the biodiversity found at Eagle Marsh, long-term seasonal surveys are necessary. Even so, this two-day survey provided a "snapshot in time" and has revealed the remarkable species richness and the inherent value of this nature preserve. An overview of the results from the thirteen taxonomic teams follows. To view the complete results, go to the Indiana Academy of Science website at *http://www.indianaacademyofscience.org/*, lay the cursor over

Table 1.—Summary of the 728 taxa reported at the 2014 Eagle Marsh Biodiversity Survey, Allen County, Indiana.

Team	Leader	Taxa observed
Aquatic Macroinvertebrates	Ross Carlson	99 taxa: representing 16 classes, 43 families, and at least 67 genera
Beetles (Coleoptera)	Jeff Holland	64 taxa; common species
Birds	Don Gorney	88 species; 6 state listed species [3 endangered]
Butterflies	Kirk Roth	18 species; common species
Dragon- and Damselflies	Paul McMurray	12 taxa; 1 Allen County record
Fish	Brant Fisher	31 species; no state/federal listed species
Freshwater Mussels	Brant Fisher	2 species; no state/federal listed species
Herpetofauna	Mark Jordan & Bruce Kingsbury	15 species; (1 state endangered)
Mammals	John Whitaker, Jr.	21 species; [6 small mammals, 15 other mammals]
Mushrooms/Fungi	Steve Russell	31 taxa [30 species]; all common
Singing Insects	Carl Strang	13 species; 3 singing insects, 10 other species
Snail-killing Flies	Bill Murphy	15 species; 12 Allen County records
Vascular Plants	Paul Rothrock	320 species; 243 native, but the non-natives have visual dominance at the site

Events at the top of the page and then click BioBlitz Archive.

Aquatic macroinvertebrates.—Aquatic macroinvertebrates recorded for the Eagle Marsh BioBlitz were collected within preserve boundaries on 31 May and 1 June 2014. Collections were made at seven sites (for location of the sites see Ruch 2014) using a D Frame dip net with a mesh size of 500 µm. Sites represented both lotic and lentic systems and comprised a variety of habitats (i.e. pools, riffles, emergent vegetation, and open water). Specimens were placed into jars of 70% isopropanol for storage. Representative voucher specimens were deposited in the Purdue Entomological Research Collections, Department of Entomology, Purdue University, West Lafayette, Indiana.

Collected specimens were identified to lowest practical taxon by use of standard texts (Merritt et al. 2008, Thorp & Covich 2001). In total 99 taxa were recorded, representing 16 classes, 43 families, and at least 67 genera. Taxa found were characteristic of aquatic wetland systems with no new or surprising species found.

Beetles (Coleoptera).—Most collecting occurred on-site during the event. To supplement these collections, several traps were set out two weeks prior to the event and trapped insects were harvested during the event. The traps consisted of two multiple-funnel Lindgren traps, two Intercept panel traps, two window traps, two purple sticky traps, and two pitfall traps. The pitfall traps were baited with dung and placed at the periphery of the parking area, while the other traps were spaced along the main trails through the wooded areas. The Lindgren, Intercept, and window traps were baited with ethanol, while the purple sticky traps were left unbaited.

Sweepnets were used during the bioblitz to capture beetles from vegetation in all areas of Eagle Marsh. During one evening of the event, several lights were used to attract beetles that were hand collected from sheets placed under the lights (Fig. 2). These lights were placed around the periphery of a pole barn that served as the base of operations for the bioblitz. The lights, which consisted of two 1000 W metal halide lights, one 175 W mercury vapor light, and four UV lights, were operated for approximately five hours. However, the mercury vapor light ran continuously until dawn with a collecting bucket under it to capture beetles. Representative voucher specimens were deposited in the Purdue Entomological Research Collection, Department of Entomology, Purdue University, West Lafayette, Indiana.

The number of species of beetles collected at Eagle Marsh was lower than first predicted, with many representatives of the same species. In hindsight this was not surprising since most species were collected at night with lights and the lighted area was effectively an island of dry

Figure 2.—Midnight collecting by the beetle team — beetles are attracted to a 1000 W metal halide light during the Eagle Marsh BioBlitz. (Photograph by Jason Kolenda)

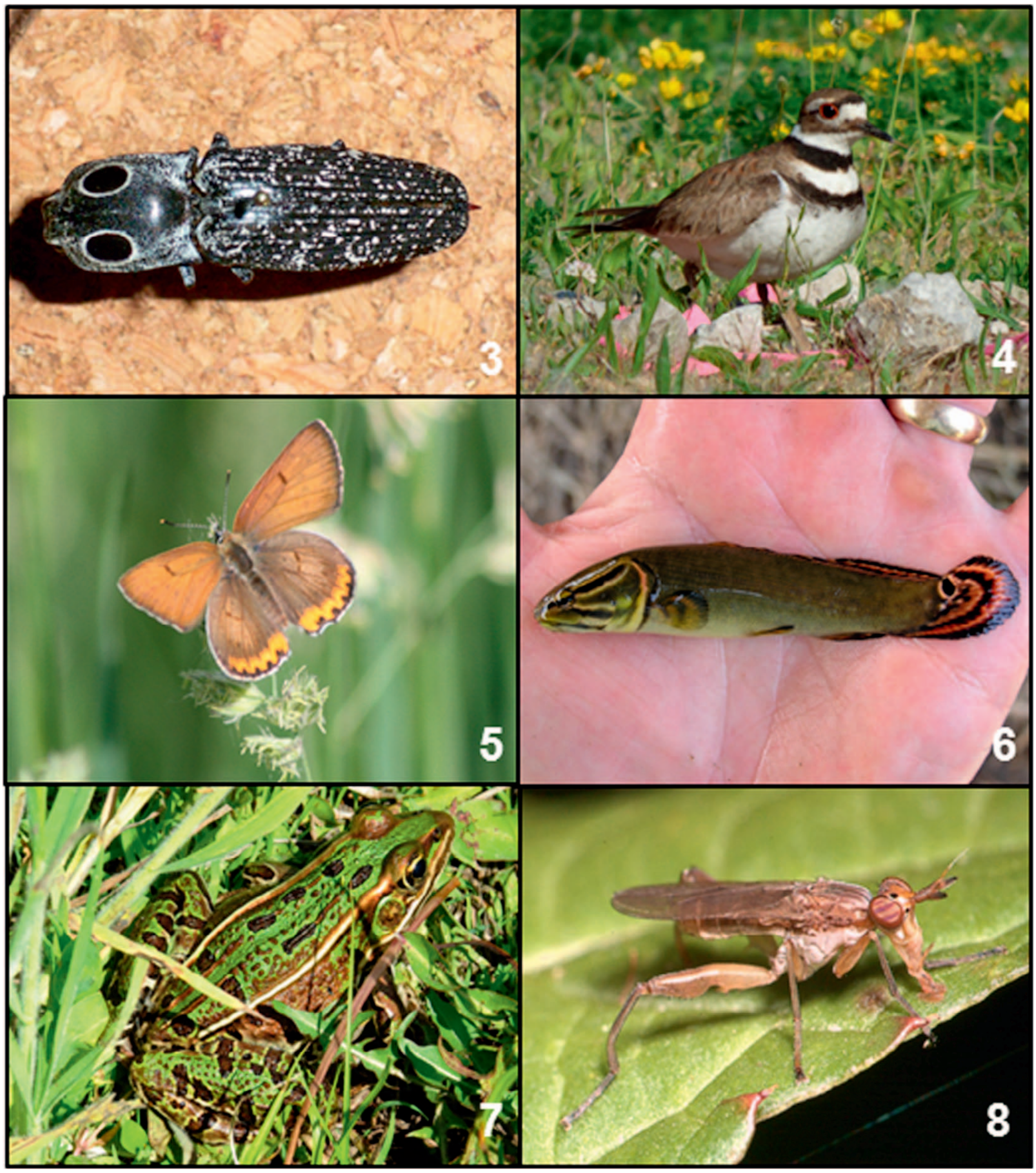
ground surrounded by marsh. Thus, collections were made on a small patch of dry ground, while the more abundant aquatic beetles were dominated by a few species. The wooded areas did contain a rich diversity of longicorn beetles and bark beetles for a rapid survey, although these mostly came from the traps left for two weeks. As with any rapid survey however, these species will represent only a small proportion of the total beetle fauna of Eagle Marsh. No rare species were found. However, some very charismatic beetles were present, including the eyed click beetle *Alaus oculatus* (Fig. 3) and the clover stem borer *Languria mozardi*. Lastly, while five species of exotic beetles were found, i.e., one exotic lady beetle and four exotic bark/ambrosia beetles, none were unusual or emerging threats.

Birds.—Eagle Marsh was divided into four territories and a team assigned to each. Teams maintained a tally of the species and the number of individuals encountered by sight or sound. On the first survey day, a few teams began before dawn and all teams finished by late afternoon. Thereafter, surveying during the evening of day one and on the morning of day two was primarily completed by the team leader who revisited locations that were productive. The focus of the team leader was to identify species new to the survey rather than counting additional individuals of species already detected. Reports of birds were also received from individuals who participated on other taxon teams. The complete effort by the team was approximately 87 person-hours.

The late May–early June date for the biodiversity survey was ideal for detecting birds as it coincided with the beginning of peak breeding activity. Nevertheless, a noteworthy number of species found were birds still migrating, using the site only for foraging or resting, or non-breeding individuals. Birds were identified by sight or by song or call note. Consequently, the survey is not limited to territorial or singing males, but this demographic constitutes the majority of the records. In an attempt to capture as much baseline data as possible, team members counted individual birds and noted any specific breeding activity by species. Bird diversity was found to be high with a total of 88 species observed on or flying over the property.

Birds at Eagle Marsh benefit from a mixture of habitats and the presence of additional suitable habitat at the adjacent Fox Island County Park. In particular, wetland- and shrubby field-dependent or associated species greatly benefit from restoration efforts and were found in robust numbers. These species include Wood Duck, Great Blue Heron, Marsh Wren (wetland species), Willow Flycatcher, Gray Catbird, Common Yellowthroat, Yellow Warbler, and Song Sparrow (grassland and shrubby field species). The Barred Owl heard calling is a new species for the property. The Killdeer (*Charadrius vociferous*) is seen in Fig. 4.

Of the 88 species detected, approximately 75 percent are presumed to be nesting at Eagle Marsh. The elevated percentage of nonbreeding species to total species is an indication that birds find the site attractive as a migratory stopover location and, for birds that nest near the site, as foraging grounds. A majority of the nonbreeding species found, including members of the duck, heron, and shorebird families, are wetland-associated species. The attractiveness of Eagle Marsh to migrating birds and individuals nesting off the property is presumed to be a lack of similar



Figures 3–8.—Images of various organisms observed during the Eagle Marsh biodiversity survey. 3. *Alaus oculatus*, the eyed elater, a large and charismatic species of click beetle. (Photograph by Jeff Holland) 4. A Killdeer, *Charadrius vociferous*, at its nest. (Photo by Don Gorney) 5. A male Bronze Copper, *Lycaena hyllus*, butterfly. (Photo by Kirk Roth) 6. A Bowfin (*Amia calva*). (Photo by Brant Fisher) 7. A Northern Leopard Frog, *Lithobates pipiens*, a species of special concern in Indiana; it was very abundant and found in most habitats across the property. (Photo by Bob Brodman) 8. A male *Sepedon armipes*, it was the most abundant sciomyzid (snail-killing fly) collected during the bioblitz. It's one of the easiest sciomyzids to identify – the male has a deep notch and some peg-like protuberances on the hind femur. The fly is about 5 mm long. (Photo by Steve Marshall, Guelph, Ontario)

large palustrine wetland sites in northeast Indiana and northwest Ohio.

Six state-listed species were detected. State-listed endangered species were Black-crowned Night Heron, Black Tern, and Marsh Wren, while species of special concern were Great Egret, Bald Eagle, and Common Nighthawk. Of the six species, only Marsh Wren is believed to be breeding on the site, although a pair of Bald Eagles nests just off the property. Virtually all 14 Marsh Wrens detected were singing males, indicating that Eagle Marsh is an important breeding location for the species in Indiana.

Butterflies.—At the time of the bioblitz, few high quality nectar sources, such as milkweeds (*Asclepias* spp.), mountain-mint (*Pycnanthemum* spp.), wild bergamot (*Monarda fistulosa*), purple coneflower (*Echinacea purpurea*) and others, were noted to be in bloom, although some of these (especially milkweed) were common. When in bloom, these plants are attractive to larger species of butterfly. Instead, most nectaring butterflies were found feeding on dame's rocket (*Hesperis matronalis*) and butterweed (*Packera glabra*), and those were almost exclusively grass skippers, with the exception of two Bronze Coppers (*Lycaena hyllus*). The types of nectar sources available during the dates of the bioblitz may account for the relative abundance of skipper observations over other types of butterflies. Cabbage Whites (*Pieris rapae*) and some Grass Skippers were noted on white clover (*Trifolium repens*) and a Red Admiral (*Vanessa atalanta*) was seen nectaring on willow (*Salix* sp.) blooms. Though red clover (*Trifolium pretense*) was blooming in several areas, the only butterfly species seen nectaring on it was Peck's Skipper (*Polites peckius*).

Bronze Copper was the third most abundant species (Fig. 5). In Indiana, this species is restricted to wetland habitats and uses dock (*Rumex* spp.) as a host plant (Belth 2013; Shull 1987). The Bronze Coppers were widely distributed throughout Eagle Marsh however, there were clusters of individuals in Management Unit I near the Engle Road Trailhead and in Unit F south of the barn adjacent to a long pond. Copulation was noted on the grasses bordering this same pond in Unit F at 5:35 p.m., May 31. Shull (1987) remarked that he had found three pairs *in copula* in Indiana in June, July, and September. All of Shull's records were in Wabash County among blue flag (*Iris versicolor*) during afternoon hours.

One Monarch (*Danaus plexippus*) egg was found on a milkweed. The single Eastern Tiger Swallowtail (*Papilio glaucous*) observation was of a dark form female. Observations of Peck's Skippers were scrutinized and photographed whenever possible for similar, rarer species, such as Indian Skipper (*Hesperia sassacus*), Leonard's Skipper (*Hesperia leonardus*), or Long Dash (*Polites mystic*), but none of these species were detected.

Dragonflies and damselflies (Odonata).—Most of the 12 odonate species observed at Eagle March were common species with distributions across northern Indiana. However, the damselfly *Ischnura hastata* (Odonata Central Record # 428032) was a new record for Allen County in which 79 species are currently recorded (Abbot 2007). *Ischnura hastata* has been previously recorded in adjacent Wells County, Indiana and Paulding County, Ohio and is widely distributed across eastern North America (Abbot 2007).

Fish.—Thirty-one species of fish representing eight families were recorded from Eagle Marsh. Only three species, Black Bullhead (*Ameiurus melas*), Central Mudminnow (*Umbra limi*), and Green Sunfish (*Lepomis cyanellus*), were collected from more than three of the seven locations sampled. Two non-native species, Goldfish (*Carassius auratus*) and Common Carp (*Cyprinus carpio*), were relatively common and reproducing, however, no Asian Carp were collected. The three lotic sites sampled were the most diverse with between 17 and 23 fish species found in each. Thirteen species were collected from all three lotic sites, i.e., Spotfin Shiner (*Cyprinella spiloptera*), Redfin Shiner (*Lythrurus umbratilis*), Bluntnose Minnow (*Pimephales notatus*), Fathead Minnow (*P. promelas*), Creek Chub (*Semotilus atromaculatus*), White Sucker (*Catostomus commersonii*), Black Bullhead, Yellow Bullhead (*Ameiurus natalis*), Blackstripe Topminnow (*Fundulus notatus*), Tadpole Madtom (*Noturus gyrinus*), Green Sunfish, Bluegill (*Lepomis macrochirus*), and Longear Sunfish (*L. megalotis*). These taxa are typical of small streams/ditches in northeast Indiana. The wetland sites sampled had low diversity (3–5 species) and were dominated by young-of-the-year individuals of the species found. Two species, Bowfin (*Amia calva*) (Fig. 6) and Redfin Pickerel (*Esox americanus*), were

Figure 9.—Male and female Blanding's Turtle (*Emydoidea blandingii*) held by Betsy Yankowiak, LRWP and bioblitz coordinator. The Blanding's Turtle is a state endangered species. (Photo by Bruce Kingsbury)

only collected from the wetland sites, but also likely inhabit the lotic sites of Eagle Marsh. No state/federal endangered or special concern fish species were collected.

Freshwater mussels.—Evidence of only two species of freshwater mussels was found from the seven locations sampled. No state/federal endangered or special concern freshwater mussel species were collected. Neither of the two species collected were found live, although the non-native Asian clam (*Corbicula fluminea*) is likely live on the property as fresh dead shell material was collected. Only weathered dead shell material was found for cylindrical paper-shell (*Anodontoides ferussacianus*) and it is not likely still living on the property. The low freshwater mussel diversity found on Eagle Marsh is not a surprise considering the ephemeral nature of many of the wetland habitats and the limited mussel habitat available in the lotic habitats.

Herpetofauna.—The herp team, a group that included 34 volunteers, observed a total of 15 species and at least 119 individuals in a survey that encompassed aquatic and terrestrial habitats representative of Eagle Marsh. Amphibians were more diverse (n = 10) than reptiles (n = 5), and the eastern side of the property appeared to have more species overall. A highlight of the survey was the capture of two Blanding's turtles (*Emydoidea blandingii*) (Fig. 9), a state-endangered species that is also in decline across much of its geographic range. Further work will need to be done to determine whether these adults are part of a breeding population. Another important observation was the Northern Leopard Frog (*Lithobates pipiens*) (Fig. 7), a species of special concern in Indiana; it was very abundant and found in most habitats across the property.

Mammals.—Mammal data collection was concentrated on small mammals. Only five species were taken during the biodiversity survey at Eagle Marsh; this was probably because up until approximately 2005 most of the land was farmland. There may be other species present, but it is likely that the five species taken are the most common on the property. Additionally, it is likely that these five were present before Eagle Marsh was formed.

The meadow vole (*Microtus pennsylvanicus*) is most commonly found in lush grassy cover and was the most common species taken at Eagle Marsh. It was most abundant in burned prairie, followed by pond edge, wet prairie, and brushy field - all had goodly amounts of cover. The burned prairie would normally provide heavy cover but during this study the cover was much less due to a recent burn of the area. The white-footed mouse (*Peromyscus leucopus*) is normally most abundant in woods and brushy field near woods. Only three were taken in woods, but there was only one trap line set out in a wooded area during the study, whereas there were six trap lines in brushy field habitat. The short-tailed shrew (*Blarina brevicauda*) is most common in woods, but is found in many habitat types. In this case three were found in woods and two were captured in other habitats. Masked shrews (*Sorex cinereus*) are found in most areas. During this study three were captured in wet prairie and another along the edge of a pond. Meadow jumping mice (*Zapus hudsonicus*) are found in grassy fields and in this case both were found in that habitat, one along a pond's edge.

Mushrooms and fungi.—The mushroom team reported 31 taxa, including 30 species, of fungi, mostly mushrooms. Early June is nearing the end of a lull in the fungal world. The spring mushrooms are coming to an end, but the summer mushrooms have yet begun to fruit in abundance. That being said, Management Unit F, the woodland at Eagle Marsh, provided a good species diversity for the time period. Most of the fungi reported are lignicolous, that is, wood rot fungi. Two collectors spent a total of about six hours on the property collecting the listed species. The majority of the species encountered are found commonly throughout much of the state. No specific genera were over-represented in numbers on the grounds.

Singing insects.—The date of the bioblitz was early in the season for singing insects. The team found all three of the common, widespread species that were likely to be found. Other possible species are much less common, and may or may not occur at the site. Green-striped grasshoppers (*Chortophaga vitripennis*) were common in all non-wetland grassy habitats. Spring field crickets (*Gryllus veletis*) were common in the railroad right-of-way and in drier habitats within easy dispersal distance from that right-of-way. The railroad bed is the likely oviposition site for this cricket, which avoids the wetlands and woodlands that dominate the Eagle Marsh site. The third species, Roesel's katydid (*Metrioptera roeselii*), was approaching maturity but not yet singing. One cluster of nymphs was found but they probably are more widespread in the site's grasslands.

Snail-killing flies (Sciomyzidae).—Ninety-one individuals of 15 species of Sciomyzidae (sciomyzid flies) were found during the event. Eagle Marsh clearly is a paradise for snail-killing flies. It offers suitable habitat that ranges from open freshwater marsh (perfect for non-operculate snails such as *Lymnaea* and *Physa*) to mature deciduous woodlands (ideal for *Succinea* snails and for slugs), with extensive edge and transitional areas dominated by sedges and cattail (*Typha* spp.). Sciomyzids were plentiful in the eastern (older) part of the marsh and quite scarce in the western (newer) part. Challenges to collecting included high daytime temperatures (sciomyzids in general are cold-adapted) and thick reed canary grass (*Phalaris arundinacea*). Canary grass slices the cloth rim of the net, destroying it within a single day of use if it is not protected by a covering of tough duct tape. The number of species found was among the highest for any site yet sampled in Indiana, higher in fact than the total number of species found so far in 76 of Indiana's 92 counties. Twelve of the species found were new for Allen County, from which 17 sciomyzid species are now known. A male *Sepedon armipes* is seen in Fig. 8.

Vascular plants.—Overall, 320 species were recorded with 243 being native to Indiana. Since most units of Eagle Marsh were restored from agricultural use during the past decade, it is of interest to note that collectively these areas had about 210 native species and three of the eight units had in excess of 100 native species. Not surprisingly, though, there still remains an abundance of non-native species, especially in the western units where *Phalaris arundinacea* and *Typha* × *glauca* were among the dominant species. A Floristic Quality Assessment of the restoration units (Units A, B, C, D, E, G, H, and I) generated a mean C for native species of 3.1, a value in keeping with the early to mid-successional status of the property. When non-native species were included in the analysis the mean C dropped to 2.3. This difference in mean C values indicates that non-natives have a meaningful impact on plant community structure and function at Eagle Marsh.

Several native graminoids were frequent in wetter habitats, including *Carex tribuloides*, *C. vulpinoidea, Leersia oryzoides, Scirpus cyperinus,* and *Sparganium eurycarpum*. The composite family member *Symphyotrichum lanceolatum* was also abundant and the sedge *Carex stipata* locally abundant. Common native species of drier habitats were *Solidago altissima* and, in eastern units, *Helianthus grosseserratus*. Native shrubs, *Cornus obliqua* and *C. racemosa,* were abundant in Unit C, a mix of oak species (*Quercus*) and *Cephalanthus occidentalis* in Unit D, and the shrubs *C. occidentalis* and *C. obliqua* in Unit H.

Within Eagle Marsh, one unit (designated as Unit F) supports a swamp woodland. The overstory was dominated by soft maples, *Acer negundo* and *A. saccharinum*. Dominant herbaceous species included *Cinna arundinacea, Geum vernum, Pilea pumila,* and *Symphyotrichum lateriflorum*. In this unit 115 native species were observed along with 20 non-native species. However, due to the presence of restoration habitat within this unit, its mean C for native species was only 3.1, somewhat below a level typically associated with remnant natural quality. On the plus side the presence of non-native species was clearly more constrained since total mean C only dropped to 2.6. Among the more conservative species present in this unit, that were lacking elsewhere at Eagle Marsh, were *Actaea pachypoda, Amphicarpaea bracteata, Arisaema dracontium, Carex conjuncta, Dryopteris carthusiana, Galium triflorum, Lindera benzoin*, and *Persicaria arifolia*.

Overall Eagle Marsh now supports a remarkable diversity of plant life and habitats given its recent intensive use as agricultural land. Many hardwoods, shrub plantings, wetland restoration species, and tall grass prairie species have been successfully established.

ACKNOWLEDGMENTS

We would like to express our sincere appreciation to the Little River Wetlands Project and the Indiana Academy of Science for hosting this event. Through their generous support, food and lodging was provided for the participants. We applaud the more than 120 scientists, naturalists, students, staff and community volunteers that participated in the event. We especially thank Betsy Yankowiak, local coordinator, for without her dedication, the event would not have occurred. Lastly, the participants express their appreciation to the Division of Nature Preserves, and especially Roger Hedge, for providing a permit allowing the event to occur.

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WATER QUALITY ASSESSMENT OF PRAIRIE CREEK RESERVOIR TRIBUTARIES IN DELAWARE COUNTY, INDIANA

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ABSTRACT. Prairie Creek Reservoir (PCR) is located in east-central Indiana within a predominantly agricultural watershed. The reservoir serves as a secondary source of drinking water for the city of Muncie and provides numerous recreational amenities. Previous studies focused on water quality in the reservoir, which led to local land management decisions. The current study was conducted to obtain baseline physical and chemical data for the five major tributaries of PCR, and to determine how agricultural, residential and commercial land use impacts water quality via tributary sub-watersheds. Water temperature, dissolved oxygen, and pH were measured; additionally, concentrations of total N, nitrate-N, ammonia-N, and phosphorus (P) species were analyzed. Dissolved oxygen concentrations were below Indiana Administrative Code (IAC) guidelines; total P and particulate P concentrations differed significantly ($p < 0.05$) between several tributaries, while total N and nitrate-N concentrations did not significantly differ. Of the five tributaries, Shave Tail Creek and Carmichael Ditch generated the greatest nutrient loads and were therefore ranked the worst tributaries in terms of overall water quality. It is recommended that best management practices be implemented at Shave Tail Creek and Carmichael Ditch to improve reservoir water quality.

Keywords: Eutrophication, nutrients, monitoring, streams, reservoir

INTRODUCTION

As of 2014, 43% of all lakes and reservoirs in the United States were classified as impaired for their designated uses (US EPA 2016). Nutrients are listed as the primary factor contributing to impairment, and agriculture is the foremost source of impairment. Commercial agricultural fertilizers inadvertently promote biological productivity in water that drains agricultural lands. An estimated 66% of nitrogen in the Mississippi River Basin originates from cultivated crops, mostly corn and soybean, grown within the Mississippi River watershed (USGS 2014). Runoff from livestock feedlots also contributes substantial N and P to surface waters (Burkholder et al. 2007). Animal manures may contain 2–5% N and P, depending on type of livestock raised (Hansen 2006; Buob & Homer no date). Such manures are often applied as a fertilizer to agricultural fields; therefore, intensive land application may result in N- and P-enriched surface runoff. Furthermore, soil-bound P may be lost via erosion (Quinton et al., 2001; Mullins 2009; Domagalski & Johnson 2013).

Cultural eutrophication of water bodies, the result of excess nutrients, reduces drinking water quality, increases sedimentation and algal blooms, and lowers or depletes dissolved oxygen (DO) concentrations. Eutrophication restricts commercial and recreational uses of water sources, reduces property values, and increases costs of water treatment for domestic consumption (Dodds & Welch 2000; US EPA 2002; Søndergaard et al. 2003; Carpenter 2005; Chislock et al. 2013; USGS 2013, 2014; Redmond et al. 2014).

Water bodies may experience eutrophication from nutrients released from domestic and municipal sources as well as from agricultural practices. Petrovic (1990) found that a small quantity of fertilizer nitrogen leaches from established turf to groundwater. Erickson et al. (2001) reported that plantings of mixed species (e.g., ornamentals) experienced ten times greater losses of nitrate than did areas planted to grass. Such landscapes may include large open spaces like parks, ornamental gardens, golf courses, as well as smaller plantings such as home gardens. Despite their prevalence, little is known about fertilizer losses from such managed landscapes (Amador et al. 2007).

Water quality in some portions of Indiana has degraded between 2002 and 2010. The US Environmental Protection Agency (US EPA

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2016) observed that 86% of assessed lakes/ reservoirs support full body contact and 81% support use for a public water supply. For streams/rivers only 63% support full body contact and 79% support use for a public water supply. The eastern corn belt region (Ecoregion 55) extends across east-central Ohio through central Indiana and includes the Prairie Creek Reservoir watershed. The region has among the highest levels of eutrophication compared to any other Indiana ecoregion. The main causes of water quality degradation are agricultural and industrial runoff (US EPA 2016).

Management of eutrophication is a complex issue and may include artificial mixing and oxygenation, sediment removal, sediment aeration, covering of sediment, phosphorus inactivation, use of algicides, light reduction, macrophyte control, and ecoremediation (Svirčev et al., 2008). In those areas most affected by nutrient pollution, best management practices (BMPs) may serve to protect water quality (US EPA 2002, 2005, 2010).

In Delaware County, Indiana, Prairie Creek Reservoir (PCR) covers 1200 acres and contains 7.2 billion gallons of water (D-MMPC 2007). The reservoir serves as a secondary source of drinking water for the City of Muncie and provides recreational amenities such as boating, fishing, and camping. The reservoir and its surrounding riparian land are owned by the Indiana-American Water Company that leases it for recreational purposes to the Muncie Department of Parks and Recreation. The Delaware Muncie-Metropolitan Commission developed a Prairie Creek Reservoir Master Plan to address future land development within the watershed, enhance park and reservoir value, and protect water quality (D-MMPC 2007; Popovičová 2008). In 2012, the City of Muncie renewed the reservoir lease for 100 years (Dick 2012).

PCR is significantly affected by agricultural land use and has shown signs of degradation (D-MMPC 2007; Popovičová 2008). Recent studies (Cescon 1997; Goward 2004; Fiallos Celi 2008; Popovičová & Fiallos Celi 2009) have recorded obvious signs of eutrophication and reduction in DO levels during summer months. Dissolved oxygen concentrations reached anoxic levels that persisted from June through September and often reached 50% of the depth at monitored sites (Popovičová & Fiallos Celi 2009).

To prevent further degradation of reservoir water quality, The Indiana Department of Environmental Management (IDEM 2004) is recommending implementation of BMPs within the watershed to reduce non-point source pollution. Previous studies, however, have not conducted monitoring and assessment of reservoir tributaries, which is essential for implementation of BMPs. Analysis of reservoir tributaries and the reservoir outflow for nitrate-N, ammonia-N, total N, particulate P and total P, and physico-chemical parameters such as temperature and DO levels can aid in implementing future land management practices to safeguard reservoir water quality.

The purpose of the reported research is to investigate water quality for the five tributaries of the PCR watershed. The ultimate goal is to determine how land use affects water quality (i.e., nutrient concentrations and loads) in PCR. Data regarding water quality of the major tributaries were collected in order to support future management decisions.

EXPERIMENTAL METHODS

Study location.—Prairie Creek Reservoir Watershed, located in Delaware County, Indiana, is classified as gently rolling and contains small lakes, prairie pothole lakes, and wetlands. The watershed is composed mainly of Crosby and Miamian soil series, both of which experience poor drainage. The watershed is dominated by agricultural land use (72%), green space (18%), and residential development (6%) (IDEM 2004). The reservoir has five major tributaries, i.e., Carmichael Ditch, Shave Tail Creek, James Huffman Ditch, Cemetery Run, and Cecil Ditch, draining 44 km^2 (17 mi^2) of New Castle Till Plains (Table 1; Fig. 1) (IDEM 2004). These streams obtain water primarily from groundwater sources and precipitation. Riparian zones are observed to be dominated by woody to herbaceous vegetation. Stream bottoms consist primarily of a silt to gravel substrate. Silty bottoms have been encountered in multiple tributaries suggesting bank erosion with streams acting as sinks for sediment.

Sample collection and analysis.—Weekly sampling and monitoring was performed at each tributary and reservoir outflow from June through October 2014. A SONTEK Flow Tracker (SONTEK, San Diego, CA) measured discharge of each stream using US Geological Survey protocols (USGS 2010). A Hydrolab DS5 Sonde (Hydrolab Inc., Austin, TX) was used to measure pH, temperature, DO, and turbidity in-situ. Water samples for nutrient

Table 1.—Tributary sub-watershed characteristics of Prairie Creek Reservoir, Delaware County, Indiana.

Location	Stream length (m)	Drainage area (ha)	Land use	Percentage of land use
Outfall	1,869	n/a	n/a	n/a
Carmichael Ditch	2,849	517	Agriculture	49%
			Commercial	46%
			Residential	5%
Shave Tail Creek	7,508	799	Agriculture	53%
			Residential	41%
			Commercial	6%
Huffman Ditch	5,336	767	Agriculture	72%
			Commercial	16%
			Residential	12%
Cemetery Run	2,402	162	Agriculture	98%
			Residential	2%
Cecil Ditch	2,345	311	Agriculture	100%

determination were collected from the center of each tributary using a grab sample technique. Samples were collected in acid-washed glass containers, transported on ice, and analyzed within 24 hours of collection. Samples were analyzed for nitrate-N using the Cadmium Reduction Method 8039; ammonia-N by the Ammonia Salicylate Method 8155; total N by the Persulfate Digestion Method 10070; particulate and soluble orthophosphate by the Ascorbic Acid Method 8048 (Reactive Phos Ver3); and total P by the Acid Persulfate Digestion Method (Hach 2015). A Hach DR/2400 Spectrophotometer (Hach, Inc., Loveland, CO) was used to determine concentrations of each analyte. One field duplicate, one lab blank, a field blank, and a laboratory fortified blank with deionized H_2O were used for quality control purposes.

The effects of location (fixed factors) for each water quality parameter (dependent variable) were determined by the use of nonparametric statistics including Spearman's rho, Kruskal-Wallis, and multiple comparisons analysis. The level of statistical significance was set at $\alpha = 0.05$. Statistical analyses were performed using Minitab® 16.2.4 statistical software (Minitab Inc., State College, PA) on a Windows-based PC.

Ranking tributaries.—A stream quality ranking system was established based on total quantity of nutrients (kg/y), with scores from 1 (best) to 6 (worst). The final ranking was determined by adding the scores for each nutrient parameter; the tributary having the highest total value was designated as the poorest quality tributary.

RESULTS AND DISCUSSION

Discharge.—The greatest discharge was measured at the outflow (0.2 m^3/s) (6.89 ft^3/s) (Table 2). The Indiana-American Water Company controls the release of water at this location. Discharge varied throughout the sampling period from 0.01 to 0.2 m^3/s. It is expected that groundwater contributes to all tributaries due to the high water table in this watershed (NRCS 2013). Shave Tail Creek had the highest discharge rate compared to the other tributaries (0.04 m^3/s) (Table 2). Carmichael Ditch had the second highest discharge (0.03 m^3/sec), while Cemetery Run had the lowest (0.01 m^3/sec). It was observed that the latter stream experienced reservoir backflow.

Table 2.—Discharge (m^3/s) measured at Prairie Creek Reservoir tributaries in 2014 (June to October). * = Significant difference at $p < 0.05$.

Location	*n*	Mean ± std dev.	Min.	Max.
Outfall	13	0.20 ± 0.44	8.5×10^{-4}	1.60
Carmichael Ditch*	18	0.03 ± 0.03	1.4×10^{-3}	0.12
Shave Tail Creek	8	0.04 ± 0.02	0.03	0.11
Huffman Ditch	18	0.02 ± 0.02	4.5×10^{-3}	0.09
Cemetery Run	17	0.01 ± 0.008	-2.8×10^{-3}	0.03
Cecil Ditch*	17	0.01 ± 0.01	4.2×10^{-3}	0.05

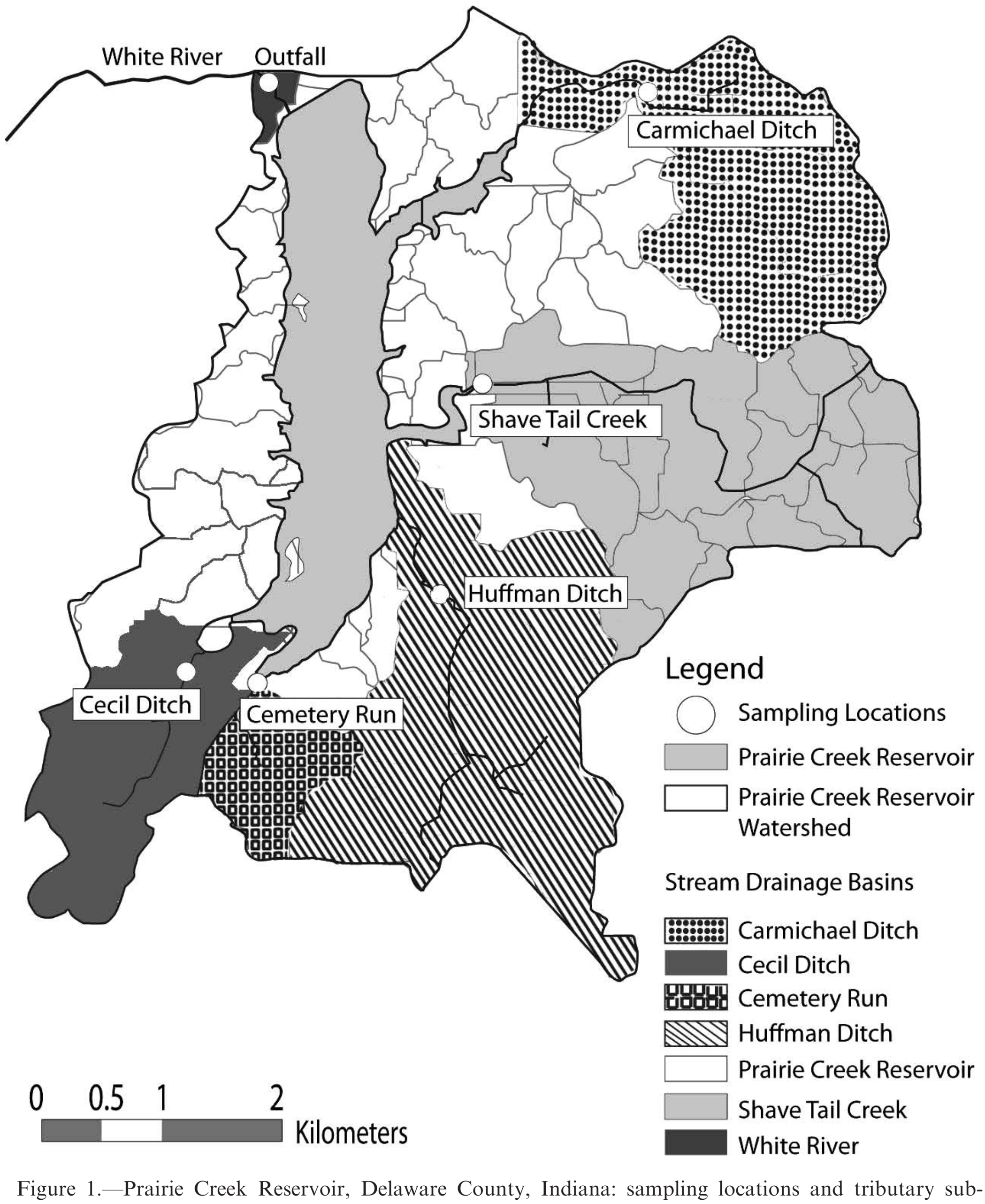

Figure 1.—Prairie Creek Reservoir, Delaware County, Indiana: sampling locations and tributary sub-watersheds.

June was the only month that had a higher mean precipitation (0.58 cm) (0.23 in.) than average for 1994–2014 (0.41 cm) (0.16 in.). It was expected that nutrient concentrations would be lower due to higher discharge rates for this month. July and September mean precipitation for 2014 was 0.25 and 0.2 cm, respectively, and was slightly less than mean precipitation for 1994–2014 (0.3 and 0.28 cm, respectively); nutrient concentrations were expected to be higher than normal concentrations (NWS 2014).

Physico-chemical characteristics.—*Reservoir outflow*: The reservoir outflow had the highest mean temperature (19.9° C) followed by Cemetery Run (17.2° C) (Table 3). The high temperature at the outflow is likely due to the

Table 3.—Physico-chemical parameters (mean ± standard deviation) measured at Prairie Creek Reservoir tributaries in 2014 (June through October). * = DO concentrations below Indiana Administrative Code (IAC) Standards; BDL = below detectable limits.

Parameter	Outfall	Carmichael Ditch	Shave Tail Creek	Huffman Ditch	Cemetery Run	Cecil Ditch
pH	7.63 ± 0.53	6.68 ± 0.26	7.16 ± 0.34	7.42 ± 0.26	7.46 ± 0.33	7.12 ± 0.41
n	9	9	9	9	9	9
median	7.53	6.69	7.22	7.47	7.51	7.23
range	7.0 - 8.4	6.16 – 7.0	6.55 - 7.7	6.8 - 7.8	6.93 - 8.1	6.22 - 7.60
DO (mg/L)	5.69 ± 3.66	3.23 ± 2.54*	2.48 ± 2.36*	2.94 ± 1.66*	3.53 ± 3.21*	2.69 ± 2.34*
n	9	9	9	9	9	9
median	5.85	3.17	2.92	3.08	3.64	2.45
range	BDL - 11.22	BDL - 6.65	BDL - 5.91	BDL - 5.23	BDL - 10.98	BDL - 5.55
Turbidity (NTU)	21.73 ± 12.67	28.28 ± 10.78	20.42 ± 7.4	38.24 ± 44.07	24.39 ± 8.14	20.32 ± 5.71
n	6	7	6	7	7	6
median	16.35	24.7	22	19.3	28.4	19.2
range	14.8 - 47.2	18.6 - 49.9	10.5 - 29.7	8.6 - 135.2	8 - 30.3	13.6 - 30
Temperature (° C)	19.92 ± 5.29	15.87 ± 3.95	15.73 ± 4.14	16.31 ± 3.19	17.24 ± 5.33	16.12 ± 4.49
n	9	9	9	9	9	9
median	20.23	16.73	17.92	17.43	18.25	17.18
range	12.1 - 25.85	10.07 - 20.96	9.8 - 20.12	10.22 - 19.25	8.81 - 25.74	9.37 - 21.29
Specific Conductivity (µS/cm)	324.4 ± 132.6	687.1 ± 116.5	647.4 ± 75.9	665.7 ± 60.0	577.8 ± 162.9	777.1 ± 32.2
n	8	9	8	9	8	7
median	349.5	690.6	667.3	670.5	643.8	780.8
range	0.1 - 471.9	410.2 - 814.9	460.8 - 685.5	532.6 - 766.5	339.5 - 754.9	738.7 - 833.6

Table 4.—Nutrient concentrations (mean ± standard deviation) for Prairie Creek Reservoir tributaries in 2014 (June through October). BDL = below detectable limits.

Parameters (mg/L)	Outfall	Carmichael Ditch	Shave Tail Creek	Huffman Ditch	Cemetery Run	Cecil Ditch
Nitrate-N	0.45 ± 0.21	1.15 ± 1.11	0.67 ± 0.43	1.07 ± 0.8	0.58 ± 0.32	1.02 ± 0.76
n	15	15	15	15	4	15
Median	0.4	0.7	0.6	0.8	0.55	0.9
Range	0.2 - 0.9	BDL - 4.2	0.2 - 1.6	0.2 - 3.2	0.1 - 1.4	BDL - 2.7
Ammonia-N	0.04 ± 0.05	0.05 ± 0.02	0.04 ± 0.06	0.01 ± 0.02	0.02 ± 0.02	0.04 ± 0.03
n	17	17	17	17	17	17
Median	0.03	0.05	0.02	0	0.03	0.03
Range	BDL - 0.14	BDL - 0.07	BDL - 0.24	BDL - 0.08	BDL - 0.07	BDL - 0.1
Total N	0.66 ± 0.84	1.61 ± 1.5	1.2 ± 1.33	1.44 ± 1.3	0.68 ± 1.04	1.55 ± 1.81
n	15	15	15	15	14	15
Median	0.2	1.3	0.9	1	0.2	0.9
Range	BDL - 2.3	BDL - 4.7	BDL - 4.4	BDL - 4	BDL - 3.6	BDL - 5.4
Soluble PO_4^{3-}	0.03 ± 0.03	0.08 ± 0.08	0.1 ± 0.13	0.08 ± 0.07	0.05 ± 0.03	0.08 ± 0.1
n	18	18	18	18	17	18
Median	0.02	0.07	0.08	0.07	0.05	0.06
Range	BDL - 0.10	BDL - 0.32	BDL - 0.61	BDL - 0.24	BDL - 0.11	BDL - 0.1
Particulate P	0.01 ± 0.02	0.03 ± 0.06	0.01 ± 0.02	0.02 ± 0.04	0.00 ± 0.00	0.01 ± 0.00
n	18	18	18	18	18	17
Median	BDL	BDL	BDL	BDL	BDL	BDL
Range	BDL - 0.12	BDL - 0.15	BDL - 0.05	BDL - 0.14	BDL - 0.01	BDL - 0.05
Total P	0.04 ± 0.06	0.09 ± 0.09	0.13 ± 0.15	0.09 ± 0.08	0.06 ± 0.07	0.04 ± 0.05
n	18	18	18	18	18	18
Median	BDL	0.06	0.06	0.05	0.05	0.01
Range	BDL - 0.19	BDL - 0.28	BDL - 0.60	BDL - 0.22	BDL - 0.22	BDL - 0.16

reservoir being exposed to direct sunlight. Specific conductivity at the outflow (324.4 μS/cm) was lowest compared to the tributaries.

The reservoir outflow had the highest pH (7.6) and DO concentration (5.69 mg/L). The high DO concentration may be a result of agitation and subsequent aeration of water discharging directly from the reservoir. pH and DO concentrations for this location were lower than those measured by Goward (2004) (7.97 and 9.06 mg/L, respectively). The lower pH and DO concentration measured in the current study may be attributed to decomposition of organic matter (Cooke et al. 2005; Jørgensen et al. 2005).

Tributaries: Carmichael Ditch had the lowest pH (6.68) and second-highest specific conductivity (687.1 μS/cm), possibly a result of algal productivity and decomposition, and mineralization of detritus; Cecil Ditch had highest specific conductivity (780.8 μS/cm) (Table 3). Fertilizer runoff likely contributed to high conductivity readings.

Nutrient concentrations.—Carmichael Ditch had the highest concentrations of total N (1.61 mg/L), nitrate-N (1.15 mg/L), and ammonia-N (0.05 mg/L) (Table 4); however, ammonia-N and nitrate-N concentrations for this sub-watershed were lower than concentrations measured a decade earlier (0.095 and 1.4 mg/L, respectively) (Goward 2004). Concentrations of nitrate-N were $<$ 0.7 mg/L in the outflow for Shave Tail Creek and Cemetery Run (Table 4).

Cecil Ditch had the highest mean concentrations of nitrite (0.78 mg/L) followed by Shave Tail Creek (0.68 mg/L) (data not shown). Nitrite concentrations were highest during warm summer months; this may be generated from nitrate-rich groundwater that discharges to the surface and is ultimately transformed via denitrification (USGS 2009, 2011).

According to the Indiana Administrative Code (IAC), concentrations of N species were within acceptable limits; however, the presence of dense algal growth in Carmichael Ditch suggests that IAC limits may be too high. Leaching from surface soil, use of tile drains, bank erosion, and stormwater runoff may have contributed to

Table 5.—Spearman correlations for tributary parameters (*r*).

Parameter	Outfall	Carmichael Ditch	Shave Tail Creek	Huffman Ditch	Cemetery Run	Cecil Ditch
Nitrate vs. Discharge	−0.64	0.77	−0.24	0.37	−0.10	0.00
Ammonia vs. Discharge	−0.22	−0.13	0.56	0.59	−0.19	0.14
Total N vs. Discharge	−0.22	0.43	−0.16	0.18	0.10	0.32
Particulate P. vs. Discharge	−0.10	−0.02	−0.08	0.13	−0.25	−0.13
Soluble P vs. Discharge	0.37	0.76	0.20	0.44	−0.17	−0.09
Total P vs. Discharge	−0.18	0.66	−0.23	0.63	0.02	0.06
pH vs. Discharge	0.88	−0.33	−0.66	0.61	0.42	0.22
Dissolved Oxygen vs. Discharge	0.82	0.30	−0.66	0.73	0.75	0.39
Specific Conductivity vs. Discharge	−0.09	0.11	0.72	−0.50	−0.38	0.63
Stream Turbidity vs. Discharge	0.73	−0.34	1.0	−0.58	0.62	−0.27
Total Phosphorus vs. Turbidity	−0.43	−0.20	−0.34	−0.16	−0.81	−0.70
Particulate Phosphorus vs. Turbidity	−0.20	−0.24	−0.11	0.81	−0.89	−0.58
Soluble Orthophosphate vs. Turbidity	0.31	0.27	0.43	−0.37	0.21	0.02
Nitrite vs. Discharge	−0.31	−0.05	−0.32	0.10	−0.37	0.30
Nitrite vs. pH	0.53	0.41	0.92	0.62	−0.30	0.25
Nitrite vs. Dissolved oxygen	0.30	−0.62	0.55	0.70	−0.17	0.48
Nitrite vs. Turbidity	−0.12	−0.48	−0.29	−0.87	0.48	−0.36
Nitrite vs. Total N.	−0.29	0.01	−0.29	−0.21	−0.21	−0.17

elevated N concentrations (Mullin 2009; Domagalski & Johnson 2013).

Shave Tail Creek had the highest total P concentration (0.13 mg/L) (Table 3), which may be caused by fertilizer runoff, soil erosion, and disturbance of the creek bed by cattle (Goward 2004). This sub-watershed consists predominantly of agricultural fields (53%). Several tributaries had similar total P concentrations compared to 2011 data (0.06 mg/L) (UWRWA 2011). Soluble orthophosphate concentrations were highest at Shave Tail Creek (0.1 mg/L) and particulate P was highest at Carmichael Ditch (0.03 mg/L) (Table 3).

Comparison of tributary properties.—Shave Tail Creek had significantly ($p < 0.05$) higher discharge and total N and P concentrations compared to Carmichael Ditch, Cemetery Run, and Cecil Ditch (Tables 2 & 4). Ammonia-N concentrations were significantly different between Carmichael Ditch and Huffman Ditch ($p = 0.02$); however, ammonia-N comprises a relatively minor fraction of total N, which did not significantly differ throughout the watershed ($p = 0.13$).

Prairie Creek Reservoir Watershed (PCRW) had lower concentrations of ammonia-N and nitrate-N (0.05 and 1.15 mg/L) (Table 4) compared to nearby Buck Creek (0.07 and 2.3 mg/L, respectively) and Killbuck Creek (0.14 and 2.4 mg/L, respectively) watersheds (Goward 2004). Soluble orthophosphate concentrations measured 0.08 mg/L each for Carmichael Ditch, Huffman Ditch, and Cecil Ditch in the PCRW, which were twice the value of Buck Creek (0.04 mg/L) but an order of magnitude less than for Killbuck Creek (0.75 mg/L) (Goward 2004).

The PCRW is less developed compared to Buck Creek and Killbuck Creek watersheds — residential use comprised 15.4 and 12.9 %, respectively, compared to 6 % for PCRW (Goward 2004; UWRWA 2011). Agricultural land use predominates in the PCRW (72%) (Goward 2004; UWRWA 2011). PCRW has the highest percentage of green space (19%) compared to the other watersheds (Buck Creek, 13% and Killbuck Creek, 7%). This may account for PCRW having better water quality than the other watersheds (Goward 2004; UWRWA 2011).

Correlations.—Concentrations of nitrate-N were strongly correlated with discharge at Carmichael Ditch ($r = 0.77$) (Table 5). Total P had a moderately positive correlation with discharge at both Carmichael Ditch ($r = 0.66$) and Huffman Ditch ($r = 0.63$).

The outflow had a moderately negative correlation with ammonia-N concentration and pH ($r = -0.63$) and a strong negative correlation with DO level ($r = -0.83$) (data not shown). Carmichael Ditch had a strongly negative correlation between ammonia-N and DO ($r = -0.75$) which may have been caused by denitrification and respiration of algae throughout the sampling period (Cooke et al. 2005; Jørgensen et al., 2005). The outflow had a

Table 6.—Nutrient loads contributed by each tributary and exported to the White River. Numbers represent kg/y.

Nutrient	Outfall	Carmichael Ditch	Shave Tail Creek	Huffman Ditch	Cemetery Run	Cecil Ditch
Nitrate-N	2790	667	953	631	188	388
Ammonia-N	265	268	60	83	81	138
Total N	4066	942	1698	849	212	577
Total P	59	14	50	14	6	4

strongly positive correlation ($r = 0.88$) and Huffman Ditch a moderately positive correlation ($r = 0.61$) for pH and discharge (Table 5). Higher rates of discharge could incorporate greater quantities of DO, reduce concentrations of CO_2, and increase pH (Araoye 2009). Higher discharge rates are capable of carrying greater quantities of particulates and ions, which result in a positive correlation with turbidity (Table 5).

Ranking tributaries and best management practices.—Among the five tributaries, Shave Tail Creek contributed the greatest annual nutrient load (total N = 1698 kg/yr and total P = 50 kg/yr) (Table 6). Comparing the sum total nutrient loads for the tributaries to the outflow, it is evident that nutrient loads in PCR are increasing due to influx of total N and P from the watershed. This study did not establish a water budget for the watershed and cannot determine an accurate mass balance for the reservoir (Walker 1999; Fetter 2001); however, it is likely that the reservoir is acting as a nutrient sink for the watershed. Eutrophication of the reservoir could impair both aquatic biodiversity and drinking water quality for Muncie.

Shave Tail Creek and Carmichael Ditch were ranked the worst tributaries within the watershed in terms of water quality parameters (Table 7). In the Shave Tail Creek subwatershed, cattle were found to have direct access to the creek (Goward 2004). Additionally, the relatively high values for N and P may be due to greater commercial and residential development (Table 1). Petrovic (1990) concluded that only a modest quantity of fertilizer nitrogen (< 10%) is typically lost from established turf. This finding is supported by Guillard & Kopp (2004). Jiang et al. (2000) have shown that turf sites retain 90% of accumulated N during the following year even if no vegetation is replanted. Erickson et al. (2001) reported that plantings of mixed species lost ten times more NO_3^- than did areas planted to grass. To assess fully the environmental impact of residential, institutional or municipal landscaping, however, all components of the landscape must be evaluated for their contribution to fertilizer losses (Amador et al. 2007). It is also possible that fewer management practices implemented for these subwatersheds allowed runoff and erosion of fertilizers and possibly septic runoff to enter the tributaries. Cecil Ditch had the lowest total P input which could be a direct result of having a riparian zone consisting largely of dense woody vegetation.

BMPs suggested for Shave Tail Creek and Carmichael Ditch include soil analysis preplanting of turf grass, ornamental, and agricultural crops (Hartz 2006) to determine soil N and P concentrations. This would allow for accurate determination of fertilizer application rates (Hartz 2006; Hartz & Smith 2009). Another

Table 7.—Ranking of reservoir outfall and tributaries based on nutrient loads. Rank: 1 = best quality, 6 = worst quality.

Parameter	Outfall	Carmichael Ditch	Shave Tail Creek	Huffman Ditch	Cemetery Run	Cecil Ditch
Ammonia	5	6	1	3	2	4
Total N	6	4	5	3	1	4
Particulate PO_4^{3-}	6	4	5	3	1	2
Soluble PO_4^{3-}	6	3	5	4	1	4
Total P	6	3	5	4	2	1
Total score	35	24	26	20	8	17
Overall rank[a]	6	4	5	3	1	2

suggested BMP is to regularly monitor soil N levels, which would provide a basis for determining accurate fertilizer application rates.

The use of cover crops could reduce nutrient loss from soils by rotating shallow-rooted crops with deep-rooted crops (Hartz 2006; Smukler et al. 2012). It is further recommended to implement catchment ponds where feasible so that runoff could be recycled onto lawns and fields (Smukler et al. 2012). The installation of vegetative buffer strips and constructed wetlands could be the best option for future land management practices due to current bank instability (US EPA 2005; MDNR 2007a, b). US EPA (2005) recommends at least a 100-foot buffer strip to efficiently remove 50% of the nutrients that may enter a stream.

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INVASIVE SPECIES IN AN URBAN FLORA: HISTORY AND CURRENT STATUS IN INDIANAPOLIS, INDIANA

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ABSTRACT. Invasive plant species are widely appreciated to cause significant ecologic and economic damage in agricultural fields and in natural areas. The presence and impact of invasives in cities is less well documented. This paper characterizes invasive plants in Indianapolis, Indiana. Based on historical records and contemporary accounts, 69 of the 120 species on the official Indiana state list are reported for the city. Most of these plants are native to Asia or Eurasia, with escape from cultivation as the most common mode of introduction. Most have been in the flora of Indianapolis for some time. Eighty percent of Indianapolis' invasive herbaceous plants were present before 1940, but only 14% of woody invasive plants were known to be present in the city at that time. The largest group of woody invasives is shrubs. Newly present invasive plants continue to be reported for Indianapolis. Expert opinion rates Callery Pear, Japanese knotweed, and Japanese stiltgrass as the greatest emerging threats.

Keywords: Indianapolis, invasive plants, invasive species, urban flora

INTRODUCTION

An invasive species is defined in the United States by Executive Order as "a species that is: 1) non-native (or alien) to the ecosystem under consideration and 2) whose introduction causes or is likely to cause economic or environmental harm or harm to human health" (USDA, NISIC 2016). Invasive species (exotic insects, plants, fish, birds, mammals, and many other organisms) are a serious threat, resulting in estimated costs and damages of more than $120 billion annually in the United States (Pimental et al. 2005) and more than €12 billion annually in Europe (van Ham et al. 2013). Human actions are the primary means of introduction.

Urban areas are often points of introduction for invasives (Pyšek 1998). In cities, invasive plants have been shown to alter species composition, resulting in loss of biodiversity and declines in primary productivity, to diminish ecosystem services (e.g., erosion control), to cause infrastructure deterioration, to alter nutrient cycling, and to contribute to declines in property value (van Ham et al. 2013). Additional social impacts include the perception of spaces overgrown with invasives as signs of urban decay, and loss of visual connection with natural features such as riparian corridors (van Ham et al. 2013).

This paper reports on invasive plants known to be present in Indianapolis/Marion County, Indiana, USA. The city and the county are the same governmental unit and so occupy the same geographic space, referred to as Indianapolis in this paper. Indianapolis is a model urban area to study invasive plants for several reasons. Much is known about its floristic composition, from the late 1800s through current times. Indianapolis was almost entirely forested in pre-European presettlement times, but forests were reduced to 13% cover by the late 1900s (Barr et al. 2002). Most of the original forest was converted into row-crop agriculture. Agriculture has declined from 80% of land use in 1922 to 72% in 1953, to 18% by 1990 (http:www.savi.org). The time period from 1953–1990 corresponds with rapid urbanization in the city. This pattern of land use change is likely a model for other cities in the American Midwest. Indianapolis is the twelfth largest city in the United States, with an estimated population of over 900,000 people and total area of 650 km^2 (105,200 ha). The city is in the Central Till Plain Natural Region of Indiana (Homoya et al. 1985), an area characterized by a terrain of gently rolling hills of glacial till.

Species richness of the Indianapolis flora has been documented by Dolan et al. (2011) at about 700 plants. This number was consistent over a 70 year period, but there has been considerable species turn-over, with a loss of rare native plants and an increase in non-native plants from 20.3% to 27.1% of the flora over the years covered by the

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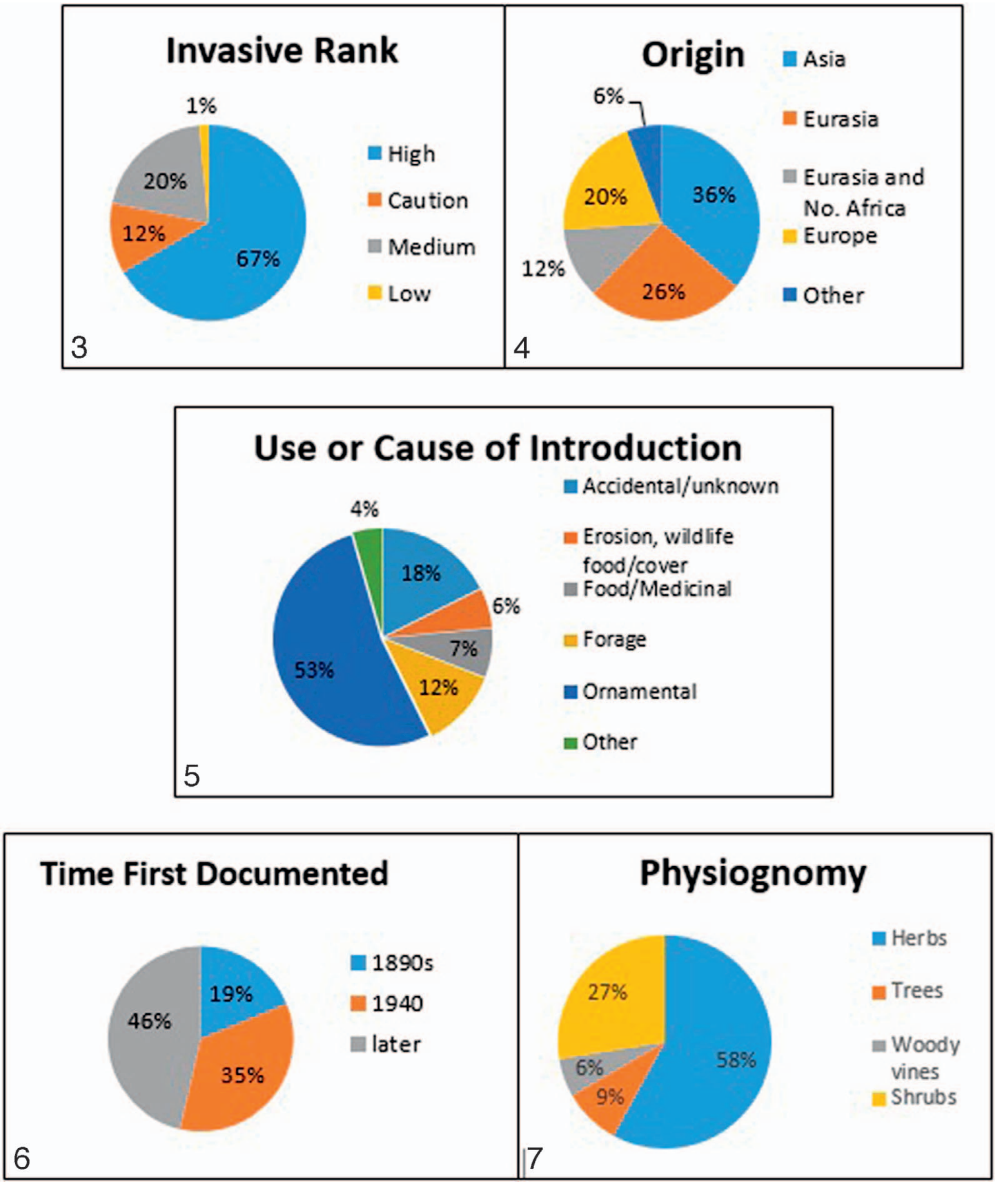
Figures 1–2.—Images of herbarium specimens. 1. Specimen of invasive plant *Lythrum salicaria* collected by Ray Friesner in Indianapolis in 1925, documenting presence of the species in the city as of that date (left). 2. Specimen of *Berberis thunbergii* collected by Charles Deam, with the comment on the label that this ornamental plant was collected far from any dwelling. Comments like these are helpful in establishing records for species that might become invasive (right).

study. These percentages are on par with urban areas across the globe (La Sorte et al. 2014). Not all non-natives in the Indianapolis flora, historically or currently, are considered invasive plants in Indiana (Indiana Invasive Species Council 2016). This report focuses upon invasive species in the Indianapolis flora, including how long they have been known to be present, their origins, and uses or causes of introduction. Finally, the invasive species that are likely to be the biggest problems in the near future for the city are discussed.

METHODS

For this study, plants were identified as invasive if they were present on the Official Indiana Invasive Plant list, established by the Indiana Invasive Species Council (2016). Nomenclature is based on scientific names used in that list. Sources of information on invasives present in the flora of Indianapolis and dates of first record range from historical journal articles to contemporary web-based records. The oldest record is a county list for Marion and adjacent Hamilton County (Wilson 1895). While not comprehensive, this paper does document species presence, often annotated with comments on abundance. Coulter (1899) produced a state-wide flora that sometimes mentions counties and ranges for plants now recognized as invasives in the state.

Deam's 1940 Flora of Indiana is the last comprehensive manual of the state's plants. Deam's flora presents county-level distribution maps based on herbarium specimen vouchers. Overlease & Overlease (2007) reported weed species distributions for Indiana at the county level, based on their own observations and compared these distributions with records from Deam (1940) and Coulter (1899). Dolan et al. (2011) compiled these and other records, including herbarium specimens (Figs. 1 & 2) from the Friesner Herbarium (BUT) of Butler University,



Figures 3–7.—Characteristics of invasive species in the Indianapolis, Indiana flora. 3. Invasive rank based on the Official Indiana Invasive Plant List (top left). 4. Continent of origin (top right). 5. Mode of introduction (middle). 6. Time of first record in the flora (bottom left). 7. Physiognomy (bottom right).

into a historical list for the city (pre-1940) and a more recent list based on species reported by botanists working in the city since that time.

Additional records for invasives in Indianapolis came from the Indiana Plant Atlas (Dolan & Moore 2016), sorted by location (Marion County) and invasiveness, and from Early Detection & Distribution Mapping System (EDDMapS 2016). Origin and mode of introduction of invasives are from Weber (2003) and Czarapata (2005).

Invasive plants that represent the largest current and emerging threats in the city were identified by polling local experts. Eight environ-

Table 1.—Herbaceous species of invasive plants in the Indianapolis, Indiana flora and time of first record. D = listed as present elsewhere in Indiana by Deam (1940); P = planting actively promoted by government agencies at some time.

Scientific name	Common name	Pre-1940	More recent	Invasive rank	Origin	Use/mode of introduction
Alliaria petiolata	garlic mustard		x	high	Europe	food, medicinal
Artemesia vulgaris	mugwort		x	high	Eurasia/N. Africa	medicinal?
Carduus nutans[D]	musk thistle		x	high	Europe/N. Africa	none/unknown
Centaurea stoebe	spotted knapweed		x	high	Europe	none/unknown
Cirsium arvense	Canada thistle	x	x	high	Eurasia	contaminated seed crop?
Cirsium vulgare	bull thistle	x	x	high	Europe	none/unknown
Clematis terniflora	sweet autumn clematis		x	caution	Asia	ornamental
Conium maculatum[D]	poison hemlock		x	high	Eurasia/N. Africa	none/unknown
Convolvulus arvense	field bindweed	x	x	high	Asia	ornamental
Coronilla varia[P]	crown vetch	x	x	high	Eurasia/N. Africa	erosion control
Cynanchum louiseae	black swallow-wort	x	x	high	Europe	ornamental
Daucus carota	Queen Anne's lace	x	x	medium	Eurasia	medicinal?
Dioscorea polystachya[D]	Chinese yam		x	high	Asia	ornamental, food
Dipsacus fullonum	common teasel	x	x	high	Eurasia/N. Africa	wool combing
Dipsacus laciniatus	cut-leaved teasel	x	x	high	Eurasia/N. Africa	wool combing
Euphorbia esula	leafy spurge	x	x	high	Eurasia	accidental contaminant, ornamental
Glechoma hederacea	creeping Charlie	x	x	medium	Europe	medicinal, food
Hesperis matronalis	dame's rocket	x	x	high	Eurasia	ornamental
Humulus japonicus[D]	Japanese hops		x	high	Asia	ornamental
Hypericum perforatum	St. John's wort	x	x	Low	Europe	medicinal
Iris pseudoacorus[D]	yellow iris		x	high	Europe/Africa	ornamental
Kummerowia stipulacea[D,P]	Korean lespedeza		x	high	Asia	forage
Kummerowia striata[P]	striate lespedeza	x	x	medium	Asia	forage
Lespedeza cuneata[P]	sericea lespedeza		x	high	Asia	erosion control, forage
Lythrum salicaria	purple loosestrife	x	x	high	Europe	ornamental, medicinal, honey
Melilotus officinale	sweet clover	x	x	medium	Eurasia	forage, honey
Microstegium vimineum	Japanese stiltgrass		x	high	Asia	accidental, in packing material
Myriophyllum spicatum[D]	Eurasian watermilfoil		x	high	Europe/N. Africa	aquarium trade, boats
Najas minor	braided naiad		x	high	Eurasia/N. Africa	ships' ballast, ornamental
Pastinaca sativa	wild parsnip	x	x	medium	Eurasia	ornamental
Phalaris arundinacea[P]	reed canarygrass	x	x	high	Europe	hay, forage
Phragmites australis	common reed	x	x	high	Global	ships' ballast?
Potamogeton crispus	curly-leaved pondweed	x	x	high	Europe	none/unknown
Ranunculus ficaria	lesser celandine		x	caution	Europe	ornamental

Table 1.—Continued.

Scientific name	Common name	Pre-1940	More recent	Invasive rank	Origin	Use/mode of introduction
Saponaria officinalis	bouncing bet	x	x	medium	Europe	ornamental, soap
Schedonorus arundinaceus[P]	tall fescue	x	x	medium	Europe	forage, erosion control
Sorghum halepense	Johnson grass	x	x	high	Europe	forage
Torilis japonica[D]	Japanese hedge parsley		x	caution	Eurasia	none/unknown
Typha angustifolia	narrow-leaved cattail	x	x	high	Eurasia	ornamental
Vicia cracca[P]	cow vetch	x	x	medium	Eurasia	forage

mental professionals and knowledgeable amateurs from government agencies, non-profit organizations, private consulting firms, and academia were asked to list the invasive plants they perceive as being the greatest current and emerging concerns in Indianapolis.

RESULTS AND DISCUSSION

Sixty-nine of the 120 species listed on the Official Invasive Plant List for Indiana are known to occur in Indianapolis. These invasives comprise approximately 10% of the flora, somewhat less than the 16% average reported for 110 cites by La Sorte et al. (2014). The majority of invasive plants reported for Indianapolis are ranked as highly invasive (Fig. 3). Most plants (62%) originated in Asia or Eurasia (Fig. 4). Escape from cultivation is, by a wide margin, the most common mode of introduction of invasives in Indianapolis (Fig. 5). Accidental or unknown modes of introduction account for 18% of species, with forage accounting for 12%. The first two introduction pathways are most common for urban floras globally as well (La Sorte et. al 2014). Forage plants may be the remnant of former wide-spread agriculture in the area, or may be the result of contemporary seed mixes used to cover bare ground during construction.

Over half of the invasive plants in Indianapolis were known in the flora prior to 1940 (Fig. 6). Nineteen percent were documented by as early as the 1890s. Most invasives are herbaceous (n = 40) (Table 1). Shrubs are the most commonly represented woody class (n = 19), followed by trees (n = 6) and vines (n = 4) (Table 2, Fig. 7).

Herbaceous invasives have long been in the flora. As early as the late 1800s, Wilson (1895) noted *Daucus carota* and *Glechoma hederacea* were very common, *Meliolotus officinale* and *Saponaria officinale* were common, and *Vinca minor* was becoming common. Most herbaceous invasives (60%) were known for Indianapolis by Deam in 1940 (Table 1). Another 20% were noted by Deam (1940) for elsewhere in Indiana, so it would not be surprising if they were present in the city (or soon would be) but had not yet been recorded. Arriving since Deam's publication are *Alliaria petiolata, Artemesia vulgaris, Centaurea stoebe, Clematis terniflora, Lespedeza cuneata, Microstegium vimineum*, and *Najas minor*.

Analysis of the dates of record for woody plants reveals a different pattern. Only 14% (4 of 29) were known historically for Indianapolis (Table 2), with another eight present elsewhere in the

Table 2.—Woody species of invasive plants in the Indianapolis, Indiana flora and time of first record. Caution means there is reason to believe the species may become a problem, but not enough data yet to support its listing. D = reported by Deam (1940) for elsewhere in Indiana; K = planted by the city in the 1920s as part of the Kessler Plan; P = planting actively promoted by government agencies at some time.

Scientific name	Common name	Pre-1940	More recent	Invasive rank	Origin	Use/mode of introduction
Trees						
Acer platanoides	Norway maple		×	high	Eurasia	ornamental
Ailanthus altissima	tree-of-heaven	×	×	high	Asia	ornamental
Alnus glutinosa[D,K,P]	black alder		×	high	Eurasia/N. Africa	ornamental
Morus alba[K]	white mulberry	×	×	high	Asia	ornamental, food, silk worms
Pyrus calleryana	Callery pear		×	high	Asia	ornamental
Ulmus pumila	Siberian elm		×	medium	Asia	ornamental
Woody vines						
Ampelopsis brevipedunculata	porcelain berry		×	caution	Asia	ornamental
Celastrus orbiculatus	oriental bittersweet		×	high	Asia	ornamental
Hedera helix	English ivy		×	medium	Asia/N. Africa	ornamental
Lonicera japonica[D]	Japanese honeysuckle		×	high	Asia	ornamental
Shrubs						
Berberis thunbergii[K]	Japanese barberry	×	×	high	Asia	ornamental
Elaeagnus angustifolia[P]	Russian olive		×	medium	Asia	erosion control, ornamental
Elaeagnus umbellata[P]	autumn olive		×	high	Asia	ornamental, wildlife food & cover
Euonymus alatus	burning bush		×	medium	Asia	ornamental
Euonymus fortunei	winter-creeper		×	high	Asia	ornamental
Fallopia japonica	Japanese knotweed		×	high	Asia	ornamental, erosion control
Frangula alnus[P?]	glossy buckthorn		×	high	Eurasia	ornamental
Ligustrum obtusifolium[D]	blunt leaved privet		×	high	Eurasia	ornamental
Ligustrum vulgare[D]	common privet		×	medium	Eurasia	ornamental
Lonicera maackii	Amur honeysuckle		×	high	Eurasia	ornamental, wildlife food & cover, erosion control
Lonicera morrowii[K]	Morrow's honeysuckle		×	high	Eurasia	ornamental
Lonicera tatarica[D,K]	Tatarian honeysuckle		×	high	Eurasia	ornamental
Lonicera × bella[K]	Bell's honeysuckle		×	high	Eurasia	ornamental
Rhamnus cathartica[D,K,P?]	common buckthorn		×	high	Eurasia	ornamental
Rhodotypos scandens	jetbead		×	caution	Asia	ornamental
Rosa multiflora[D,K,P]	multiflora rose		×	high	Asia	ornamental, living fence, wildlife cover & food
Rubus phoenicolasius[D]	wine raspberry		×	caution	Asia	ornamental
Viburnum opulus var. *opulus*	highbush cranberry		×	caution	Europe	ornamental
Vinca minor	periwinkle	×	×	medium	Europe	ornamental

Table 3.—The top five ranked invasive plant species, including ties, that pose the biggest current and emerging threats in Indianapolis, Indiana, based on expert opinion. Rankings represent the frequency of citation, with one (1) being the most frequently cited.

Current			Emerging		
Rank	Scientific name	Common name	Rank	Scientific name	Common name
1	*Euonymus fortunei*	purple winter-creeper	1	*Fallopia japonica*	Japanese knotweed
1	*Lonicera maackii*	Amur honeysuckle	1	*Microstegium vimineum*	Japanese stiltgrass
3	*Alliaria petiolata*	garlic mustard	1	*Pyrus calleryana*	Callery pear
4	*Celastrus obiculatus*	oriental bittersweet	4	*Berberis thunbergii*	Japanese barberry
5	*Euonymus alatus*	burning-bush	5	*Clematis terniflora*	sweet autumn clematis
5	*Lonicera japonica*	Japanese honeysuckle	5	*Euonymus alatus*	burning bush
5	*Pyrus calleryana*	Callery pear			

state based on Deam (1940). As noted by Dolan et al. (2011), non-native shrubs escaped from landscaping are the physiognomic group with the largest increase in numbers of species in the Indianapolis flora over the last 70 years. Many of these plants were planted by the city as part of the Kessler Plan, a parkway and boulevard beautification plan during the 1920s (Table 2). Others were actively promoted by the USDA and other government agencies in the past for wildlife food and cover, erosion control, and other purported benefits (Tables 1 & 2).

Five of the seven species that were most frequently cited by restoration experts surveyed for this study as the biggest current invasive plant problems in Indianapolis are woody (Table 3). *Euonymous fortunei* and *Lonicera maackii* tied for first as the biggest current problem. Among plants that were perceived as emerging problems, three (one shrub, one grass and one tree) tied for first: *Fallopia japonica*, *Microstegium vimineum*, and *Pyrus calleryana*.

Early detection and rapid response protocols are the most effective means of preventing the spread of invasive species into new territories. It is widely recognized to be easier to eradicate and control invasive species before they become widely established (e.g., Allendorf & Lundquist 2003). Reports of new sightings posted to EDDMapS and other online sites by consultants, naturalists, academics, and the general public provide the opportunity to track new records and to eradicate plants before they can spread. Greater awareness of invasive plants and their modes or pathways of introduction will hopefully lead to more careful vetting and selection of plants for large-scale landscaping projects in the city and elsewhere.

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POPULATION ECOLOGY STUDY OF *EPIFAGUS VIRGINIANA* (L.) W.P.C. BARTON (BEECHDROPS) IN CENTRAL INDIANA

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ABSTRACT. *Epifagus virginiana* (Beechdrops) is a holoparasitic plant that is obligate on the roots of *Fagus grandifolia* (American Beech) throughout mesic forests in the Midwest. This parasite has a coefficient of conservatism of 8, indicating it requires high-quality plant communities and tolerates little disturbance. *Epifagus virginiana* resembles twigs, producing inconspicuous flowers from August to October. An unexpectedly large population of this species, comprised of 886 plants growing on 17 *F. grandifolia* trees, was found in Hougham Woods Biological Field Station (HWBFS) in Johnson County, Indiana. Plants were morphologically similar to descriptions in literature, with an average height of 16.1 cm and many were commonly observed growing in association with large *Fagus grandifolia* trees (DBH $>$ 40 cm). A statistic previously undocumented was that these plants had cleistogamous and chasmogamous flowers in a 20:1 ratio. Chasmogamous flowers in this population proved sterile. However, since each cleistogamous flower produced an average of 827 seeds, the *E. virginiana* population in HWBFS displays a very large reproductive potential for the coming years. Monitoring this population could provide a way to assess the health of this forest remnant.

Keywords: *Epifagus virginiana*, beechdrops, beech trees, *Fagus grandifolia*, mesic woods, root parasite

INTRODUCTION

Epifagus virginiana (L.) W.P.C. Barton is a holoparasitic plant that lacks chlorophyll (Porcher & Rayner 2001; Tsai & Manos 2010; Weakley et al. 2012; Yatskievych 2013) and is an obligate parasite interacting solely with the roots of *Fagus grandifolia* Ehrh. (Deam 1940; Brooks 1960; Gleason & Cronquist 1991; Yatskievych 2000; Mohlenbrock 2002; Jones 2005; Rhoads & Block 2007; Tsai & Manos 2010; Homoya 2012; Abbate & Campbell 2013). These plants grow in mesic, eastern deciduous forests, occupying the same range as their host (Thieret 1969; Smith 1994; Chapman et al. 1998, 2008; Yatskievych 2000; Porcher & Rayner 2001; Tsai & Manos 2010; Homoya 2012; Abbate & Campbell 2013). Both plants have a coefficient of conservatism of 8, indicating they grow in high-quality plant communities and tolerate little disturbance (Rothrock 2004).

Epifagus virginiana is a member of the Orobanchaceae or Broomrape family. Traditionally, species in this family were non-green, root parasites that relied entirely on deciduous host trees. These herbaceous plants were characterized by having small, scale-like leaves, irregular flowers, and fruit capsules that produced an abundance of small seeds (Radford et al. 1968; Jones 2005). However, today, Orobanchaceae also includes hemiparasitic species and is comprised of 89 genera and 2061 species (Bennett & Mathews 2006). These species are distributed worldwide, but are most predominant throughout the Mediterranean, Southern Africa, the Himalayas, and North America (Bennett & Mathews 2006).

Most botanists describe *Epifagus virginiana* as an annual (Thieret 1969; Yatskievych 2013; Porcher & Rayner 2001; Tsai & Manos 2010; Abbate & Campbell 2013); however, they have also been reported as a perennial (Homoya 2012; Weakley et al. 2012). These non-showy, brown, twig-like plants are 10–15 cm tall (Yatskievych 2000; Homoya 2012) with branches bearing alternate scale-like leaves and two types of flowers (Homoya 2012) (Fig. 1). *Epifagus* flowers from August to October (Gleason & Cronquist 1991; Chapman et al. 1998, 2008; Mohlenbrock 2002; Jones 2005; Homoya 2012; Yatskievych 2013); however, the dried plant stalks persist throughout winter (Porcher & Rayner 2001).

The open, chasmogamous flowers (1 cm) of *Epifagus* are tubular, having four purple and white petals and are located on the upper portion of the stem (Radford et al. 1968; Homoya 2012;

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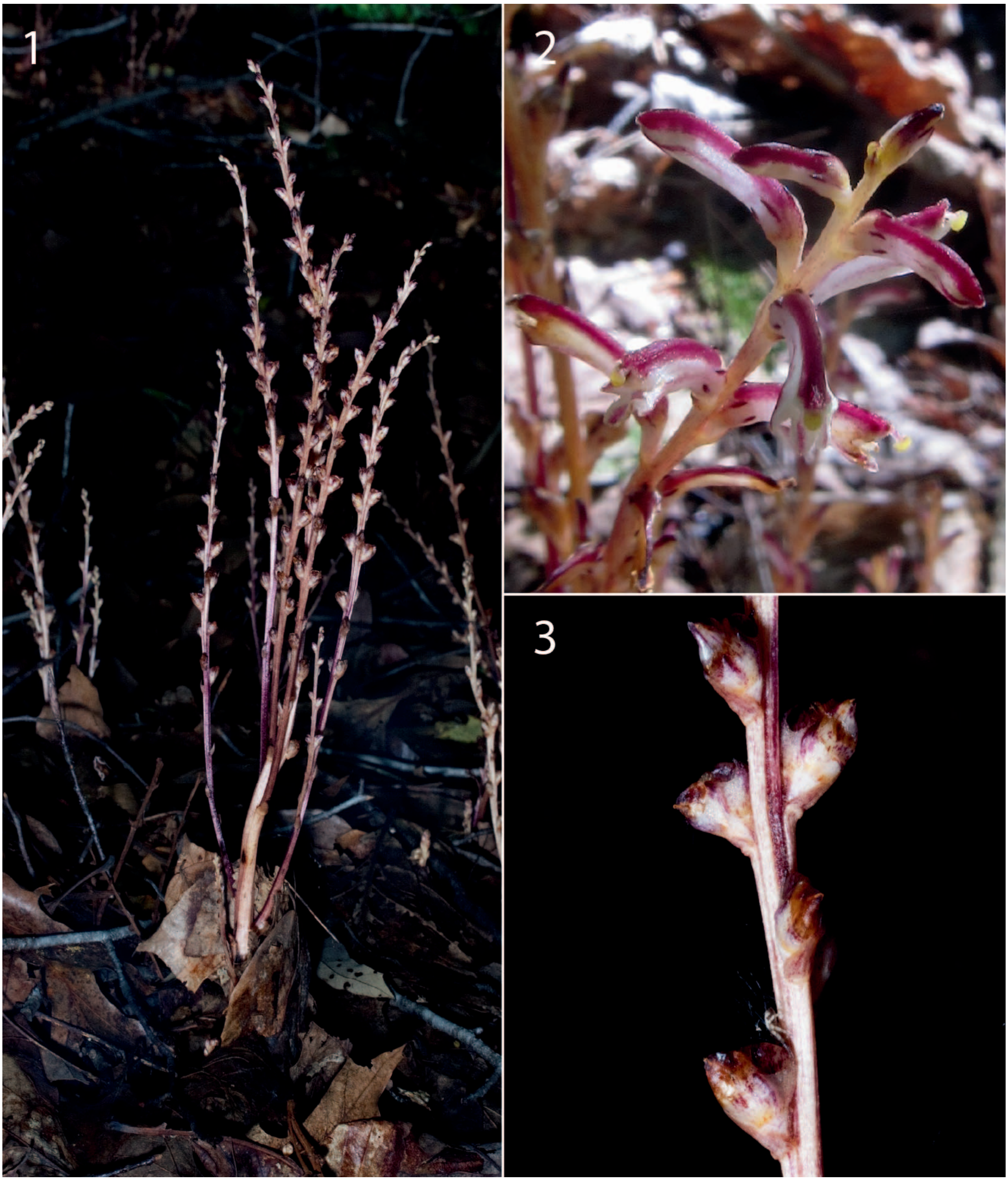

Figures 1–3.—*Epifagus virginiana*. 1. Entire plant. 2. Chasmogamous flower. 3. Cleistogamous flower. (Photos 1 & 2 by Kay Yatskievych; photo 3 by Charles de Mille-Isles (2011).)

Abbate & Campbell 2013) (Fig. 2). These flowers are considered to be sterile (Radford et al. 1968; Gleason & Cronquist 1991; Porcher & Rayner 2001; Homoya 2012; Weakley et al. 2012; Yatskievych 2013). Occasionally, however, long-tongued bees and ants have been observed visiting them, possibly attracted to a nectary found near the ovary of the chasmogamous flower. Infrequently, these flowers produce fruits and seeds (Abbate & Campbell 2013). Normally, chasmogamous flowers are few in comparison to the self-fertile, closed cleistogamous flowers.

Cleistogamous, closed, spike-like flowers (4–6 mm) are found near the base of the plant and produce seeds (Radford et al. 1968; Jones 2005; Homoya 2012; Yatskievych 2013) (Fig. 3). The small, dust-like seeds are washed deep into the soil, in close proximity to *F. grandifolia* roots (Porcher & Rayner 2001; Jones 2005). These roots release a chemical, signaling the seeds to germinate in spring (Porcher & Rayner 2001; Jones 2005; Grafton 2008).

Epifagus virginiana, which is found throughout southern Indiana counties (Fig. 4), is scattered throughout northern Indiana (Deam 1940; Yatskievych 2000) with a recent new record for Johnson County (specimen #155310 housed in Friesner Herbarium (BUT) at Butler University). As high quality forests are degraded by habitat fragmentation, invasive species, and anthropogenic disturbances, the high quality sites where *F. grandifolia* and *E. virginiana* occur are at high risk for declining populations. There is also a lack of information about this relatively inconspicuous species and its population ecology. The objectives of this research are to establish baseline information about the size and locations of *E. virginiana* within Hougham Woods Biological Field Station (HWBFS) in Johnson County, Indiana, to determine the size and morphological characteristics of these plants, to examine the reproductive potential of the *Epifagus* population, and to examine host tree size and location.

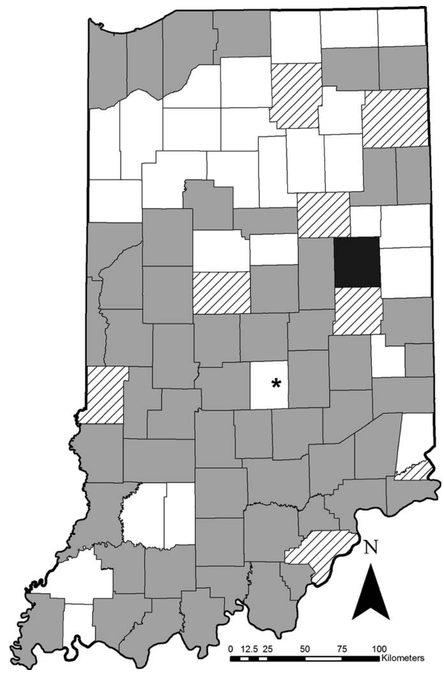

Figure 4.—Distribution of *Epifagus virginiana* in Indiana. Light shading indicates distribution from BONAP (2014), hashing indicates new records in several counties (K. Yatskievych Per. Comm.), dark shading indicates the Delaware County Record (Ruch et al. 1999 specimen BSUH 8227), and the star indicates the county record for Johnson County.

METHODS

Study site.—HWBFS is a 12 ha relatively flat, mesic forest that was donated to Franklin College in 2006. Dominant canopy species include *Acer saccharum* Marshall, *F. grandifolia*, and *Quercus* spp. (Smith & Heikens 2014). The forest is located in Johnson County east of Franklin, Indiana in the Tipton Till Plain Section of the Central Till Plain Natural Region (Fig. 4). In this region, soil types are often neutral silt and silty clay loams (Homoya et al. 1985; Smith & Heikens 2014). HWBFS is surrounded by agricultural field and an industrial park, and has experienced disturbances, including selective cutting and wind throw (Smith & Heikens 2014). Despite these disturbances, a few species with high coefficients of conservatism, such as *F. grandifolia* and *Aplectrum hyemale* (Muhl. ex Willd.) Torr., persist in the forest.

From September to November 2013, *E. virginiana* and their associated host trees were located, flagged, and numbered in HWBFS and marked using GPS. The locations were mapped using ArcGIS software. To establish baseline data on the size of *Epifagus* plants, large populations ($\geq$ 40 plants) were randomly sampled (all plant numbers ending in 2) and measured for the following morphological characteristics: plant height (cm), number of branches, number of chasmogamous and cleistogamous flowers, and distance (cm) to nearest host tree. In small populations ($<$ 40 plants), this data was gathered for all plants. In total, 225 plants were examined.

Twenty plants from the entire population were randomly selected for seed production. The 10th capsule from the bottom of the plant was examined if the capsule was intact. The capsules were weighed, then opened and the seeds were weighed and counted.

To investigate chasmogamous flowers, in the largest population (470 plants), 32 plants were randomly selected and examined for chasmogamous and cleistogamous flower ratios. In addition, 50 plants were randomly selected from the three largest populations to determine if seeds

Table 1.—Morphological characteristics and seed production in *Epifagus virginiana* in 2013 in Hougham Woods Biological Field Station, Franklin, IN. (n = 225).

	Mean	Range	Standard deviation
Height (cm)	16.1	4.3 – 40.0	7.3
Distance from Host Trunk (cm)	195.0	18 – 912	5.1
Number of Branches	6	1 – 40	6.9
Number of Flowers	61	3 – 471	80.6
Capsule Weight (mg)	14.00	8.00 – 20.80	0.0
Seeds	6.18	1.00 – 12.00	0.0
Number of Seeds	827	188 – 1799	344.6
Weight per Seed (µg)	7.38	3.89 – 10.74	1.81×10^{-6}

were produced in chasmogamous flowers. Plants taller than 25 cm were examined because chasmogamous flowers were not found on plants shorter than 16 cm.

In the summer of 2014, all *Fagus* trees in HWBFS were flagged and marked using GPS. The trees were grouped into the following DBH (cm) size classes arbitrarily: 0-10, 11-20, 21-30, 31-40, 40+ cm. Host trees were examined for number of *Epifagus* plants, average height (cm), and average distance to host trunk (cm). *Epifagus* plants were assumed to parasitize the nearest *Fagus* tree. Minitab 17 was used for the Pearson Correlations.

RESULTS AND DISCUSSION

In 2013, 886 *Epifagus* plants were found growing on 17 host trees in the interior of HWBFS. The 225 *Epifagus* plants that were measured in HWBFS were larger (16.1 cm) than the size documented in Indiana (10–15 cm) (Yatskievych 2000) (Table 1); however, the observed heights were similar to ranges reported throughout the Midwest (15–60 cm) (Chapman et al. 1998, 2008; Porcher & Rayner 2001; Homoya 2012; Weakley et al. 2012). Distances between *Epifagus* and their host trunks varied widely (Table 1). Large variations were also discovered when examining the morphological characteristics of the plants, i.e., number of branches and number of flowers (Table 1). Plants had multi-branched stems averaging 6 stems and 61 flowers per plant. Flower totals varied from 3 to 471 per plant (Table 1).

Epifagus is reported as producing an abundance of small seeds (Radford et al. 1968; Jones 2005; Homoya 2012; Yatskievych 2013) and the results from this study are consistent with this (Table 1). In 2013, 20 cleistogamous flowers from 20 different plants produced approximately 16,500 seeds with an average weight of 7.38 µg per seed (Table 1). The large number of seeds per cleistogamous capsule, combined with the high cleistogamous flower presence per plant (Table 2), suggests that the overall *Epifagus* population in HWBFS has a high reproductive potential. Chasmogamous flowers occurred in much smaller numbers per plant and were limited to larger plants (Table 2). While these flowers have been reported as being pollinated and producing seeds (Abbate & Campbell 2013), this study supports the more common findings that these flowers are sterile (Radford et al. 1968; Gleason & Cronquist 1991; Porcher & Rayner 2001; Homoya 2012; Weakley et al. 2012; Yatskievych 2013). In an examination of 50 chasmogamous flowers from large *Epifagus* (average height 29.4 cm), no seeds were discovered. The ratio of cleistogamous to chasmogamous flowers in this study was 20:1.

In 2014, 415 *Fagus* trees were located throughout HWBFS, 17 of which were associated with *Epifagus* populations (Table 3). The majority of *Epifagus* were associated with trees in the 40+ DBH size classes and none were found in the smallest size class of 0-10.9 cm (Table 3). Also, the largest *Epifagus* populations were supported by trees that had a DBH of 40+ cm. However, there was not a significant ($p \leq 0.05$) correlation

Table 2.—Presence of chasmogamous and cleistogamous flowers on *Epifagus virginiana* in 2013 in Hougham Woods Biological Field Station, Franklin, IN. (n = 32).

Plant height (cm)	Flower type	
	Chasmogamous	Cleistogamous
5.0 – 10.9	0	12
11.0 – 15.9	0	34
16.0 – 20.9	3	68
21.0 – 25.9	8	114
26.0 – 30.9	18	249

Table 3.—Host tree metrics and *Epifagus virginiana* association in Hougham Woods Biological Field Station, Franklin, IN. DBH = diameter at breast height.

DBH (cm)	*Epifagus virginiana* population size	Mean height of plants (cm)	Mean distance from host trunk (cm)
13.3	13	15.0	89.7
14.9	2	16.2	254.2
16.1	16	15.3	185.6
17.1	1	18.2	237.2
18.9	7	13.0	242.3
20.6	1	17.4	276.1
24.3	3	19.3	36.2
33.7	3	7.9	43.2
36.5	34	15.9	191.4
39.9	1	16.5	295.6
46.4	190	19.9	267.0
48.6	18	15.6	57.5
48.8	23	10.1	75.7
55.8	18	17.1	220.1
57.9	40	15.5	139.9
61.5	15	24.6	286.5
63.5	470	16.0	281.7

between tree size and size of the *Epifagus* population ($R^2 = 0.47$, $p = 0.053$). This lack of a significant correlation may be due to the shortest *Epifagus* plants being associated with trees that were 33.7 and 48.4 cm DBH, respectively. It appears that factors other than tree size impact *Epifagus* growth. There was also a lack of a significant correlation between mean height of *Epifagus* and tree size ($R^2 = 0.18$, $p = 0.47$), or tree size and distance of *Epifagus* from the host trunk ($R^2 = 0.10$, $p = 0.71$). It is possible that large *Fagus* trees produce an abundance of chemicals that trigger *Epifagus* germination (Porcher & Rayner 2001; Jones 2005; Grafton 2008). It is unknown if younger trees are more resistant to *E. virginiana*, if it takes a number of years for above ground stems to form, or what environmental factors impact germination.

In conclusion, the large population of *E. virginiana* at HWBFS has a high reproductive potential due to its abundance of plants, number of flowering plants, and number of seeds per capsule. Additional research is needed to determine the impact of chemicals released by the host species on germination and how environmental conditions impact *Epifagus* population sizes. The abundance of *E. virginiana* and *F. grandifolia*, is one indicator that HWBFS is a relatively high-quality forest remnant and the continued monitoring of these populations in HWBFS may be one way to determine the quality of the forest overtime.

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SPIDERS IN INDIANA: SEVENTY-ONE NEW AND UPDATED DISTRIBUTION RECORDS

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ABSTRACT. Spiders are an integral part of many ecosystems, yet their diversity is understudied in Indiana. To uncover spider diversity within the state, spiders were collected from several ecosystems in many counties across the state. Moreover, spiders were re-identified within the collection of a former Indiana arachnologist, Thomas Parker. Herein we report sixty-four new state records for spider species and update seven species distribution records. These new records represent a 19% increase in the number of spider species known from Indiana, from 383 to 454. Some notable discoveries: 1) *Antrodiaetus unicolor*, *Cybaeus giganteus*, *Oecobius cellariorum*, and *Ummidia tuobita* represent species within four new families of spiders for the state (Antrodiaetidae, Cybaeidae, Oecobiidae, and Ctenizidae, respectively); and 2) *Dipoena nigra*, *Eidmannella pallida, Idionella rugosa*, *Neon nelli*, *Styloctetor purpurescens*, *Paracornicularia bicapillata*, *Oreonetides beattyi*, *Larinia directa, Microneta viaria*, *Spintharus flavidus*, *Lepthyphantes turbatrix*, *Lupettiana mordax*, *Tapinocyba emertoni*, *Phylloneta pictipes*, *Ceratinopsidis formosa*, *Talanites exlineae*, and *Epiceraticelus fluvialis* represent species within 17 new genera for the state.

Keywords: Araneae, arachnid, range, extension, faunistics, state record

INTRODUCTION

Spiders are a diverse group of arthropods, with approximately 46,000 species known worldwide (World Spider Catalog 2015). Within North America, approximately 3,800 species have been described (Bradley 2013). The latest inventory of spiders in Indiana recorded 383 species from the state (Sierwald et al. 2005). However, this estimate is ten years old, and compared to its closest neighbors, the state of Indiana has lagged in inventorying its spider diversity. The best evidence of this comes from an examination of spiders known from states surrounding the Great Lakes (Sierwald et al. 2005); either Indiana is depauperate in relation to Illinois, Ohio, Wisconsin, and Michigan or there has been inadequate study of spider diversity within the state (383 spider species known from Indiana in comparison to 646, 571, 479, and 563 from Illinois, Ohio, Wisconsin, and Michigan, respectively). Although there may be some environmental differences among the states, we predict that continued inventory of spiders within the state will indicate that the latter explanation is more likely.

The earliest comprehensive work on Indiana spiders was a published talk at the Washington Entomological Society in 1891 by Fox that listed 77 species. A very detailed explanation of publications dealing with Indiana spiders prior to 1952 can be found in Elliot (1953). Published accounts by Banks (1907), Elliot (1932, 1953), Parker (1969), and Beatty (2002) brought the number of known spider species in Indiana to 383. Herein, we add 71 species to Beatty's (2002) list to

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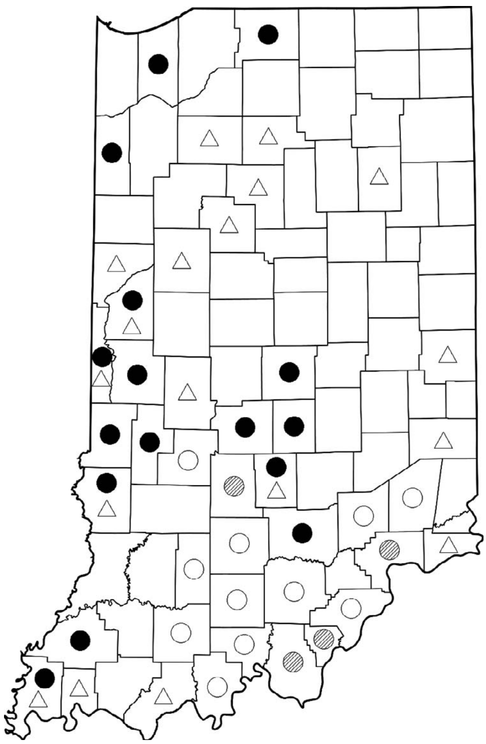

Figure 1.—Sampling locations and museum specimen locales by county. Closed circles represent counties in which only surface sampling was conducted; open circles represent counties in which only subterranean sampling was conducted (including sinkholes); cross-hatched circles represent counties in which both surface and subterranean sampling was conducted; triangles represent counties from which specimens were examined that were from the collections of T.A. Parker housed at the Purdue Entomological Research Collection.

bring the total number of spider species that are known to occur in Indiana to 454.

METHODS

Spiders were collected from multiple localities using a variety of methods over the past ten years (Fig. 1). The majority of specimens were collected from 2014 to 2015. Many spiders were collected from Morgan-Monroe State Forest (Morgan Co. and Monroe Co., Indiana; MMSF henceforth) and Yellowwood State Forest (Brown Co., Indiana; YSF henceforth) as part of the Hardwood Ecosystem Experiment (HEE) from 15 January 2015 to 6 February 2016. Spiders at these locations were collected from pitfall traps or from leaf litter using Berlese funnels in the lab. Pitfall traps consisted of 88.7 ml (3 oz) plastic cups recessed into the soil so that the top was flush with the soil surface. Pitfall traps were half-filled with soapy water and allowed to collect arthropods for two weeks before being collected. Pitfall traps were established in pairs approximately 10 m apart. Sites for pitfall trap pairs were at least 80 m apart. Twelve pitfall traps were set and collected (at six sites) in MMSF and 36 were set and collected (at 18 sites) in YSF on six occasions: 1/17/2015–1/31/2015, 3/15/2015–3/29/2015, 5/18/2015–5/31/2015, 7/25/2015–8/8/2015, 9/20/2015–10/4/2015, and 1/23/2016–2/6/2016. Three or four handfuls (2–3 L) of leaf litter were collected from each of the pitfall trap sites and transferred to the lab in plastic bags. Leaf litter from each site was placed into a Collapsible Berlese Funnel Trap (BioQuip, Product No. 2832). An LED light (Triangle Bulbs, 13-Watt PAR30 Flood Light Bulb, Model No. T97005-4) was placed at the top and a vial of ethanol was placed at the bottom of each funnel trap. Each trap was allowed to extract for two to three days before being inspected.

Other spiders were collected from Glacier's End Nature Preserve (Johnson Co., Indiana; GENP henceforth) during a bioinventory on 16 April 2015 and supplemental sampling on 17 March 2016. More collections were done at MMSF during bioinventories on 22 June 2014 and 13 September 2014. These spiders were collected by hand using manual aspirators (BioQuip, Product No. 1135B) and by litter sifting using a litter reducer (BioQuip, Product No. 2834) and a canvas beating sheet (BioQuip, Product No. 2840C).

Further sampling of spiders was done by sweeping vegetation, sifting leaf litter, and hand collecting in various wooded areas in Clay, Floyd, Fountain, Gibson, Harrison, Jefferson, Monroe, Newton, Parke, Posey, Sullivan, Vermillion, and Vigo Counties in July and August 2015. Additional sampling in these counties was done at night using a headlamp. Moreover, four pitfall traps were set in Vigo Co. during the summer of 2015.

Several new spider distribution records came from cave and sinkhole habitats. These records were provided by J. Lewis from his exhaustive sampling of over 750 Indiana caves and sinkholes during 1976–2015. We examined 631 spiders from these sampling efforts. Spiders were collected from caves by hand collecting and pitfall trapping using 118 ml (4 oz) containers baited with limburger cheese.

In addition to collecting, a small part of Parker's (1969) spider collection (his "miscellaneous" spider specimens) at the Purdue Entomological Research Collection (PERC) was examined and re-identified. These specimens were examined because the latest list of Indiana spiders (Sierwald et al. 2005) unquestioningly used Beatty's (2002) list. However, Beatty did not re-examine Parker's (1969) collection and therefore could not have caught misidentifications or partial identifications (see Beatty 2002). All specimens from PERC were kept there as vouchers.

Spiders were identified by the lead author using Ubick et al.'s *Spiders of North America* (2005) and hundreds of associated keys and records. Only adult specimens were considered for records unless identification of the immature could be completed with certainty. All nomenclature follows that used by the World Spider Catalog (World Spider Catalog 2015). Distribution records of all species found were compared against the most recent peer-reviewed article in a scientific journal that contained a list of species located in Indiana (see Sierwald et al. 2005). As explained by Sierwald et al. (2005), their distribution list of spiders in Indiana was taken directly from Beatty (2002) with no modification. There is a newly-released online database of species distributions throughout North America hosted by the American Arachnological Society (see Bradley 2015), but we have decided not to use this list due to its lack of peer-review and frequent conflict (over omission and inclusion) with Sierwald et al.'s (2005) published list.

Sierwald et al. (2005) predicted the presence of certain species in Midwestern states based on their distribution in other nearby states. If a species was predicted by Sierwald et al. (2005) to be present in Indiana, we have indicated that prediction with a "**P**" next to the listing. We have also found and examined spiders from Indiana that were reported to occur in the state by other authors but were not included in Sierwald et al.'s (2005) list. These records have been denoted by an asterisk (*) before the listing and the author of the original record is stated. Voucher specimens for all epigean (surface-dwelling) collected species were deposited in the spider collection at Indiana State University. Cave-collected spiders were deposited in the entomological collection at Southern Illinois University until they were transferred to the Illinois Natural History Survey (INHS) in the mid-2000s.

RESULTS

Of 3,917 spider specimens examined, 71 species were new or updated records for Indiana. Within these new records are four new families (Antrodiaetidae, Ctenizidae, Cybaeidae, and Oecobiidae) and 17 new genera (*Ceratinopsidis*, *Dipoena*, *Eidmannella, Epiceraticelus, Idionella*, *Larinia, Lepthyphantes*, *Lupettiana*, *Microneta*, *Neon, Oreonetides*, *Paracornicularia*, *Phylloneta*, *Spintharus*, *Styloctetor*, *Tapinocyba*, and *Talanites*) for the state. Approximately 55% (39/71) of the species we located within the state were predicted to exist in Indiana by Sierwald et al. (2005). With these added and updated records, Indiana now has 454 known spider species within the state, yet is still ranked last among states in the Midwest in the number of identified spider species. Each new or updated species record is listed below alphabetically by taxonomic family and then alphabetically by scientific name within the family. Ranges are largely taken from the American Arachnological Society website (Bradley 2015).

Agelenidae

Agelenopsis emertoni Chamberlin & Ivie, 1935 **P** – occurs from Nova Scotia to Florida and west to Colorado. One male specimen was collected in a forested area in West Terre Haute, Vigo Co. on 28 August 2015.

Antrodiaetidae

Antrodiaetus unicolor* Hentz, 1842 **P – is known from the eastern United States, from Georgia to New York and west to Arkansas. A single female specimen was found in Brown Co. in 2013. This species was previously captured by Coyle (1971) in Jefferson Co.

Anyphaenidae

Lupettiana mordax O. Pickard-Cambridge, 1896 – occurs in the southeastern United States and California. One female was captured on Blunk Knob, south of Georgetown, Floyd Co. on 24 August 2015.

Araneidae

Acanthepeira cherokee Levi, 1976 – occurs in the southeastern United States, from Texas to Virginia. One male was collected from

Not So Grand Cave II near Laconia in Harrison Co. in March 1997. One immature specimen was also collected from MMSF (Monroe Co.) in September 2015.

Araneus cingulatus Walckenaer, 1841 **P** – ranges from New York south to Florida and west to Texas and Illinois. One female was found near a woodland road northwest of Brazil, Clay Co. in July 2015.

Cyclosa conica Pallas, 1772 **P** – is distributed throughout Canada and most of the United States except for the southeast and some central states. A male and female were collected from MMSF and YSF (Monroe and Brown Co.) in August and June of 2015, respectively.

Eustala emertoni Banks, 1904 **P** – occurs from New York south to Florida and west to Texas and Kansas. One female was found in a grassy field in Vigo Co. in July 2015.

Larinia directa Hentz, 1847 – occurs in the southern United States, from California to Florida. One immature specimen was found in Vigo Co. in September 2015.

Atypidae

Sphodros coylei Gertsch & Platnick, 1980 – is only known from South Carolina, Virginia, and Ohio. This specimen's location is the most western known occurrence of the species. One male specimen misidentified as "*Atypus milberti*" (*Sphodros rufipes*) was examined and re-identified as this species within Parker's (1969) collection at PERC. This single specimen was collected by Gary Finni from South Pine Creek in Warren Co. in May 1968.

Sphodros rufipes Latreille, 1829 **P** – occurs on the East Coast of the United States, from New York to Florida and west to Texas. Six females were collected from Buzzard Roost in Hoosier National Forest, Perry Co., in September 2015.

Corinnidae

Castianiera variata Gertsch, 1942 **P** – has a Midwestern distribution, but extends south to Louisiana, west to Kansas, east to Pennsylvania, and north to Ontario. We collected one female beneath discarded cardboard from a yard in Terre Haute and a second female from another residential yard in West Terre Haute, Vigo Co. in July 2015.

Ctenizidae

Ummidia tuobita Chamberlin, 1917 – is known from Illinois, Arkansas, Missouri, and Texas. A female and male were collected in Harrison Co. in September 2015. Minton (1955) described capturing *U. audouini* from Floyd Co., but this specimen may actually have been *U. tuobita* since *U. audouini* is only known from Louisiana and Texas.

Cybaeidae

Cybaeus giganteus Barrows, 1919 **P** – is known from Illinois, Ohio, New York, and North Carolina. One female was collected from Wools Whim Cave in Jennings Co. and more specimens from Bernice Chandler Cave (GC 06) in Ripley Co.

Dictynidae

Cicurina itasca Chamberlin & Ivie, 1940 – is largely confined to the states and provinces surrounding the Great Lakes with the exception of Quebec. This is the most southerly report of this species on record and extends this species' range south from Wisconsin by approximately 656 km from Fish Creek, Wisconsin. One female was collected at YSF (Brown Co.; N39.121056°, W-86.366832°) in February 2015 and another female was found in a sinkhole at HNF in Lawrence Co. in September 2015.

Lathys immaculata Chamberlin & Ivie, 1944 (Araneae, Dictynidae) – is largely restricted to the southeastern United States, but is also known to exist in Illinois. Two males and three females were collected at YSF and MMSF (Monroe and Brown Co.) March–May 2015.

Eutichuridae

Cheiracanthium mildei L. Koch, 1864 **P** – is an introduced species from Europe and has a spotty distribution, being present in the Midwest, East Coast, and West Coast, but only in certain states. Three specimens were collected from buildings in Indianapolis, Marion Co., in July 2009, May 2015, and June 2015.

Gnaphosidae

Drassyllus covensis Exline, 1962 – is known to occur in only six states in the southeastern United States, from Texas to New Jersey. Nine males and four females were found at MMSF and YSF (Monroe and Brown Co.) January–May 2015.

Drassyllus dixinus Chamberlin, 1922 – is known from the southeastern United States. One female was found in St. Joseph Co. in June 2015.

Drassyllus fallens Chamberlin, 1922 **P** – is widespread on the East Coast, from Quebec to Georgia and as far into the Midwest as Wisconsin and Illinois. Two males were found at YSF (Brown Co.) in April and June 2015.

Gnaphosa fontinalis Keyserling, 1887 **P** – occurs in the eastern and central United States, as far west as Texas and Oklahoma. Two males and one female were found at MMSF and YSF (Monroe and Brown Co.) in May 2015. Moreover, two males were found in Vigo Co. in July 2015.

Micaria longipes Emerton, 1890 **P** – is known throughout the United States and Canada. We found one male at MMSF (Monroe Co.) in June 2014.

Sergiolus minutus Banks, 1898 – is distributed largely in the southern United States, but can be found as far north as New York and occurs in Wisconsin and Illinois as well. One male specimen was collected at a landfill site in Terre Haute, Vigo Co. in May 2015.

Talanites exlineae Platnick and Shadab, 1976 – occurs throughout the southeastern United States. Multiple specimens were collected from Heron Cave in Crawford Co., Liar's Bluff Cave in Orange Co., and Rocky Hollow Hole in Perry Co. in September 1996.

Hahniidae

Hahnia flaviceps* Emerton, 1913 **P – has a southeastern distribution, occurring from Texas to North Carolina and north to Illinois. Three males were found in MMSF (Monroe Co.) in March 2015 and four females and two males from YSF (Brown Co.) in March, May, and October 2015. This species was recorded from Indiana by Opell & Beatty (1976) but is not present in the lists of Beatty (2002) and Sierwald et al. (2005).

Linyphiidae

Agyneta angulata Emerton, 1882 – occurs along the East Coast of North America, from Nova Scotia to Georgia and as far west as Missouri. One male was found at YSF (Brown Co.) in May 2015.

Agyneta barrowsi Chamberlin & Ivie, 1944 **P** – occurs in the southeastern United States, north into the Midwest and Ontario, and as far west as Nebraska. Two males and seven females were found at YSF (Brown Co.) February–June 2015.

Agyneta evadens Chamberlin, 1925 – is largely found in the central East Coast and Midwest, but also occurs in Alabama and Ontario. Two females were found at GENP (Johnson Co.) in April 2015.

Agyneta parva Banks, 1896 – occurs in the south central and southeastern United States from Texas to Florida and north to Virginia. The Indiana collection extends its range approximately 316 km NW of its Kentucky locality. One female was found at GENP (Johnson Co.; N 39.357780°, W-86.167365°) in April 2015. A second specimen identified as "*Meioneta* sp." was examined and re-identified as this species within Parker's (1969) collection at PERC. This PERC specimen was collected from Santa Claus, Spencer Co., in May 1966.

Agyneta serrata Emerton, 1909 – has an eastern distribution, ranging from Texas to Florida and north to Ontario and Nova Scotia. Two male specimens identified as "*Meioneta* sp." were examined and re-identified as this species within Parker's (1969) collection at PERC. One specimen was collected by Gary Finni from South Pine Creek, Warren Co., in April 1968 and the other at Nussmeier Plantation near Santa Claus, Spencer Co., in May 1966.

Bathyphantes alboventris* Banks, 1892 **P – has a northeastern distribution, ranging from Tennessee north to Quebec. One male and nine females were recorded from MMSF

(Monroe Co.) in September 2015 and three males and eight females from Fuzzy Hole Cave, Lawrence Co., in November 2015. Additional specimens were collected from Dryden Cave, Dryden Sinks Cave, Hammerhead Pit, and Meek Cave in Jennings Co. and Tincher Swallowhole Cave in Lawrence Co. This species was recorded on a distribution map by Ivie (1969), but was not stated as being from Indiana in the text of the manuscript. It is not clear if the distribution map specimens were collected by Ivie or were from the American Museum of Natural History.

Ceraticelus similis Banks, 1892 **P** – can be found from Quebec south to Georgia and west to Arizona. An unidentified female specimen was in Parker's (1969) collection at PERC. This specimen was collected by R.W. Meyer from Merritt's Pine Plantation, Tippecanoe Co., in July 1968.

Ceratinops crenatus Emerton, 1882 **P** – has an easterly distribution, occurring from Quebec south to Florida and west to Texas. Parker's (1969) collection at PERC had two male specimens. These were collected via pitfall traps from Merritt's Pine Plantation, Tippecanoe Co., in April 1968.

Ceratinopsidis formosa Banks, 1892 **P** – occurs from Ontario south to North Carolina and Tennessee. One male was found in Harrison Co. in August 2015.

Epiceraticelus fluvialis Crosby & Bishop, 1931 – is known from only three states: New Jersey, New York, and Ohio. Four males and one female were located at YSF and MMSF (Brown and Monroe Co.) during February–April 2015.

Idionella rugosa Crosby, 1905 – occurs in eastern Canada, New York, Connecticut, and Illinois. Two male specimens within Parker's (1969) collection at PERC that were misidentified as "*Ceraticelus laetus*" were examined and re-identified as this species. Both specimens were collected from Attica, Fountain Co., in April 1968.

Islandiana cavealis Ivie, 1965 – is only known from caves in Kentucky. We found multiple specimens in Stygian River Cave in Harrison Co. in September 1996. This record is approximately 145 km WNW of the closest known location in Kentucky.

Islandiana flavoides Ivie, 1965 – is only known from New York and Illinois. Two male specimens within Parker's (1969) collection at PERC, marked as unidentified, were examined and identified as this species. One specimen was collected from Vanderburgh Co. in June 1966 and the other by R.W. Meyer from Merritt Pine Plantation, Tippecanoe Co., in August 1968.

Lepthyphantes turbatrix O. Pickard-Cambridge, 1877 **P** – is a northern species, known from throughout Canada, the Midwest, and northeastern United States. One male was found at MMSF (Monroe Co.) in September 2014.

Mermessus maculatus* Banks, 1892 **P – exists throughout eastern United States and Canada and can be found as far west as Arizona. One male and 10 females were collected from MMSF (Monroe Co.) and 11 males and 13 females from YSF (Brown Co.) January 2015–February 2016. We also found one male and three females from GENP (Johnson Co.) in April 2015. Additional specimens were collected from Bordens Pit, Limekiln Hollow Pit, and Webers Swallowhole in Harrison Co., John Samples Cave in Jefferson Co., and Williams Cave in Lawrence Co. Millidge (1987) records this species from Montgomery Co.

Mermessus trilobatus* Emerton, 1882 **P – can be found throughout the United States and Canada. Two males from PERC that were misidentified as "*Eperigone contorta*" and "*Eperigone indicabilis*" were identified as this species. Both of these specimens were collected from Americus in Tippecanoe Co. One was collected in August 1968 and the other was collected on an unknown date. Moreover, one unidentified female specimen from PERC was identified as this species and was collected from Lafayette in October 1968. Millidge (1987) records this species from Adams and Marion Co.

Microneta viaria Blackwall, 1841 **P** – occurs throughout Canada, the Midwest, and as far south as New Mexico. Two males and five females were found at Eagle Creek Park, Indianapolis, Marion Co., in September 2015.

**Oreonetides beattyi* Paquin et al., 2009 – is known from Appalachian caves and west to southern Indiana. Two males and 11 females of this recently described species (Paquin et al. 2009) were collected from caves throughout southern Indiana during 2000–2007 (see collections by Lewis in Paquin et al. 2009).

Paracornicularia bicapillata Crosby & Bishop, 1931 – is only known from three states: Illinois, Missouri, and Mississippi. One male was discovered in YSF (Brown Co.) in March 2015.

Styloctetor purpurescens Keyserling, 1886 **P** – has an eastern distribution, but can be found as far west as Texas. Four females were found from MMSF (Monroe Co.) and GENP (Johnson Co.) in June 2014 and April 2015, respectively.

Tapinocyba emertoni Barrows & Ivie, 1942 – is only known from Ohio. Thirty females and 13 males were found at GENP (Johnson Co.), YSF (Brown Co.), and MMSF (Monroe Co.) February–October 2015.

Tenuiphantes sabulosus Keyserling, 1886 **P** – is distributed throughout North America, but is more prevalent in the East and Midwest. Eight females were found at GENP (Johnson Co.) and YSF (Brown Co.) from January to April 2015. In addition, multiple specimens were found in Hoosier National Forest's Swallow Hole, JJ's Sister Cave, and Williams Cave in Lawrence Co.

Walckenaeria brevicornis Emerton, 1882 **P** – occurs largely in the eastern United States but has been found as far west as Kansas. One male was hand collected at GENP (Johnson Co.) in March 2016.

Walckenaeria communis Emerton, 1882 **P** – has a wide distribution throughout Canada and most of the United States. One female was found within the leaf litter in a sinkhole at Hoosier National Forest, Orange Co., in November 2015.

Walckenaeria directa O. Pickard-Cambridge, 1874 **P** – is known to exist throughout Canada and most of the United States, especially in the Northeast. One female was found at YSF (Brown Co.) in February 2016.

Lycosidae

Arctosa virgo Chamberlin, 1925 – is distributed in several East Coast and Midwest states. Two females were found in August 2015: one at MMSF (Monroe Co.) and another at YSF (Brown Co.).

Gladicosa pulchra Keyserling, 1877 **P** – is distributed throughout the southern United States, from Colorado to Florida, north to New Jersey. One female was collected from a sinkhole 1.1 km west of Deuchars in Hoosier National Forest, Crawford Co., in November 2015. An additional six specimens were collected from Floyd Co. (one male in August 2015), Orange Co. (one female in September 2014), Perry Co. (two males and one female in September 2015), Brown Co. (one female in September 2015), Harrison Co. (one female 25 August 2015), and Crawford Co. (one male in October 2015).

Schizocosa crassipes Walckanaer, 1837 – occurs along the East Coast, Midwest, and central portions of the United States. Two females were found at MMSF (Monroe Co.) in June 2015.

Varacosa shenandoa Chamberlin & Ivie, 1942 – occurs on the eastern and central region of the United States in addition to Quebec in Canada. One female was found at the Indiana Dunes National Lakeshore in Porter Co. in September 2015.

Nesticidae

**Eidmannella pallida* Emerton, 1875 – can be found throughout the United States and eastern Canada. One female was collected from Porter Cave, Owen Co., in September 2014. Other specimens were found in Binkley Cave (Harrison Co.), Crew 88 Cave (Orange Co.), and Guetig Cave (Washington Co.). This species was recorded from Mayfield's Cave (Monroe Co.) and Porter Cave (Owen Co.) by Gertsch (1984).

Oecobiidae

Oecobius cellariorum Dugès, 1836 **P** – is a non-native species that occurs throughout the southern United States, from Arizona to Maryland. Two immature specimens were collected in a greenhouse in Terre Haute in Vigo Co. in August 2015.

Phrurolithidae

Phrurolithus singulus Gertsch, 1941 – has previously been recorded from Virginia, Tennessee, Alabama, Georgia, and Florida. One male was found from Mill Creek Sink at Hoosier National Forest, Crawford Co., in September 2015. This is a northwestern range extension and this record is approximately 382 km NW of its closest previously recorded location in Tennessee.

Scotinella fratrella Gertsch, 1935 **P** – is only known from three states (Texas, Illinois, and Ohio) and one Canadian province (Ontario). One male specimen within Parker's (1969) collection at PERC identified as "*Phrurolithus* sp.?" was examined and re-identified as this species. This specimen was collected by R.W. Meyer from Santa Claus, Spencer Co., in June 1968.

Scotinella redempta Gertsch, 1941 **P** – occurs in the southeastern United States and north through Virginia, Ohio, and into Ontario. One male and six females were captured at GENP (Johnson Co.) and YSF (Brown Co.) February–May 2015. Multiple specimens were also found in Pikes Peak Pit in Washington Co.

Pisauridae

Dolomedes albineus Hentz, 1845 – is distributed in the southeastern United States from Texas to Florida and north to Virginia and Missouri. Fourteen specimens were collected from a variety of locations. A male was collected from MMSF (Monroe Co.) in June 2015. Five females were found in West Terre Haute in Vigo Co. in June 2015. Two females and a male were found in Vigo Co. in June and July 2015. One juvenile was located in Floyd Co. in August 2015 and another in Perry Co. in September 2015. Finally, one juvenile was found in Putnam Co. and two more in Clay Co. in October 2015.

Pisaurina dubia Hentz, 1847 **P** – occurs largely in the eastern United States, from Kansas to Massachusetts and south to Florida. Two males and one female were found near an urban structure in West Terre Haute, Vigo Co., in May 2015.

Salticidae

Colonus puerperus Hentz, 1846 – can be found in the eastern and central United States. Four males were found in September 2015 from MMSF (1; Monroe Co.), YSF (2; Brown Co.), and Eagle Creek Park (1; Marion Co.).

Neon nelli Peckham & Peckham, 1888 **P** – is distributed across Canada and along the Eastern Seaboard and in the central United States. One female was found at GENP (Johnson Co.) in April 2015.

Loxosceles rufescens Dufour, 1820 **P** – is a non-native species that occurs throughout the United States, from Pennsylvania to California. Twenty-one specimens (11 males, 9 females, 1 immature) were found in a building in Terre Haute, Vigo Co. during 2005–2015.

Theridiidae

Dipoena nigra Emerton, 1882 **P** – is distributed throughout the United States and Canada. One female was found at YSF (Brown Co.) in May 2015.

Enoplognatha caricis Fickert, 1876 **P** – occurs largely in the northern United States and Canada, though specimens have been found in Texas and Colorado. One female was found at MMSF (Monroe Co.) in June 2014.

Parasteatoda tabulata Levi, 1980 – is a non-native species that is largely restricted to eastern Canada, but has been found in Illinois. One female was found at MMSF (Monroe Co.) in June 2014.

Phylloneta pictipes Keyserling, 1884 **P** – is known to occur in the southeastern United States, Illinois, and Ohio. One female was found at MMSF (Monroe Co.) in September 2015.

Robertus frontatus Banks, 1892 **P** – is largely restricted to the East Coast except for a record from Manitoba, Canada. Two males and three females were found from MMSF (Monroe Co.) in September and October 2015. We also located two males and seven females in YSF (Brown Co.) in March, April, June, and August 2015.

Spintharus flavidus Hentz, 1850 **P** – occurs in the southeastern United States, from Texas to Florida, north to New York. One female was found at MMSF (Monroe Co.) in September 2015.

Theridion cheimatos Gertsch & Archer, 1942 – occurs on the East Coast from Ohio to

Florida. This record is a western range extension. The closest previously documented location of this species is in Ohio, 326 km ENE of our record. One male was found from YSF (Brown Co.) in September 2015.

Thymoites marxi Crosby, 1906 – occurs in the southeastern United States, from Texas to Florida, north to New York. Two females were found at MMSF (Monroe Co.) in September 2015 and one female at GENP (Johnson Co.) in March 2016. These records represent a NW range extensions for the species. The closest previously documented location of this species is in Tennessee, 329 km S of our record.

DISCUSSION

Although this study slightly extends the range of *Epiceraticelus fluvialis* and *Tapinocyba emertoni* (west), *Phrurolithus singulus* and *Islandiana cavealis* (north), and *Paracornicularia bicapillata* and *Ummidia tuobita* (east), most of these records are "gap-filling" ones whereby Indiana lacks records of these species' existence although surrounding states do hold such records. For example, *Araneus cingulatus*, *Agelenopsis emertoni*, *Cyclosa conica*, *Drassyllus fallens*, *Dipoena nigra*, *Enoplognatha caricis*, *Microneta viaria, Neon nelli*, *Tenuiphantes sabulosus*, *Walckenaeria brevicornis*, and *Walckenaeria directa* are known from every Midwestern state surrounding Indiana (MI, WI, IL, and OH). An additional piece of evidence for this point is that Sierwald et al. (2005) predicted that 203 new species distribution records would eventually be found in Indiana. Approximately 55% (39/71) of the species we located within the state were on this list of predicted species. These patterns suggest that the lack of records for these species in Indiana is more an artifact of insufficient sampling rather than differences in habitat.

Some of the new records are associated with cave and sinkhole habitats in the karst regions of southern Indiana (*E. pallida*, *I. cavealis,* and *O. beattyi*), where Lewis (2012) reported sampling over 600 caves as well as other karst habitats. In North America, the erigonine (a subfamily of Linyphiidae) linyphiids are well-established in the cooler, northerly latitudes. It is thus not surprising that these spiders would constitute an important part of terrestrial cave communities in Indiana. The troglobite (obligate cave inhabitant) *Phanetta subterranea* is nearly ubiquitous in Indiana caves and *Porrhomma cavernicola* is equally widespread, but more sporadic in occurrence. New records for other troglobitic linyphiids in Indiana have been slow to accumulate as the spiders are difficult to find, but persistence in sampling has resulted in the discovery of a number of rare species. *Oreonitides beattyi* was discovered in caves in the Hoosier National Forest (Lewis et al. 2002) and Big Oaks National Wildlife Refuge (Lewis & Rafail 2002; Paquin et al. 2009). Although rare and seemingly patchy in distribution, *O. beattyi* has proved to be relatively widespread in the east-central United States. *Islandiana cavealis* has been found in Indiana only in Stygian River Cave, despite searching in nearly 200 caves in the Blue River area (Lewis 1998). Likewise, in Indiana *Bathyphantes weyeri* has only been found in Guetig Hole, a one-room cave in Washington County (Lewis 1998). This species is cave-limited in a range spanning Arkansas to Virginia (Ivie 1969), but has been reported from surface habitats in eastern Canada (Paquin et al. 2010) and Illinois (Sierwald et al. 2005).

In contrast to the cryophilic erigonines, the presence of another assemblage of spiders is emerging with sampling in Indiana cave entrances and sinkhole floors. These habitats are apparently sufficiently buffered from the extremes of the surface climate in Indiana to provide acceptable habitat for spiders otherwise known from more southerly localities (e.g., *P. singulus*). Unlike the troglobitic linyphiids that are able to thrive in the food-poor environments of deep cave habitats, none of this assemblage of species is particularly cave-adapted and in Indiana these spiders occur in sheltered sinkhole floors and cave entrances, but not deep into caves. In this way, these spiders profit from the sheltered environments while remaining in habitats where prey is abundant.

About half of the species (~ 44%) mentioned in this study were discovered to exist in Indiana after only 14 months of sampling two locations (MMSF and YSF). While this may be because arachnology in Indiana is a fairly nascent field, the characteristics of several species in our list may have contributed to our knowledge of their recent local existence. For instance, many of the spiders on our list are tiny (< 2 mm), rarely seen, and difficult to identify, even when proper keys are available. For example, the first definitive key to North American linyphiid genera by Draney & Buckle (2005) opened the door to the identification of many smaller spiders in the US. Similarly, a key to the female erigonine linyphiids still does not exist (but see Sandlin 2015).

Sierwald et al. (2005) predicted the occurrence of 203 more spider species in Indiana than were known at the time. In this study, we discovered only 39 of those 203 species (19%), indicating that the list of Indiana spiders is far from complete. Moreover, our discovery of 32 spider species that were not even predicted to occur in the state further suggests that there is much more spider diversity present in Indiana than is thought to exist. Our hope is that more extraordinary discoveries about spider diversity will emerge with greater sampling of the diverse array of Indiana habitats and an increase in taxonomic clarity.

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