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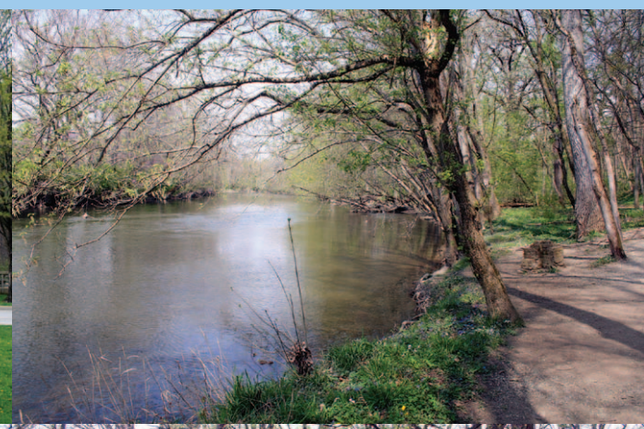

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Cover: Mounds State Park received its named because it contains some of the finest examples of earthwork and mound building in the state of Indiana. These structures were built around 150 B.C. by the Adena-Hopewell people primarily for religious ceremonies. The park, located just to the east of Anderson, Indiana, is one of the finest sites of habitat diversity and species richness in east-central Indiana. The Nature Center [top left] is located at the main entrance on the east side of the park. The White River flows along the entire western border of the park [top right]. This view of the river faces north, thus the park is on the right side of the picture. Although the park features 10 unique earthworks, only two are featured. The largest earthwork and mound is the Great Mound [bottom left]. This picture is a view down the entrance path. The Great Mound is a circular earth enclosure with an internal ditch [seen in the photograph] and measure 120 m from bank to bank. The embankment is 2.7 m high and 19 m wide at its base. The internal ditch is 3.2 m deep and 18 m across at its top. The central platform [center left of the photograph] is 42 m across. Although considerable smaller, Circle Mound [bottom right] is an excellent example of mound building. [Photographs by Don Ruch]

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ADDITIONS TO THE FLORA OF MOUNDS STATE PARK AND PRESERVE, MADISON COUNTY, INDIANA

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ABSTRACT. Rothrock et al. (1993) documented 455 species of vascular plants at Mounds State Park and Preserve, Madison County, Indiana. During the 2009 growing season, an additional floristic survey was conducted, including a 16.6 hectare annex to the south end of the park. An additional 129 species of vascular plants, representing 98 genera in 46 families, were vouchered. Of these species, 94 (~73%) were native, 35 (~27%) were adventives, and 87 represent Madison County records. Combining the results of the two studies yielded 584 total species, 478 (~82%) native species, and 106 (~18%) adventive species. For native species, the Floristic Quality Index (FQI) = 96.2 and the mean Coefficient of Conservatism (C) = 4.4, whereas for all species, the FQI = 87.1 and the mean C = 3.6. The floristic quality data indicate that the conservation and richness of Mounds State Park and Preserve are of paramount importance from a regional perspective.

Keywords: County records — vascular plants, Madison County – flora, Mounds State Park – flora, Indiana – flora, mean C, floristic quality index, FQI

INTRODUCTION

Established in 1930, Mounds State Park is a 117.4 hectare (290 acres) property located on the east edge of the city of Anderson, Indiana (N 40°05′44.7″ and W 85°37′12″, entrance to the Visitor Center). The entire western side of the park is border by the White River. The park received it name because it contains some of the finest examples of earthwork and mound building in the state of Indiana. These structures were built around 150 B.C. by the Adena and Hopewell cultures primarily for religious ceremonies (Mounds State Park Brochure).

Although Mounds State Park is recognized for its botanical richness, few formal botanical studies had been conducted there prior to 1993. Most notable was the compilation of a list of wetland species sighted within the nature preserve by the Indiana Department of Natural Resources (Casebere 1990). Rothrock et al. (1993) published an inventory of the vascular flora vouchered in the park and nature preserve and documented 455 species, including 189 Madison County records. Of the 455 species, 388 (85%) were native and 67 (15%) were exotics. Since 1993, one of the authors of this paper (KT) has kept a list of species occurring in the park that have not previously been reported. Once the number of species on the list exceeded 10% of the documented species for the park and nature preserve, an inventory of additional species was deemed appropriate.

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DESCRIPTION OF THE STUDY AREA

Rothrock et al. (1993) provided a description of the physical features and topography, geology and soils, and human history of the park. They also presented a description of the plant associations within the park, including floodplain woods, wet sedge meadow (and fen), mesic ravine woods, dry to mesic upland woods, and various cultural communities, such as lawns. These associations are still present today. For community types and distribution, as well as reference points, see Figure 1 of Rothrock et al. (1993).

Since 1993, there have been a number of alterations to the landscape and purchases of new acreage within the park, and the majority

of the additional species reported in this paper are the result of these changes or additions. The most notable of the alterations to the landscape are described here. First, the nature preserve has undergone at least six prescribed burns. Both the sedge meadow/fen and the slope woods on the eastside of the preserve were burned. These burns removed organic debris from the slope woods, increased habitat, and opened the woods to additional sunlight. Second, just south of the public swimming pool, a large, well manicured field was allowed to undergo old-field succession. This field has been in succession long enough to allow many perennial species to become established. Third, in the field around the old horse barn, several large piles of soil, which were removed from various locations within the park, are stored for future use. These piles provide disturbed soil habitat and are colonized by early successional annuals and biennials.

In addition to alternations in the landscape, a tract of forested land, approximately 16.6 hectares (41 acres), was added to the southern end of the original park and extends to Rangeline Road. The habitats within this wooded area are variable. Several habitats are similar to those in the original park, including mesic ravine woods, dry to mesic upland and slope woods, and a narrow floodplain woods close to the White River. However, this new addition includes several large, well-vegetated seeps and a young successional woodland dominated by hawthorn, honeysuckle, and osage-orange. This young woodland occurs on the hillside and uplands to the east of the White River. Lastly, there is a dry hillside, old-field community along Rangeline Road.

Within the unaltered portion of the park originally surveyed by Rothrock et al. (1993), the one habitat that was under sampled was the riverbank, sandbars, and gravel bars along the White River. Many species reported here were from these sites on the river near the boat ramp. The remaining species reported here were found in the unaltered sections of the original park.

METHODS

During the 2009 growing season, approximately one foray every 7–10 days was made into the park and nature preserve. In the early season, forays were made into every major habitat type and efforts were made to cover all areas within these habitats. However, latter in the season, more time was spent in the altered habitats or the new woodland addition (see Description of the Study Area). Voucher specimens for each species were collected and initially deposited in the Ball State University Herbarium (BSUH). Upon publication, the voucher specimens from this study will be stored in the Friesner Herbarium, Butler University, with the specimens from the Rothrock et al. (1993) study. Notes on vegetation consisted of species lists with visual estimates of their distribution and abundance (see catalog of vascular plants, Appendix 1). Additionally, we noted the habitat(s) for each species collected. Nomenclature follows the USDA Plants Database (USDA 2010). The floristic quality index (FQI) for the site was determined using the program developed by the Conservation Research Institution (Wilhelm & Masters 2000) in conjunction with Rothrock (2004).

RESULTS

This floristic survey identified 129 new species of vascular plants in Mound State Park and Preserve, representing 98 genera in 46 families (Appendix 1). Of the 129 new species, 94 (~73%) were native, 35 (~27%) were adventives, and 87 were recorded for the first time in Madison County (county records). Of the native species, 17 were woody (eight trees, six shrubs, three woody vines), 56 were forbs, 18 were graminoids (eight grasses, ten sedges), and three were ferns. Of the exotic species, seven were woody (two trees, four shrubs, one woody vine), 18 were forbs, and 10 were graminoids (all grasses). For all 129 species, the floristic quality index (FQI) was 34.0 and the mean coefficient of conservatism (C_{avg}) was 3.0. For just the 93 native species, the FQI was 39.8 and the C_{avg} was 4.1. Twenty-one of the 93 native species have C values of 7 or higher, including *Carex careyana*, *Iris brevicaulis* and *Oligoneuron riddellii* with C = 9, *Amelanchier laevis*, *Aristolochia serpentaria*, *Asclepias exaltata*, *Carya laciniosa*, *Epifagus virginiana*, *Erigeron pulchellus*, *Trillium grandiflorum* and *Veronicastrum virginicum* with C = 8, and 10 species with C = 7 (Appendix 1). Of the 129 additional species, 23 were in the Asteraceae, 18 were in the Poaceae, 10 were in the Cyperaceae, and three were in the Orchidaceae (Appendix 1).

Table 1.—Floristic Quality summary resulting from the combination of results from the current study with those of Rothrock et al. (1993). C_{avg} = mean Coefficient of Conservatism, FQI = Floristic Quality Index. Total Species is native plus exotic species.

	Species Count	C_{avg}	FQI
Native species	478	4.4	96.2
Total species	584	3.6	87.1

Tungesvik (pers. comm.) reported other vascular plants occurring in the park since the Rothrock et al. (1993) inventory, but there are no permanent records of their occurrence, neither vouchered or photography, nor were they found in the current study. These include *Acorus calamus* L., *Frasera caroliniensis* Walter, *Lonicera reticulata* Raf., *Oxalis violacea* L., *Sabatia angularis* (L.) Pursh, and *Triodanis perfoliata* (L.) Nieuwl.

Lastly, although little effort was made to find the 455 species reported earlier, 441 of these species were observed. Thus, only 14 of the species reported in the 1993 study were not observed during this study.

DISCUSSION

Combining the results of the current study with those of the Rothrock et al. (1993) study yielded the floristic quality summary seen in Table 1 and the physiognomic analysis seen in Table 2. Swink & Wilhelm (1994) have suggested that land with an FQI less than 20 essentially has no significance from a natural area prospective, while areas with an FQI greater than 35 possess sufficient conservation and richness to be of profound importance from a regional perspective, and areas registering in the 50s and higher are of paramount importance. The FQI = 96.2 for the native flora (FQI = 87.1 for all species) clearly signifies the "paramount importance" of the floral natural heritage of Mounds State Park and the enclosed nature preserve.

The only other site in Indiana with an FQI for the native flora that approaches that of Mounds State Park and Preserve is Yellow Birch Ravine Nature Preserve, Crawford County [Shawnee Hills Natural Region, Crawford Upland Section] with an FQI = 90.4 (Yatskievych & Yatskievych 1987). Certainly for east-central Indiana (ECI), there are no sites with such a high FQI value. The highest quality sites for ECI include Wilbur Wright Fish and Wildlife Area, Henry County, FQI = 77.3, Ginn Woods, Delaware County, FQI =74.1, and Botany Glen, Grant County, FQI = 68.5 (Rothrock & Homoya 2005). It should be noted that the total number of species reported from a site does influence the FQI value (see Rothrock 2004, Ruch et al. 2010, Swink & Wilhelm 1994, and Taft et al. 1997). All the sites listed here have high species numbers, e.g., Mounds State Park and Preserve (478 native species/584 total species), Yellow Birch Ravine Nature Preserve (385/420), Wilbur Wright Fish

Table 2.—Physiognomic analysis resulting from the combination of results from the current study with those of Rothrock et al. (1993). A = annual, B = biennial, H = herbaceous, P = perennial, W = woody.

	Native Species Summary		Adventive Species Summary	
	Number	% of Total	Number	% of Total
# of species	478	81.8%	106	18.2%
Tree	49	8.4%	7	1.2%
Shrub	30	5.1%	14	2.4%
W-Vine	10	1.7%	2	0.3%
H-Vine	5	0.9%	0	0.0%
P-Forbs	230	39.4%	27	4.6%
B-Forbs	13	2.2%	14	2.4%
A-Forbs	45	7.7%	19	3.3%
P-Grass	25	4.3%	12	2.1%
A-Grass	5	0.9%	11	1.9%
P-Sedge	54	9.2%	0	0.0%
A-Sedge	2	0.3%	0	0.0%
Fern	10	1.7%	0	0.0%

and Wildlife Area (388/536), Ginn Woods (364/441), and Botany Glen (295/357) (Rothrock & Homoya 2005).

Swink & Wilhelm (1994) state that species inventories from sites of natural quality will attain a C_{avg} of 3.5 or higher, while those with high natural quality might be expected to have a C_{avg} of 4.5 or greater. Because the average C values in Indiana are 1.2 cohorts lower than those of the Chicago region (Rothrock & Homoya 2005), it is reasonable to anticipate that Indiana sites with high natural quality may have somewhat lower C_{avg} values than those suggested by Swink & Wilhelm (1994). Rothrock & Homoya (2005) state that the best quality reference sites in central Indiana had C_{avg} ranging from 3.8–4.1. Thus, the C_{avg} = 4.4 (Table 1) clearly denotes the high natural quality of Mounds State Park and its nature preserve. In comparison, the highest C_{avg} values for native flora reported for any site in ECI are 4.0 for Botany Glen in Grant County and 3.9 for the wetland complex on the IMI property in Henry County, Wilbur Wright Fish & Wildlife Area in Henry County, and Ginn Woods in Delaware County (Rothrock & Homoya 2005, Ruch et al. 2008, Stonehouse et al. 2005). Higher C_{avg} values are typically found for sites outside of ECI, such as Lime Lake Nature Preserve in Steuben County (6.3), Fox Lake in Steuben County (5.4), Wening-Sherrit Seep Springs in Dubois County (5.3), Plaster Creek Seep Springs in Martin County (5.2) and Perry County Limestone Glade (5.2) (Rothrock & Homoya 2005). The lower C_{avg} values for ECI are probably due to heavy anthropogenic impact through agriculture and urbanization.

As would be predicted, the high C_{avg} at Mounds State Park is due to the number of native species with $C \geq 7$, i.e., 113 (~23%) of the 478 native species. There are 56 species with C = 7, 30 species with C = 8, nine species with C = 9, and 15 species with C = 10 (Appendix 1). The species with C = 10 include *Arnoglossum plantagineum*, *Calopogon tuberosus*, *Carex bromoides*, *C. prairea*, *Deschampsia cespitosa*, *Eleocharis elliptica*, *Lobelia kalmii*, *Oligoneuron ohioense*, *Parnassia glauca*, *Ranunculus hispidus* var. *caricetorum*, *Rhynchospora capillacea*, *Saxifraga pensylvanica*, *Scleria verticillata*, *Solidago uliginosa*, and *Spiranthes lucida*.

Rothrock & Homoya (2005) state that the natural quality of a site is compromised when the adventive diversity lowers C_{avg} by more than 0.7 units. At Mounds State Park the difference between the native C_{avg} and the total C_{avg} is 0.8 units (Table 1). Plot data were not gathered in either this study or the earlier study of Rothrock et al. (1993), but based on our visual observations, the adventives species do not appear to be compromising native species except in very specific sites, such as the various cultural communities (i.e., lawns and the campground), the disturb ground near the old horse barn, the old-field in succession south of the swimming pool, the woodland edges bordering the lawns and roads, and the successional woodland at the southern end. Once into the interior of the woodland along the White River, which is most of the site, there are few adventives species. The habitats with little adventive species impact include the floodplain woods (though the recent spread of *Alliaria petiolata* is worrisome), the wet sedge meadow (and fen), mesic ravine woods, dry to mesic upland woods, and the circumneutral seeps in the recently purchased woodland.

In summary, the floristic quality data, with native FQI = 96.2 and C_{avg} = 4.4, reveal that Mounds State Park and Preserve retain noteworthy remnants of the region's natural heritage. In fact, the floristic quality data indicate that Mounds State Park and Preserve is the highest quality site reported to date in east-central Indiana and that plant diversity there is comparable to the best sites anywhere in Indiana.

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APPENDIX 1

CATALOG OF THE ADDITIONAL VASCULAR PLANTS AT MOUNDS STATE PARK AND NATURE PRESERVE

(Arranged alphabetically by family)

Listed are the 129 additional species vouchered from the park and enclosed nature preserve, and special notes on two species, e.g., *Arnoglossum atriplicifolium* and *Trillium recurvatum*, previously documented from the site (Rothrock et al. 1993). Additionally, three species, e.g., *Asclepias exaltata*, *Gentianella quinquefolia*, and *Silphium integrifolium*, which were not found during the current study but had been documented previously with photography, are included in the list. Lastly, six have been observed at various times in the park since 1993, but none were observed during this study nor have they been documented with photographs. These plants, which are excluded from this list, include *Acorus calamus* L., *Lonicera reticulata* Raf., *Oxalis violacea* L., *Sabatia angularis* (L.) Pursh, *Triodanis perfoliata* (L.) Nieuwl., and *Frasera caroliniensis* Walter.

Nomenclature follows the USDA Plants Database (USDA 2010). Each species report contains the following information: (1) current scientific name based on the USDA Plants Database; (2) current taxonomic synonyms, if appropriate; (3) common name(s), based primarily on Gleason & Cronquist (1991), Swink & Wilhelm (1994), and Yatskievych (2000); (4) typical habitat(s) within the study site; (5) a visual estimate of its relative abundance; (6) its coefficient of conservatism (C-value) for Indiana (Rothrock 2004); and (7) the Ball State University Herbarium (BSUH) number(s).

The relative abundance for species is defined as follows: rare = < 5 sites, although a species may be abundant at one or two sites; infrequent = occasional, not widespread throughout its potential habitats, but may be locally abundant at a site; common = frequent throughout its potential habitats and may be locally abundant at one or more sites; and abundant = common and numerous throughout its potential habitats.

The symbols in parentheses immediately preceding each species refer to the following: * = naturalized, non-native (exotic) species, # = Madison County record, and z = vouchered as photograph only. Species were deemed unreported for Madison County (and hence considered a county record) if they did not appear in the computer database of Keller et al. (1984), which is the same list of plants for Madison County as the one at the Indiana Natural Heritage Data Center, IDNR.). Two plants documented in this study, *Carex trichocarpa* and *Prenanthes crepidinea*, are on the Divisions of Nature Preserves, Indiana Department of Natural Resources' ''Watch List'' (Division of Nature Preserves 2007). There are 87 Madison County records.

DIVISION POLYPODIOPHYTA

Ferns

Aspleniaceae (Spleenwort Family)

(#) *Asplenium platyneuron* (L.) Britton, Sterns & Poggenb. var. *platyneuron*; Ebony Spleenwort; Woods; Infrequent; C = 3; BSUH 16701.v

Dryopteridaceae (Wood Fern Family)

(#) *Polystichum acrostichoides* (Michx.) Schott var. *acrostichoides*; Christmas Fern; Woods; Infrequent; C = 5; BSUH 16751.

Thelypteridaceae (Marsh Fern Family)

Phegopteris hexagonoptera (Michx.) Fée; SYN: *Thelypteris hexagonoptera* (Michx.) Weath.; Broad or Southern Beech Fern; Woods in the new addition; Rare; C = 7; BSUH 16712.

DIVISION MAGNOLIOPHYTA

Angiosperms

Acanthaceae (Acanthus Family)

(#) *Ruellia humilis* Nutt.; Sessile-Leaved Hairy Ruellia, Fringeleaf Wild Petunia; Dry hillside/roadside along Rangeline Road at southwestern end of property; Rare but locally common; C = 5; BSUH 16732.

Aceraceae (Maple Family)

(#) *Acer nigrum* Michx. f.; SYN: *Acer saccharum* Marsh. ssp. *nigrum* (Michx. f.) Desmarais, *Acer saccharum* Marsh. var. *nigrum* (Michx. f.) Britton; Black Maple; Floodplain forest; Common; C = 6; BSUH 16773.

(* #) *Acer platanoides* L.; Norway Maple; Woods along Mounds Road north of entrance; Common; C = 0; BSUH 16765.

Agavaceae (Century-Plant Family)

(* #) *Yucca filamentosa* L.; SYN: *Yucca smalliana* Fernald, *Y. flaccida* Haw.; Spanish Bayonet, Adam's Needle, Weak-Leaf Yucca; Dry hillside along Rangeline Road at southwestern end of property; Rare; C = 0; BSUH 16735.

Alismataceae (Water Plantain Family)

Sagittaria latifolia Willd.; Common or Broad-Leaf Arrowhead; Fen; Common; C = 3; BSUH 16801.

Annonaceae (Custard-Apple Family)

Asimina triloba (L.) Dunal; Pawpaw; Floodplain forest; Infrequent; C = 6; BSUH 16700, 16766.

Apiaceae (Carrot Family)

(* #) *Conium maculatum* L.; Poison Hemlock; Floodplain woods; Infrequent; C = 0; BSUH 16946.

Aristolochiaceae (Birthwort Family)

(#) *Aristolochia serpentaria* L.; Virginia Snakeroot; Woods; Infrequent; C = 8; BSUH 16738.

Asclepiadaceae (Milkweed Family)

(z) *Asclepias exaltata* L.; SYN: *Asclepias phytolaccoides* Pursh; Poke Milkweed; Slope woods in preserve above fen; C = 8; BSUH 16986. Special Note: Not found in this study but photographed between 1993 and 2009.

(#) *Asclepias incarnata* L. ssp. *incarnata*; Swamp Milkweed; Fen; Infrequent; C = 4; BSUH 16752.

(#) *Cynanchum laeve* (Michx.) Pers.; SYN: *Ampelamus albidus* (Nutt.) Britton, *Ampelamus laevis* (Michx.) Krings; Sandvine, Bluevine, Honeyvine; Old field; Infrequent; C = 1; BSUH 16784.

Asteraceae (Aster Family)

Arnoglossum atriplicifolium (L.) H. Rob.; SYN: *Cacalia atriplicifolia* L.; Pale Indian Plantain; Wooded ravine in preserve woods above fen; Rare but locally abundant; C = 6; BSUH 16770, 16788. Special Note: Rothrock et al. (1993) reported the following, ''A single plant was sighted (but not collected) by John Bacone and HS (Helena Starks) in May 1980 on a wooded bluff; C.C. Deam (2459) collected this species on 'dry banks of wooded ravine, south side of White River about 2 mi. N of Anderson,' 11 August 1907; not seen during our 1990–1992 survey.'' Currently, the plant is rare (one site) but locally abundant on a wooded ravine above the fen in the preserve.

(*) *Cichorium intybus* L.; Chicory; Dry hillside/roadside along Rangeline Road at southwestern end of property; Rare but locally common; C = 0; BSUH 16734.

Cirsium discolor (Muhl. ex Willd.) Spreng.; Field or Pasture Thistle; Old field; Infrequent; C = 3; BSUH 16783, 16791.

(* #) *Cirsium vulgare* (Savi) Ten.; Bull Thistle; Old field; Common; C = 0; BSUH 16744, 16754.

(#) *Erechtites hieraciifolia* (L.) Raf. ex DC. var. *hieraciifolia*; White Fireweed, American Burnweed; Old field; Abundant; C = 2; BSUH 16785, 16789.

(#) *Erigeron pulchellus* Michx. var. *pulchellus*; Robin's Plantain; Slope woods of preserve; Common; C = 8; BSUH 16714.

(#) *Eupatorium altissimum* L.; Tall Boneset; Tall Thoroughwort; Dry hillside/roadside along Rangeline Road at southwestern end of property; Rare but locally common; C = 1; BSUH 16805.

Heliopsis helianthoides (L.) Sweet var. *helianthoides*; False Sunflower, Smooth Oxeye; Floodplain forest near boat launch; Rare; C = 4; BSUH 16809.

(#) *Helianthus tuberosus* L.; Jerusalem Artichoke; Floodplain forest near boat launch; Rare; C = 2; BSUH 16811.

Lactuca biennis (Moench) Fernald; SYN: *Lactuca spicata* auct. non (Lam.) Hitchc.; Tall Blue Lettuce; Old field; Infrequent; C = 2; BSUH 16807.

(*) *Lactuca serriola* L.; *Lactuca scariola* L.; Prickly Lettuce; Old field; Infrequent; C = 0; BSUH 16781.

(#) *Oligoneuron riddellii* (Frank ex Riddell) Rydb.; SYN: *Solidago riddellii* Frank ex. Riddell; Riddell's Goldenrod; Fen; Common; C = 9; BSUH 16772.

(#) *Prenanthes crepidinea* Michx.; Great White Lettuce, Nodding Rattlesnake-Root; Floodplain forest; Common; C = 7; BSUH 16695. [Watch List; S2]

Rudbeckia triloba L. var. *triloba*; Three-Lobed Coneflower, Brown-Eyed Susan; Old field; Rare; C = 3; BSUH 16790.

(#) *Silphium integrifolium* Michx. var. *integrifolium*; Entire-Leaved Rosinweed, Wholeleaf Rosinweed; Fen; Rare; C = 7; BSUH 17030.

Silphium perfoliatum L. var. *perfoliatum*; Cup Plant; Floodplain forest near boat launch; Infrequent but locally common; C = 4; BSUH 16786.

Solidago gigantea Aiton; Late or Giant Goldenrod; Floodplain woods west of fen; Rare but locally common; C = 4; BSUH 16804, 16818.

(#) *Solidago nemoralis* Aiton var. *nemoralis*; Gray or Old-Field Goldenrod; Dry hillside/roadside along Rangeline Road at southwestern end of property; Rare but locally abundant; C = 3; BSUH 16806.

Solidago ulmifolia Muhl. ex Willd. var. *ulmifolia*; Elm-Leaved Goldenrod; Slope woods in the preserve above fen; Rare but locally common; C = 5; BSUH 16796.

(* #) *Sonchus asper* (L.) Hill; Prickly or Spiny Sow Thistle; Old field; infrequent; C = 0; BSUH 16759.

(* #) *Sonchus oleraceus* L.; Common or Store-Front Sow Thistle; Riverbank; Rare; C = 0; BSUH 16780.

(#) *Symphyotrichum firmum* (Nees) G.L. Nesom; SYN: *Aster firmus* Nees, *Symphyotrichum puniceum* (L.) A. Löve & D. Löve var. *puniceum*;

Shining Aster; Fen; Common; C = 4; BSUH 16771.

Symphyotrichum pilosum (Willd.) G.L. Nesom var. *pilosum*; SYN: *Aster pilosus* Willd.; Hairy White Old-Field Aster; Old field; Common; C = 0; BSUH 16820.

(#) *Xanthium strumarium* L. var. *glabratum* (DC.) Cronquist; SYN: *Xanthium chinense* Mill., *Xanthium strumarium* L. var. *wootonii* (Cockerell) M. Peck; Common or Rough Cocklebur; Floodplain forest and riverbank; Infrequent; C = 0; BSUH 16690.

Brassicaceae (Mustard Family)

(#) *Arabis hirsuta* (L.) Scop. var. *pycnocarpa* (M. Hopkins) Rollins; SYN: *Arabis hirsuta* (L.) Scop. var. *adpressipilis* (M. Hopkins) Rollins; Hairy Rockcress, Creamflower Rockcress; Slope woods in the new addition; Infrequent; C = 5; BSUH 16710.

(* #) *Brassica nigra* (L.) W.D.J. Koch; SYN: *Sinapis nigra* L.; Black Mustard; Riverbank and open floodplain forest; Infrequent; C = 0; BSUH 16761.

Lepidium virginicum L. var. *virginicum*; Common Peppergrass, Poor Man's Pepper, Virginia Pepperweed; Lawns and open woods near shelters; Infrequent; C = 0; BSUH 16721.

Caprifoliaceae (Honeysuckle Family)

(* #) *Lonicera morrowii* A. Gray; Morrow's Honeysuckle; Floodplain forest around fen; Common; C = 0; BSUH 16697, 16698.

Caryophyllaceae (Pink Family)

Silene virginica L. var. *virginica*; Fire Pink; Woods; Infrequent but locally common; C = 7; BSUH 16722.

Celastraceae (Staff-tree Family)

(#) *Celastrus scandens* L.; American Bittersweet; Woodland edge along Mounds Road, south of entrance; Rare; C = 2; BSUH 16731.

(#) *Euonymus atropurpureus* Jacq.; Eastern Wahoo; Floodplain forest near boat launch; Rare; C = 5; BSUH 16797.

(* #) *Euonymus fortunei* (Turcz.) Hand.-Maz. var. *radicans* (Siebold ex Miq.) Rehder; Chinese Spindle Tree, Winter Creeper; Edge of preserve along path next to river; Rare; C = 0: BSUH 16711.

Euonymus obovatus Nutt.; Running Strawberry Bush; Woods; Infrequent; C = 7; BSUH 16702.

Chenopodiaceae (Goosefoot Family)

(* #) *Chenopodium album* L. var. *album*; Lamb's Quarters, Pigweed; Disturbed soil near pool; Rare but locally common; C = 0; BSUH 16939.

Convolvulaceae (Morning-glory Family)

(#) *Calystegia sepium* (L.) R. Br.; Typical Hedge-Bindweed; Old field; Infrequent but locally common; C = 1; BSUH 16782.

Cornaceae (Dogwood Family)

(#) *Cornus obliqua* Raf.; SYN: *Cornus amomum* Mill. var. *schuetzeana* (C.A. Mey.) Rickett; Gray Dogwood; Knob-Styled Dogwood, Silky Dogwood; Floodplain woods within preserve; Rare; C = 5; BSUH 16764.

Cyperaceae (Sedge)

(#) *Carex aggregata* Mack.; SYN: *Carex sparganioides* Muhl. ex Willd. var. *aggregata* (Mack.) Gleason; Smooth Clustered Sedge, Glomerate Sedge; Moist unmowed woodland edge near Nature Center; Rare; C = 2; BSUH 16673.

(#) *Carex careyana* Torr. ex Dewey; Carey's Wood Sedge; Slope woods; Rare, one small colony; C = 9; BSUH 16705.

(#) *Carex grayi* Carey; Common Bur Sedge; Floodplain forest near the boat ramp; Rare; C = 5; BSUH 16988.

(#) *Carex lupulina* Muhl. ex Willd.; Common Hop Sedge; Floodplain forest within preserve; Infrequent but locally common; C = 4; BSUH 16762.

Carex tribuloides Wahlenb var. *tribuloides*; Awl-Fruited Oval Sedge, Blunt Broom Sedge; Floodplain woods with preserve; Common; C = 5; BSUH 16669.

(#) *Carex trichocarpa* Muhl. ex Willd.; Hairy-Fruited Lake Sedge; Woodland seep near river; Rare but locally common; C = 4; BSUH 16670. [Watch List; S3]

(#) *Cyperus esculentus* L. var. *leptostachyus* Boeckeler; Yellow or Field Nut-Sedge; Disturbed soil in old field near former horse barn; Rare; C = 0; BSUH 16674.

(#) *Cyperus odoratus* L.; Fragrant Flatsedge; Riverbank and sand/rock bar near boat launch; Rare; C = 1; BSUH 16675.

(#) *Cyperus strigosus* L.; False Nutsedge, Strawed-Colored Nutsedge; Riverbank and sand/rock bar near boat launch; Infrequent; C = 0; BSUH 16676.

Eleocharis erythropoda Steud.; Red-Rooted Spike Rush; Riverbank and sand/rock bar near boat launch; Rare but locally common; C = 2; BSUH 16668.

Elaeagnaceae (Oleaster Family)

(* #) *Elaeagnus umbellata* Thunb. var. *parvifolia* (Wall. ex Royle) C.K. Schneid.; Autumn Olive; Edge of woods, woods along Mounds Road north of entrance; Rare; C = 0; BSUH 16942.

Euphorbiaceae (Spurge Family)

(#) *Chamaesyce maculata* (L.) Small; SYN: *Euphorbia maculata* L., *Euphorbia supina* Raf.; Creeping

Spurge; Spotted Sandmat; Roadside near pool; Infrequent; C = 0; BSUH 16757.

(#) *Euphorbia corollata* L.; Flowering Spurge; Slope woods in preserve at edge of fen; Rare; C = 4; BSUH 16755, 16800.

Euphorbia dentata Michx. var. *dentata*; Toothed Spurge; Old field near former horse barn; Rare; C = 0; BSUH 16793.

Fabaceae (Pea or Bean Family)

(* #) *Securigera varia* (L.) Lassen; SYN: *Coronilla varia* L.; Crown Vetch; Disturbed ground and old field; Infrequent; C = 0; BSUH 16794.

(* #) *Trifolium hybridum* L.; Alsike Clover; Open woods along river near boat launch; Infrequent; C = 0; BSUH 16948.

(* #) *Trifolium pratense* L.; Red Clover; Old field; Rare; C = 0; BSUH 16940.

(#) *Wisteria frutescens* (L.) Poir.; American Wisteria; Dry hillside/roadside along Rangeline Road at southwestern end of property; Rare; C = 4; BSUH 16733.

Fagaceae (Beech Family)

(#) *Quercus imbricaria* Michx.; Shingle Oak; Woods along Mounds Road north of entrance; Rare (several trees); C = 3; BSUH 16769.

(#) *Quercus palustris* Münchh.; Pin Oak; Edge of woods, woods along Mounds Road north of entrance; Rare (one tree); C = 3; BSUH 16943.

Quercus shumardii Buckley; Shumard Oak; Woods in the new addition; Infrequent; C = 7; BSUH 16728.

Fumariaceae (Fumitory Family)

(#) *Dicentra canadensis* (Goldie) Walp.; Squirrel Corn; Woods in the new addition; Rare (one colony); C = 7; BSUH 16696.

(#) *Dicentra cucullaria* (L.) Bernh.; Dutchman's Breeches; Woods in the new addition and on the southern ravine in the original acreage; Common; C = 6; BSUH 16694.

Gentianaceae (Gentian Family)

(z) *Gentianella quinquefolia* (L.) Small ssp. *occidentalis* (A. Gray) J.M. Gille; SYN: *Gentiana quinquefolia* L.; Stiff Gentian, Agueweed; Border between slope woods and fen in preserve; Rare; C = 5; BSUH 16985. Special Note: Not found in this study but photographed between 1993 and 2009.

Hamamelidaceae (Witch Hazel Family)

(#) *Hamamelis virginiana* L.; American Witch Hazel; Steep slope woods between preserve and boat launch; Infrequent; C = 5; BSUH 16813.

Iridaceae (Iris Family)

(#) *Iris brevicaulis* Raf.; SYN: *Iris brevipes* Small; Short-Stemmed Iris, Lamance Iris, Zigzag Iris; Floodplain woods in preserve; Rare but locally common; C = 9; BSUH 16716.

(#) *Sisyrinchium albidum* Raf.; Common or White Blue-Eyed-Grass; Steep slope woods; Rare; C = 4; BSUH 16709.

Juglandaceae (Walnut Family)

(#) *Carya laciniosa* (Michx. f.) G. Don; Shellbark Hickory; Floodplain woods; Rare; C = 8; BSUH 16936.

Lamiaceae (Mint Family)

(* #) *Chaiturus marrubiastrum* (L.) Rchb.; SYN: *Leonurus marrubiastrum* L.; Horehound Motherwort, Lion's Tail; Floodplain woods; Rare; C = 0; BSUH 16810.

Scutellaria incana Biehler var. *incana*; Downy or Hoary Skullcap; Floodplain woods in preserve; Common; C = 4; BSUH 16749.

Scutellaria lateriflora L. var. *lateriflora*; Mad-Dog or Blue Skullcap; Slope woods; Infrequent but locally common; C = 4; BSUH 16774.

Liliaceae (Lily Family)

(#) *Lilium michiganense* Farw.; Michigan Lily; Floodplain forest; Infrequent; C = 5; BSUH 16703, 16746.

(#) *Maianthemum stellatum* (L.) Link; SYN: *Smilacina stellata* (L.) Desf.; Starry Solomon's Plume, starry false lily of the valley; Around a large seep in the new addition; Rare but locally abundant; C = 6; BSUH 16715.

Polygonatum biflorum (Walter) Elliott var. *commutatum* (Schult. & Schult. f.) Morong; SYN: *Polygonatum commutatum* (Schult. & Schult. f.) A. Dietr.; Great Smooth Solomon's Seal; Woods along Mounds Road north of entrance; Infrequent; C = 4; BSUH 16938.

(#) *Trillium grandiflorum* (Michx.) Salisb.; Large-Flowered White Trillium; Woods near Nature Center; Rare; C = 8; BSUH 16707.

Trillium recurvatum Beck; Prairie Trillium (Yellow Form), Bloody Butcher; C = 4; BSUH 16706. Special Note: Rothrock et al. (1993) reported this species. We are reporting the yellow form of the species, which is uncommon in woods.

Trillium sessile L.; Sessile Trillium, Toadshade; C = 4; Woods in the new addition; Common; BSUH 16693.

Malvaceae (Mallow Family)

(*) *Malva neglecta* Wallr.; Common Mallow, Cheeses; Lawn near Nature Center; Rare; C = 0; BSUH 16737.

Moraceae (Mulberry Family)

Morus rubra L. var. *rubra*; Red Mulberry; Woods in the new addition; Infrequent; C = 4; BSUH 16727.

Orchidaceae (Orchid Family)

Aplectrum hyemale (Muhl. ex Willd.) Torr.; Putty Root, Adam-and-Eve; Slope woods near river; Rare; C = 7; BSUH 16692.

(#) *Galearis spectabilis* (L.) Raf.; SYN: (*Orchis spectabilis* L., *Galeorchis spectabilis* (L.) Rydb.; Showy Orchis, Showy Orchid; Woods near creek; Rare; C = 7; BSUH 16713.

(#) *Liparis liliifolia* (L.) Rich. ex Ker Gawl.; Purple Twayblade, Brown Widelip Orchid; Dry hilltop woods near river; Rare; C = 3; BSUH 16725.

Orobanchaceae (Broom-Rape Family)

Epifagus virginiana (L.) W. Barton; SYN: *Leptamnium virginianum* (L.) Raf.; Beech-Drops; Woods; Frequent; C = 8; BSUH 16814, 16815.

Poaceae (Grass Family)

(* #) *Agrostis gigantea* Roth; SYN: *Agrostis alba* auct. non L.; Redtop; Old field; Infrequent but locally abundant; C = 0; BSUH 16743.

(*) *Bromus arvensis* L.; SYN: *Bromus japonicus* Thunb.; Field Brome, Japanese Chess; Dry hillside/roadside along Rangeline Road at southwestern end of property; Infrequent; C = 0; BSUH 16729.

(*) *Bromus racemosus* L.; SYN: *Bromus commutatus* Schrad.; Hairy Chess, Bald Brome; Disturbed soil in old field near former horse barn; Rare but locally common; C = 0; BSUH 16756.

Dichanthelium latifolium (L.) Gould & C.A. Clark; SYN: *Panicum latifolium* L.; Broad-Leaved Panic Grass, Broad-Leave Rosette Grass; Slope woods of the preserve; Common at this site; C = 6; BSUH 16679.

(*) *Digitaria sanguinalis* (L.) Scop.; Hairy or Northern Crab-Grass; Disturbed ground near former horse barn; Infrequent; C = 0; BSUH 16755.

(#) *Echinochloa muricata* (P. Beauv.) Fernald var. *muricata*; Rough Barnyard Grass; Sandy/gravel sandbar near boat launch; Rare; C = 1; BSUH 16686.

(#) *Eragrostis hypnoides* (Lam.) BSP.; Creeping or Teal Love Grass; Muddy areas and sandbars of the White River; Infrequent but locally common; C = 3; BSUH 16684, 16685.

(* #) *Eragrostis minor* Host; SYN: *Eragrostis poaeoides* P. Beauv. ex Roem. & Schult.; Low or Little Love-Grass; Disturbed soil in old field near former horse barn; Rare; C = 0; BSUH 16681.

Eragrostis pectinacea (Michx.) Nees ex Steud.; Small, Tufted or Carolina Lovegrass; Disturbed soil in old field near former horse barn; Infrequent; C = 0; BSUH 16682.

(*) *Hordeum jubatum* L.; Foxtail Barley; Dry hillside/roadside along Rangeline Road at southwestern end of property; Rare; C = 0; BSUH 16730.

Leersia oryzoides (L.) Sw.; Rice Cut-Grass; Floodplain woods near boat launch; Rare; C = 2; BSUH 16817.

(#) *Muhlenbergia tenuiflora* (Willd.) BSP.; Slender Satin Grass, Slim-Flower Muhly; Slope woods of preserve; Abundant; C = 7; BSUH 16937.

(#) *Panicum dichotomiflorum* Michx. var. *dichotomiflorum*; Knee Grass, Fall Panic Grass; Floodplain woods along riverbank in open areas; Common; C = 0; BSUH 16819.

(#) *Panicum philadelphicum* Benth. ex Trin.; Philadelphia Panic Grass; Floodplain woods along path and riverbank in open areas; Common; C = 4; BSUH 16680.

(*) *Poa compressa* L.; Canada Bluegrass; Woods; Abundant; C = 0; BSUH 16720.

(* #) *Poa trivialis* L.; Rough Bluegrass; Floodplain woods in new addition; Common; C = 0; BSUH 16677.

(* #) *Schedonorus phoenix* (Scop.) Holub; SYN: *Festuca arundinacea* Schreb., *Festuca elatior* L. ssp. *arundinacea* (Schreb.) Hack., *Lolium arundinaceum* (Schreb.) S.J. Darbyshire, *Schedonorus arundinaceus* (Schreb.) Dumort., nom. illeg.; Tall Fescue; Old fields; Common and locally abundant; C = 0; BSUH 16726.

(* #) *Setaria viridis* (L.) P. Beauv. var. *viridis*; Green Foxtail, Green Bristle-Grass; Disturbed soil in old field near former horse barn; Infrequent; C = 0; BSHU 16792.

Polygonaceae (Smartweed Family)

(#) *Polygonum lapathifolium* L. var. *lapthifolium*; SYN: *Persicaria lapathifolia* (L.) Gray; Dock-Leaved Smartweed, Curly-Top Knotweed, Heart's-Ease; Floodplain forest along river; Infrequent; C = 0; BSUH 16799.

Primulaceae (Primrose Family)

(#) *Samolus valerandi* L. ssp. *parviflorus* (Raf.) Hultén; SYN: *Samolus floribundus* Kunth, *Samolus parviflorus* Raf.; Water Pimpernel, Brookweed, Seaside Brookweed; Floodplain woods around boat launch; Infrequent; C = 5; BSUH 16760.

Ranunculaceae (Buttercup Family)

(#) *Ranunculus sceleratus* L. var. *sceleratus*; Cursed Buttercup, Cursed Crowfoot; Sandy riverbank; Rare; C = 3; BSUH 16808.

Rosaceae (Rose Family)

(#) *Agrimonia gryposepala* Wallr.; Common or Tall Agrimony; Woods; Infrequent; C = 2; BSUH 16750.

(#) *Amelanchier laevis* Wiegand; SYN: *Amelanchier arborea* (Michx. f.) Fernald ssp. *laevis* (Wiegand) S. McKay ex Landry; Smooth or Allegheny Serviceberry; Steep slope woods next to river; Rare; C = 8; BSUH 16691.

(* #) *Duchesnea indica* (Andrews) Focke; SYN: *Fragaria indica* Andrews; Indian or Mock Strawberry; Roadside and fields; Common; C = 0; BSUH 16947.

(* #) *Prunus cerasus* L.; Sour Cherry; Edge of woods, woods along Mounds Road, north of entrance; Rare (two trees); Woodland edge, woods along Mounds Road north of entrance; Rare; C = 0; BSUH 16945.

(* #) *Rhodotypos scandens* (Thunb.) Makino; SYN: *Rhodotypos tetrapetalus* (Siebold) Makino; Jetbead; Woods along Mounds Road north of entrance; Rare; C = 0; BSUH 16768. Special Note: this species was incorrectly reported as *Philadelphus inodorus* L. in the earlier inventory (Rothrock et al. 1993).

Rubiaceae (Madder Family)

(#) *Cephalanthus occidentalis* L.; Common Buttonbush; Swamp woods between fen and river; Rare; C = 5; BSUH 16767.

Salicaceae (Willow Family)

(#) *Salix interior* Rowlee; SYN: *Salix exigua* Nutt. ssp. *interior* (Rowlee) Cronquist; Sandbar Willow; Riverbank and sandy shore; Rare; C = 1; BSUH 16798.

Santalaceae (Sandalwood Family)

(#) *Comandra umbellata* (L.) Nutt. ssp. *umbellata*; Bastard Toadflax; Slope woods in preserve above fen; Rare but locally common; C = 7; BSUH 16719.

Scrophulariaceae (Figwort Family)

(*) *Chaenorrhinum minus* (L.) Lange; SYN: *Antirrhinum minus* L.; Small or Dwarf Snapdragon, Lesser Toadflax; Woodland edge along Mounds Road; Common; C = 0; BSUH 16736.

Mimulus alatus Aiton; Winged or Sharpwing Monkey-Flower; Floodplain forest and riverbank; Infrequent; C = 4; BSUH 16776.

(* #) *Verbascum thapsus* L.; Common or Woolly Mullein; Disturbed ground near former horse barn; Rare; C = 0; BSUH 16944.

(*) *Veronica arvensis* L.; Corn Speedwell; Open woods and lawns near shelters; Infrequent; Lawns near shelters; Infrequent; C = 0; BSUH 16708.

(#) *Veronicastrum virginicum* (L.) Farw.; SYN: *Veronica virginica* L., *Leptandra virginica* (L.) Nutt.; Culver's-Root; Eastern edge of fen; Rare; C = 8; BSUH 16775, 16987.

Smilacaceae (Catbrier Family)

(#) *Smilax tamnoides* L.; SYN: *Smilax hispida* Muhl. ex. Torr., *Smilax tamnoides* L. var. *hispida* (Muhl. ex Torr.) Fernald; Bristly or Hispid Greenbrier; Woods; Common; C = 3; BSUH 16699. Special Note: this species was incorrectly reported as *Smilax rotundifolia* L. in the earlier inventory (Rothrock et al. 1993).

Solanaceae (Nightshade Family)

Physalis longifolia Nutt. var. *subglabrata* (Mack. & Bush) Cronquist; SYN: *Physalis subglabrata* Mack. & Bush; Long-Leaved Ground Cherry; Floodplain forest along the river; Infrequent; C = 0; BSUH 16742.

(#) *Solanum carolinense* L. var. *carolinense*; Carolina Horse Nettle, Carolina Poppy; Old Fields and campground; Infrequent; C = 0; BSUH 16745.

(* #) *Solanum dulcamara* L. var. *dulcamara*; Bittersweet Nightshade, Climbing Nightshade; Edge of woods, woods along Mounds Road, north of entrance; Rare (one site); C = 0; BSUH 16941.

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FLORISTIC QUALITY ASSESSMENT ALONG AN OLD-FIELD CHRONOSEQUENCE

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ABSTRACT. Floristic Quality Assessment (FQA), a protocol for quantifying plant community quality relative to one that retains remnant natural condition, has been successfully applied to a suite of natural community types and ecological restoration projects. This study, performed in east central Indiana, examined floristic quality in a natural old-field succession chronosequence. Sites ranged in age from 1 to 50 years. Differences in species richness, mean conservatism, and floristic quality index were assessed at both transect and quadrat level. As hypothesized, richness of non-native species decreased while all other metrics increased with post-disturbance time. The mean conservatism for native species (at both transect and quadrat level) was ca. 2–3 for sites over 30 years of age. Floristic quality index for native species ranged from ca. 9–15 (transect level) and 4–7 (quadrat level). Given FQA expectations in the literature, 50 years of old-field succession was insufficient for mean conservatism or the floristic quality index to even reach levels associated with a degraded remnant natural community.

Keywords: Floristic Quality Assessment, old-field, ecological succession, mean C, FQI, vegetation monitoring

Floristic Quality Assessment (FQA), developed by Swink & Wilhem (1994), is a protocol used for quantifying the ecological condition of plant communities, i.e., the degree to which a community retains the species composition and richness characteristic of the pre-European settlement landscape. Uses for FQA include identifying areas of high natural value (Young 1994), monitoring change of remnant natural communities (Bacone et al. 2007), and monitoring habitat restorations (McIndoe et al. 2008).

In order to implement FQA, each species known to occur in a regional flora must be assigned a coefficient of conservatism or C value (Swink & Wilhelm 1994; Rothrock 2004). Conservatism is the likelihood that an individual species comes from a habitat with little modification by human activity from pre-European settlement condition. A species with a high fidelity to undisturbed presettlement conditions is assigned the maximum ranking of ten. A species that tolerates much disturbance and may, in fact, thrive under human disturbance would be ranked at zero. Conservatism values should be calibrated by state or region and are available for Indiana (Rothrock 2004), the location of this study.

Based upon the species present within a habitat, several basic metrics may be calculated, namely mean C (MC) and floristic quality index (FQI). Typically MC ranges from 3.5–5 or higher in sites that retain remnant natural quality (Swink & Wilhelm 1994; Rothrock 2004). FQI is a function of MC and species richness. As a result, its interpretation is dependent upon size of area being evaluated and habitat diversity at a site. Small, high quality remnant communities are likely to have FQI of 30 or higher, while a large site with a mosaic of communities may have an FQI of over 60 (Rothrock & Homoya 2005).

In most studies FQA has proven efficacious in assessing plant community quality relative to gradients of human impact. FQA has been applied to wetlands (Fennessy et al. 1998; Lopez & Fennessy 2002; Mushet et al. 2002; Matthews 2003; Herman 2005), woodlands (Francis et al. 2000), grasslands (Bowles & Jones 2006; Jog et al. 2006), and lakes (Alix & Scribailo 2006; Bourdaghs et al. 2006). In dry tallgrass prairie, FQA responded to a disturbance gradient but with less sensitivity than alternative metrics (Bowles & Jones 2006). In

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some studies the concepts implicit to FQA have been supported but modifications to the standard FQA protocol have been recommended (e.g., Alix & Scribailo 2006; Bourdaghs et al. 2006). Since its development for Indiana, the regionalized database of C values has been tested for nature preserve checklists (Rothrock & Homoya 2005) and with a longitudinal dataset from a tallgrass prairie restoration (McIndoe et al. 2008). Additional examples validating the performance of FQA both in Indiana and elsewhere are needed.

The 145-acre (59-hectare) Taylor University Arboretum in east central Indiana affords an excellent opportunity to apply FQA protocol to a series of sites in stages of old-field succession ranging from 1 year to approximately 50 years. Details of ecological succession vary from one geographic region to the next due to individualistic life histories of species in varying climates and competitive environments (Walker & Chapin 1987). Nonetheless, a general pattern emerges (Keever 1983; Vankat & Snyder 1991). In the Midwestern region, one finds that annual and biennial species (e.g., *Ambrosia artemisiifolia, Setaria* spp. and *Erigeron annuus*) dominate during the first two years after release from cultivation, perennials (e.g., *Symphyotrichum pilosum* and *Solidago altissima*) by year five, and woody plants (e.g., *Rubus* spp., *Rosa* spp., *Fraxinus americana,* and *Prunus serotina*) after several decades. Given the concept of conservatism and the expected sequence of plant species, we hypothesize that FQA metrics, MC and FQI, will increase with years since cultivation. The results of this study will also provide performance expectations for FQA metrics that can serve as benchmarks for other monitoring studies.

METHODS

Study sites.—The study sites were located west of the central Taylor University campus in Upland, Grant County, Indiana (T23N, R9E, Sec. 9; N40° 27.5′ W85° 30.7′; Fig. 1). Plots of 1–5 years post-disturbance were created by spring rototilling to a depth of ca. 12 cm of an area immediately west of the Randall Environmental Center over a 5-year cycle. In 1980 two fields, approximately 20 acres (8 hectares) in size, were allowed to go fallow after a history of cultivation in row crops, rotating among corn, soybeans, and winter wheat. The north field experienced natural recovery over a 27-year period, when the sampling was performed for this study. A portion of the south field experienced a single deep tilling in 1990 followed by 17 years of natural recovery before sampling. Two additional sites (located in the Arboretum Addition) were chosen. These support an abundance of woody species characteristic of early successional stages. The species include *Carya ovata, Cornus drummondii, Crataegus mollis, Fraxinus americana, Prunus serotina, Elaeagnus umbellata,* and *Rosa multiflora.* Based upon estimated counts of annual growth rings from selected woody species, the ranges of post-disturbance age were estimated at 35–40 years for one site and 50 years for the second.

Sampling and data analysis.—Sampling for species and aerial cover was performed during June 2006 (old field plots) and 2007 (woody plots). Transects consisted of 20 quadrats. Herbaceous species were sampled with 0.25 m^2 quadrats at 5 m intervals. Woody plants (shrubs above 1.5 m and trees greater than 2.5 cm dbh) were sampled from contiguous 5×5 m quadrats. A total of 21 transects were sampled: 1 for each of the first five year plots; 2 for the 17-year site; 10 for the 27-year sites; 3 for the 35–40-year site; and 2 for the 50-year site. The year-4 transect was deleted since its proximity to a prairie planting added atypical species into the old-field succession sequence. Ten transects were sampled in the 27-years sites since site size allowed an assessment of variation due to factors such as proximity to potential seed sources. Nomenclature and coefficients of conservatism follow Rothrock (2004). Data analysis was performed using *Floristic Quality Assessment Computer Programs*, Version 1.0 (Wilhelm & Masters 2000). The program generates the following metrics: species richness, MC, and FQI. These are computed with and without non-native species and at both transect as well as quadrat levels. For transect level metrics, MC and related metrics are based upon the roster of species observed along the entire transect. For quadrat level analyses, metrics are first calculated for each quadrat independently, then averaged. Thus, in quadrat level analysis, but not in transect level analysis, species frequency is important. Linear regression analyses were performed in Excel®.

RESULTS

Community descriptions.—The pattern of community change and the species encountered across the 50-year chronosequence were as expected. During Years 1–3 of the chronosequence the

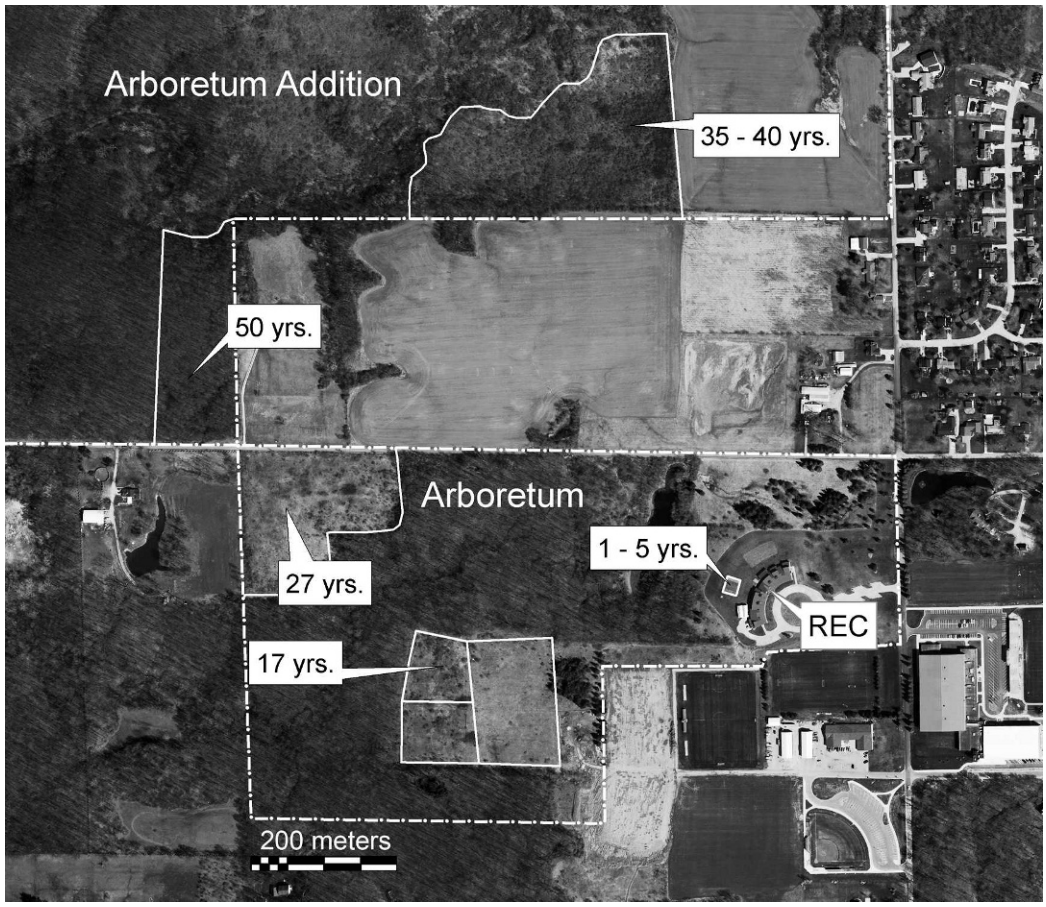
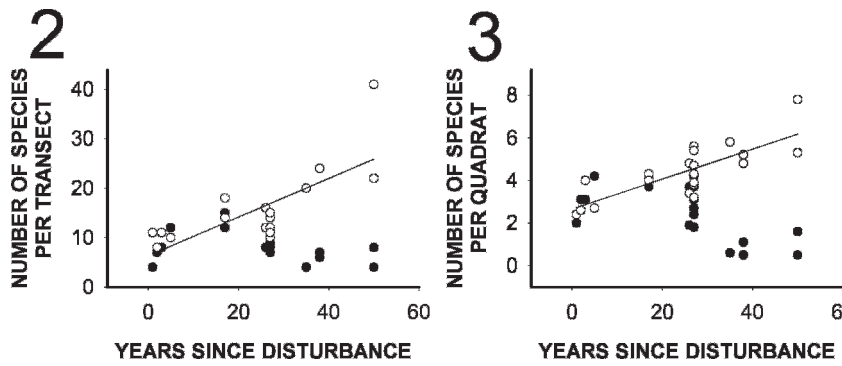


Figure 1.—Aerial photograph of Taylor University Arboretum study area. REC = Randall Environmental Center situated on the west-edge of campus and east side of the Arboretum. The 2006 Arboretum Addition is north and west of the original Arboretum. Years indicates estimated lapse between fallow and sampling.

dominant plant species included the annuals *Setaria faberi, Ambrosia artemisiifolia* and biennials *Daucus carota* and *Dipsacus fullonum*. The perennials *Symphyotrichum pilosum* and *Melilotus officinale* were among the dominant species by Years 2–3. By the fifth year, annual species were replaced by perennials, especially *Poa pratensis, Solidago altissima,* and *S. pilosum.* Transects in the 17-year old-field remain dominated by *Solidago*. Woody species also were present, but none were among the dominant species. By Year 27, some woody species – *Rubus occidentalis* and *Toxicodendron radicans* – were locally dominant. Sites 35–40 years of age displayed high cover by *Crataegus* spp. and, in one transect, *Ulmus americana.* The oldest site, an estimated 50 years post-disturbance, had developing canopies of either *Carya ovata* or a mix of *Fraxinus americana* and *Prunus serotina*. In the oldest site the common woodland species *Sanicula odorata* and *Parthenocissus quinquefolia* were among ground layer dominants and the common old-field perennial *Solidago altissima* was almost completely absent. Across the chronosequence we observed an increase in the number of native species both per transect (Fig. 2) and per quadrat (Fig. 3). Initially, non-native species were as numerous as native species (Figs. 2,3) but after about 20 years of old-field succession, the numbers of these non-shade tolerant species declined.

Changes in mean C and FQI.—MC at both the transect and the quadrat level increased across the chronosequence (Figs. 4,5; $r^2 = 0.68$–0.78, $P < 0.0001$). During the first five years, transect MC ranged between 0.8–1.3 for native species (Fig. 4). After 35 years this same metric



Figures 2,3.—Changes in number of species during 50 years of old-field succession. 2. Species per transect: open circles = native species, closed circles = non-native species; regression line for native species has $r = 0.74$ ($P = 0.0001$). 3. Average number of species per quadrat: open circles = native species, closed circles = non-native species; regression line for native species has $r = 0.78$ ($P < 0.0001$).

had increased to 2.3–3.1. When non-native species were included in the calculation, MC along the chronosequence was lowered on average by 0.6 units. At the quadrat level, the trends were similar (Fig. 5). MC during the first five years was low, 0.3–0.8, and increased to 1.3–3.0 after 35 year. Inclusion of non-native species lowered the MC an average of 0.4 units.

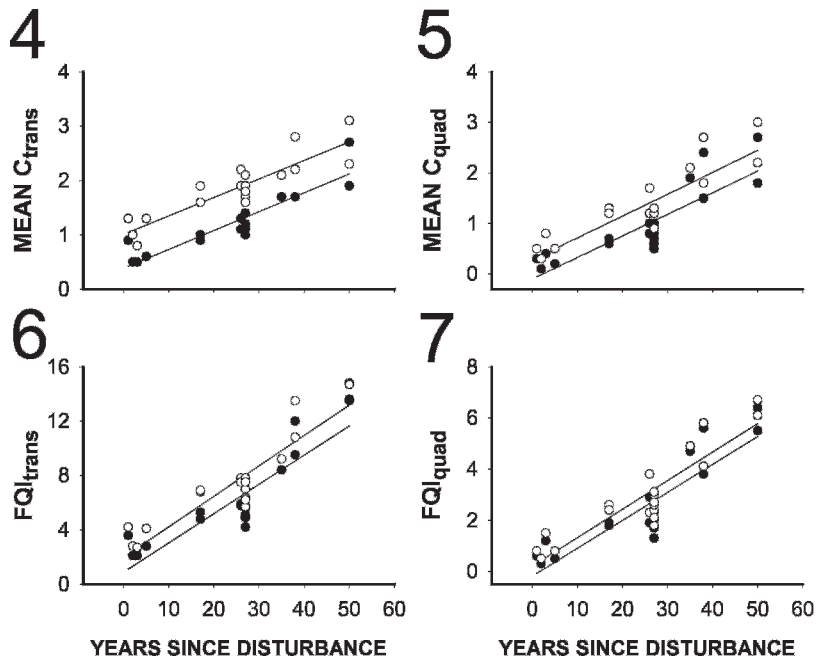
Since MC and native species richness both increased across the successional chronosequence, FQI also increased (Figs. 6,7; $r^2 = 0.75–0.81$, $P < 0.0001$). At the transect level (Fig. 6), sites five years or younger had an FQI for native species that ranged from 2.7–4.2. After 35 years this increased to 9.2–14.8. The inclusion of non-native species in the metric lowered transect FQI values along the chronosequence by approximately 1.5 units. Quadrat FQI values (Fig. 7) were approximately 0.5–1.5 during early succession and increased to 3.8 and above after 35 years.

DISCUSSION

The old-field chronosequence in the Taylor University Arboretum exhibited a pattern of species change similar to that previously reported for the Midwestern U.S. region (Quarterman 1957; Hopkins & Wilson 1974; Hoyle et al. 1979; Vankat & Snyder 1991). The earliest successional species were common ruderal annuals (especially non-native species) followed by perennials, *Solidago altissima* and *Symphyotrichum pilosum*. All had C values of 0–1 (Rothrock 2004). After several decades, dominant species were still those with very low C values (C = 0–1). Less frequent species contributed to the slow rise in MC. These included perennials such as *Fragaria virginiana*, *Parthenocissus quinquefolia*, *Carex cephalophora*, and *Vitis vulpina* (C = 2–3) and a few species with C = 4 (Table 1). Woody species (e.g., *Fraxinus americana* and *Carya ovata*) and several woodland perennials (including *Galium circaezans* and *Packera obovatus*) with C values as high as 7 were observed in the oldest sites (Table 1). As the time since disturbance increased, the number of native species also increased; this further contributed to the increases observed in FQI over time.

Our results indicate a strong relationship between MC or FQI and time since disturbance. Swink & Wilhelm (1994) have suggested that transect MC = 3.5 or higher signifies that a site possesses some remnant natural quality. The highest MC observed in the Taylor University Arboretum successional sites was 3.1, clearly below this threshold. However, given the linear regression trend in our study, we would estimate that the 3.5 threshold potentially could be reached over a period of 70–90 years post-disturbance.

The rate of change of MC and FQI can be influenced by availability of seed rain and post-disturbance management. For example, within our 27-year transects the lowest FQI value was recorded for a transect furthest from woodland seed sources. We also observed that an area of the TU Arboretum planted with *Poa pratensis* shortly after fallowing has had low rates of perennial and woody invasion after 27 years. Here quadrat MC and FQI do not exceed a meager 0.6 (unpublished data). In contrast, in a study of the floristic quality of a prairie restoration where a species-rich seed mix was



Figures 4–7.—Changes in mean C and FQI during 50 years of old-field succession; open circles = native species, closed circles = total species (native + non-native). 4. Mean C per transect: both regression lines have $r = 0.87$ ($P < 0.0001$). 5. Average mean C per quadrat: regression lines have $r = 0.84$ for native species and 0.86 for total species ($P < 0.0001$). 6. FQI per transect: regression lines have $r = 0.88$–0.90 ($P < 0.0001$). 7. Average FQI per quadrat: regression lines have $r = 0.87$–0.89 ($P < 0.0001$).

Table 1.—Species with C value of 4 or higher and years when present in transect sample.

Species	Years when present	C value
None in years 1–5		
Acer saccharum	17–50	4
Crataegus crus-galli	17–50	4
Liparis loeselii	17	4
Galium triflorum	27–50	5
Ophioglossum vulgatum	27	4
Lactuca floridana	35–50	5
Carex amphibola	38	8
Carex radiata	38	4
Rubus occidentalis	38	4
Smilax lasioneuron	38	4
Fraxinum americana	38–50	4
Leersia virginica	38–50	4
Asimina triloba	50	6
Carya ovata	50	4
Galium circaezans	50	7
Packera obovata	50	7
Prunus serotina	50	4
Ranunculus recurvatus	50	5
Symphyotrichum shortii	50	6

sown, McIndoe et al. (2008) reported MC and FQI values for a prairie restoration whose floristic quality after 6 years were comparable to our old-field after 35 or more years.

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EVALUATING THE QUALITY OF A DISTURBED WETLAND IN SOUTHWESTERN INDIANA: A SURVEY OF NATIVE AND EXOTIC FLORA AT VECTREN CONSERVATION PARK

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ABSTRACT. Wetland stability promotes ecosystem services such as water purification and maintenance of biodiversity. These ecosystem services have been disrupted by anthropogenic degradation of natural habitats resulting in decreased biodiversity and the spread of introduced species. In Indiana, more than 87% of wetlands have been destroyed or degraded; those wetlands that remain are threatened by invasive species. To assess the need for restoration at Vectren Conservation Park in Southwest Indiana, a survey of the floral species present, as well as a study of the relative abundance of native and exotic species, was performed. The site includes more than 1100 acres of wetland habitat, including riparian forest, recently planted trees, and abandoned agricultural land. We collected 144 species from 109 genera, with 31 of the species being non-native to Indiana. When including all native and non-native species, the floristic quality index (FQI) of the site was 23.5 and the mean coefficient of conservatism (C_{av}) was 2.0. The FQI and mean coefficient of conservatism (C_{av}) were relatively low compared to other sites found in Indiana, indicating few natural remnants remain at the site. Although highly degraded, the site is capable of supporting high quality native wetland species, which would result in the improvement of ecosystem services and buffer against more extensive establishment of non-native species.

Keywords: coefficient of conservatism, floristic quality index, invasive species, restoration, species diversity, wetlands

INTRODUCTION

Ecosystem stability contributes to processes such as water purification, flood control, ground water recharge, and even maintenance of biodiversity. Anthropogenic degradation and destruction of natural habitats negatively impacts ecosystem stability (Pearson 1972; Van Auken 2000), which in turn threatens biodiversity and creates opportunities for colonization by invasive species (Burke & Grime 1996). In the United States, more than 50% of wetlands (Nichols 1988; McCorvie & Lant 1993) and up to 99% of prairies (Samson & Knopf 1994; Samson *et al.* 2004) have been destroyed or degraded, resulting in imperilment of native species.

Because so little high quality native habitat remains in Indiana, restoration of degraded habitats must be a priority. Efforts aimed at restoring degraded habits have become more widespread as awareness regarding the benefits of natural habitats has increased (Rood *et al.* 2003; Clark 2003). Restoration can reestablish ecosystem services in degraded habitats (Gratton & Denno 2006; Benayas *et al.* 2009), as well as enhance biodiversity. In turn, biodiversity can have a positive feedback on ecosystem function (Vernberg 1993; Lehman & Tilman 2000; Benayas *et al.* 2009). For example, diverse wetland plant communities purify water and provide flood control (Vernberg 1993; Benayas *et al.* 2009). Increasing diversity may also buffer against species invasions (Naeem *et al.* 2000; Kennedy *et al.* 2002, but see Foster *et al.* 2002; Eriksson *et al.* 2006).

After habitat destruction, biological invasions represent the second greatest threat to biodiversity (Vitousek *et al.* 1996; Knight & Reich 2005), so enhancing native diversity needs to be a priority when performing restoration ecology. Invasive species diminish biodiversity by outcompeting and excluding native species resulting in homogenization of habitats (Kaufman 1992). In some U.S. states, up to 47% of the flora is composed of exotic

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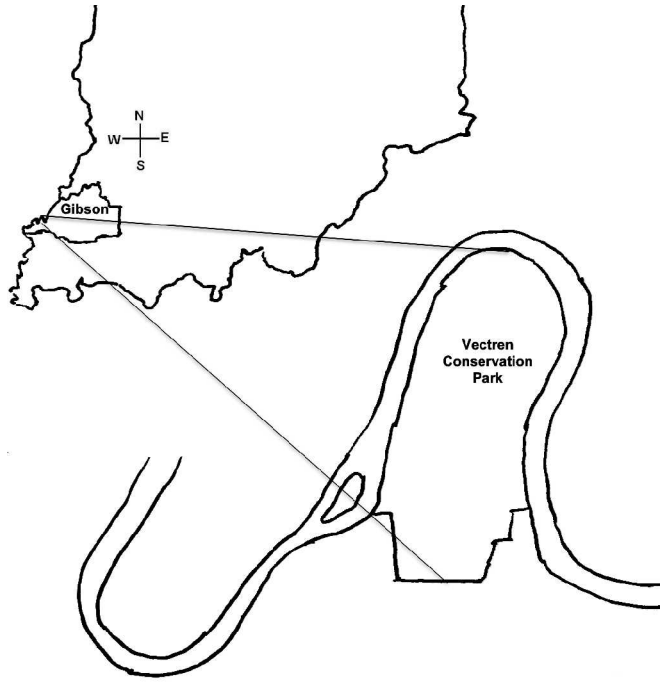

Figure 1.—Vectren Conservation Park in Gibson County, Indiana.

species. (Rejmanek & Randall 1994; Vitousek *et al.* 1997). In Indiana, invasive species pose a significant threat to the state's native flora (Weber & Gibson 2007), with approximately 39% of the 2800-plus species occurring in Indiana being categorized as non-native (Rothrock & Homoya 2005)

Special attention should be paid to biodiversity when restoring Indiana wetland habitat. First, wetlands are of particular interest when considering restoration because they support diverse habitats containing many rare species; in Indiana, wetlands lay claim to some of the highest levels of species diversity of any ecosystem type (Myers 1997). Second, more than 87% of Indiana's wetlands have been drained or destroyed (Dahl 1990; Miller & MacGowan 2004). Finally, wetland areas are especially susceptible to invasions; nearly 25% of the world's most invasive plants occur in wetlands (Zedler & Kercher 2004).

Vectren Conservation Park (**VCP**), a wetland next to the Wabash River in southern Indiana, may be a candidate for conservation or restoration. Comprised of abandoned agricultural fields and riparian forests, qualitative and quantitative surveys of native and exotic flora are necessary to evaluate the quality of the habitat in order to make conservation and restoration decisions for the site. One measure of plant diversity, species richness (i.e., the number of species in an area), provides a means to evaluate ecosystem quality. Examination of native versus exotic plants provides a second assessment tool. In this study, we performed a qualitative survey of the property to identify the plant species at the site, as well as conducting a quantitative study to determine the relative abundance of native and exotic species at the site. In addition, to provide metrics of floristic and ecological quality comparable to other Indiana sites, a floristic quality index (**FQI**) and a coefficient of conservatism (**C**) were generated for the site.

METHODS

Study Site.—VCP is an 1118 acre property located in southwestern Indiana (38°17′ N, 87°52′W) (Fig. 1). Found 6.75 miles north-northeast of the town of Griffin in Posey County, the property is approximately 380 feet above sea level. In 2007, Vectren Corporation

provided the University of Evansville (**UE**) with a long-term lease to the property in order to provide a research site for undergraduate students and UE faculty. Surrounded on three sides by the Wabash River, VCP regularly floods, occasionally being entirely inundated with water. The soil composition of the site varies. (McWilliams 1989). The soils at the northern end of the property are characterized as frequently flooded silt loams, while the southern half of the property includes a wide range of frequently and occasionally flooded silt loams, silty clay loams, and fine sandy loams. The site consists of 157 acres of riparian forest, 454 acres of recently restored forest (see below for details), and 508 acres of meadow (Woodburn 2001). In addition, an operating agricultural field of 81 acres exists in the middle of the meadow. The remains of a levee erected by farmers follow the path of the river, and most of the mature riparian forest is bounded by the farmer's levee. The meadow resides in the interior of the site.

Based on U.S. census data, most of VCP was farmed from the early 1800's until 2001 when the Vectren Corporation purchased the property. In 2002, Vectren Corporation planted 454 acres with trees and shrubs, including five species of oak and three species of dogwood, as well as sycamore, black walnut, sweetgum, spicebush, and button bush (see Appendix 1 for a complete list of species planted and the number of each species planted). The trees and bushes were purchased from Vallonia State Nursery in Vallonia, Indiana. Of the trees planted by Vectren Corp., sweetgum, black walnut, and sycamore have had the most success establishing at the site. In addition to the 136,100 trees and shrubs that were planted, selected areas including roadway easements and the area surrounding the 81-acre agricultural field, were planted with warm and cool season grasses. Except for the 81-acre agricultural plot, the land has remained mostly unmodified by human activity after Vectren planted the trees, shrubs and grasses.

Plant Survey.—In 2007, we initiated a survey of the flora of VCP. On a semi-weekly basis from May to October in 2007 and 2009, trips were made to VCP to document the flora present, both in the meadow and the forest. The recently replanted forests were avoided in the surveys. During each visit to the site, surveyors collected, pressed, and later identified any previously unidentified plant that was observed. The collected species were identified using a variety of identification keys and field guides (Deam 1940; Steyermark 1963; Gleason & Cronquist 1991; Holmgren 1998; Yatskievych 1999, 2006; Yatskievych 2000). The nomenclature from Gleason and Cronquist (1991) is reported for all species. The voucher specimens are being held at UE's herbarium.

Using plant information from the survey, each species was rated for level of wetland habitat preference, with categories including obligate and facultative wetland plants, as well as categories related to preference for upland habitat (U.S. Army Corps of Engineers 1996; see Appendix 2 for more details). The wetness scale proposed by Swink and Wilhelm (1994) was used to calculate a mean wetness value for VCP. In this scale, OBL = −5, FACW = −2.5, FAC = 0, FACU = 2.5, and UPL = 5; a site with a mean wetness below 0 is considered predominantly to have wetland plants.

In addition, each species was given a coefficient of conservatism (C-value) for Indiana (Rothrock 2004). C-values range from zero to 10, with lower values representing plants that are highly tolerant of disturbance and higher values representing plants that are usually restricted to high quality plant community remnants. Introduced plants are often not categorized for C-values, but they can be considered to have C-values of zero. A mean C-value (C_{av}) was calculated for the native species at the site. In addition, a C_{av} for the combined values of native species and introduced species was calculated (after attributing a C-value of zero to the introduced species). To provide a floristic quality index (**FQI**), the two C_{av}'s were multiplied by the square root of the number of plant species.

Plot Samples.—In addition to the qualitative survey, a study examining the relative abundance of native and exotic plants occurring in meadow and forest environments was conducted during the summer of 2007. Three 20×20 m plots were randomly selected both in the meadow and in the forest habitat. The plots were constructed at least 100 meters from any farmlands, tree plantings, or access roads to minimize the effects of adjacent small-scale habitats. Within each plot, 12 subplots (0.5×1.0 m) were randomly sampled. If the subplot chosen included a large tree (whether standing or fallen), the subplot was moved to

the next 0.5×1.0 m location directly to the left. In the forest, if a randomly selected subplot contained high concentrations of *Toxicodendron radicans* (poison ivy), the plot was moved to an area relatively free of this species. All forest plots were on the eastern edge of VCP. For all plots, ramet density was calculated by counting the number of stems of each plant species encountered in each subplot. Each species was identified as either native or non-native to the United States and to Indiana. Any species that could not be identified to species was identified to the lowest taxonomic level possible.

RESULTS

Plant Survey.—In total, 144 species from 109 genera within 54 families were collected from VCP (Appendix 2). Of the 144 species, 113 were native species and 31 were introduced species. Eight species were collected but identified only to genus because of a lack of morphological characteristics; these species were not classified as native or invasive. Seven additional species could not be identified to genus. Of those seven, one was a member of the Brassicaceae family, one was a member of the Poaceae family, and one was a member of the Apiaceae family. The final four species could not be attributed to a family because of a lack of identifying features.

Of the 113 native species at our site, twelve species (*Asclepias incarnata, Senecio glabellus, Rorippa palustris, Rorippa sessiliflora, Cephalanthus occidentalis, Hibiscus laevis, Salix nigra, Phyla lanceolata, Forestiera acuminata, Amorpha fruticosa, Saururus cernuus, and Mimulus alatus*) are almost always found in wetlands in our region. Overall, 42% of the species occur naturally at higher frequency in wetland habitat (FACW or OBL), and 36% are found equally as often in wetland habitats as in non-wetland habitats (FAC) (seed Appendix 2 for notation). The remaining 22% of native species are more commonly associated with upland habitats (FACU or UPL). The non-native species were plants less commonly associated with wetlands; 57% of the non-native species that had a wetland designation are more commonly found in upland habitat (FACU or UPL). Using Swink and Wilhelm's wetness scale (1994), the plants at VCP had a mean wetness value of −0.37, suggesting that the site is only weakly associated with wetland plants.

Few species had both high coefficients of conservatism (C-values of six or greater) and high fidelity to wetland habitats. The exceptions included *Aristolochia tomentosa*, *Aster praealtus, Carex conjuncta*, *Celtis laevigata,* and *Forestiera acuminata* (see Appendix 2 for details). Of the 107 native species with C-values, 49.5% had C-values of 0–2, and 43.9% had C-values of 3–5. The mean C-value (C_{av}) for the native species was 2.5, and the C_{av} for all species (native and non-native species combined) was 2.0. The FQI for native species was 26.6, while the FQI for all species was 23.5.

Plot Samples.—The composition of the three forest plots and the three meadow plots differed greatly. In general, woody species were more abundant in the forest plots, while the meadow plots were dominated by herbaceous species. In total, 17 families and 21 genera were represented in the forest plots, while 14 families and 26 genera of plants occurred in the meadow plots. Four families (Apiaceae, Cyperaceae, Poaceae, and Urticaceae) characterized the forest plots, accounting for over 91% of the species collected. The families characterizing the meadow plots differed from those of the forest; three families (Asteraceae, Fabaceae, and Poaceae) accounted for over 88% of the species in the meadow. Of the 23 species collected in the forest plots, only one was introduced (although four species were unidentified). Of the 29 species collected in the meadow plots, eight species were introduced (although four species were identified only to genus, and thus not classified as native or introduced). In the meadow plots, invasive species accounted for the majority of the stems counted (59.8%) (Table 1A). Together, *Medicago lupulina* (black medic), *Melilotus officinalis* (yellow sweet clover), *Melilotus albus* (white sweet clover), and *Sorghum halepense* (Johnson grass) accounted for almost all of the introduced stems counted in the meadow plots (98.4%).

In Meadow Plot I, native species accounted for almost all of the stems (98.1%). The plot was characterized by *Aster praealtus* (56.8% of the stems) and *Elymus virginicus* (25.6%). The majority of stems in Plot II belonged to introduced species, with *Melilotus albus* and *Melilotus officinalis* accounting for over 51% of the stems. The native species in Plot II primarily consisted of *Aster praealtus* (14.5% of the stems) and *Solidago canadensis* (15.7%). Plot III was largely characterized by introduced

Table 1.—Species encountered in (A) meadow and (B) forest plots at Vectren Conservation Park. Family, origin (native or introduced), scientific and common name, and number of stems are given. A dash in the origin column indicates uncertain origin; these species were not included in abundance calculations.

A. Meadow							
Family	Origin	Scientific Name	Common Name	Plot I	Plot II	Plot III	Total
Aceraceae	Native	*Acer saccharinum*	Silver maple (sapling)	1	0	0	1
Anacardiaceae	Native	*Toxicodendron radicans*	Poison ivy	1	0	18	19
Apiaceae	Introduced	*Torilis arvensis*	Field hedge parsley	0	1	0	1
Asteraceae	Native	*Ambrosia artemisifolia*	Common ragweed	0	1	16	17
Asteraceae	Native	*Ambrosia trifida*	Great ragweed	40	6	4	50
Asteraceae	Native	*Aster praealtus*	Veiny-line aster	870	328	87	1285
Asteraceae	–	*Aster* sp. 1	Unidentified long-leaf aster	145	3	1	149
Asteraceae	Native	*Conyza canadensis*	Horseweed	0	5	92	97
Asteraceae	Native	*Erigeron annuus*	Daisy fleabane	1	0	0	1
Asteraceae	Native	*Lactuca canadensis*	Wild lettuce	0	11	2	13
Asteraceae	Introduced	*Lactuca serriola*	Prickly lettuce	0	0	8	8
Asteraceae	Native	*Pyrrhopappus carolinianus*	False dandelion	0	1	0	1
Asteraceae	Native	*Solidago canadensis*	Common goldenrod	162	353	55	570
Asteraceae	Native	*Taraxacum officinale*	Common dandelion	11	41	85	137
Brassicaceae	Introduced	*Capsella bursa-pastoris*	Shepherd's purse	0	1	0	1
Convolvulaceae	Native	*Calystegia sepium*	Hedge bindweed	1	1	2	4
Chenopodiaceae	–	*Chenopodium* sp.	Lamb's quarters	0	0	519	519
Fabaceae	Introduced	*Lespedeza cuneata*	Silky bushclover	0	48	0	48
Fabaceae	Introduced	*Medicago lupulina*	Black medic	1	0	3234	3235
Fabaceae	Introduced	*Melilotus albus and Melilotus officinalis*	Sweet clover	0	1159	70	1229
Fabaceae	Introduced	*Trifolium campestre*	Low hop clover	5	20	0	25
Onagraceae	Native	*Oenothera biennis*	Biennial evening primrose	1	40	466	507
Oxalidaceae	–	*Oxalis* sp.	Woodsorrel	1	0	0	1
Polygonaceae	–	*Polygonum* sp.	Various smartweeds	0	0	8	8
Poaceae	Native	*Andropogon virginicus*	Broom sedge	22	0	0	22
Poaceae	Native	*Elymus virginicus*	Virginia wildrye	392	79	155	626
Poaceae	Introduced	*Sorghum halepense*	Johnson grass	23	156	274	453
Ulmaceae	Native	*Ulmus rubra* and *U. americana*	Elm (sapling)	0	4	0	4
Verbenaceae	Native	*Verbena urticifolia*	White vervain	1	0	3	4

Table 1.— Continued.

B. Forest Family	Origin	Scientific Name	Common Name	Plot I	Plot II	Plot III	Total
Anacardiaceae	Native	*Toxicodendron radicans*	Poison ivy	16	14	17	47
Apiaceae	Native	*Cryptotaenia canadensis*	Honewort	690	245	7	942
Asteraceae	Native	*Ambrosia trifida*	Great ragweed	2	0	0	2
Asteraceae	–	*Aster* sp. 2	Unidentified forest aster 1	42	0	36	78
Asteraceae	–	*Aster* sp. 3	Unidentified forest aster 2	27	3	0	30
Asteraceae	Native	*Solidago canadensis*	Common goldenrod	0	0	3	3
Bigoniaceae	Native	*Campis radicans*	Trumpet creeper	0	2	1	3
Convolvulaceae	Introduced	*Ipomea hederacea*	Ivy-leaved morning glory	2	0	0	2
Cyperaceae	Native	*Carex grayi*	Globe sedge	22	175	351	548
Menispermaceae	Native	*Menispermum canadense*	Common moonseed	0	0	1	1
Oleaceae	Native	*Fraxinus pennsylvania*	Green ash	0	0	1	1
Phytolaccaceae	Native	*Phytolacca americana*	American pokeweed	26	0	0	26
Polygonaceae	Native	*Polygonum virginianum*	Jumpseed	1	5	0	6
Poaceae	Native	*Elymus virginicus*	Virginia wildrye	490	758	627	1875
Poaceae	Native	*Festuca subverticillata*	Nodding fescue	0	0	8	8
Smilaceae	Native	*Smilax herbacea*	Smooth carrion-flower	1	1	1	3
Smilaceae	–, but native	*Smilax* sp.	Unidentified smilax	3	13	0	16
Solanaceae	Native	*Solanum ptycanthum*	Eastern black nightshade	0	0	4	4
Ulmaceae	Native	*Celtis laevigata*	Sugarberry (sapling)	0	0	5	5
Urticaceae	Native	*Laportea canadensis*	Wood nettle	97	112	1	210
Urticaceae	Native	*Pilea pumila*	Clearweed	317	14	342	673
Violaceae	–	*Viola* sp.	Unidentified violet	3	12	4	19
Vitaceae	Native	*Parthenocissus quinquefolia*	Virginia creeper	38	44	59	141

species, with *Medicago lupulina* accounting for over 70% of the stems. *Oenothera biennis* (10.2% of the stems) was the only native species commonly found in Plot III. Some species were found throughout all three plots, while others were clustered in one or two of the three plots. For example, all but one specimen of *Medicago lupulina* was found in Plot III, while most of the *Melilotus* stems were found in Plot II. *Sorghum halepense* was the only introduced species commonly found throughout the three meadow plots. The native species commonly found in all three plots included *Aster praealtus*, *Solidago canadensis*, and *Elymus virginicus*.

In contrast to the meadow plots, native species represented almost all of the species present in the forest plots. In fact, only two introduced specimens were found across the three plots—two specimens of *Ipomea hederecea* (ivy-leaved morning glory) in forest Plot I (Table 1B). Five species (*Cryptotaenia canadensis* (honewort), *Carex grayi* (globe sedge), *Elymus virginicus* (Virginia wildrye), *Laportea canadensis* (wood nettle), *Pilea pumila* (clearweed), and *Parthenocissus quinquefolia* (Virginia creeper)) accounted for more than 95% of the stems counted in the forest plots. The most common species in forest plots were fairly consistent across plots. Forest Plot I was represented by *Cryptotaenia canadensis* (40.5%), *Elymus virginicus* (28.8%), and *Pilea pumila* (18.6%). *Elymus virginicus* (55.3%), *Cryptotaenia canadensis* (17.9%), *Carex grayi* (12.8%) accounted for most of the stems found in forest Plot II. In Plot III, *Elymus virginicus* (43.9%), *Carex grayi* (24.6%), and *Pilea pumila* (23.9%) were the most common species.

DISCUSSION

Plant Survey.—The quality of habitat (C_{av} = 2.5, FQI = 26.6) at VCP is relatively low compared to other wetland sites in Indiana. For example, Bennett Wetland Complex (BWC) in Henry County has a C_{av} for native species of 3.8 (Ruch *et al.* 2009) and an FQI of 54.6. Turkey Run State Park in Parke County, Indiana also contains two habitats of higher quality. Two seep areas in the park are categorized as high quality habitats (C_{av1} = 5.4, FQI_1 = 29.8, C_{av2} = 5.1 FQI_2 = 32.1) (Rothrock & Homoya 2005). The C_{av} of VCP fails to fall within the suggested range of values for a natural habitat (>3.5), indicating that few natural remnants remain at this site. Although the site is degraded, it is still capable of sustaining species that require a wetland habitat. This is evident by the presence of *Aristolochia tomentosa*, *Aster praealtus, Carex conjuncta*, *Forestiera acuminaa*, and *Celtis laevigata,* which all have C-values equal to or greater than six and are commonly found in wetlands.

Plot Samples.—The proportion of non-native species in the meadow and forest plots differed greatly. The meadow has been used for agricultural purposes as recently as 2001, so the higher the abundance of invasive species was expected because disturbance promotes colonization by non-native species (Burke & Grime 1996). Although the invasive species accounted for over 50% of stems present in meadow plots, the presence of some non-native species (e.g., *Medicago lupulina* and *Melilotus spp.*) was localized, suggesting that concentrated efforts to control these non-natives may be successful.

In contrast, the widespread presence of Johnson grass, which was found in all plots, represents a pressing concern. Johnson grass produces large quantities of viable seed, while also spreading rhizomatously (Oyer *et al.* 1959). Furthermore, the formation of rhizomes as early as 50 days after seed planting causes the plants to become increasingly difficult to control because the entire rhizomatous system must be eradicated as opposed to simply destroying the aerial foliage. In addition, large quantities of herbicide are generally required to control Johnson grass (Frans *et al.* 1991), and some biotypes of Johnson grass have become resistant to certain herbicides (Smeda *et al.* 1997). For these reasons Johnson grass presents a major challenge in site restoration.

Restoration efforts have demonstrated that reestablishing biodiversity and ecosystem services can be effective. While VCP is a degraded site, restoring this environment could lead to improved ecosystem function, enhanced biodiversity, and reduced abundance of non-native plants (Vernberg 1993; Lehman & Tilman 2000; Benayas *et al.* 2009). Waterways, such as the Wabash River that borders VCP, expedite the spread of invasive species by acting as corridors for dispersal (Thebaud & Debussche 1991; Parendes & Jones 2000). So yearly floods currently wash in invasive seed banks and receding waters export invasive seed from the property's established invasive populations. In addition to restoring the ecosystem of VCP, establishment of a stable native wetland should

result in exportation of native seed instead of non-native seed. For example, a few recently discovered species (i.e., *Rudbeckia laciniata* and *Vernonia gigantea*) may have come from an attempted prairie planting in 2008, where floods swept the seeds far from the planting.

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APPENDIX 1:
Species planted by Vectren Corporation at Vectren Conservation Park in 2002.

Family	Scientific Name	Common Name	Inds. Planted
Caesalpiniaceae	*Cercis canadensis*	redbud	2300
Cornaceae	*Cornus amomum*	silky dogwood	2000
Cornaceae	*Cornus florida*	flowering dogwood	2300
Cornaceae	*Cornus racemosa*	gray dogwood	2300
Cornaceae	*Nyssa sylvatica*	black gum	7000
Fagaceae	*Quercus alba*	white oak	20900
Fagaceae	*Quercus imbricaria*	shingle oak	4600
Fagaceae	*Quercus macrocarpa*	bur oak	10000
Fagaceae	*Quercus michauxii*	swamp chestnut oak	4500
Fagaceae	*Quercus palustris*	pin oak	10000
Hamamelidaceae	*Liquidambar styraciflua*	sweetgum	20000
Juglandaceae	*Carya illinoinensis*	pecan	10000
Juglandaceae	*Juglans nigra*	black walnut	10000
Lauraceae	*Lindera benzoin*	spicebush	2300
Oleaceae	*Fraxinus pennsylvanica*	green ash	10000
Plantanaceae	*Plantanus occidentalis*	sycamore	13000
Rosaceae	*Crataegus phaenopyrum*	Washington hawthorne	2300
Rosaceae	*Physocarpus opulifoius*	ninebark	700
Rubiaceae	*Cephalanthus occidentalis*	buttonbush	1900
		Total trees planted	**136100**

APPENDIX 2:

Species list of flora present at Vectren Conservation Park (arranged alphabetically by family). Each species report includes the following information: (1) scientific name based on Gleason and Cronquist (1991), (2) common name, (3) origin (native or introduced), (4) wetland indicator category (U.S. Army Corps of Engineers 1996), and (5) coefficient of conservatism (C-value) for Indiana (Rothrock 2004). For the wetland indicator categories, OBL represents obligate wetland plants (with plants almost always occurring in wetlands (>99%)), FACW represents facultative wetland plants (with plants usually occurring in wetlands (67%–99%)), FAC represents facultative plants (with plants being equally likely to occur in wetlands or non-wetlands (34%–66%)), FACU represents facultative upland plants (with plants usually occurring in non-wetlands (67%–99%)), and UPL represents Obligate Upland plants (with plants almost always occurring in non-wetlands in our region (>99%))(Reed 1996). NI represents plants with insufficient information available to determine indicator status. Signs (+/−) represent discrimination within categories, with a positive sign representing a greater frequency in that habitat and a negative sign representing a lower frequency). C-values range from zero to 10, with lower values representing plants that are highly tolerant of disturbance and higher values representing plants that are restricted to high quality plant community remnants. In addition to the species listed only to the level of genus, eight species were identified only at higher taxonomic levels; four specimens belonged to three families—Brassicaceae, Poaceae, and Asteraceae, and four were not placed to any family. Many introduced plants were neither categorized for wetland category nor for C-values. Still, introduced species are often given C-values of zero.

Acanthaceae (Acanthus Family)
- *Ruellia strepens* L.: Wild petunia; native; FAC+; 4

Aceraceae (Maple Family)
- *Acer negundo* L.: Boxelder; native; FACW-; 1
- *Acer saccharinum* L.: Silver maple; native; FACW; 1

Amaranthaceae (Amaranth Family)
- *Amaranthus sp.*

Apiaceae (Carrot Family)
- *Cryptotaenia canadensis* (L.) DC.: Honewort; native; FAC; 3
- *Torilis arvensis* (Huds.) Link: Field hedge-parsley; introduced, Europe

Apocynaceae (Dogbane Family)
- *Apocynum cannabinum* L.: Indian hemp; native; FAC; 2

Araceae (Arum Family)
- *Arisaema dracontium* (L.) Schott: Green dragon; native; FACW; 5

Aristolochiaceae (Birthwort Family)
- *Aristolochia tomentosa* Sims: Pipe vine; native; FAC; 7

Asclepiadaceae (Milkweed Family)
- *Ampelamus albidus (Nutt.) Britton:* Bluevine; native; - ; 1
- *Asclepias incarnata* L.: Swamp milkweed; native; OBL; 4
- *Asclepias syriaca* L.: Common milkweed; native; NI; 0

Asteraceae (Aster Family)
- *Achillea millefolium* L.: Yarrow; native; FACU; 0
- *Ambrosia artemisiifolia* L.: Common ragweed; native; FACU; 0
- *Ambrosia trifida* L.: Giant ragweed; native; FAC+; 0
- *Aster praealtus Poir.*: Veiny lined aster; native; FACW; 6
- *Aster pilosus* Willd.: Heath aster; native; 0
- *Bidens comosa* (A. Gray) Wiegand: Strawstem burmarigold; native; - ; 1
- *Carduus nutans* L.: Musk thistle; introduced, Europe
- *Conyza canadensis* (L.) Cronquist: Horseweed; native; FAC-; 0
- *Erigeron annuus* (L.) Pers.: Daisy fleabane; native; FAC-; 0
- *Erigeron philadelphicus* L.: Philadelphia fleabane; native; FACW; 3
- *Eupatorium coelestinum* L.: Mistflower; native; FACW; 2
- *Eupatorium serotinum* Michx.: Late boneset; native; FAC+; 0
- *Iva annua* L.: Rough marsh elder; native; FAC; 0
- *Lactuca serriola* L.: Prickly lettuce; introduced, Europe; FAC; 0
- *Pyrrhopappus carolinianus* (Walter) DC.: False dandelion; introduced; 2
- *Rudbeckia hirta* L.: Black-eyed Susan; native; FACU; 2
- *Rudbeckia laciniata* L.: Cutleaf coneflower; native; FACW; 3
- *Rudbeckia triloba* L.: 3-lobed coneflower; native; FAC 3
- *Senecio glabellus* Poir.: Butterweed; native; OBL; 0
- *Solidago canadensis* L.: Common goldenrod; native; FACU; 0
- *Tragopogon dubius* Scop.: Yellow salsify; introduced, Europe
- *Verbesina alternifolia* (L.) Britton: Wingstem; native; FACW; 3
- *Vernonia gigantea* (Walter) Trel.: Tall ironweed; native; FAC; 2

Bignoniaceae (Trumpet Creeper Family)
- *Campsis radicans* (L.) Seem.: Trumpet creeper; native; FAC; 1
- *Catalpa speciosa* Warder: Northern catalpa; native; FACU; 0

Brassicaceae (Mustard Family)
- *Capsella bursa-pastoris* (L.) Medik.: Shepherd's purse; introduced, Europe; FAC-
- *Cardamine rhomboidea* (Pers.) DC.: Springcress; native

Lepidium virginicum L.: Poor man's pepper; native; FACU-; 0
Rorippa palustris (L.) Besser: Common yellow cress; native; OBL; 2
Rorippa sessiliflora (Nutt.) Hitchc.: Southern yellow cress; native; OBL; 3
Caesalpiniaceae (Caesalpinia Family)
Cercis canadensis L.: Redbud; native; FACU; 3
Gleditsia triacanthos L.: Honey-locust; native; FAC; 1
Gymnocladus dioicus Lam.: Kentucky coffee-tree; native; 3
Campanulaceae (Bellflower Family)
Campanula americana L.: Tall bellflower; native; FAC; 4
Caprifoliaceae (Honeysuckle Family)
Symphoricarpos sp.
Caryophyllaceae (Pink Family)
Dianthus armeria L.: Deptford pink; introduced, Europe; NI
Chenopodiaceae (Goosefoot Family)
Chenopodium sp.
Convolvulaceae (Morning-glory Family)
Calystegia sepium (L.) R. Br.: Hedge bindweed; native; FAC; 4
Ipomoea hederacea Jacq.: Ivy-leaved morning glory; introduced; FAC
Ipomoea pandurata (L.) G.Mey.: Wild potato; native; FACU; 3
Cornaceae (Dogwood Family)
Cornus drummondii C. A. Mey.: Rough-leaved dogwood; native; FAC; 2
Cucurbitaceae (Gourd Family)
Sicyos angulatus L.: Bur cucumber; native; FACW-; 3
Cuscutaceae (Dodder Family)
Cuscuta gronovii Willd. Common dodder; native; 2
Cyperaceae (Sedge Family)
Carex conjuncta Boott: Green-headed fox sedge; native; FACW; 6
Carex cristatella Britton: Crested sedge; native; FACW+; 3
Carex digitalis Willd.: Slender woodland sedge; native; UPL; 7
Carex grayi J. Carey: Globe sedge; native; FACW+; 5
Carex muehlenbergii Schkuhr ex Willd.: Muehlenberg's sedge; native; - ; 5
Cyperus strigosus L.: False nutsedge; native; FACW; 0
Elaeagnaceae (Oleaster Family)
Elaeagnus angustifolia L.: Russian olive; introduced, native of Eurasia; FACU-
Fabaceae (Pea Family)
Amorpha fruticosa L.: False indigo; native; OBL; 3
Desmodium paniculatum (L.) DC.: Panicled tick-clover; native; FACU; 2
Lespedeza cuneata (Dum. Cours.) G. Don: Silky bushclover; introduced, Asia; UPL
Melilotus albus Medik: White sweet clover; introduced, Asia;
Melilotus officinalis (L.) Pall.: Yellow sweet clover; introduced, Eurasia; FACU
Trifolium campestre Schreb.: Low hop clover; introduced, Eurasia and N. Africa
Fagaceae (Beech Family)
Quercus macrocarpa Michx.: Bur oak; native; FAC-; 5
Geraniaceae (Geranium Family)
Geranium carolinianum L.: Carolina crane's bill; native; - ; 2
Juglandaceae (Walnut Family)
Juglans cinerea L.: Butternut; native; FACU+; 5
Juglans nigra L.: Black walnut; native; FACU; 2
Lamiaceae (Mint Family)
Lamium amplexicaule L.: Henbit deadnettle; introduced, Eurasia and Africa
Teucrium canadense L.: American germander; native; FACW-; 3
Liliaceae (Lily Family)
Allium vineale L.: Field garlic; introduced, native of Europe; FACU
Malvaceae (Mallow Family)
Hibiscus laevis All.: Smooth rose mallow; native; OBL; 4
Sida spinosa L.: Prickly mallow; native; FACU
Menispermaceae (Moonseed Family)
Menispermum canadense L.: Common moonseed; native; FAC; 3
Mimosaceae (Mimosa Family)
Desmanthus illinoensis (Michx.) MacMill.: Bundle-flower; native; FACU; 3
Moraceae (Mulberry Family)
Maclura pomifera (Raf.) Schneid: Osage orange; native; FACU
Morus alba L.: White mulberry; introduced, Asia; FAC
Morus rubra L.: Red mulberry; native; FAC-; 4
Oleaceae (Olive Family)
Forestiera acuminata (Michx.) Poir.: Swamp privet; native; OBL; 8
Fraxinus americana L.: White ash; native; FACU; 4
Fraxinus pennsylvanica Marshall: Green ash; native; FACW; 1
Onagraceae (Evening Primrose Family)
Oenothera biennis L.: Common evening primrose; native; FACU; 0
Oenothera laciniata Hill: Ragged evening primrose; native; FACU; 2
Oenothera sp.
Oxalidaceae (Wood-sorrel Family)
Oxalis dillinii Jacq.: Southern yellow wood-sorrel; native; NI; 0
Phytolaccaceae (Pokeweed Family)
Phytolacca americana L.: American pokeweed; native; FAC-; 0

Plantaginaceae (Plantain Family)
Plantago rugelii Decne.: American plantain; native; FAC; 0
Platanaceae (Plane-tree Family)
Platanus occidentalis L.: American sycamore; native; FACW; 3
Poaceae (Grass Family)
Agrostis gigantea Roth: Redtop; introduced, Europe; FACW
Andropogon virginicus L.: Broomsedge; native; FAC-; 1
Arundinaria gigantea (Walter) Muhl.: Giant cane; native; NI, 5
Bromus racemosus L.: Bald brome; introduced, Europe
Chasmanthium latifolium (Michx.) H.O. Yates: Wild oats; native; FAC; 4
Dactylis glomerata L. Orchard grass; introduced, Europe; FACU
Echinochloa crusgalli (L.) P.Beauv.: Barnyard grass; introduced, Eurasia; FACW
Elymus canadensis L.: Canada wildrye; native; FAC-; 5
Elymus virginicus L.: Virginia wildrye; native; FACW-; 3
Festuca pratensis Huds.: Meadow fescue; introduced, Europe; FACU-
Festuca subverticillata (Pers.) E.B. Alexeev: Nodding fescue; native; FACU+; 4
Hordeum jubatum L.: Foxtail barley; native; FAC+
Hordeum pusillum Nutt.: Little barley; introduced, N. America; FAC; 0
Koeleria pyramidata (Lam.) P. Beauv.: Junegrass; native; - ; 8
Lolium perenne L.: Perennial rye; introduced, Europe; FACU
Phleum pratense L.: Common timothy; introduced, Europe; FACU
Setaria viridis (L.) P. Beauv.: Green foxtail; introduced, Europe
Sorghum bicolor (L.) Moench: Sorghum; introduced, Africa; UPL
Sorghum halepense (L.) Pers.: Johnson grass; introduced, Europe and Africa; FACU
Polygonaceae (Smartweed Family)
Polygonum aviculare L.: Knotweed; native; FAC-
Polygonum cuspidatum Siebold & Zucc: Japanese knotweed; introduced; Japan; FACU
Polygonum erectum L.: Erect knotweed; native; FACU; 0
Polygonum pennsylvanicum L.: Pinkweed; native; FACW+; 0
Polygonum persicaria L.: Spotted lady's thumb; introduced, Europe; FACW
Polygonum virginianum L.: Jumpseed; native; FAC; 3
Rumex altissimus A. W. Wood: Pale dock; native; FACW-; 2
Rumex crispus L.: Curley dock; introduced, Europe; FAC+
Ranunculaceae (Buttercup Family)
Ranunculus micranthus Nutt.: Small-flowered crowfoot; native; FAC-; 4
Rosaceae (Rose Family)
Crataegus mollis (Torr. & Gray) Scheele: Downy hawthorn; native; FACW-; 2
Geum canadense Jacq.: White avens; native; FAC; 1
Potentilla norvegica L.: Rough cinquefoil, native; FAC; 0
Prunus sp.
Rubus sp.
Rubiaceae (Madder Family)
Cephalanthus occidentalis L.: Common buttonbush; native; OBL; 5
Salicaceae (Willow Family)
Populus deltoides Marshall: Cottonwood; native; FAC+; 1
Salix exigua Nutt.: Sandbar willow; native; FACW+; 1
Salix nigra Marshall: Black willow; native; OBL; 3
Saururaceae (Lizard's tail Family)
Saururus cernuus L.: Lizard's tail; native; OBL; 4
Scrophulariaceae (Figwort Family)
Verbascum blattaria L.: Moth mullein; introduced, Eurasia; FACU-
Mimulus alatus Aiton: Sharpwing monkey-flower; native; OBL; 4
Smilacaceae (Catbriar Family)
Smilax herbacea L.: Smooth carrion flower; native; FAC; 4
Smilax sp.
Solanaceae (Nightshade Family)
Solanum carolinense L.: Horse-nettle; native; FACU-; 0
Ulmaceae (Elm Family)
Celtis laevigata L.: Southern hackberry; native; FACW; 7
Celtis occidentalis L.: Northern hackberry; native; FAC-; 3
Ulmus americana L.: American elm; native; FACW-; 3
Ulmus rubra Muhl.: Slippery elm; native; FAC; 3
Urticaceae (Nettle Family)
Laportea canadensis (L.) Wedd.: Canada nettle; native; FACW; 2
Pilea pumila (L.) A. Gray: Clearweed; native; FACW; 2
Urtica dioica L. Stinging nettle; native; FACW-; 1
Valerianaceae (Valerian Family)
Valerianella radiata (L.) Dufr.: Beaked cornsalad; native; FAC; 1
Verbenaceae (Vervain Family)
Verbena urticifolia L.: White vervain; native; FAC+; 3
Phyla lanceolata (Michx.) Greene: Fogfruit; native; OBL; 2
Violaceae
Viola sp.

Vitaceae (Grape Family)

Parthenocissus quinquefolia (L.) Planch.: Virginia creeper; native; FAC-; 2

Vitis aestivalis Michx.: Summer grape; native; FACU; 4

Vitis cinerea Engelm.: Sweet winter grape; native; FACW-; 4

Vitis riparia Michx.: Riverbank grape; native; FACW-; 1

Vitis vulpina L.: Winter grape; native; FACW-; 3

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OCCURENCE OF HERBICIDES IN CENTRAL INDIANA STREAMS

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ABSTRACT. Agricultural chemicals, such as pesticides and herbicides, play an important role in agricultural operations to control unwanted pests and maximize crop yield. However, these chemicals may also threaten aquatic life. The abundance of agricultural chemicals, specifically atrazine and metolachlor, was measured in eighteen headwater streams of the Upper White River Watershed (UWRW) of central Indiana. Sites were selected to represent a range of agriculture activity within the watershed. Sites were sampled seasonally over one year (N=4) to assess temporal variation in herbicide abundance and the influence of physiochemical factors. Pearson correlation coefficients were used to assess independent variables influencing stream herbicide concentrations. All sites had measurable concentrations of atrazine and metolachlor during June 2010 sampling; no herbicides were above the detection limit in August; only two sites had measureable concentrations of metolachlor in November; and, one site had measurable concentrations of atrazine and metolachlor in May. These data indicate concentrations of atrazine and metolachlor in central Indiana streams are temporally variable, being highest in late spring. Concentrations measured were comparable to other studies in agricultural areas and frequently exceeded concentrations known to have adverse effects on aquatic organisms.

Keywords: Agriculture, herbicides, streams

Freshwater is a valuable resource to both surrounding ecosystems and human activities. Streams play an important role linking terrestrial ecosystems to downstream ecosystems because they assist in energy and nutrient exchange among adjacent terrestrial, atmospheric, and downstream ecosystems (Likens et al. 1974). In addition, they provide fundamental resources including drinking water, sanitation, and flood control. Freshwater ecosystems also provide habitat for a variety of flora and fauna that can improve or maintain water quality (Meyer et al. 2007). Since terrestrial and aquatic ecosystems are fundamentally linked, agricultural activities can influence the integrity of receiving waters within a watershed.

Agricultural activities can influence water quality and the organisms that depend on freshwater habitats in a number of ways. For example, agricultural activities can inflate erosion rates, especially after rain events, increasing sediments entering waterways (Al-Kaisi 2009). This sediment runoff is one of the leading impairments to streams and rivers in the United States (Miller et al. 2011). Increased sediment in turn provides more surface area for pathogens and yields light limitation to autotrophic organisms (Matson et al. 1978; Vidon et al. 2008). Agricultural activity can also increase nutrient concentrations, primarily as nitrogen and phosphorus, resulting in algal blooms and subsequent eutrophication (Paerl 1997). Eutrophication decreases dissolved oxygen concentrations threatening fish and reducing diversity of other aquatic organisms (Nixon 1995). Decades of research have highlighted adverse effects of sediment and nutrients associated with agricultural activity on freshwater ecosystems (Wolman 1967; Paerl 1997; Turner et al. 2003; Schoonover et al. 2006; Miller et al. 2011). However, agricultural activity can also influence freshwater ecosystems via introduction of herbicides and pesticides and less is known about the potential effects of these contaminants on freshwater integrity.

Herbicides, in particular atrazine and metolachlor, are commonly used in central Indiana and the Midwestern United States (Fenelon et al. 1996; Pratt et al. 1997; Fenelon 1998; Fuhrer et al. 1999; USGS 2011) but comprehensive data on concentrations and effects are lacking (Thurman et al. 1992; Crawford 1995). According to 2007 United States Census data, 64% of Indiana is classified as cropland (United States Department

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of Agriculture 2009). Indiana also lies within the Mississippi River Watershed yielding agricultural inputs draining into the Mississippi River and subsequently the Gulf of Mexico. Historically, the Gulf of Mexico has experienced hypoxic areas partly due to the increased nutrient loading from nonpoint sources within the Midwest (Ongley 1996; Kanwar et al. 2005). It is likely that other agricultural contaminants, like herbicides and pesticides are also being transported to downstream ecosystems.

Abundance of herbicides and pesticides, especially atrazine and metolachlor, can differ substantially with regards to individual studies conducted. In studies conducted in Midwestern streams, atrazine, a pre-emergence herbicide, can reach concentrations exceeding 1 mg/L in nonpoint sources (agricultural runoff), and up to 40 μg/L in precipitation (Hayes et al. 2002). Atrazine can also persist in streams at high concentrations for weeks (Pratt et al. 1997). Metolachlor can reach concentrations as high as 138 μg/L in surface waters, with increases in concentration primarily associated with runoff events in agricultural areas (Rivard 2003; Extoxnet 2000a). Another study performed in Illinois over eight years (1992–2000), measured atrazine concentrations as high as 49 μg/L and metolachlor at 8.2 μg/L in freshwaters draining agricultural areas (David et al. 2003).

Amount of precipitation, terrain, soil characteristics, and type of tillage system may all influence export of agricultural chemicals into receiving waters (Kalkhoff et al. 2003; Miller et al. 2011). Soil physical and chemical characteristics can foster chemical degradation as well as transport to ground water and receiving streams. For example, as the permeability of soil increases, ground water infiltration and movement into streams also increases (Burkart et al. 1999; Kalkhoff et al. 2003). In contrast, when soil permeability is low, agricultural chemicals are more likely to be retained on the landscape and enter freshwater primarily during runoff events (Burkart et al. 1999; Kalkhoff et al. 2003). Runoff events frequently introduce higher concentrations of contaminants into receiving waters (Burkart et al. 1999; Kalkhoff et al. 2003). Type of tillage system used in the surrounding agricultural fields can also affect the abundance of herbicides and pesticides in receiving waters. Conventional tillage and crop harvesting exposes the ground to the elements, increasing the rate of erosion and resulting in greater runoff of herbicides and pesticides to receiving streams (Miller et al. 2011).

Once in the freshwater ecosystem, pesticides and herbicides may have adverse effects on aquatic organisms. Atrazine is considered to be lethal to invertebrates at concentrations ranging from 6–22 mg/L (Mayer et al. 1986; Pratt 1997) and from 0.8–2.7 mg/L for fishes (Mayer et al. 1986; Pratt 1997). Even though agricultural chemicals may not be lethal at environmentally-relevant concentrations, sub-lethal effects on organismal growth, reproduction, or behavior may also occur (Fleeger et al. 2003). For example, atrazine can act as an endocrine disrupter in frogs at 1 μg/L (Hayes et al. 2002). Herbicides can also alter the behavior of lobsters and crayfish by impairing their ability to locate food sources (Wolf and Moore 2002; Cook et al. 2008).

Criteria used to determine how a chemical may influence water quality include toxicity, persistence, degradates, and environmental fate (Ongley 1996). Further, effects at the organism or ecosystem level are usually considered to be an early warning indicator of potential human health impacts (Ongley 1996). However, many times these criteria are established using isolated samples not representative of spatial and temporal variability in ecosystem contaminants or target aquatic communities. To protect water resources, more comprehensive research is needed to assess the distribution and concentrations of agricultural chemicals in central Indiana freshwaters.

METHODS

Study sites.—Sampling was conducted in the Upper White River Watershed (UWRW) of central Indiana (Figure 1). The UWRW covers 2,720 square miles across 16 counties with a gradient of agricultural activities (UWRWA 2010). The UWRW supplies 85% of the surface water needed for human use in Indianapolis and central Indiana (Crawford 1995). Within the UWRW, 17 headwater stream sites were selected for sampling, representing a range of agricultural activity (Table 1). One site in another nearby watershed (Sugar Creek) was also sampled for a total of 18 sites. Sites were sampled seasonally over one year (N=4) in June, August, and November 2010 and May 2011.

Water sampling.—At each sampling event, two filtered water samples were collected at each site. All water samples were collected using a rinsed 60 mm syringe placed in the

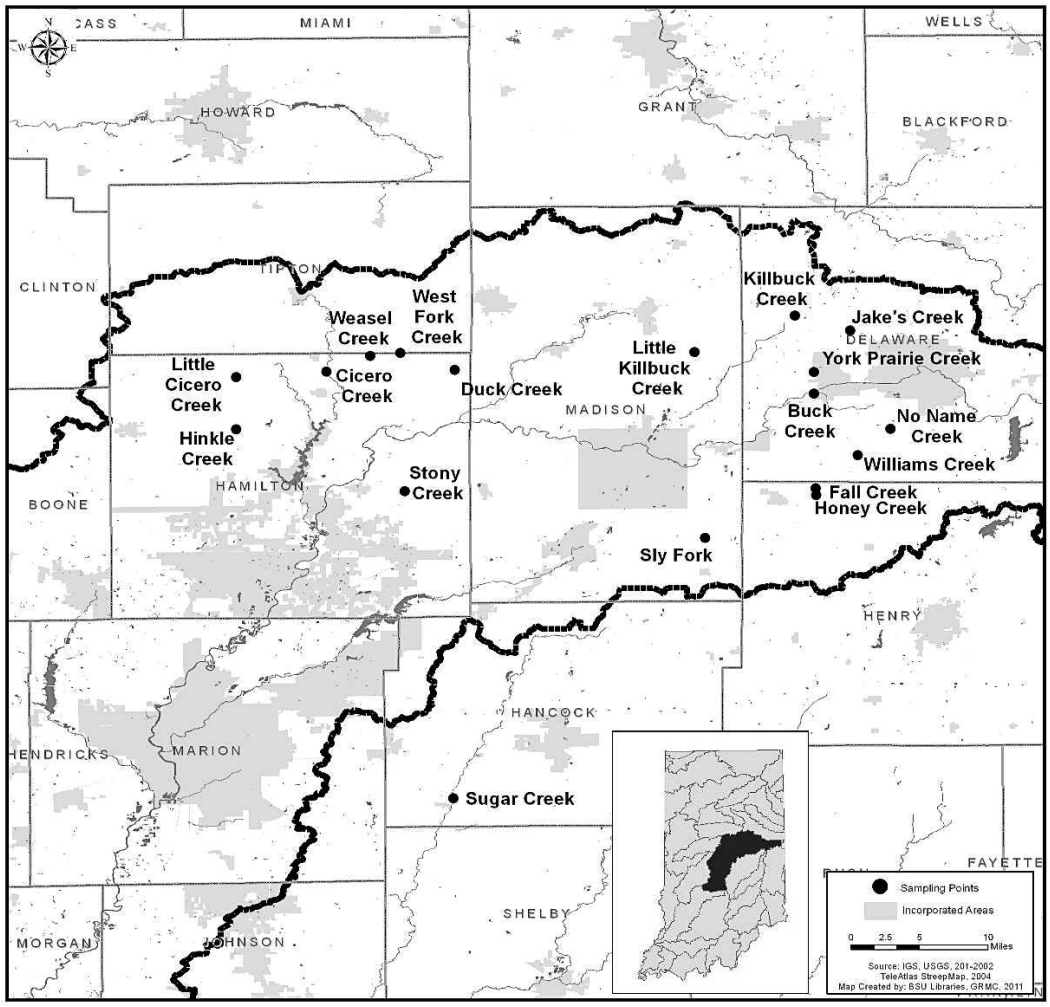

Figure 1.—The Upper White River Watershed (UWRW) in central Indiana with sampling locations (black circles). Surrounding land use is identified within the watershed.

Table 1.—Mean site physiochemical characteristics values for June, August, and November 2010 and May 2011 with standard deviation in parenthesis.

Site	Width (m)	Mean depth (m)	Mean velocity (m/s)	Discharge (m^3/s)	Temperature (C°)	pH	Specific Conductivity (µS/cm)	Total Dissolved Solids (g/L)	DO (% sat)	DO (mg/L)	Salinity (ppt)	% Organic Matter
Buck Creek	20.13 (3.04)	0.30 (0.12)	0.55 (0.27)	3.71 (2.78)	15.96 (4.53)	9.33 (0.28)	661.30 (134.25)	0.42 (0.09)	99.78 (7.60)	11.40 (1.66)	0.34 (0.07)	1.52 0.92)
Cicero Creek	15.00 (2.58)	0.54 (0.20)	0.36 (0.15)	3.14 (2.17)	17.39 (5.71)	8.18 (1.85)	538.90 (101.82)	0.34 (0.07)	99.00 (15.69)	11.07 (2.90)	0.28 (0.06)	1.13 (0.22)
Duck Creek	12.53 (2.49)	0.44 (0.27)	0.33 (0.11)	1.84 (1.20)	17.03 (4.93)	8.75 (0.22)	768.78 (331.33)	0.48 (0.19)	79.35 (14.76)	9.04 (2.97)	0.39 (0.16)	2.66 (1.83)
Fall Creek	1.80 (1.24)	0.31 (0.23)	0.06 (0.08)	0.07 (0.12)	12.76 (9.44)	6.88 (4.59)	492.45 (332.73)	0.32 (0.21)	77.43 (52.17)	7.83 (5.46)	0.26 (0.17)	2.07 (1.47)
Hinkle Creek	2.23 (1.10)	0.27 (0.18)	0.16 (0.17)	0.17 (0.23)	16.09 (6.88)	8.87 (0.71)	596.63 (128.78)	0.38 (0.08)	89.85 (45.47)	10.34 (6.16)	0.30 (0.07)	0.95 (0.33)
Honey Creek	4.83 (1.02)	0.14 (0.06)	0.23 (0.22)	0.21 (0.24)	16.23 (3.11)	9.06 (0.09)	661.85 (127.78)	0.40 (0.07)	109.30 (10.26)	11.92 (2.93)	0.33 (0.05)	0.59 (0.19)
Jake's Creek	5.63 (2.41)	0.33 (0.22)	0.25 (0.24)	0.96 (1.59)	17.29 (5.17)	8.82 (0.34)	422.30 (297.19)	0.27 (0.19)	72.43 (9.41)	8.17 (1.91)	0.22 (0.15)	2.20 (1.58)
Killbuck Creek	9.60 (1.55)	0.39 (0.22)	0.18 (0.12)	0.79 (0.78)	16.33 (4.51)	8.80 (0.38)	700.08 (126.05)	0.45 (0.08)	77.40 (8.41)	8.87 (1.99)	0.36 (0.07)	2.54 (1.19)
Little Cicero Creek	7.93 (1.18)	0.33 (0.05)	0.20 (0.14)	0.59 (0.50)	17.38 (5.98)	9.04 (0.35)	635.18 (121.50)	0.41 (0.08)	97.03 (20.85)	10.91 (3.46)	0.33 (0.07)	3.19 (2.87)
Little Killbuck	4.60 (1.25)	0.24 (0.14)	0.08 (0.09)	0.16 (0.24)	14.41 (4.48)	8.52 (0.64)	613.23 (124.69)	0.39 (0.08)	69.43 (10.33)	8.36 (2.59)	0.32 (0.07)	2.66 (2.04)
No Name Creek	3.35 (0.62)	0.28 (0.12)	0.14 (0.15)	0.19 (0.23)	15.33 (3.69)	9.23 (0.35)	676.10 (113.95)	0.43 (0.07)	97.05 (14.39)	11.49 (3.58)	0.35 (0.06)	1.41 (0.43)
Sly Fork	6.95 (2.28)	0.36 (0.15)	0.15 (0.08)	0.48 (0.46)	17.59 (4.01)	9.03 (0.25)	675.75 (93.43)	0.44 (0.04)	105.68 (16.26)	11.63 (1.83)	0.36 (0.04)	4.65 (3.09)
Stony Creek	4.40 (1.71)	0.25 (0.13)	0.37 (0.28)	0.41 (0.44)	17.01 (4.83)	8.78 (0.40)	594.53 (130.18)	0.38 (0.08)	102.60 (15.01)	11.60 (3.23)	0.31 (0.07)	1.72 (1.58)
Sugar Creek	15.30 (9.83)	0.58 (0.15)	0.34 (0.22)	4.51 (5.88)	18.42 (5.88)	9.28 (0.35)	587.30 (136.08)	0.38 (0.09)	110.25 (16.26)	12.02 (2.42)	0.30 (0.07)	1.14 (0.39)
Weasel Creek	2.40 (0.70)	0.41 (0.34)	0.09 (0.03)	0.11 (0.10)	16.21 (6.35)	8.88 (0.20)	643.30 (118.93)	0.41 (0.08)	77.38 (49.94)	9.39 (7.12)	0.33 (0.06)	5.27 (2.43)
West Fork	5.53 (2.36)	0.58 (0.06)	0.03 (0.01)	0.16 (0.21)	16.41 (6.10)	8.79 (0.50)	596.20 (119.95)	0.38 (0.08)	72.25 (35.59)	8.67 (5.08)	0.31 (0.06)	11.72 (5.07)
Williams Creek	6.68 (1.49)	0.17 (0.09)	0.22 (0.19)	0.37 (0.47)	15.68 (4.34)	9.12 (0.12)	629.00 (87.40)	0.40 (0.06)	95.28 (12.46)	10.77 (3.11)	0.33 (0.05)	1.07 (0.72)
York Prairie Creek	5.08 (0.81)	0.33 (0.32)	0.35 (0.16)	0.43 (0.21)	16.46 (4.17)	9.02 (0.50)	744.70 (116.04)	0.48 (0.07)	79.73 (6.49)	9.01 (1.24)	0.39 (0.06)	1.85 (1.91)

thalweg of each stream and subsequently filtered through a glass fiber filter (Whatman GF/F; 0.6 μm nominal pore size) into acid-washed sample bottles. The first filtered water sample was collected into a 1000 mL amber glass bottle containing preservative, which was then analyzed within 24h for 33 specific herbicides at the Indiana State Department of Health Analytical Laboratory, Indianapolis, using liquid chromatography followed by mass spectrometry (ISDH SOP: 525.2 SVOC). Herbicides analyzed included atrazine (detection limit=0.20 μg/L), metolachlor (0.15 μg/L), simazine (0.15 μg/L), acetochlor (0.16 μg/L), alachlor (0.12 μg/L), hexachlorocycolpentadiene (0.044 μg/L), propachlor (0.075 μg/L), desethylatrazine (0.062 μg/L), trifluralin (0.054 μg/L), desisopropylatrazine (0.54 μg/L), hexachlorobenzene (0.10 μg/L), clomazone (0.072 μg/L), pentachlorophenol (0.59 μg/L), lindane (0.098 μg/L), terbufos (0.083 μg/L), heptachlor (0.11 μg/L), chlorpyrifos (0.088 μg/L), cyanazine (0.40 μg/L), aldrin (0.11 μg/L), pendimethalin (0.13 μg/L), heptachlor epoxide (0.094 μg/L), oxychlordane (0.030 μg/L), gamma-Chlordane (0.068 μg/L), alpha-Chlordane (0.090 μg/L), trans-Nonachlor (0.060 μg/L), dieldrin (0.11 μg/L), endrin (0.23 μg/L), cis-Nonachlor (0.067 μg/L), p,p'-DDT (0.10 μg/L), bis (2-ethylhexyl) adipate (0.020 μg/L), methoxychlor (0.32 μg/L), bis (2-ethylhexyl) phthalate (0.090 μg/L), and benzo[a]pyrene (0.13 μg/L). Three additional 1000 mL amber glass bottles were collected, at a randomly chosen site, during each sampling event to be used for matrix assessments in analytical procedures. The second filtered water sample was collected into a 125 mL acid-washed Nalgene bottle and frozen within 8 h for subsequent analysis of cations (ammonium, calcium, lithium, magnesium, potassium, sodium) and anions (bromide, chloride, nitrate, nitrite, phosphate, sulfate) using ion chromatography (DIONEX, ICS-3000).

Sediment sampling.—In addition to water samples, sediment was also collected at each sampling site by collecting a composite sample, along an established transect, of the top 5–10 cm of sediment and placing into a 120 mL specimen cup. Sediment was transported on ice to the laboratory, and subsequently dried (60°C). Once dried, each sample was then further processed to determine percent organic matter. Three subsamples were taken from each sediment sample and weighed, followed by combustion in a Barnstead Thermolyne© FB 1400 muffle furnace for at least 2 h followed by measurement of ash weight. Sediment % organic matter for each sampling event was calculated as the mean of the three sub-samples.

Ancillary variables.—Stream physiochemical and channel characteristics were also measured at each location and sampling period. Water quality parameters were measured using a Hydrolab© minisonde equipped with a Luminescent Dissolved Oxygen (LDO) sensor, temperature probe, conductivity sensor, and pH sensor. Stream discharge, mean depth, velocity, and wetted width were measured using a Marsh McBirney flow meter.

Statistics.—Differences in herbicide concentrations among seasons and sites were assessed using analysis of variance (ANOVA). Factors influencing measured herbicide concentrations were analyzed using Bonferroni-corrected Pearson correlation statistics.

RESULTS

Physiochemical characteristics.—The 18 sites selected for this study represented a range of headwater streams with variation among average width (1.80–20.13 m), depth (0.17–0.58), velocity (0.03–0.28), and discharge (0.07–4.51 m/s) (Table 1). In addition, specific conductivity (422–769 μS/cm), dissolved oxygen (7.83–12.02 mg/L), and percent organic matter (0.59–11.72%) also varied among sites. Temperature (12.76–18.42 C °), pH (8.18–9.33), and salinity (0.26–0.39 ppt) were the least variable characteristics among sites.

Sugar Creek had the highest mean discharge (4.51 m^3/s) and DO (110%; 12.02) of all sampling sites; whereas, Fall Creek and Weasel Creek had the lowest mean discharge (0.07 m^3/s; 0.11 m^3/s, respectively). West Fork had 2× benthic organic matter in sediment relative to Weasel Creek (11.72% compared to 5.27%). Specific conductivity varied significantly across the four sampling events (standard deviation was >87 for all sites), while variation in salinity was lower (standard deviation <0.15).

Herbicide concentrations.—Across all sampling periods, only four herbicides were at or above the detection limits including atrazine, metolachlor, acetochlor, and simazine. Herbicides which were not detected during any sampling event included hexachlorocycolpentadiene, propachlor, desethylatrazine, trifluralin, desisopropylatrazine, hexachlorobenzene, clomazone, pentachlorophenol, lindane, terbufos,

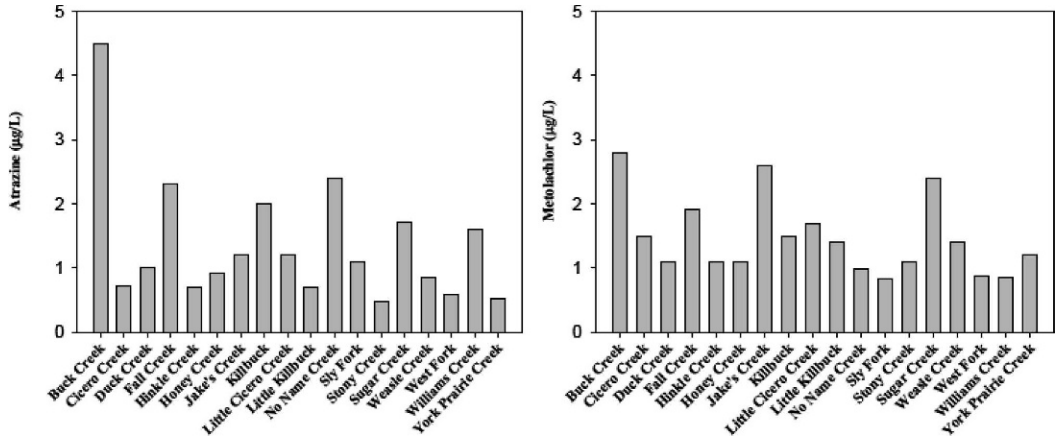

Figure 2.—Concentrations (µg/L) of atrazine and metolachlor for June 2010 sampling.

heptachlor, chlorpyrifos, cyanazine, aldrin, pendimethalin, heptachlor epoxide, oxychlordane, gamma-Chlordane, alpha-chlordane, trans -Nonachlor, dieldrin, endrin, cis-Nonachlor, p,p'-DDT, bis (2-ethylhexyl) adipate, methoxychlor, bis (2-ethylhexyl) phthalate, and benzo[-a]pyrene. In the June 2010 sampling, atrazine (0.52–4.5 µg/L) and metolachlor (0.86–2.8 µg/L) were found detected at all sites (Figure 2). Acetochlor was detected at 10 sites (56%

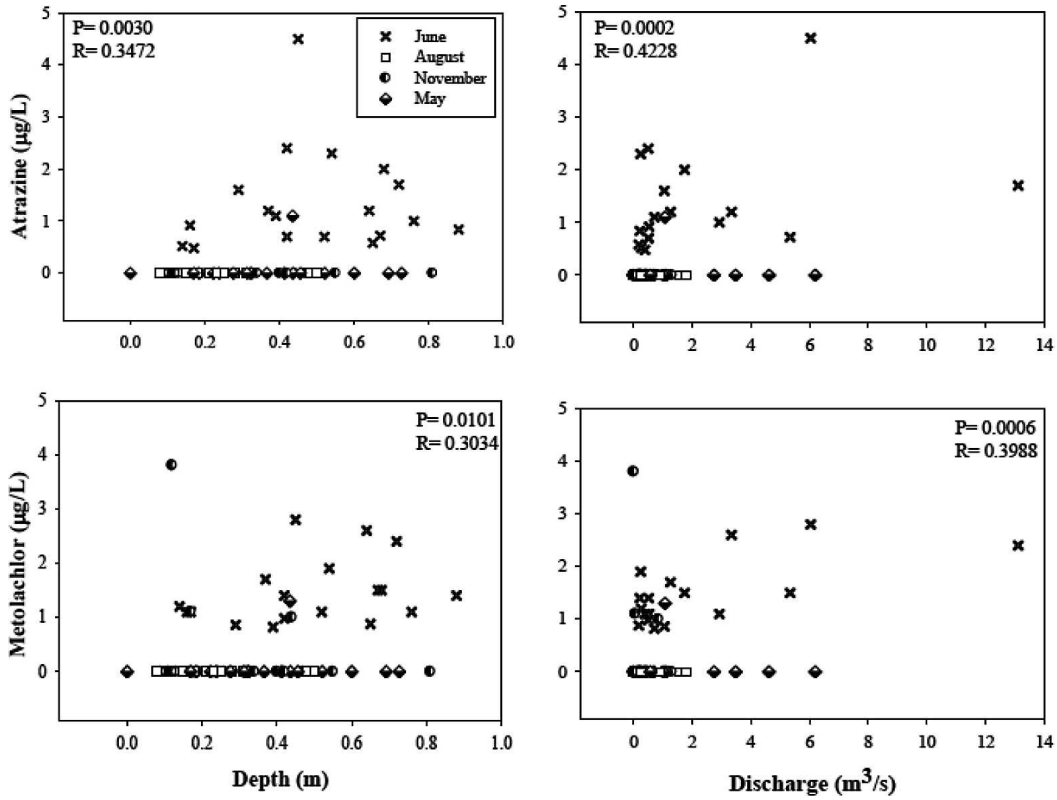

Figure 3.—The relationship between stream discharge and depth with atrazine and metolachlor concentrations for all sampling events. Pearson Correlation statistics noted.

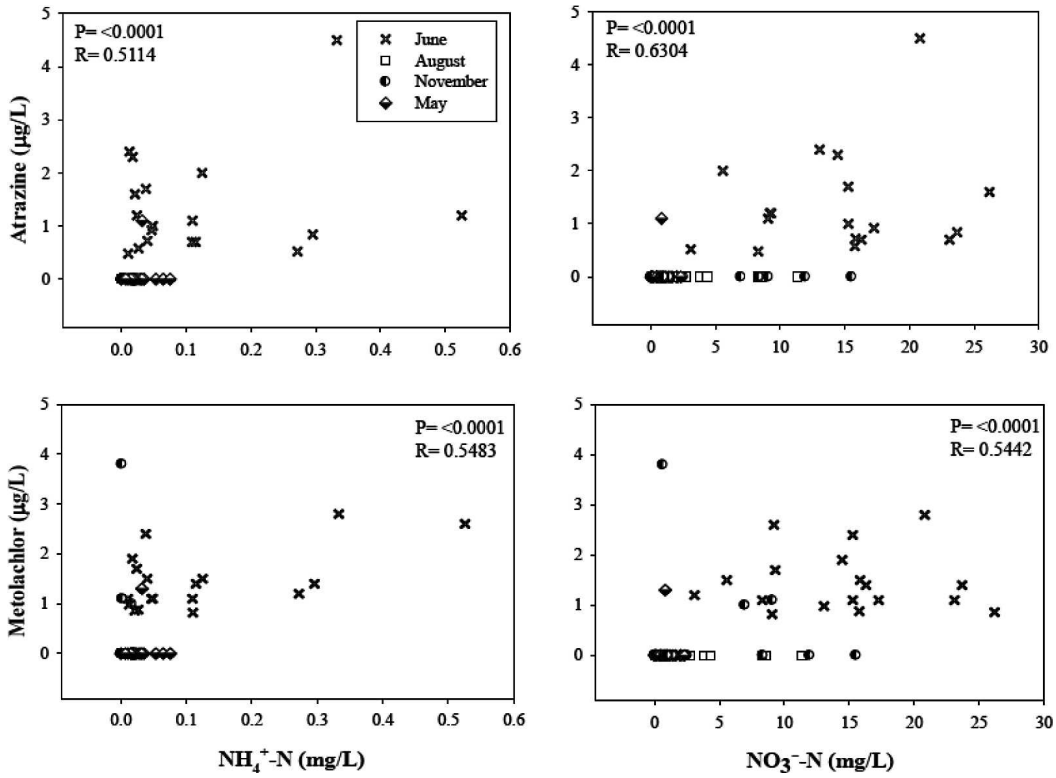

Figure 4.—The relationship between ammonium (NH_4^+-N) and nitrate (NO_3^--N) with atrazine and metolachlor concentrations for all sampling events. Pearson Correlation statistics noted.

detection frequency; 0.68–2.1 µg/L) during the June sampling event and simazine was detected at 1 site (0.05% detection frequency; 0.92 µg/L) only during the June sampling event. The highest concentration of atrazine was also found during the June sampling event (4.5 µg/L) and was measured at Buck Creek, which was characterized by both urban and agricultural inputs. The August 2010 sampling event yielded concentrations that were below detection limits for all herbicides tested. In November 2010, all herbicides were below detection limits except for metolachlor. Little Killbuck (3.8 µg/L) and Stony Creek (1.1 µg/L) had metolachlor concentrations above detection limits. Across all sites and sampling events, the highest concentration of metolachlor was measured at Little Killbuck in November 2010. In May 2011, only one site, Stony Creek, had any herbicides that were above detection limits with measureable concentrations of atrazine (1.1 µg/L) and metolachlor (1.3 µg/L).

Factors influencing herbicide concentrations.—Depth and discharge were positively correlated with both atrazine ($R=0.3472$ $p=0.0030$; $R=0.4228$ $p=0.0002$, respectively) and metolachlor concentrations ($R=0.3034$ $p=0.0101$; $R=0.3988$ $p=0.006$, respectively; Figure 3). Dissolved nutrient concentrations were also correlated with herbicide concentrations. Specifically, nitrate (NO_3^--N) and ammonium (NH_4^+-N) were positively correlated with atrazine ($R=0.6304$ $p=<0.0001$; $R=0.5114$ $p=<0.0001$, respectively) and metolachlor concentration ($R=0.5442$ $p=<0.0001$; $R=0.5483$ $p=<0.0001$, respectively; Figure 4). Temperature, pH, dissolved phosphate concentrations and sediment percent organic matter were not correlated with herbicide concentrations across sites ($p>0.1$).

DISCUSSION

Herbicide concentrations found in this study were comparable to other studies conducted within the United States. Specifically, Fenelon (1998) measured atrazine concentrations between 0.04–>10 µg/L and metolachlor concentrations between 0.005–10 µg/L in streams.

Similarly, David et al. (2003) measured atrazine concentrations between 0–17 μg/L and metolachlor between 0–3.4 μg/L in Illinois streams influenced by agriculture.

Although concentrations measured in this study were comparable to previous studies, metolachlor concentrations were higher than atrazine for 71% of the sampling sites across all sampling events, inconsistent with previous studies. Atrazine is thought to be more widely used in the United States, relative to metolachlor and previous studies of freshwater have yielded higher atrazine concentrations, relative to metolachlor. These higher metolachlor concentrations may either be due to higher usage rates or variability in sample collection timing (Thurman et al. 1992; David et al. 2003). However, one previous study conducted in Illinois, Iowa and Minnesota, found herbicide concentrations above detection limits even during base-flow conditions (Kalkhoff et al. 2003). This was not supported in our study as no herbicides were found above detection limits during the August sampling.

Higher concentrations of herbicides typically occur after high-flow conditions, particularly in spring (Thurman et al. 1992). These data support this observation as the greatest concentrations of herbicides were measured during the June 2010 sampling period. However, lower concentrations were measured in early spring (May) indicating a delay in herbicide runoff following early spring runoff. Land use and agricultural practices can also influence the abundance of pesticides and herbicides in streams (Fenelon 1998; David 2003).

Herbicide concentrations as low as 1 μg/L can have non-lethal effects on aquatic life (Hayes et al. 2002). These effects can range from being an endocrine disruptor to changing the behavior of a particular organism (Hayes et al. 2002; Wolf and Moore 2002; Cook et al. 2008). In this study, atrazine and metolachlor concentrations were measured at these effective concentrations in 58% and 81% of samples, respectively. Continued study of the abundance of herbicides in these streams is needed to identify periods of time of greatest concern.

ACKNOWLEDGEMENTS

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EFFECTS OF MULTIPLE AGRICULTURAL CHEMICALS ON NORTHERN LEOPARD FROG, *LITHOBATES PIPIENS*, LARVAE

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ABSTRACT. A primary factor contributing to amphibian declines is the application and accumulation of agricultural chemicals. We examined how a common fungicide, chlorothalonil, affects development in larval northern leopard frogs (*Lithobates pipiens*) in conjunction with atrazine and increased nitrate concentrations in laboratory containers. No synergistic or antagonistic interactions between the treatments were identified. Further, there was no significant difference in weight and Gosner stage between tadpoles exposed to different chlorothalonil concentrations. However, nitrate increased tadpole mortality and decreased growth. According to our results, environmentally relevant concentrations of chlorothalonil may not be directly toxic to leopard frogs but may indirectly influence development.

Keywords: amphibian toxicology, chlorothalonil, mortality, agricultural chemicals

Amphibians' semi-permeable skin, dependence on aquatic habitats, and limited dispersal ability are among the many factors that make these organisms susceptible to environmental contaminants (Blaustein et al. 2003). There is evidence that reduced abundance, lower diversity, and increased instances of malformations occur in intensive agricultural areas (Bonin et al. 1997; Hayes et al. 2002). Therefore, the monitoring of amphibian reactions to agriculturally-derived pollutants is important not only for the retention of biodiversity, but also as an indicator of biotic response to human activities. Chemical contamination is a primary contributor to decline of amphibian populations (Blaustein et al. 2003). Amphibians may be affected by these contaminants either directly, through increased mortality, or indirectly such as through changes in immune response (Blaustein et al. 2003) or sexual morphology (Hayes et al. 2003).

In 2008, Boone showed that interactive effects between agricultural chemicals can be present when individual effects are not. These data necessitate an assessment of synergistic and antagonistic toxicities to understand the complexity of threats to amphibian development. Because there is a complex group of potential stressors for amphibian populations, the control laboratory experiments afford is beneficial for identifying effects of agricultural chemicals on amphibians.

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The objective of this research was to identify effects of three common agricultural pollutants on amphibian development as well as synergistic and antagonistic interactions of these pollutants. Target compounds were selected to evaluate effects of nutrients (as nitrate), a common herbicide (atrazine), and a common fungicide (chloranthalonil). Although several studies have independently evaluated toxicity of atrazine and nitrate, few data examine interactions of these contaminants on amphibian development. Chlorothalonil is the second most used fungicide in the United States (Gianessi and Anderson 1995), and there is no data available analyzing chlorothalonil's synergistic and antagonistic effects with other agricultural chemicals (Winkler et al. 1996).

METHODS

Methods were similar to those used by Allran and Karasov (2000). 180 northern leopard frog (*Lithobates pipiens*) tadpoles were randomly distributed to laboratory containers (5 tadpoles per replicate) associated with twelve treatment groups (Table 1). Each treatment container was replicated three times for a total of 36 containers.

Table 1.—Chemical treatments and corresponding concentrations used in laboratory container experiments.

Chemical Treatment	Treatment Concentration	Reference
control	0	
chlorothalonil	0.5μg/L	Caux et al. 1996
chlorothalonil	1.0μg/L	
chlorothalonil	1.8μg/L	
atrazine	18μg/L	Solomon et al. 1996
nitrate	10mg/L	Allran and Karasov 2000
chlorothalonil + atrazine	0.5μg/L + 18μg/L	Caux et al. 1996; Solomon et al. 1996
chlorothalonil + atrazine	1.0μg/L + 18μg/L	
chlorothalonil + atrazine	1.8μg/L + 18μg/L	
chlorothalonil + nitrate	0.5μg/L + 10mg/L	Caux et al. 1996; Allran and Karasov 2000
chlorothalonil + nitrate	1.0μg/L + 10mg/L	
chlorothalonil + nitrate	1.8μg/L + 10mg/L	

Each replicate container consisted of a 1L HDPE container filled with 1000 ml deionized water. Water was changed every three days to ensure that chemical concentrations remained consistent and to remove waste. During water changes, a bulb syringe was used to suction out solid waste at the bottom of the containers. Then, water was decanted out of the tub until it reached a volume of 500ml. At this point, 500ml of fresh water was poured into the containers down the wall in order to minimalize splashing and stress to tadpoles. Target compounds were then added evenly across the water's surface. All containers were placed in an environmental incubator maintained at 23°C for the duration of the experiment. The light cycle consisted of 14 h of fluorescent lighting and 10 h darkness.

Treatment chemical concentrations (Table 1) were chosen to reflect typical ranges previously measured in freshwater ecosystems. Atrazine concentrations seldom exceed 20 μg/L *in situ* (Solomon et al. 1996). Nitrate concentrations are based on concentrations measured by Allran and Karasov (2000). Chlorothalonil concentrations have been measured up to 1.8 μg/L in agricultural areas (Caux et al. 1996). Chemical stressors were prepared using deionized water and stored at room temperature in acid-washed HDPE containers. Chemicals were added to the containers one day before tadpoles emerged from eggs. Tadpoles were added to laboratory containers immediately after they hatched from their eggs. All containers were checked at least every other day for deceased larvae, which were removed immediately. Tadpoles were fed *Xenopus* ground meal *ad libitum*.

Tadpoles were euthanatized by immersion in a tricaine solution at 250mg/L for 10 minutes. The experiment was terminated at day 35 and each tadpole was examined for any noticeable abnormality. Tadpoles were also weighed for wet body mass and assessed for Gosner developmental stage (Gosner 1960) using a dissection scope. All statistical comparisons of means were made with two-way ANOVA tests using IBM's Statistical Package for the Social Sciences (SPSS) v.18. Chlorothalonil concentration and additional chemical treatment (Atrazine, Nitrate) were treated as two separate factors. Separate two-way ANOVA tests were executed for each of the three dependent variables: mortality, Gosner stage, and weight. Least Significant Difference pairwise comparisons between treatments and chlorothalonil concentrations were used to elucidate differences between specific treatments and levels.

RESULTS

Terminated tadpoles ranged in weight from 0.4 to 4.8 grams. Gosner stage ranged from 27 to 38. There were no externally visible deformities. There was, as expected, a positive correlation between stage and weight (exponential regression, $p<0.001$, $F = 168.91$, $df = 1.81$, $R^2 = 0.676$).

Mortality was 53% during the 35 d experiment period. All chemical treatments produced higher mortality than the control groups (Fig. 1). The mortality rates were highest in treatments containing nitrate ($n = 4$) and lowest in chlorothalonil only groups ($n = 8$). All tadpoles in the nitrate-only treatment had died by day 35. All tadpoles in control

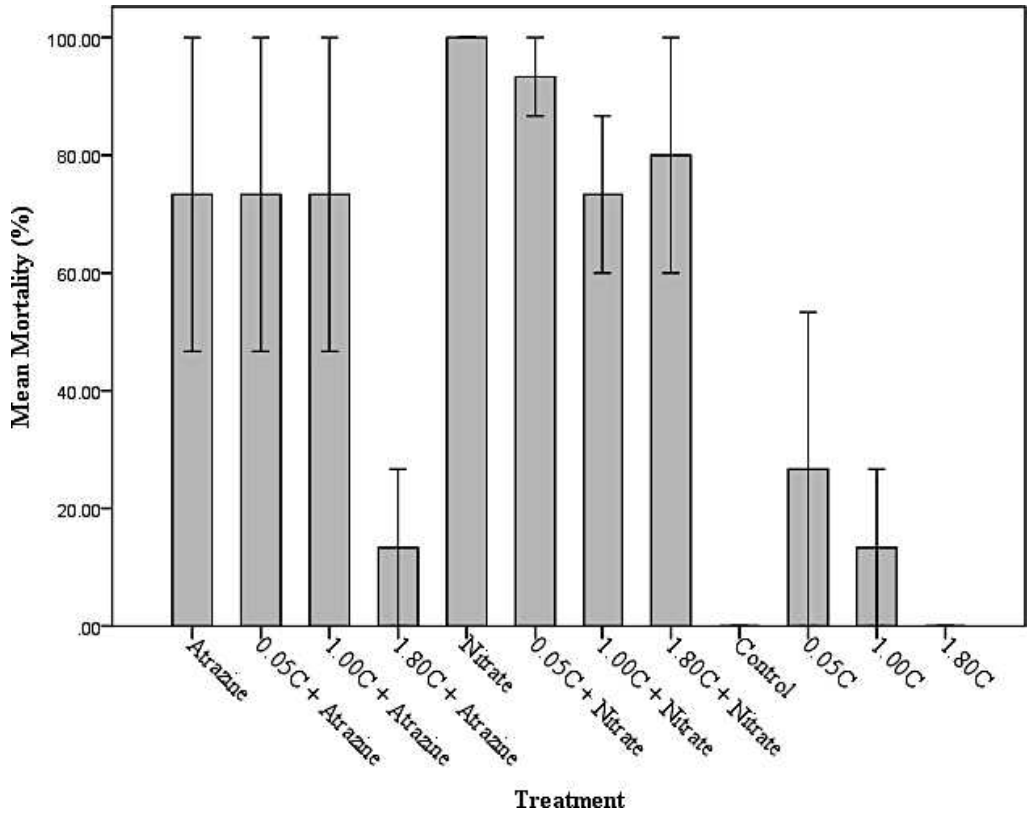

Figure 1.—Average mortality ± one standard error of *R. pipiens* tadpoles for all treatments with one standard error. For concentrations of all chemicals, see Table 1. C=chlorothalonil.

treatments survived the duration of the experiment. Mean mortality was significantly different between treatment groups ($F_{2,11} = 18.87$, $p < 0.001$). Chlorothalonil concentration had no significant effect on mortality ($F_{3,11} = 1.97$, $p = 0.146$). In addition, the interaction between treatment and chlorothalonil concentration had no significant ffect on mortality ($F_{6,11} = 0.92$, $p = 0.50$). Pairwise comparisons indicated that groups treated with Nitrate had higher mortality than groups treated with either Atrazine (Mean Difference = 28.33, SE = 12.62, $p = 0.034$) or chlorothalonil (MD = 76.67, SE = 12.62, $p < 0.001$). Additionally, chlorothalonil treatments had significantly lower mortality than Atrazine groups (MD = −48.33, SE = 12.62, $p = 0.001$).

Because only four containers that included nitrate held surviving tadpoles, nitrate was not included as a factor in both weight and Gosner stage analyses due to unacceptable sample size. The surviving individuals in chlorothalonil groups showed little variation in weight or Gosner stage between the treatments (Fig. 2). Tadpole weight was not significantly affected by treatment ($F_{1,7} = 3.39$, $p = 0.07$), chlorothalonil concentration ($F_{3,7} = 01.03$, $p = 0.383$), or the interaction between treatment and chlorothalonil concentration ($F_{3,7} = 0.51$, $p = 0.679$). Likewise, tadpole Gosner stage was not significantly affected by treatment ($F_{1,7} = 0.97$, $p = 0.33$), chlorothalonil concentration ($F_{3,7} = 0.22$, $p = 0.89$), or the interaction between treatment and chlorothalonil ($F_{3,7} = 1.37$, $p = 0.26$).

DISCUSSION

Our experiment identified no synergistic or antagonistic interactions between atrazine, nitrate, and chlorothalonil concentration. However, our study demonstrated that these three chemicals do influence amphibian mortality at environmentally-relevant concentrations. Our data indicate nitrate can cause direct mortality

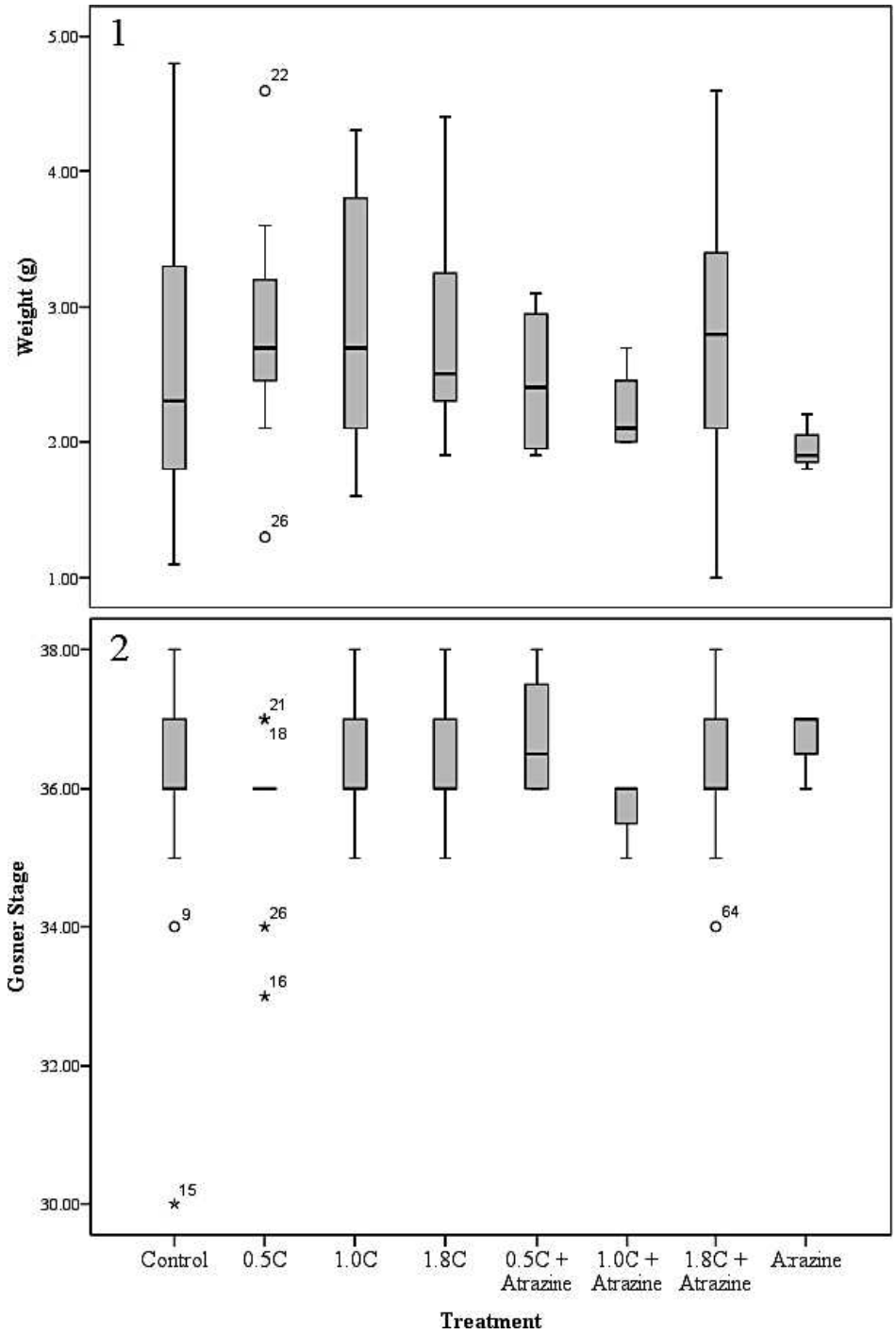

Figure 2.—Mean tadpole weight (1) and Gosner stage (2) of surviving tadpoles across chemical treatment treatments. For chemical treatment concentrations see Table 1. C=chlorothalonil, circles indicate outliers, asterisks indicate extreme values (> 3x the interquartile range), and all numbers represent individual cases.

in *L. pipiens*. Other studies using comparable nitrate concentrations identified variable responses among species (reviewed in Rouse et al. 1999). Allran and Karasov (2000, 2001) also demonstrated that nitrate slows development of *L. pipiens* larvae.

There was no significant difference in mortality, weight, or Gosner stage between tadpoles with different chlorothalonil concentrations. Our data show no evidence that low environmental concentrations of chlorothalonil in freshwater influence *L. pipiens* development. Previous field documentation of amphibian declines in response to chlorothalonil focused on cranberry bogs, where the chemical can bind to humic material and increase chlorothalonil exposure (Winkler et al. 1996). At the time of this experiment, the chlorothalonil concentration of 1.0μg/L was estimated to be the lowest observable effect concentration for several fish species. Larger chlorothalonil concentrations have been identified in runoff (~ 272μg/L, Shuman et al. 2000), and expected environmental concentrations have been calculated (164 μg/L, McMahon et al. 2011). McMahon et al. (2011) found significant mortality in *L. sphenocephalus* (Southern leopard frog) and *Hyla cinerea* (green treefrog) at concentrations as low as 0.0164 μg/L in laboratory experiments with single doses of chlorothalonil. However, McMahon et al. showed that *L. sphenocephalus* had significantly higher mortality at low and high concentrations compared to intermediate concentrations (~1.64 μg/L). The concentrations used in our experiment could have fallen in this intermediate zone of tolerance. Similar to chlorothalonil, atrazine treatments did not influence Gosner stage and tadpole weight when compared to nitrate and chlorothalonil treatments. This result is consistent with previous studies (Allran and Karasov 2000, 2001). Studies that have shown developmental deformities in *L. pipiens* from atrazine exposure have assessed higher concentrations (Hayes et al. 2003) or have been descriptive field assessments (Bonin et al. 1997), potentially influencing observed effects. However, atrazine is known to have indirect and sublethal effects that were not measured in the experiment (reviewed in Rohr and McCoy 2010).

One possible explanation for why there were no synergistic or antagonistic effects of chemical treatment could be the dissimilar modes of action of the three contaminants. Boone and Bridges-Britton (2006) suggested that non-additive results may be due to chemicals that show different modes of action. Given that other studies have found greater additive effect on *L. pipiens* with atrazine and alachlor (Howe et al. 1988), it was surprising that atrazine when combined with chlorothalonil had little effect on the weight and Gosner stage when compared to chlorothalonil-only treatments. From an ecological perspective, these chemicals also affect amphibians indirectly. Greater mortality in a natural wetland setting could be facilitated by direct chemical effects on both individual larvae and their food sources (Caux et al. 1996). Additionally, agrochemicals can increase amphibian susceptibility to parasites (Rohr et al. 2008). Future testing is required using chlorothalonil, but our data supports theories that chlorothalonil tolerance is highly species specific (McMahon et al. 2011), and that amphibian mortality is most likely in environments where chlorothalonil is allowed to bind to humic material, resulting in very large bioconcentrations (Winkler et al. 1996). The lack of synergistic and antagonistic effects of atrazine and nitrate is consistent with previous work (Allran and Karasov 2001).

Atrazine, nitrate, and recently chlorothalonil have both been hypothesized to be factors in amphibian reductions, but studies, including our data, indicate these chemicals do not cause negative consequences by themselves. However, there are many factors that influence amphibian larval development and many environmental situations that can lead to higher concentrations than those used in this experiment. For management purposes it is vital that contaminant studies continue to be done both in the field and in the laboratory to discover any and all negative consequences these chemicals may have on freshwater integrity.

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DIEL PATTERNS OF DISSOLVED OXYGEN AND SELECT CHEMICAL PARAMETERS IN THREE LAKES WITHIN HOOSIER NATIONAL FOREST THAT HAVE EXPERIENCED RECENT FISH KILLS

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ABSTRACT. Three lakes in Hoosier National Forest, Perry County, southern Indiana, were studied for changes in dissolved oxygen, temperature, pH, oxidation-reduction potential, salinity, specific conductance, and total dissolved solids because they have experienced recent fish kills. These lakes were evaluated for patterns in their chemical limnology over a 24–hr period during September 2005. The shallow nature of these three lakes has caused dramatic shifts in pH, dissolved oxygen, oxidation-reduction potential, and specific conductance. The largest changes in dissolved oxygen and pH occurred at dawn and dusk when aquatic plants switched from photosynthesis to respiration. Changes in oxidation-reduction and specific conductance were the result of the loss of dissolved oxygen in the lake sediment microzone. As the lake substrate changed from a reducing environment to an oxygenated environment, total dissolved solids and specific conductance increased as these materials were released into the water column. Low night-time dissolved oxygen levels and associated chemical stressors associated with aquatic plant respiration could explain the recent fish kills.

Keywords: oxidation-reduction, physio-chemical limnology, reservoirs, water quality

INTRODUCTION

Oxygen is an important attribute that is necessary for sustaining life. The decrease in dissolved oxygen during critical periods of species life history or during diel periods may cause stunting, a loss of reproductive capacity by adults, or mortality as a result of summer or winter kill (Brett 1979; Chapman 1986; Wesolowski 1996). Chapman (1986) found that prolonged exposures to less than 60% oxygen saturation may result in altered behavior, reduced growth, adverse reproductive effects, and mortality. Exposure to less than 30% saturation (~ 2 mg/L at summer temperatures) for one to four days causes mortality to most biota, especially during summer months, when metabolic rates are high. Stresses that can occur in conjunction with low dissolved oxygen (e.g., exposure to hydrogen sulfide or ammonia) may cause as much, if not more, harm to aquatic biota than exposure to low dissolved oxygen concentration alone (Chapman 1986). In addition, aquatic populations exposed to low dissolved oxygen concentration may be more susceptible to adverse effects of other stressors (e.g., disease, toxic substances) (Evans et al. 2004).

As biological assemblages of lakes are confronted with oxygen deficits during seasonal changes that may be due to increased temperature during summer, decreased depth due to lake eutrophication, and diel changes resulting in lower oxygen concentration because of plant respiration, known changes in assemblage structure are effected (Wetzel 2001). Numerous studies have shown that lower dissolved oxygen concentrations affect species composition, relative abundance, growth, maturation, and reproductive capacity.

Hoosier National Forest has experienced a series of summer fish kills (Anne Timm, U.S.

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Forest Service, personal communication). The exact causes of these fish kills were unknown; however, evidence did not suggest a species-specific effect since all species and all sizes were observed among the mortality.

Our objective was to describe the changes occurring in select chemical characteristics of three reservoirs in southern Indiana. Knowledge of this information influences our understanding of many aspects of the biota and many of the indices of productivity. This survey was designed to collect data on the chemical characteristics of three reservoirs in the Hoosier National Forest and to determine possible explanations for the fish kills.

MATERIALS AND METHODS

Description of the Study Area.—This study was conducted in the littoral zone of three lakes on Hoosier National Forest (Simon submitted). Measurements were made over a 24-hr period near the boat launch of Lakes Celina, Indian, and Tipsaw, Perry County, Indiana. Many of the lakes in southern Indiana were created by the damming of small streams and moderate sized rivers. These artificial systems were formed when steep valley ridges were closed and stream channels naturally flooded these areas. Maximum depths of the lakes (IDEM, 1996) range from 15 feet (Tipsaw Lake) to 38 feet (Celina Lake). The area is part of the Mitchell Plain (Schneider 1966) of the Interior Plateau Ecoregion (Omernik & Gallant 1988). The area is primarily natural and without anthropogenic impact, and is managed by the U.S. Forest Service, Hoosier National Forest.

Water Parameter Measurement and Study Design.—All measurements were made at the terminal end of the boat dock for each lake. Boat docks were well marked and were easy to navigate to in the dark. By providing a consistent position we ensured that the sample would be made at the same exact location throughout the study period. Since multiple crews were involved in the data collection it was necessary to ensure that measurements were comparable. The reservoirs of Hoosier National Forest comprise the only significant lakes occurring in Perry County. Measurements were made at the lake surface every two hours beginning at 15:00 on September 16, 2005 and concluding at 14:00 on September 17, 2005. Crews were divided into two-person units and were assigned specific times that the circuit would be collected. Lakes were sampled beginning at Lake Celina, then Indian Lake, and concluding at Lake Tipsaw. Each crew provided training and oversight of the crew following to ensure comparability and quality assurance of the information.

Water chemistry.—Two of the three lakes were too shallow to develop a stratified profile. Since most portions of each lake are less than 2 m in maximum depth it was not necessary to evaluate changes occurring in the deepest portion of the lake. These areas would have not been used by fish assemblages.

A digital meter (Dow Corning, Inc, Pocket Meter M90) was used to measure dissolved oxygen (precision and accuracy DO 0.0-20.00 $\pm$ 0.1 mg/L), temperature (-0.5 °C to 100 °C $\pm$ 0.1 °C), pH (0 to 14 $\pm$ 0.1 SU), salinity (0 to 20 $\pm$ 0.1 ppt), specific conductance (0.0 to 1999 $\pm$ 1 μS), and total dissolved solids (TDS; 0.0 to 1000 $\pm$ 0.1 mg/L). The oxidation-reduction potential (E_h) was measured using a digital meter (LaMotte, Inc, ORPTestr, -200 to 1100 $\pm$ 5 mv). Dissolved oxygen was calibrated using a Winkler titration (American Public Health Association 1989). Water samples were taken along shore near the boat launch within each lake.

RESULTS AND DISCUSSION

Patterns in Temperature and Oxygen.—Dissolved oxygen levels were highest during the late morning in Lake Celina and mid-afternoon in Lakes Tipsaw and Indian (Figures 1–3). Supersaturated dissolved oxygen levels were observed during mid-morning in Lakes Tipsaw (102% saturation at 9:45) and Celina (110% saturation at 10:08). The levels of dissolved oxygen generally followed predictable relationships with temperature, but differed during late afternoon in Lake Celina when saturation levels declined to 55.8% saturation during nocturnal periods (measured at 4:06). Indian Lake showed the lowest concentrations of dissolved oxygen with levels near or less than 50% saturation from midnight to dawn periods. Levels of dissolved oxygen followed predictable patterns with temperature for Lake Tipsaw. The lowest dissolved oxygen level was measured from Indian Lake from 2:06 to 3:10 when levels measured 0.8 mg/L.

Temperature and dissolved oxygen profiles typically show declining dissolved oxygen levels with increasing temperatures (Wetzel 2001). There are a variety of complex reasons that can

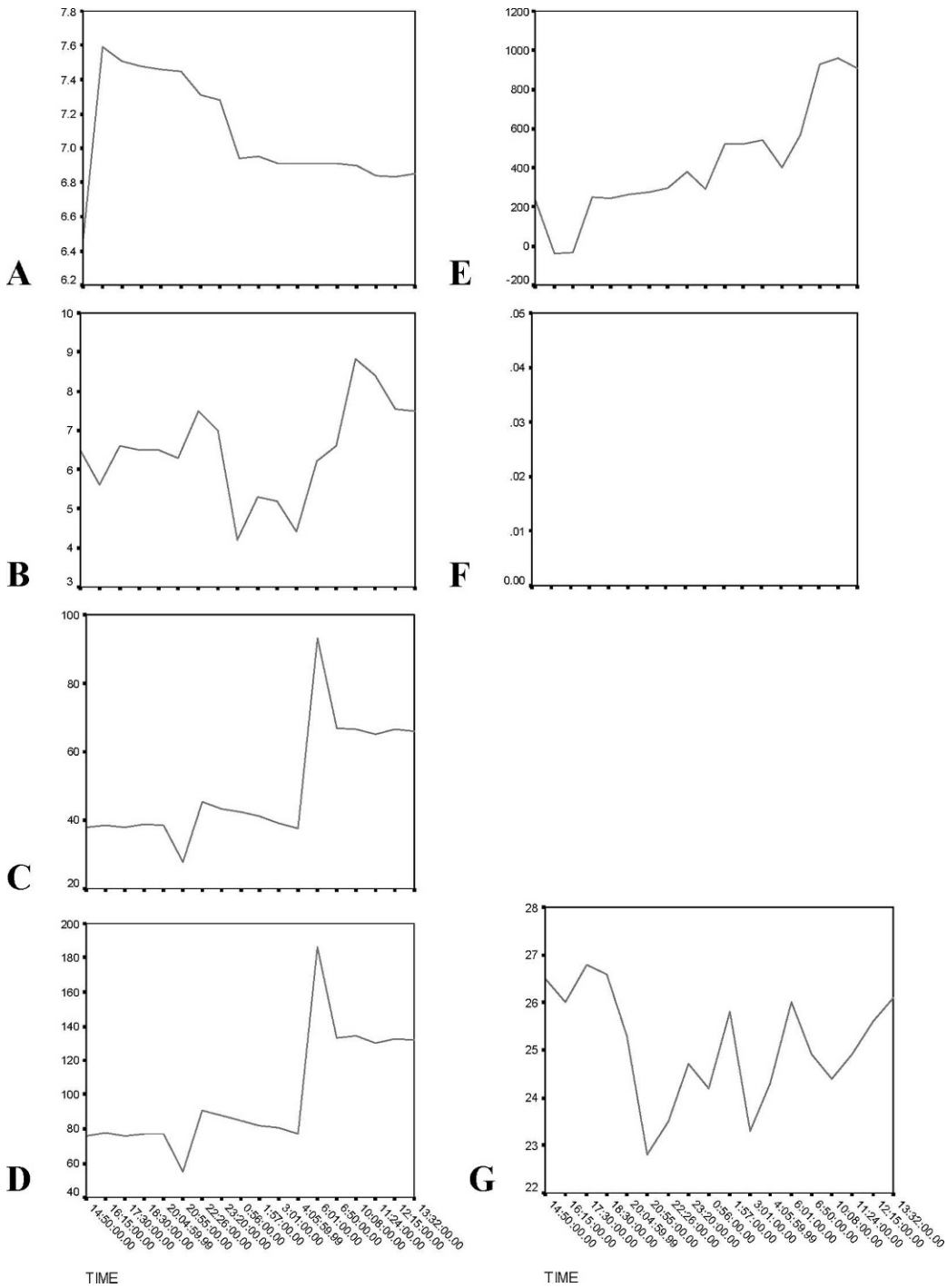

Figure 1.—Diel profiles for seven water quality variables in Lake Celina in September. A. pH (standard units), B. Dissolved oxygen (mg/L), C. Total dissolved solids (mg/L), D. Specific conductance (μS), E. oxidation-reduction potential (E_h), F. Salinity (ppt), and G. temperature (°C).

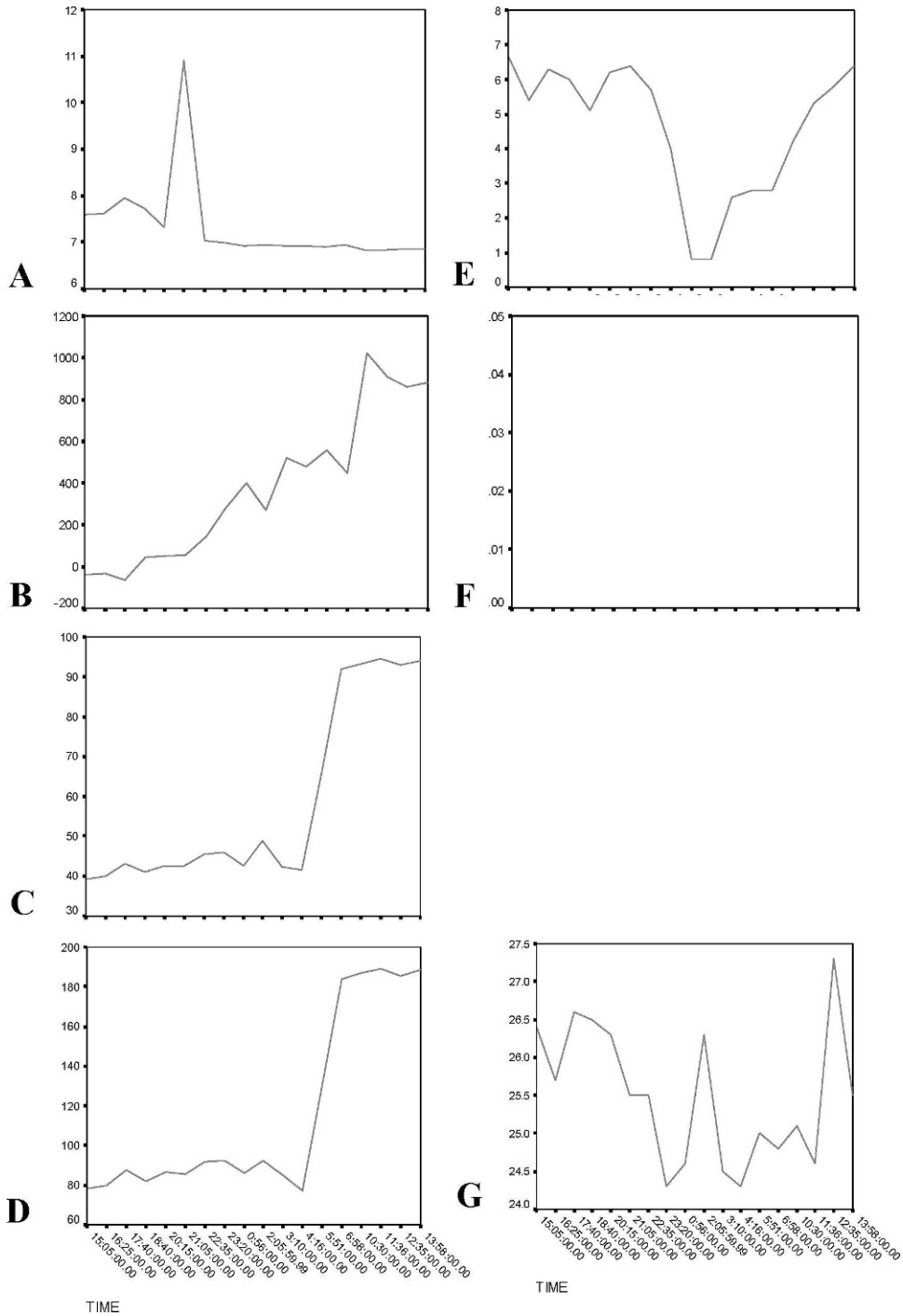

Figure 2.—Diel profiles for seven water quality variables in Indian Lake in September. A. pH (standard units), B. Dissolved oxygen (mg/L), C. Total dissolved solids (mg/L), D. Specific conductance (μS), E. oxidation-reduction potential (E_h), F. Salinity (ppt), and G. temperature (°C).

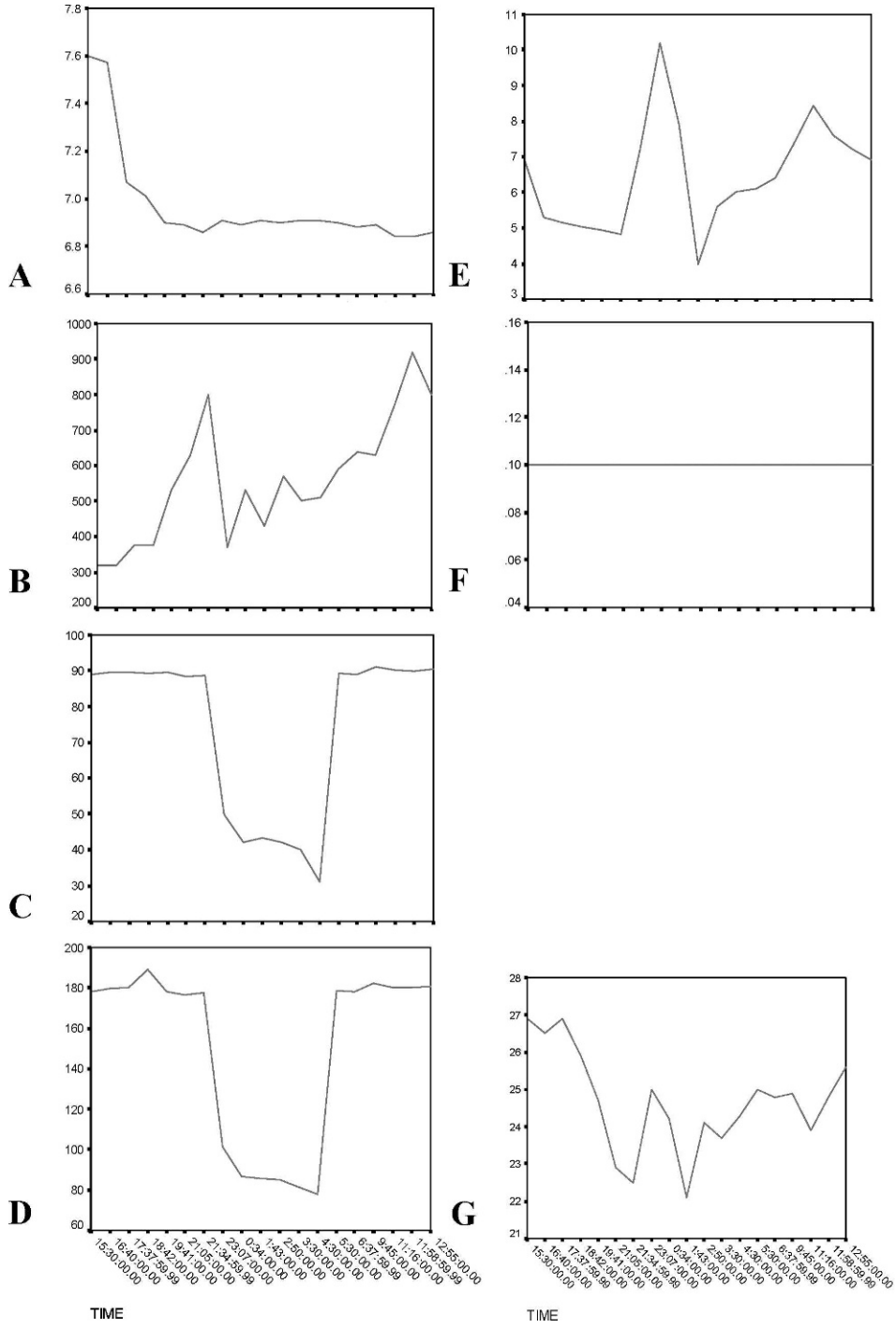

Figure 3.—Diel profiles for seven water quality variables in Tipsaw Lake in September. A. pH (standard units), B. Dissolved oxygen (mg/L), C. Total dissolved solids (mg/L), D. Specific conductance (μS), E. oxidation-reduction potential (E_h), F. Salinity (ppt), and G. temperature (°C).

explain dissolved oxygen profiles being significantly lower than predicted from temperature. These can be a result of increasing chemical or biological oxygen demand, chemical contaminants, biological respiration or a combination of these factors. Respiration of aquatic plants causes the greatest declines during nocturnal periods and increasing levels of dissolved oxygen levels during the peak photosynthetic periods. These dissolved oxygen patterns seem to follow most closely the patterns predicted by aquatic plant respiration. Phytoplankton levels are usually quite low in these lakes (IDEM, 1996), so macrophytic vegetation may be more important in regulating the diel variations.

During the 24-hr diel study, temperatures ranged from 24.3 to 27.3 °C in Indian Lake, 22.8 to 26.8 °C in Lake Celina, and 21.8 to 26.9 °C in Tipsaw Lake. The lowest temperature was measured during nocturnal periods in Indian Lake (4:16) and Tipsaw Lake (6:38), and dusk periods in Lake Celina (20:55). The highest temperature was measured during late afternoon in Lake Celina (17:30) and Tipsaw Lake (15:30 to 17:38), and mid-afternoon in Indian Lake (12:35).

Patterns in pH, Oxidation-Reduction Potential, Total Dissolved Solids, Specific Conductivity, and Salinity.—Diel variations in pH levels were observed in all three lakes (Figs. 1–3) with highest pH levels observed in all three lakes during mid-afternoon on 16 September and declining to lowest levels during the nocturnal and dawn periods. A single pH spike was observed in Indian Lake during dusk period (21:05) with a recorded pH of 10.91. This extreme measurement is not considered an outlier since measurements later dropped to normal pH levels by midnight. Standard units of pH ranged from 6.82 (10:30) to 7.95 (17:40) in Indian Lake, 6.84 (11:16 to 11:59) to 7.57 (16:40) in Tipsaw Lake, and from 6.46 (14:50) to 7.59 (16:15) in Lake Celina.

Oxidation-reduction potential (E_h) is an index of the exchange activity of electrons among elements in solution. A positive potential indicates oxidizing conditions in the water; a negative potential indicates reducing conditions, which determines the valence state of metals (Hem 1985). Reducing conditions were measured in all three lakes (Figures 1–3). The lowest reducing condition during the longest period of time was measured in Tipsaw Lake with reducing conditions during mid-afternoon to dusk periods (16:40–23:07). Reducing conditions were measured during late-afternoon in both Lake Celina (16:15–17:30) and Indian Lake (16:25–17:40). It is not known why extreme alkaline pH conditions were measured in Indian Lake; however, this corresponded to the change from reducing to oxidized conditions during dusk to nocturnal periods. This shift from reducing to oxidized conditions may have caused a valence change and a release of autochthonous materials from the sediment-water interface.

Specific conductance is a measure of the ability of a substance to conduct electricity across a unit length at a specific temperature. Dissolved substances increase the conductivity of water; measurements of specific conductance provide an indication of the amount of dissolved substances in water (Hem 1985). Specific conductance and total dissolved solids increased in Lake Celina (Fig. 1) and Indian Lake (Figure 2) from dawn to mid-afternoon and declined during dusk to dawn periods in Tipsaw Lake (Figure 3).

Salinity did not show any diel changes in any of the three lakes during this study (Figs. 1–3). Salinity was stable during the entire measurement period and showed no variability.

CONCLUSIONS

Three lakes in Hoosier National Forest, Perry County, southern Indiana, were studied for diel changes in dissolved oxygen, temperature, pH, oxidation-reduction potential, salinity, specific conductance, and total dissolved solids during the fall of 2005. The shallow nature of these three lakes has caused dramatic shifts in pH, dissolved oxygen, oxidation-reduction potential, and specific conductance. The largest changes in dissolved oxygen and pH occurred at dawn and dusk when aquatic plants switched from photosynthesis to respiration. Changes in oxidation-reduction and specific conductance were the result of the loss of dissolved oxygen in the lake sediment microzone. As the lake substrate changed from a reducing environment to an oxygenated environment, total dissolved solids and specific conductance increased as these materials were released into the water column. Low dissolved oxygen levels and associated chemical stressors associated with aquatic plant respiration could have been responsible for recent fish kills observed in the lakes.

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LAKE FISH ASSEMBLAGES IN HOOSIER NATIONAL FOREST, PERRY COUNTY, INDIANA, WITH EMPHASIS ON LAKE CONDITION ASSESSMENT

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ABSTRACT. Three lakes in Hoosier National Forest were created by the impounding of several creeks within the Middle Fork Anderson River watershed. All three lakes were created during the early 1960's to provide recreational opportunities for the public. Only a single study by the Indiana Department of Natural Resources provides data about a decade after the lakes were created on the historical fish assemblage of the lakes prior to reclamation. The current study found that the three reservoirs possess simple fish assemblage structure and are dominated by sunfish species. The lakes do not possess many benthic species or obligate lake species. The index of biotic sustainability classified the lakes as ''poor'' for all three lakes. Scores ranged from 28 to 31, with the mode 29. The majority of the sites in Hoosier National Forest lakes scored between 29 and 30 IBS points. Both Indian and Tipsaw lakes had sustainability scores of 30, while Celina Lake had a mean IBS score of 28.5. Tipsaw Lake ranked the highest with an IBS score of 31.

Keywords: Lacustrine wetlands, reservoirs, community structure, ecosystem function, sustainability

INTRODUCTION

Limited information concerning species composition and relative abundance of small reservoir fish assemblages is available for most areas of North America (Frey 1986). The Indiana Lakes and Streams Survey sampled many lakes in northeastern Indiana (Johnson 1945; Ricker 1945a,b; Ricker 1942a,b; Wohlschlag 1950; Gerking 1945, 1950a,b), but small reservoirs in southern Indiana were not surveyed. Many of these small reservoir lakes were created during the early- to mid-1960's for recreation, water storage, and drinking water prior to the golden days of the Lake and Stream Survey.

Small reservoirs in Hoosier National Forest have experienced an increasing number of fish kills, affecting species diversity and biological condition. Haas & Boston (1998) did not cite anthropogenic disturbance as the cause for concern; rather, they suggested that water level drawdown has influenced these effects across similar small recreational lakes. Generally, as reservoirs age, changes in the stability and sustainability of the biological assemblages occur. Productivity of reservoirs increases during the first decade as river and lacustrine fish species co-occur in the reservoir (Wetzel 1983). As recruitment of river species fails with time, lacustrine species dominate and increase until the carrying capacity of the reservoir is exceeded and declines are observed.

Fisheries management studies of Hoosier National Forest lakes were done between 1972 and 1987 by the Indiana Department of Natural Resources (Hottell 1977a,b; 1978a,b,c; Glander 1984; Glander & Burch 1988). Celina, Tipsaw, and Indian lakes were surveyed the most frequently; however, Saddle Lake and English Reservoir were also surveyed (Hottell 1977b, 1978c). With an increasing need to provide recreational opportunities for sport anglers, management of black basses, such as largemouth and smallmouth bass (*Micropterus salmoides* and *M. dolomieu*) and sunfish populations have received increasing attention. These species are dominant members of the fish assemblages of small reservoirs in southern Indiana. Recently, sampling intensity has increased providing the opportunity to evaluate trends in water quality and fish assemblage stability. Sampling was conducted between 1970–2005 for several projects, including the monitoring of game species populations by the

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Indiana Department of Natural Resources and the current study.

The purpose of this study is to document the distribution of the fish assemblages in small reservoirs of Hoosier National Forest; describe the species richness, structure, and function of fish assemblages; and document invasive species threats. In addition, there are many environmental threats that require further management such as sport fishing, water quality, watershed nutrients, and biological pollution of the lakes.

METHODS

Study area.—The need for recreation opportunities on public lands provided the impetus for creating reservoir lakes on public lands (Haas & Boston 1998). Lakes in the Hoosier National Forest are found in Perry County and impound a variety of stream tributaries including the Middle Fork Anderson River.

For this manuscript, I describe the fish assemblages of Celina, Tipsaw, and Indian lakes, which are part of the Middle Fork Anderson River watershed (Fig. 1). Many of the smaller ponds and shallow wetlands within the area were not sampled. I recognize that increased sampling in these small ponds and shallow wetland habitat may result in additional fish species being found, but I believe that the majority of species are documented in our studies from the main lakes.

Sample collection and reach selection.—Fish were collected using a representative sampling approach. Species are sampled in their relative abundance and not true abundance. Most fish sampling approaches are generally incomplete since individual fish cannot be seen, and rather rare species are usually under-sampled. My sampling approach used boat-mounted electrofishing equipment capable of applying 250–300 v, pulsed DC current, with 2–3 amps into the water. A single netter was positioned on the bow of the boat and, using a long-handled dip net, attempted to collect every individual fish that was seen. Fish were placed into a live-well until completion of the reach. All fish were identified using Gerking (1955), Smith (1979), or Becker (1983). Fish were counted and the maximum and minimum lengths were recorded (mm TL). Batch weights (g) were recorded for each species and each individual was inspected for deformities, eroded fins, lesions, and tumors (DELT) anomalies. Voucher specimens were deposited at the Division of Fishes, Aquatic Research Center, Indiana Biological Survey, Bloomington, Indiana.

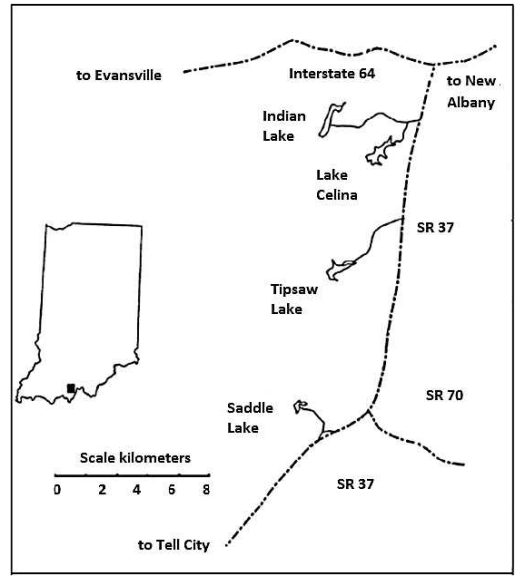

Figure 1.—Location of Celina, Tipsaw, and Indian lakes in Hoosier National Forest, Perry County, Indiana.

Reach selection was based on natural shoreline features of each water body. For example, intact riparian corridors consisting of wetlands, natural vegetation, or deciduous trees were preferred habitats over anthropogenically modified habitats such as boat slips, steel sheet piling, manicured lawns, or rock rip-rap. A project that developed reference conditions for southern Indiana lakes showed that areas with natural features had the greatest diversity and most natural fish assemblage attributes (Simon 2004). The number of reaches sampled within a single lake was determined by lake surface area (ha). Each lake reach was 500 m, which in the smallest lakes (Indian and Tipsaw) almost covered the entire shoreline. The smallest lakes (20–100 ha) were sampled at a minimum of two reaches.

Habitat parameters were measured using a qualitative measure of habitat that was based on a modified qualitative habitat evaluation index (Rankin 1989). The index is a composite of scores for each category based on a total of 100 points. Measures include substrate quality, instream habitat cover, watershed characteristics, shoreline development, littoral and profundal zone development and quality, depth, and measures of erosion and embeddedness.

Biological sustainability.—Lake management benefits from an estimate of condition, so that a reference or standard can be used to determine the waterbody quality. Karr et al. (1986) developed a quality assessment index for streams and flowing waters that relies on 12 attributes of stream fish assemblages. This same approach was instituted to evaluate the quality of lake-fish assemblages in southern Indiana reservoirs in the Interior River Lowland and Interior Plateau Ecoregions. Ten attributes of lake-fish assemblages were tested to develop a reference condition for lakes greater than 20 ha (Simon 2004); however since reservoirs are not natural systems an index of biological sustainability (IBS) was created consistent with reservoirs on the Tennessee River (McDonough & Hickman 1999).

The index attributes for each fish species occurring in northern Indiana lakes were based on published reproductive guilds (Simon 1999), trophic dynamics (Goldstein & Simon 1999), tolerance (Simon 1991), and habitat specialization characteristics. Each species was classified into the respective guild and species associations. These species memberships were then calibrated to formulate the sustainability or "least-impacted" condition.

RESULTS AND DISCUSSION

Historical changes.—Limited information is available to evaluate changes in the species richness of small reservoirs on Hoosier National Forest. The Indiana Department of Natural Resources (Hottell 1977a; Hottell 1978a,b; Glander 1984; Glander & Burch 1988) sampled Tipsaw, Celina, and Indian lakes. They found 14 species in Tipsaw Lake, 9 species in Lake Celina, and 13 species in Indian Lake (Table 1). Our unpublished sampling for Tipsaw Lake found 10 species in 2002 and 8 species in 2005, in Indian Lake 13 species in 2002 and 10 species in 2005, and in Lake Celina 10 species in 2002 and 6 species in 2005. The total combined fish list for Tipsaw Lake includes 15 species, for Lake Celina 14 species, and for Indian Lake 14 species. Upon completion of the dam for each lake, occurring in 1967, all fish in the watershed were eradicated. The Department of Natural Resources stocked largemouth bass, bluegill, redear sunfish (*Lepomis microlophus*), black crappie (*Pomoxis nigromaculatus*), and channel catfish (*Ictalurus punctatus*) in 1968 (Glander 1984).

Fish in Hoosier National Forest Lakes.—Twenty fish species in seven families have been collected from the three lakes in Hoosier National Forest (Table 1). The dominant family was the sunfish family, which was represented by eight species. Species diversity of the Hoosier National Forest has not changed appreciably since the lakes were created in the mid-1960's.

The three reservoirs have become more similar as a result of recreational requirements, patterns in eutrophication (which has caused the decline of former native species resident in the impounded creeks), and the decline of depth in the three lakes. All three lakes have a managed fish assemblage that reflects recreational fishing objectives. All three lakes are dominated by bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*). These two species are found throughout the three lakes.

Structure and function of fish assemblages in Hoosier National Forest reservoirs.—The managed fish assemblage of the Hoosier National Forest consists of a simple community structure that is designed to enhance sunfish and top-level carnivore production (Glander 1984). With the goal of increasing the numbers of bluegill, while largemouth bass were often highest in relative abundance, the remainder of the fish assemblage has become stunted. This has resulted in a loss of biological diversity, community structure, and biological sustainability for these water bodies. For example, the highest number of species based on a single collection in any of the lakes is 13 species in Lake Celina. Compared to regional expectations, the highest diversity observed in Lake Celina is equivalent to an average condition for a lake of similar size (Simon 2004).

The management of these three Hoosier National Forest lakes has caused an unstable community structure that is not sustainable. As species are managed towards panfish and top level carnivore production, larger numbers of forage base species and greater competition are needed to enhance growth. However, since carrying capacity restricts that number, the food chain becomes an inverted trophic pyramid. This inverted pyramid, instead of possessing a large forage base supporting a small top carnivore base, has a large number of top carnivores that are forced to feed on a small forage base. This type of stability can be

Table 1.—Listof fishes collected in three lakes in Hoosier National Forest by time periods. IDNR = Indiana Department of Natural Resources, INBS = Indiana Biological Survey. Numbers refer to relative abundance of each species while data in parentheses refer to lake (see below) and year collected. Lake Celina site 1 = 1, Lake Celina site 2 = 2, Tipsaw Lake site 1 = 3, Tipsaw Lake site 2 = 4, Indian Lake site 1 = 5, and Indian Lake site 2 = 6.

	IDNR		INBS	
Scientific name	1970–1976	1983–1987	2002	2005
Esocidae, pickerel & pikes				
Esox americanus, Grass pickerel			2(5)	1(6)
E. lucius, Northern pike	1 (5-1976)			
Cyprinidae, carps, minnows, & shiners				
Notemigonus crysoleucas, Golden shiner	2 (1-1976); 1 (5-1976)		6(3); 3(5)	2(6)
Pimephales notatus, Bluntnose minnow			6(1); 6(2)	
Catostomidae, suckers				
Catostomus commersonii, White sucker	45 (5-1976)		10(5)	
Ictaluridae, bullheads, madtoms, & catfish				
Ameiurus melas, Black bullhead		2(3-1983)	3(1); 1(6)	1(6)
A. natalis, Yellow bullhead	4 (1-1976); 6 (3-1976); 35 (5-1976)	1(3-1983); 4(5-1987); 2(3-1987)	11(5); 6(3)	1(5); 3(6)
A. nebulosus, Brown bullhead	17 (1-1976); 8 (3-1976)		1(2); 3(4)	
Ictalurus punctatus, Channel catfish	10 (1-1970); 19 (1-1972); 10 (1-1976); 10 (3-1972); 9 (3-1976); 2 (5-1976)	102 (3-1983); 1(5-1987)	7(1); 9(2); 2(5); 3(3); 5(4)	
Pylodictis olivaris, Flathead catfish		1(3-1987)		
Fundulidae. Topminnows				
Fundulus notatus, Blackstripe topminnow	1 (3-1976)			18(1); 9(2)

Table 1.—Continued.

Scientific name	IDNR 1970–1976	IDNR 1983–1987	INBS 2002	INBS 2005
Poeciliidae, livebearers				
Gambusia affinis, Eastern mosquitofish	1 (3-1976)			34(1); 15(2); 3(3); 2(4)
Centrarchidae, sunfish & black bass				
Lepomis cyanellus, Green sunfish	28 (1-1976); 9 (3-1976); 10 (5-1976)	16(3-1983)	16(2); 27(3); 8(4); 12(5)	6(3); 17(4); 14(5); 18(6)
L. gulosus, Warmouth	24 (3-1976); 57 (5-1976)	5(3-1983); 29(1-1987); 35(3-1987); 30(5-1987)	3(1); 9(2); 5 (3); 7(4); (5); 14(6)	3(6)
L. macrochirus, Bluegill	184(1-1970); 281(1-1972); 473(1-1976); 235(3-1972); 384(3-1976); 453(5-1976)	174(3-1983); 257(1-1987); 276(3-1987); 486(5-1987)	41(1); 56(2); 38(3); 37(4); 32(5); 78(6)	128(1); 221(2); 207(3); 132(4); 106(5); 59(6)
L. megalotis, Longear sunfish	19 (5-1976)	1(3-1983); 15(5-1987)	11(3); 14(5); 31(6)	2(4)
L. microlophus, Redear sunfish	48 (1-1976); 22 (3-1972); 12 (3-1976); 38 (5-1976)	23(3-1983); 21(1-1987); 65(3-1987); 74(5-1987)	17(1); 12(2); 12(3); 10(4); 17(6)	24(1); 16(2); 6(3); 4(4); 12(5); 18(6)
Micropterus salmoides, Largemouth bass	236(1-1970); 115(1-1972); 523(1-1976); 282(3-1972); 252(3-1976); 302(5-1976)	182(3-1983); 103(1-1987); 228(3-1987); 185(5-1987)	9(1); 17(2); 7 (4); 6(5); 14(6)	16(1); 19(2); 8(3); 11(4); 11(5); 7(6)
M. punctulatus, Spotted bass	2(5-1976)			
Pomoxis nigromaculatus, Black crappie	11(3-1976); 56(5-1976)	1(3-1983)	5(2); 1 (3); 3(4); 4(5)	2(3); 3(4); 1(6)

sustained for short periods of time (<10 years); however, such a system cannot produce continued top-level fish without causing cannibalism of recruits. The pyramid is a simple three-tiered structure with the fish community comprised of insectivores and top carnivores. The transfer of energy between the various levels of the pyramid is greatly reduced. The number of benthic species such as channel catfish (*Ictalurus punctatus*) and bullhead (*Ameiurus* spp.), and obligate lake species such as bowfin (*Amia calva*), has been reduced. As benthic species and obligate lake species decline in relative abundance, a need exists to conserve and recover these species. Promoting the return of biological species richness will assist in the maintenance of biological sustainability.

Local extirpations and new records.—Local extirpations observed in the three Hoosier National Forest lakes were a result of changes that occurred with reservoir aging. Species lost during the first decade were not commonly found and were probably a result of stocking from adjacent lakes or small streams in the area. For example, northern pike (*Esox lucius*) and spotted bass (*Micropterus punctulatus*) were first and last collected in 1976 from Indian Lake. Northern pike is a coolwater species and would not be expected to occur this far south naturally, while spotted bass would be the native black bass occurring in the Anderson River watershed (Simon 1997). The only record is based on a single individual. Flathead catfish (*Pylodictis olivaris*) is another species, typical of large rivers, that was first and last collected in 1987 as a single individual from Tipsaw Lake. As habitat changed from riverine to lacustrine, changes in substrate and dissolved oxygen concentration caused the extirpation of creek species. No new records of species have been recorded from any of the three watersheds since the preliminary study, suggesting that the rotenone treatment of the waterbodies prior to restocking was completely effective.

Condition of Hoosier National Forest lakes.—The condition of the three Hoosier National Forest lakes is based on biological sustainability of these systems. Biological sustainability is different from biological integrity since reservoirs are not natural systems. The index of biotic sustainability classification rated as "poor" for all three lakes (Fig. 2), while the scores ranged from 28 to 31, with the mode 29. The majority of the sites in Hoosier National Forest lakes scored 29 or 30 IBS points (66.7% of IBI scores, n = 6 collection events). Both Indian and Tipsaw lakes had sustainability scores of 30, while Celina Lake had a mean IBS score of 28.5. Tipsaw Lake ranked the highest with an IBS score of 31. The loss of high quality biological conditions among the lakes in the Hoosier National Forest is not a result of littoral habitat loss, but rather a loss of profundal zone due to the loss of depth. All three lakes are experiencing rapid depth loss due to sedimentation of the highly erodible soils in the upper portions of the watersheds. All three lakes have a larger percentage of littoral habitat compared to profundal habitat, which provides increasing area for aquatic plants (Simon et al. submitted, a). Rapid cycling of dissolved oxygen concentrations in these areas, as plants change from photosynthesis to respiration during the night, has caused low dissolved oxygen levels that have resulted in summer kill (Simon et al. submitted, b). All three lakes are dominated by sunfish species and few other species were collected during the 2005 survey.

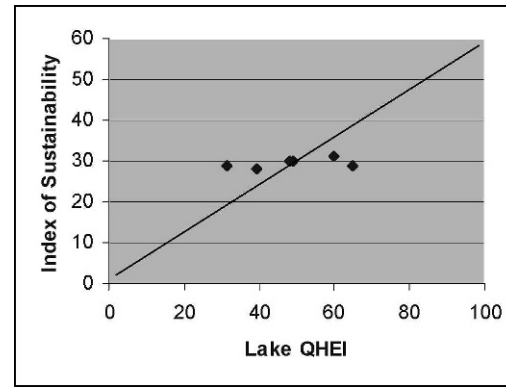

Figure 2.—Hypothesized relationship between index of biological sustainability and lake qualitative habitat evaluation index (QHEI) (black line) and observed scores for three lakes in Hoosier National Forest. The observed relationship between fish diversity and habitat does not follow the expected model.

Trends in lake condition showed that all three lakes have remained stable during the last 35 years (see appendix A–C). Indian Lake had the highest sustainability with scores ranging from 28 to 38, followed by Tipsaw Lake (25–38) and Lake Celina (25–34). Although the sustainability scores are declining for all three

lakes, the decline is not significant from the mean for each lake. This suggests that the variability associated with each lake is sufficient to explain the variability between years.

Relationships with habitat.—Barbour & Stribling (1991) showed that an index of biological integrity was directly related with habitat quality in streams and flowing water. In the absence of pollutants or other anthropogenic disturbance, the quality of fish assemblages should be directly related to habitat. It would not be possible for fish assemblages in lakes to exhibit high quality condition without the presence of natural conditions. The three lakes in Hoosier National Forest showed no relationship with habitat consistent with the model proposed by Barbour & Stribling (1991)(Fig. 2). Habitat and biological sustainability was skewed for the three lakes. Although riparian habitat scored the highest for regionally similar reservoirs in the Interior River Lowland or Interior Plateau Ecoregions, substrates, profundal zone quality, and instream cover were reduced compared to other reservoirs.

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APPENDIX A

Celina Lake metrics for all available years from 1970–2005. DELT = Deformities, Eroded fins, Lesions, and Tumors (DELT), VP = Very Poor, P = Poor.

Metrics	1970	1972	1976	1987	2002	2005		
Total number of species	3 (1)	3 (1)	9 (3)	4 (1)	7 (1)	9 (3)	6 (1)	6 (1)
Number benthic species	1 (1)	1 (1)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Number centrarchid species	2 (1)	2 (1)	4 (3)	2 (1)	4 (3)	5 (3)	4 (3)	4 (3)
Percent tolerant individuals	0 (5)	0 (5)	4.6 (5)	0 (5)	11 (5)	17 (5)	0 (5)	0 (5)
Percent omnivore individual	0 (5)	0 (5)	0 (5)	0 (5)	7 (5)	5 (5)	0 (5)	0 (5)
Percent insectivore individual	45 (3)	72 (5)	53 (3)	75 (5)	90 (5)	87 (5)	94 (5)	92 (5)
Percent carnivore individuals	55 (1)	28 (5)	47 (1)	25 (5)	10 (2)	13 (3)	6.7 (1)	6.6 (1)
Relative abundance	430(3)	415(3)	1105(5)	410(3)	86 (1)	131 (1)	229 (2)	288 (3)
Percent lake obligate individuals	0 (0)	0 (0)	1.6 (1)	7.1 (1)	3.5 (1)	7.6 (1)	3.9 (1)	2.8 (1)
Percent DELT	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)
TOTAL Sustainability score	**25**	**31**	**34**	**31**	**29**	**31**	**28**	**29**
Classification	**P-VP**	**P**	**P**	**P**	**P**	**P**	**P**	**P**

APPENDIX B

Tipsaw Lake metrics for all available years from 1970–2005. DELT = Deformities, Eroded fins, Lesions, and Tumors (DELT), VP = Very Poor, P = Poor, F = Fair.

Metrics	1972	1976	1983	1987	2002	2005		
Total number of species	4 (1)	10 (3)	9 (3)	6 (1)	10 (3)	8 (3)	7 (2)	8 (3)
Number benthic species	1 (1)	3 (3)	3 (3)	1 (1)	2 (1)	2 (1)	0 (0)	0 (0)
Number centrarchid species	3 (1)	7 (5)	7 (5)	3 (1)	5 (3)	5 (3)	6 (3)	7 (5)
Percent tolerant individuals	0 (5)	2.5 (5)	6.7 (5)	0.4 (5)	39 (1)	7.3 (5)	2.5 (5)	9.6 (5)
Percent omnivore individuals	0 (5)	0 (5)	0 (5)	7 (5)	5 (5)	0 (5)	0 (5)	0 (5)
Percent insectivore individuals	49 (3)	63 (3)	41 (3)	58 (3)	100 (5)	90 (5)	97 (5)	94 (5)
Percent carnivore individuals	51 (1)	37 (3)	59 (1)	42 (1)	0 (0)	10 (1)	3.4 (1)	6.2 (1)
Relative abundance	549(5)	685(5)	310(3)	541 (5)	100 (1)	67 (1)	236 (2)	177 (1)
Percent lake obligate individuals	0 (0)	0.8 (1)	1.6 (1)	6.5 (1)	5 (1)	15 (3)	1.7 (1)	3.4 (1)
Percent DELT	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)
TOTAL Sustainability score	**27**	**38**	**34**	**28**	**25**	**32**	**29**	**31**
Classification	**P-VP**	**P-F**	**P**	**P**	**P-VP**	**P**	**P**	**P**

APPENDIX C

Indian Lake metrics for all available years from 1970–2005. DELT = Deformities, Eroded fins, Lesions, and Tumors (DELT), F= Fair, P = Poor, VP = Very poor.

Metrics	1976	1987	2002		2005	
Total number of species	12 (3)	7 (3)	12 (3)	6 (1)	8 (3)	10 (3)
Number benthic species	3 (3)	2 (1)	4 (3)	1 (1)	1 (1)	2 (1)
Number centrarchid species	8 (5)	2 (1)	4 (3)	5 (3)	6 (3)	6 (3)
Percent tolerant individuals	1 (5)	0 (5)	13 (5)	0 (5)	9 (5)	18 (5)
Percent omnivore individuals	4.4 (5)	0 (5)	10.8(5)	0 (5)	0 (5)	1.7 (5)
Percent insectivore individuals	66 (3)	77 (5)	81 (5)	91 (5)	93 (5)	92 (5)
Percent carnivore individuals	30 (3)	23 (5)	10 (3)	9 (1)	6.5 (1)	6.7 (1)
Relative abundance	1021(5)	795(5)	115 (1)	155 (1)	169 (1)	120 (1)
Percent lake obligate individuals	5.6 (1)	3.8 (1)	7.8 (1)	9.0 (1)	5.3 (1)	2.5 (1)
Percent DELT	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)
TOTAL Sustainability score	**38**	**36**	**34**	**28**	**30**	**30**
Classification	**F-P**	**F-P**	**P**	**P**	**P**	**P**

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PHYLOGENETIC SPECIES IDENTIFICATION OF *PILOBOLUS* ASSOCIATED WITH HORSES IN INDIANA AND OHIO

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ABSTRACT. *Pilobolus*, a coprophilous zygomycete, is associated with herbivores. The various species of this fungus have been collected from a wide range of hosts. Most species have been isolated from multiple hosts. However, *P. longipes* has been reported exclusively associated with *Equus* spp. This study was undertaken to examine the species specific relationship of *P. longipes* and members of *Equus,* specifically *E. caballus* (horses). Dung samples were collected from horses located within 25 miles of Richmond, Indiana for isolates of *Pilobolus*. Sporangiospores from these fungal isolates were used as the source of DNA. Sequences of taxonomically informative 18S and ITS regions of rDNA were obtained using previously published protocols. DNA sequences were aligned with BLAST and compared with sequences deposited in GenBank. Sequences of DNA from the isolates in this study were examined using MEGA 4 with Clustal W. By comparing the sequences of DNA from isolates in this study with those in GenBank, it was determined that *P. longipes*, *P. sphaerosporus*, *P. kleinii* and *P. pullus* are associated with horses in Indiana and Ohio. All isolates recovered in this study are large-spore producing species of *Pilobolus*.

Keywords: *Pilobolus*, horses, DNA, ITS, 18S

INTRODUCTION

The coprophilous fungus, *Pilobolus*, is a well known, but little studied organism. Because of its dramatic ballistic spore discharge mechanism and its photogenic qualities, *Pilobolus* is mentioned in many general biology texts and is often given a lot of space in mycology texts (Buller 1934, Yafetto et al. 2008, Page 1962, 1964). However, other than these characteristics, little is known about the environmental conditions most conducive to its growth, and while having been collected and described from many locations worldwide, few correlations have been drawn about the growth of the fungus in relationship to its hosts, climate, nutrition, economic value, parasitic or symbiotic relationships with other organisms.

Pilobolus has been reported associated with many herbivores including virtually all ungulates, a large number of rodents, and various other mammals (Hu et al. 1989, Santiago et al. 2008). However, there seems to be almost no correlation between most species of *Pilobolus* and a particular species of host. However, *P. longipes* has been reported only associated with the genus *Equus* (horses, zebras, donkeys). This study was designed to examine the specificity of this relationship of the various species of *Pilobolus* and *Equus caballus* (horses) and to determine whether *P. longipes* is the most frequently occurring species found in association with horses. *Pilobolus* isolates associated with each of the horses in this study were identified to species using molecular techniques.

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METHODS AND MATERIALS

Isolates of *Pilobolus* were collected from the dung of horses in Ohio and Indiana and cultivated in microcosms until sporangia were produced. Mature sporangia were collected and maintained in collecting water using techniques described previously (Foos 1989, Foos & Royer 1989, Foos et al. 2001).

It has been shown that multiple species of *Pilobolus* exist simultaneously in dung (Foos 1997), so multiple sporangia were collected from each dung sample. When two or more isolates from the same dung sample were morphologically identical, only one was maintained for further study.

Pure cultures were obtained using single sporangium transfers to plastic Petri dishes containing dung agar or synthetic hemin medium (SHM) (Levetin &Caroselli 1976). Cultures arising from sporangial transfers were

maintained on SHM or dung agar in disposable plastic Petri dishes sealed with parafilm at 22 ± 2 °C with alternating 12 h light and dark periods of 2000 lux, cool white fluorescent illumination. Sporangia from pure culture isolates were examined microscopically and tentatively identified to species using morphological characteristics.

Individual sporangia which adhered to the lids of the Petri dishes were collected using sterile inoculating needles and placed in 0.2 ml microcentrifuge tubes containing 20 µl sterile collecting water (with 3% penicillin, 3% streptomycin, and 1% Tween 20), labeled and stored at 4 °C. DNA was extracted from the sporangiospores using techniques described previously (Foos et al. 2011).

The primers designed specifically to amplify and sequence taxonomically significant DNA fragments were used (White et al. 1990). Primers NS1 – NS8 were used to amplify the nuclear 18S small subunit (SSU) of rRNA and primers ITS5 and ITS4 were used to amplify the entire ITS region of nuclear rRNA, specifically the 5.8S region, the associated internal transcribed spacers (ITS1 and ITS2), with terminal portions of the 18S and 28S regions of rRNA.

DNA was amplified using AmpliTaq® Gold DNA polymerase (Applied Biosystems, Foster City, California) and a dNTP mix (Promega Corporation, Fitchburg, Wisconsin). Thermal cycling was conducted in a Perkin Elmer GeneAmp® PCR System 2400. PCR reaction conditions for thermal cycling were 94 °C for 5 min, followed by 36 cycles of 94 °C for 1 min, 50 °C for 1 min 30 sec, 72 °C for 2 min, followed by an extension at 72 °C for 7 min. PCR products were purified with QIAquick® PCR Purification Kit (Qiagen, Inc., Valencia, California).

PCR amplified DNA fragments were electrophoresed prior to and following the clean up process, on 1% agarose gels in 1X TBE buffer (50 mM Tris-HCl, 50 mM boric acid, and 1 mM EDTA) containing ethidium bromide and visualized using a ChemiImager™ 4400 Imaging System (Alpha Innotech, San Leandro, California). A 100 bp DNA ladder (Takara Mirus Bio, Madison, Wisconsin) was used as a size marker.

PCR products were sequenced with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and a sequence reaction mix comprised of 2 µl H_2O, 3 µl 5X buffer, 1 µl BigDye, 2 µl 10 mM primer, and 2 µL fungal DNA. Thermal cycling conditions for sequencing were 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Sequences were analyzed using an Applied Biosystems 3700 automated fluorescence system at the Indiana Molecular Biology Institute.

DNA sequences were examined and compared using CodonCode Aligner (CodonCode Corp., Dedham, Massachusetts) containing PHRED and PHRAP (Ewing &Green 1998, Ewing et al. 1998) for base calling, sequence comparisons and sequence assembly. Contigs created in CodonCode were oriented with BLAST (Altschul et al. 1990) and aligned using Clustal W (Thompson et al. 1994, 1997). Phylogenetic and molecular evolutionary analyses were conducted and trees constructed using MEGA version 4 (Tamura et al. 2007).

Evolutionary histories of both 18S and ITS rDNA sequences were inferred using the neighbor-joining method (Saitou &Nei 1987). The bootstrap consensus tree inferred from 2000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test were shown next to the branches (Felsenstein 1985). The trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using maximum composite likelihood (Tamura et al. 2004) and are in the units of the number of base substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons.

RESULTS

Fifteen isolates of *Pilobolus* were obtained from horse dung in Ohio and Indiana. Locations, collection dates, and voucher numbers of these isolates are listed in Table 1. *Pilobolus kleinii* Tiegh., *P. longipes* Tiegh., *P. pullus* Massee and *P. shpaerosporus* Palla were isolated from this dung. DNA sequences of both 18S and ITS regions were analyzed from each isolate, except that the sequences from the 18S region of IUE 0018 and the ITS regions of IUE 0006 and IUE 0021 could not be recovered.

Table 1.—Specimens of *Pilobolus* isolated from horse dung in Ohio and Indiana listed by IUE voucher numbers, collection dates and locations.

Voucher Number	Date	Location	GPS-N	GPS-W	Species
IUE0002	5/2/2004	Fayette Co., IN	39° 38.798′	85° 12.860′	*P. sphaerosporus*
IUE0004	5/8/2004	Union Co., IN	39° 38.570′	84° 49.920′	*P. kleinii*
IUE0005	5/8/2004	Union Co., IN	39° 36.709′	84° 53.225′	*P. kleinii*
IUE0006	5/8/2004	Union Co., IN	39° 38.815′	84° 54.507′	*P. kleinii*
IUE0007	5/22/2004	Wayne Co., IN	39° 52.008′	85° 09.714′	*P. sphaerosporus*
IUE0009	5/24/2004	Preble Co., OH	39° 51.976′	84° 30.283′	*P. kleinii*
IUE0010	6/14/2004	Wayne Co., IN	39° 57.501′	85° 06.160′	*P. pullus*
IUE0013	6/14/2004	Wayne Co., IN	39° 57.869′	85° 05.983′	*P. sphaerosporus*
IUE0014	6/14/2004	Wayne Co., IN	39° 59.574′	85° 05.329′	*P. pullus*
IUE0015	6/14/2004	Wayne Co., IN	39° 56.831′	85° 04.044′	*P. sphaerosporus*
IUE0016	7/12/2004	Darke Co., OH	39° 56.079′	84° 30.305′	*P. longipes*
IUE0017	7/12/2004	Darke Co., OH	39° 55.947′	84° 29.859′	*P. pullus*
IUE0018	7/12/2004	Darke Co., OH	39° 58.577′	84° 32.364′	*P. sphaerosporus*
IUE0020	7/12/2004	Darke Co., OH	39° 57.774′	84° 33.122′	*P. longipes*
IUE0021	7/12/2004	Preble Co., OH	39° 52.144′	84° 43.396′	*P. sphaerosporus*

Phylogenetic species identification using phylograms.—Phylograms were created using orthologous sequences from six species of *Pilobolus* obtained from GenBank as controls. These DNA sequences from these GenBank ex-type specimens were used as a phylogenetic species key.

The phylogram (Fig. 1) is inferred from the rDNA sequences that code for the 18S region of rRNA of the isolates. Sequences from the isolates in this study form a clade with large-spore producing species of *Pilobolus* from GenBank (*P. kleinii*, *P. longipes* and *P. sphaerosporus*), distinct from the clade with small-spore producing species from GenBank (*P. crystallinus*, *P. roridus* and *P. umbonatus*). Two subordinate or sister clades formed within the major clade of large-spore producing species. One contains *P. sphaerosporus* (DQ211052) from GenBank and isolates of *P. sphaerosporus* and *P. pullus* from this study. *Pilobolus pullus* forms a subordinate clade distinct from that of *P. sphaerosporus*. The other clade contains *P. longipes* (DQ211053), and *P. kleinii* (EU595656) from GenBank and specimens from this study. There is strong bootstrap support [100%] for these clades as represented in the phylogram.

The phylogram (Fig. 2) inferred from the rDNA sequences that code for the ITS region of rRNA is very similar to the phylogram inferred from the sequences for the 18S region (Fig. 1). One major clade includes all of the large-spore producing species from GenBank (*P. kleinii*, *P. sphaerosporus*, *P. longipes* and *P. heterosporus*) and sequences from specimens from this study. (The ITS sequence from *P. heterosporus* (HM049582) in GenBank is included. There is no 18S sequence of this species in GenBank or other DNA sequence repository.) The small spore-producing species from GenBank (*P. crystallinus*, *P. roridus* and *P. umbonatus*) are outside this clade. This major clade includes subordinate clades which contain all of the specimens from this study. *Pilobolus sphaerosporus* (DQ059382) from GenBank and specimens from this study form one subordinate clade. The isolates of *P. kleinii* and *P. pullus* are in a second subordinate clade, while *P. longipes* (FJ160950) and *P. heterosporus* (HM049582) from GenBank and specimens from this study form a third clade. The inferences from these distinct clades are supported by strong bootstrap values.

Phylogenetic species identification using homology.—Species identification using sequence identity of homologous regions was recently reported for *Pilobolus* (Foos & Sheehan 2011). Representative sequences of all species of *Pilobolus* deposited in GenBank were used as controls representing these species. Percentage identity of both the homologous 18S and ITS regions were examined.

When comparing specimens of various species of *Pilobolus*, homologous 18S regions of rDNA from GenBank representatives had $>$ 97% identity. Table 2 shows the percentage

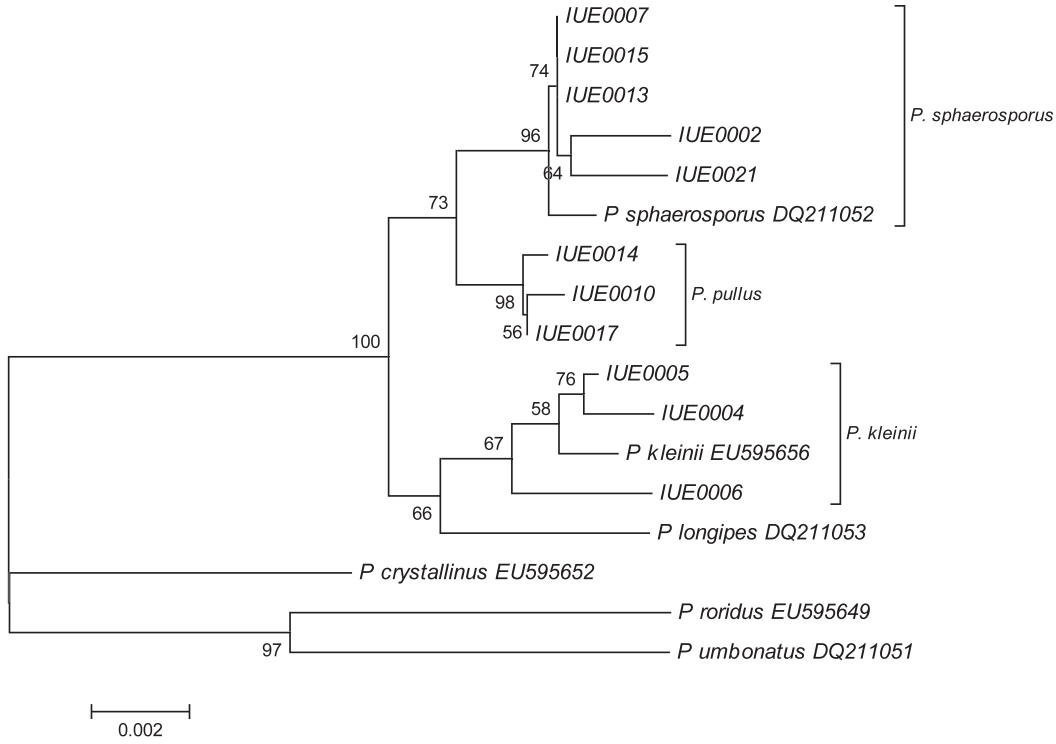

Figure 1.—Evolutionary relationships of *Pilobolus* based on 18S rDNA. The analysis involved 17 nucleotide sequences; ambiguous positions were removed for each sequence pair. There were a total of 1768 positions in the final dataset.

identities of the homologous 18S regions of rDNA for specimens from this study compared with the orthologous regions of GenBank specimens. The sequence homology of the three isolates of *P. kleinii* from this study and *P. kleinii* (EU595656) from GenBank displayed $>$ 99.6% identity. Some variation may be attributed to particular strains of the species or associated with the molecular techniques used to amplify and sequence the DNA.

The three isolates of *P. sphaerosporus* were identical to each other and had $>$ 99.8% identity with *P. sphaerosporus* (DQ211052) from GenBank. The other two isolates of this species were unique, yet had $\geq$ 99.6% identity with the GenBank control.

In all instances the identities of intraspecies homologous 18S rDNA regions from isolates from this study and from GenBank sequences were $\geq$ 99.6%. Interspecies homologies of the same 18S regions from the GenBank specimens had $<$ 99.3% identity.

Table 3 shows the percentage identities of the homologous ITS regions of rDNA for specimens from this study compared with the orthologous regions of GenBank specimens.

The identities of the homologous ITS regions of the three specimens of *P. kleinii* from this study and the GenBank control (FJ160957) were $>$ 95.4%. The homologous ITS regions of the two specimens of *P. longipes* from this study and GenBank (FJ160950) displayed $>$ 97.7% identity. The homologous ITS regions of *P. sphaerosporus* specimens from this study had $>$ 89.4% identity with GenBank (DQ059382). The homologous ITS regions of the three isolates of *P. pullus* had $>$ 99.8% identity to each other. No sequences from isolates of *P. pullus* are present in GenBank or other DNA sequence repository, so these isolates were compared with sequences from all species of *Pilobolus* for which sequences were available. When compared to the homologous ITS regions of the four large-spore producing species of *Pilobolus* in GenBank, including *P. heterosporus* (HM049582), the *P. pullus* sequences displayed 78.3 – 83.2% identity.

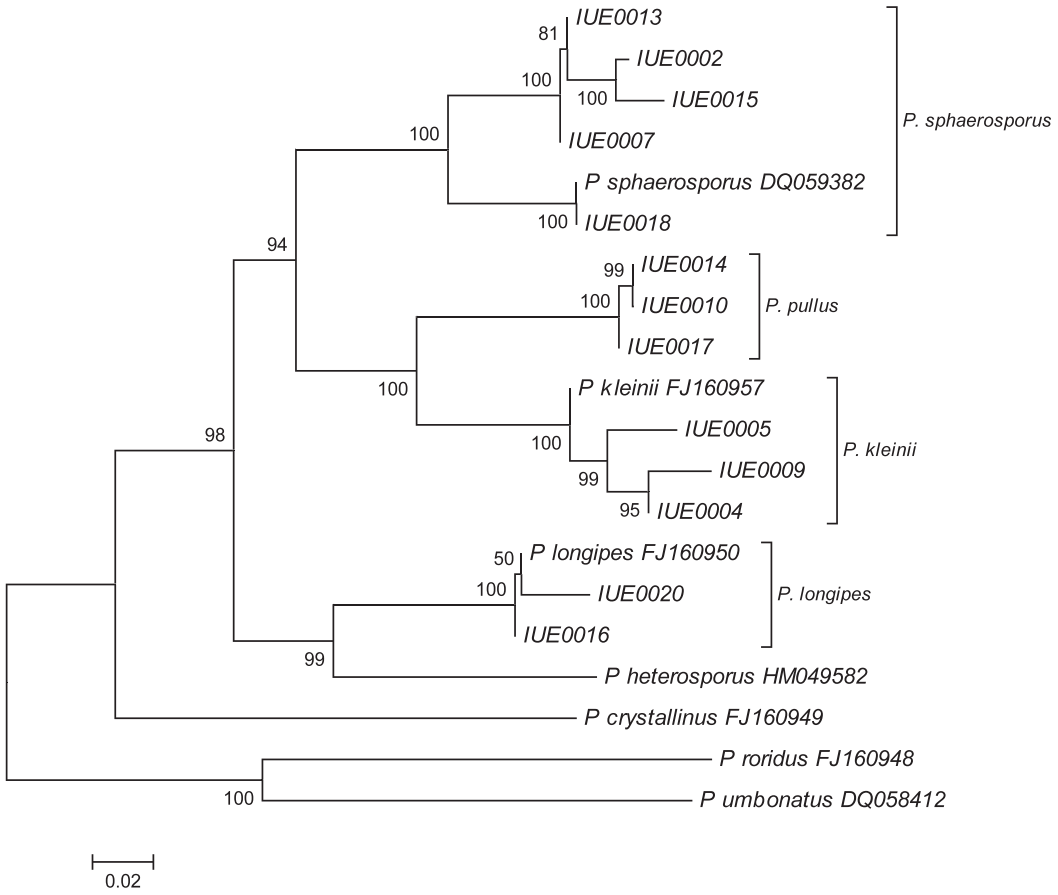

Figure 2.—Evolutionary relationships of *Pilobolus* based on ITS rDNA. The analysis involved 20 nucleotide sequences; ambiguous positions were removed for each sequence pair. There were a total of 811 positions in the final dataset.

DISCUSSION

Specimens of *Pilobolus* collected from horses in Indiana and Ohio were identified by phylogenetic species identification techniques using the 18S regions and the ITS regions of rRNA as taxonomic markers. Orthologous regions of DNA sequences from ex-type isolates of species of *Pilobolus* from GenBank were used for comparison.

Inferred evolutionary histories and taxonomically important characteristics used for identification do not always agree. However, the use of phylograms to show relationships among DNA sequences from various organisms permits an opportunity to distinguish among species and strains in which these DNA sequences differ. The phylogram in Figure 1 displays the inferred evolutionary distance based upon 18S rDNA sequences in this study and shows a great evolutionary distance between the major clade containing *P. sphaerosporus*, *P. pullus*, *P. kleinii* and *P. longipes*, all large-spore producing species, from *P. crystallinus*, *P. roridus* and *P. umbonatus*, all small-spore producing species. Figure 2, a phylogram illustrating the inferred evolutionary distance based upon ITS rDNA sequences shows the same pattern. The relationship of *P. pullus* to *P. kleinii* and *P. sphaerosporus* differs in the two inferred evolutionary histories and supports the concept of a "*Pilobolus kleinii* group" suggested earlier (Palla 1900, Massee 1901, Grove 1934).

The high percentage identities of the homologous 18S regions of rDNA make these good sequences to use to identify specimens of *Pilobolus* to genus. Intraspecies identities of the homologous 18S region of rDNA from specimens of *Pilobolus* were $> 99\%$. The most

Table 2.—Comparison of 18S homologous rDNA sequences from known species of *Pilobolus* in GenBank with sequences from isolates collected in this study in percent identity. No GenBank representative of *P. pullus* existed prior to this study. Here *P. pullus* is compared with representatives of all *Pilobolus* species from GenBank.

GenBank Control Species	bp	GenBank Number	This Study Isolate	bp	IUE ID	GenBank Number	Identity %
P. kleinii	1763	EU595656	*P. kleinii*	1763	0004	HQ682649	100.00
			P. kleinii	1763	0005	AY823738	99.98
			P. kleinii	1764	0006	DQ363379	99.60
			P. pullus	1764	0017	HQ877880	99.32
P. longipes	1763	DQ211053	*P. pullus*	1764	0017	HQ877880	99.21
P. sphaerosporus	1764	DQ211052	*P. sphaerosporus*	1764	0002	HQ682648	99.89
			P. sphaerosporus	1764	0007	HQ682650	99.89
			P. sphaerosporus	1764	0013	HQ682651	99.89
			P. sphaerosporus	1764	0015	HQ682652	99.89
			P. sphaerosporus	1763	0021	HQ682653	99.60
			P. pullus	1764	0017	HQ877880	99.55
P. pullus	1764	HQ877880	*P. pullus*	1764	0014	HQ877879	99.94
			P. pullus	1674	0010	HQ877878	99.76
P. crystallinus	1763	EU595652	*P. pullus*	1764	0017	HQ877880	98.13
P. roridus	1762	EU595649	*P. pullus*	1764	0017	HQ877880	97.34
P. umbonatus	1764	DQ211051	*P. pullus*	1764	0017	HQ877880	97.51

similar orthologous 18S sequence from a genus other than *Pilobolus* found in GenBank was 94.18% identity from *Pilaira anomala* (EU-595659). From this study we can infer that homologous identities of the 18S region between isolates of *Pilobolus* > 99% indicate the same species, identities of 95 – 99% indicate both are the same genus, but different species.

The rDNA that codes for the complete ITS region of rRNA contains two non-coding internal transcribed spacers that are more variable than coding regions (Nilsson et al. 2008). Because of this homologous identities within ITS regions of rDNA are much lower than those of the 18S regions.

Intraspecies identities of homologous ITS regions from the specimens collected in this study and the GenBank controls ranged from 95.4 – 100% for *P. kleinii*, 97.7 – 100% for *P. longipes*, 89.4 – 100% for *P. sphaerosporus* and > 99.8% among the specimens of *P. pullus*. The relative low percentage identity (89.4%) of intraspecies homologies of the ITS region of *Pilobolus sphaerosporus* isolates in this study indicates multiple strains.

Homologous identities of ITS regions of rDNA from isolates of different species of *Pilobolus* deposited in GenBank range from 59.7 – 83%. The most similar orthologous ITS sequence from a genus other than *Pilobolus* found in GenBank was from *Mucor heimalis* (DQ888726) with 62.0% identity. Interspecies homologous identities of the ITS regions of rDNA of isolates are more variable than those of 18S regions. Two isolates of *Pilobolus* having homologous identities of > 85% would indicate that they are the same species.

Variability of taxonomically valuable morphological characteristics is at the core of the difficulty identifying cryptic species of *Pilobolus*, thus requiring the use of phylogenetic species identification. The ephemeral nature of specimens, the propensity for multiple species of the same genus to grow in the same habitat, and the difficulty in culturing cryptic organisms have caused multiple field studies to use phylogenetic species identifications to report the presence of species that have not been isolated or cultured (Smit et al. 1999, Anderson & Cairney 2004, Bonito et al. 2010, Poitelon et al. 2009). This creates a taxonomic problem. If DNA from all taxa were to have been sequenced and reported, then the discovery of a new DNA sequence would indicate the presence of an undescribed organism. However, DNA sequences from most organisms, particularly cryptic organisms and microorganisms that were collected and identified many years ago, have not been identified. So, when new phylogenetic species are identified using DNA

Table 3.—Comparison of ITS homologous rDNA sequences from known species of *Pilobolus* in GenBank with sequences from isolates collected in this study in percent identity. No GenBank representative of *P. pullus* existed prior to this study. Here *P. pullus* is compared with representatives of all *Pilobolus* species from GenBank.

GenBank Control Species	bp	GenBank Number	This Study Isolate	bp	IUE ID	GenBank Number	Identity %
P. kleinii	704	FJ160957	*P. kleinii*	613	0004	HQ682655	100.00
			P. kleinii	700	0005	HQ682656	95.43
			P. kleinii	690	0009	HQ682658	96.52
			P. pullus	689	0017	HQ877877	83.16
P. longipes	688	FJ160950	*P. longipes*	688	0016	HQ682661	100.00
			P. longipes	671	0020	HQ682663	97.76
			P. pullus	689	0017	HQ877877	78.37
P. sphaerosporus	694	DQ059382	*P. sphaerosporus*	618	0002	HQ682654	89.48
			P. sphaerosporus	695	0007	HQ682657	90.94
			P. sphaerosporus	694	0013	HQ682659	90.78
			P. sphaerosporus	696	0015	HQ682660	91.24
			P. sphaerosporus	694	0018	HQ682662	100.00
			P. pullus	689	0017	HQ877877	78.66
P. pullus	662	HQ877877	*P. pullus*	662	0014	HQ877876	100.00
		HQ877877	*P. pullus*	661	0010	HQ877875	99.85
P. heterosporus	698	HM049582	*P. pullus*	689	0017	HQ877877	82.50
P. crystallinus	657	FJ160949	*P. pullus*	689	0017	HQ877877	75.38
P. roridus	694	FJ160948	*P. pullus*	689	0017	HQ877877	70.42
P. umbonatus	707	DQ058412	*P. pullus*	689	0017	HQ877877	68.43

sequences, there is no way to know whether the organisms are undescribed morphological species, or the phylogenetic species identity of a morphological species described previously. As organisms for which we have phylogenetic species identifications are cultured and identified morphologically, we can reconcile the morphological species identity with the phylogenetic species identity of an organism.

In this study the specimens of *P. pullus* isolated at three locations are morphologically very similar to *P. kleinii*. However DNA sequences of taxonomically significant rRNA regions of *P. pullus* do not align with the homologous sequences of any known *Pilobolus* species. *Pilobolus pullus* from this study might be described as *P. kleinii* if only morphological characteristics were considered. However, the significant difference between the rDNA sequences of *P. pullus* and *P. kleinii* show them to be distinct cryptic species.

DNA sequences of only 7 of 59 species of *Pilobolus* described in the literature have been deposited in GenBank. Most of these species were originally described many years ago, the most recent by Buller (1934). Even though multiple species have been redescribed more recently (Hu et al. 1989), no molecular data were included. Specimens of *Pilobolus* are ephemeral, microscopic, and fragile. No type cultures exist. These conditions make the revision of this genus necessary. In fact, the very reasons that revisions are necessary make revisions very difficult.

The example of *P. pullus* from this study represents a chasm between phylogenetic species recognition (PSR) and morphological species recognition (MSR) and demonstrates the challenge represented the need MSR to be correlated with PSR (Taylor et al. 2006). When viable type cultures of species described in the nineteenth century and earlier are unavailable, and there is no specimen that can be examined using molecular methods, there is little likelihood that it will be possible to determine the PSR identity for most of the species that have been described.

We found four species of *Pilobolus* growing on dung of horses in Indiana and Ohio. *Pilobolus longipes*, often specifically associated with horses, was isolated. But, it did not constitute the only species nor was it the most commonly found species. The identities of the various species were supported by both

phylograms and sequence homology. These phylogenetic techniques correlated with tentative morphological species identifications. However, the phylogenetic techniques distinguished between specimens of cryptic species (*P. kleinii* and *P. pullus*) that would probably have been identified as a single species using morphological techniques.

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CONSERVATION STATUS OF NORTH AMERICAN FRESHWATER CRAYFISH (*DECAPODA: CAMBARIDAE*) FROM THE SOUTHERN UNITED STATES

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ABSTRACT. A list is provided of all crayfishes (family Cambaridae) in the southern United States, which includes common names, global conservation status, an alternative review of the conservation status based on the IUCN red list criteria, and state distribution. This list includes 357 native crayfishes, of which 12 (3.4%) are critically endangered, 37 (10.4%) are endangered, 126 (35.3%) are vulnerable, 181 (50.7%) are lower risk, and 1 (0.3%) is not evaluated. The leading factors causing imperilment are restricted ranges caused by anthropogenic impacts from changes in land use, contaminants, invasion by non-indigenous species, and habitat fragmentation. In order to conserve and manage diversity of native crayfish, consistency is needed in determining conservation status and more complete distribution and life history information are needed for about 60% of species.

Keywords: imperiled species, global vulnerability, International Union for Conservation of Nature, biodiversity, threatened species

INTRODUCTION

North American aquatic biodiversity has been disproportionately affected by anthropogenic influences. A major focus is to fill gaps in research on crayfish ecology and present new information on imperilment of aquatic species. This need has benefited from the focus produced by the Natural Heritage Global (G) compilation of conservation status ranks (Master 1991). As a result of the Global status ranks, other groups have increased their emphasis on assessing other freshwater faunal diversity. The American Fisheries Society (AFS) Endangered Species Committee evaluated the conservation status of North America's freshwater fish fauna (Deacon et al. 1979; Williams et al. 1989; Warren et al. 2000), freshwater mussels (Williams et al. 1993), and North American freshwater crayfishes (Taylor et al. 1996, 2007). Taylor et al. (2007) assessed the conservation status and threats to native crayfishes in the United States and Canada using the best information available, provided updated state/provincial distributions, updated the list of references on the biology, conservation, and distribution of crayfishes in the United States and Canada, and assigned standardized common names. All of the AFS conservation assessments used the best professional judgment of taxonomic group experts to determine imperilment.

Crayfishes are closely related to marine lobsters (Crandall et al. 2000) and are members of the order Decapoda, which includes crabs, lobsters, and shrimps. Crayfishes are included in three families, and native inhabitants occur in freshwater ecosystems on every continent except Africa and Antarctica. Two families, Astacidae and Cambaridae, are native to North America and include about 408 species and subspecies, representing about 77% of the global species diversity in North America (Taylor 2002). The majority of the North American fauna (99%) is assigned to the family Cambaridae with over two-thirds of its species endemic to the southeastern United States.

Crayfishes occur in every seasonally wet and terrestrial habitat including a variety of aquatic habitats. They possess unique life-history traits adapted for these habitats, including alteration of reproductive form and burrowing abilities that allow colonization (Hobbs 1981; Welch & Eversole 2006). Our purpose for this research is to report on the conservation status of the North American crayfish fauna distributed in the southeastern United States using the International Union for Conservation of Nature and Natural Resources (IUCN) red list

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criteria. The purpose is to report the status and effects that might cause future declines in relative abundance and loss of species richness (Huner 1994; Taylor et al. 1996; Holdich 2002).

METHODS

Our review of the conservation status of crayfishes includes all species and subspecies from the southern United States. We do not recognize *Cambarus ornatus* following Taylor (1997), we continue to recognize *Procambarus ferrugineus* until the work by Robison and Crandall is published in peer-reviewed literature, and *Cambarus bartonii carinirostris* is recognized as *C. carinirostris* Hay (Rock Crawfish) following Thoma & Jezerinac (1999). Additional southern taxa included in our work were described subsequent to Taylor et al. (1996, 2007).

Conservation status.—The current assessment of southern crayfish includes several rating systems that are used by the Heritage Database and the International Union for Conservation of Nature and Natural Resources (IUCN) red list criteria. Ranking categories for conservation status follow Master (1991) and are defined as follows: G1 = critically imperiled, G2 = imperiled, G3 = vulnerable to extirpation or extinction, G4 = apparently secure, G5 = demonstrably widespread, abundant, and secure, GH = possibly extinct, known only from historical collections, T = threatened, and GX = presumed extinct. The conservation ranks for each taxon follow the system developed by The Nature Conservancy/ NatureServe and the Network of Natural Heritage Programs (Master 1991, www.natureserve.org/explorer/ranking.htm). The International Union for Conservation of Nature and Natural Resources (IUCN) includes the following six conservation categories: EXTINCT (EX) – when there is no reasonable doubt that the last individual has died. EXTINCT IN THE WILD (EW) – when it is known only to survive in cultivation, in captivity or as a naturalized population (or populations) well outside the past range. A taxon is presumed EW when exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), throughout its historic range have failed to record an individual. Surveys should be over a time frame appropriate to the taxon's life cycle and life form (Appendix 1). CRITICALLY ENDANGERED (CR) – A taxon is CR when it is facing an extremely high risk of extinction in the wild in the immediate future, as defined by any of the criteria (A to E) as described in Appendix 1. ENDANGERED (EN) – A taxon is EN when it is not CR, but is facing a very high risk of EW in the near future, as defined by any of the criteria (A to E) as described in Appendix 1. VULNERABLE (VU) – A taxon is VU when it is not CR or EN but is facing a high risk of extinction in the wild in the medium-term future, as defined by any of the criteria (A to E) as described in Appendix 1. LOWER RISK (LR) – A taxon is LR when it has been evaluated and does not satisfy the criteria for any of the categories CR, EN, or VU. Taxa included in the LR category can be separated into three subcategories: (1) Conservation Dependent (cd) – Taxa which are the focus of a continuing taxon-specific or habitat-specific conservation program targeted towards the taxon in question, the cessation of which would result in the taxon qualifying for one of the threatened categories above within a period of five years; (2) Near Threatened (nt) – Taxa which do not qualify for cd, but which are close to qualifying for VU; and (3) Least Concern (lc) – Taxa which do not qualify for cd or nt. DATA DEFICIENT (DD) – A taxon is DD when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status. A taxon in this category may be well studied, and its biology well known, but appropriate data on abundance and/or distribution are lacking. Data Deficient is therefore not a category of threat or Lower Risk. Listing of taxa in this category indicates that more information is required and acknowledges the possibility that future research will show that threatened classification is appropriate. It is important to make positive use of whatever data are available. In many cases great care should be exercised in choosing between DD and threatened status. If the range of a taxon is suspected to be relatively circumscribed, and if a considerable period of time has elapsed since the last record of the taxon, threatened status may well be justified. NOT EVALUATED (NE) – A taxon is NE when it is has not yet been assessed against the criteria (Appendix 1).

List of taxa.—The list of crayfish species and subspecies is arranged alphabetically by genus and by species and subspecies within genus (Table 1). Following scientific names, common

names are based primarily on those provided by two internet sites; the Crayfish Tree of Life – crayfish.byu.edu, and the Global Crayfish and Lobster taxonomy browser – iz.carnegiemnh.org/crayfish/NewAstacidea/index.aspwww.crayfish.byu.edu. However, we also used common names as reported in state lists, taxonomic descriptions, McLaughlin et al. (2005), and Taylor et al. (2007).

In determining conservation status and distribution, a variety of sources were used including state and federal endangered species lists, government agency reports and websites, research publications, and books. Global Heritage Rank criteria were based on numerals G1 through G5 and correspond to those defined in the Methods. Global Heritage ranks immediately follow common name. Following the Global Heritage ranks is the conservation status based on the IUCN conservation formula. Finally, the distribution of each taxon is indicated by an alphabetical listing of USA states within the Southern Division of the American Fisheries Society where it occurs.

Distribution.—The distribution reflected for each species is based only on its range within the Southern United States. This geographic state inclusion in based on the American Fisheries Society Southern Division membership (Fig. 1). Distribution information is based on reports for Alabama (Bouchard 1976; Harris 1990; McGregor et al. 1999; Ratcliffe & DeVries 2004; Schuster & Taylor 2004; Heath et al. 2010), Arkansas (Williams 1954; Bouchard & Robison 1980; Hobbs & Robison 1988; Hobbs 1989a), Florida (Hobbs 1942; Deyrup & Franz 1994; Franz & Franz 1990; Hobbs & Hobbs 1991), Georgia (Hobbs 1981; Skelton 2010), Kentucky (Rhoades 1944; Burr & Hobbs 1984; Taylor & Schuster 2004), Louisiana (Penn 1950, 1952, 1956, 1959; Penn & Marlow 1959; Walls & Black 1991; Walls and Shively 2003; Walls 2009), Maryland (Meredith & Schwartz 1959, 1960; Kilian et al. 2010; Loughman 2010), Mississippi (Adams 2008), North Carolina (Cooper & Braswell 1995; Cooper et al. 1998; Cooper 2002; LeGrand et al. 2006; Simmons and Fraley 2010), Oklahoma (Creaser & Ortenburger 1933; Dunlap 1951; Reimer 1969; Jones et al. 2005; Taylor et al. 2004), South Carolina (Hobbs et al. 1976; Eversole 1995; Eversole & Jones 2004), Tennessee (Bouchard 1972), Texas (Penn & Hobbs 1958; Albaugh & Black 1973; Hobbs 1990; Johnson & Johnson 2008), Washington District of Columbia (Loughman 2009), and West Virginia (Jezerinac et al. 1995; Loughman et al. 2009; Jones et al. 2010; Loughman & Welsh 2010). Each state is designated by the two-digit postal code. Parentheses around states indicate known or suspected introductions.

RESULTS AND DISCUSSION

The list of southern crayfishes includes 357 taxa (Table 1). This list includes 357 native crayfishes, of which 12 (3.4%) are critically endangered, 37 (10.4%) are endangered, 126 (35.3%) are vulnerable, 181 (50.7%) are lower risk, and 1 (0.3%) was not evaluated. Taxonomic efforts since Hobbs' (1989b) checklist of North American crayfishes have resulted in the description of many new crayfish species in the United States. New descriptions have averaged slightly less than two new species per year. This demonstrates the need to train taxonomists to further study biodiversity that continues to be undiscovered in North America. The number of imperiled crayfishes (49.0%) ranks intermediate between the high levels observed in fishes and freshwater mussels, almost 33% and 72%, respectively (Williams et al. 1989; Williams et al. 1993; Warren & Burr 1994; Warren et al. 2000). Our assessment is similar to the findings of Taylor et al. (2007), who assessed 48% of crayfish as imperiled. This assessment confirms the assertion that North American diversity in aquatic systems is in poorer condition than that in terrestrial systems (Master 1991; Master et al. 2000). The benefit of this assessment is the validation of the assessment of conservation status by Taylor et al. (2007) and the stabilization of the assessment process by removing best professional judgment from the analysis. Taylor et al. (1996) used as part of the American Fisheries Society's review process the recognition of either the actual or potential imperilment of crayfishes between governmental agencies charged with protecting natural resources and non-profit conservation organizations as a rationale for listing. Only four crayfish species (*Pacifastacus fortis*, *Cambarus aculabrum, Cambarus zophonastes,* and *Orconectes shoupi*) receive protection under the Federal Endangered Species Act of 1973 (ESA) and 66 species received varying levels of protection at the state level, which is lower than the 197 species listed

Table 1.—Checklist of crayfishes of the Southeastern United States including common name, Global Heritage rank, International Union for Conservation of Nature and Natural Resources (IUCN) red list, and distribution.

Species	Global Rank	Red List	Distribution
Barbicambarus cornutus (Faxon) (Bottle Brush Crayfish)	G4	LR lc	KY, TN
Barbicambarus simmonsi Taylor and Shuster (Tennessee Bottle Brush Crayfish)	G3	VU D2	TN
Bouchardina robisoni Hobbs (Bayou Bodcau Crayfish)	G2	VU B1+2c	AR
Cambarellus blacki Hobbs (Cypress Crayfish)	G1	EN B1+2c	FL
Cambarellus diminutus Hobbs (Least Crayfish)	G3	VU B1+2c	AL, MS
Cambarellus lesliei Fitzpatrick and Laning (Angular Dwarf Crayfish)	G3	VU B1+2c	AL, MS
Cambarellus ninae Hobbs (Aransas Dwarf Crawfish)	G3	VU B1+2c	TX
Cambarellus puer Hobbs (Swamp Dwarf Crayfish)	G5	LR lc	AR, KY, LA, MS, OK, TN, TX
Cambarellus schmitti Hobbs (Fontal Dwarf Crawfish)	G3	LR lc	FL
Cambarellus shufeldtii (Faxon) (Cajun Dwarf Crayfish)	G5	LR lc	AL, AR, KY, LA, MS, TN, TX
Cambarellus texanus Albaugh and Black (Brazos Dwarf Crawfish)	G3G4	LR lc	TX
Cambarus acanthura Hobbs (Thornytail Crayfish)	G4G5	LR lc	AL, GA, NC, TN
Cambarus aculabrum Hobbs and Brown (Benton County Cave Crayfish)	G1	EN B1+2c	AR
Cambarus acuminatus Faxon (Acuminate Crayfish)	G4	LR nt	MD, NC, SC, VA
Cambarus aldermanorum Cooper and Price (Carolina needlenose crayfish)	G3	VU B1, D1	SC
Cambarus angularis Hobbs and Bouchard (Angled Crayfish)	G3	LR lc	TN, VA
Cambarus asperimanus Faxon (Mitten Crayfish)	G4	LR lc	GA, NC, SC, TN
Cambarus bartonii bartonii (Fabricius) (Common Crayfish)	G5T5	LR lc	AL, GA, KY, MD, NC, SC, TN, VA, WV
Cambarus bartonii cavatus Hay (Appalachian Brook Crayfish)	G5T5	LR lc	AL, KY, WV
Cambarus batchi Schuster (Bluegrass Crayfish)	G3	VU B1+2c, D1	KY
Cambarus bouchardi Hobbs (Big South Fork Crayfish)	G2	VU B1+2c, D1	KY, TN
Cambarus brachydactylus Hobbs (Shortfinger Crayfish)	G4	LR lc	TN
Cambarus brimleyorum Cooper (Valley River Crayfish)	G3	VU B1+2c	NC

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Cambarus buntingi Bouchard (Bunting Crayfish)	G4	LR nt	KY, TN, VA
Cambarus carinirostris Hay (Rock Crawfish)	G5	LR lc	MD, VA, WV
Cambarus carolinus (Erichson) (Red Burrowing Crayfish)	G4	LR lc	NC, SC, TN
Cambarus catagius Hobbs and Perkins (Greensboro Burrowing Crayfish)	G3	VU B1+2c	NC
Cambarus causeyi Reimer (Boston Mountains Crayfish)	G2	VU A1,B1+2c	AR
Cambarus chasmodactylus James (New River Crayfish)	G4	LR lc	NC, VA, WV
Cambarus chaugaensis Prins and Hobbs (Chauga Crayfish)	G2	VU B1+2c, D1	GA, NC, SC
Cambarus clivosus Taylor, Soucek, and Organ (Short Mountain Crayfish)	G2	VU B1+2c, D1	TN
Cambarus conasaugaensis Hobbs and Hobbs (Mountain Crayfish)	G3	VU B1+2c	GA, TN
Cambarus coosae Hobbs (Coosa Crayfish)	G5	LR lc	AL, GA, TN
Cambarus coosawattae Hobbs (Coosawattae Crayfish)	G1	VU B1+2c, D1	GA
Cambarus cracens Bouchard and Hobbs (Slenderclaw Crayfish)	G1	EN B1+2c	AL
Cambarus crinipes Bouchard (Bouchard's Crayfish)	G3	LR lc	TN
Cambarus cryptodytes Hobbs (Dougherty Plain Cave Crayfish)	G2G3	VU B1+2c	FL, GA
Cambarus cumberlandensis Hobbs and Bouchard (Cumberland Crayfish)	G5	LR lc	KY, TN
Cambarus cymatilis Hobbs (Conasauga Blue Burrower Crayfish)	G1	VU B1+2c, D1	GA, TN
Cambarus davidi Cooper (Carolina Ladle Crayfish)	G3	LR lc	NC
Cambarus deweesae Bouchard and Etnier (Valley Flame Crayfish)	G4	EN B1+2c	KY, TN
Cambarus diogenes Girard (Devil Crawfish)	G5	LR lc	AL, AR, FL, GA, KY, LA, MD, MS, NC, OK, SC, TN, TX, VA
Cambarus distans Rhoades (Boxclaw Crawfish)	G5	LR lc	AL, GA, KY, TN
Cambarus doughertyensis Cooper and Skelton (Dougherty Burrowing Crayfish)	G1G2	EN B1+2c	GA
Cambarus dubius Faxon (Upland Burrowing Crayfish)	G5	LR lc	KY, MD, NC, TN, VA, WV
Cambarus eeseeohensis Thoma (Linville River Crayfish)	G1	EN B1+2c	NC
Cambarus elkensis Jezerinac and Stocker (Elk River Crayfish)	G2	VU B1+2c	WV

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Cambarus englishi Hobbs and Hall (Tallapoosa Crayfish)	G3	VU B1+2c	AL, GA
Cambarus erythrodactylus Simon and Morris (Warpaint Mudbug)	G5	LR lc	AL, GA, MS
Cambarus extraneus Hagen (Chickamauga Crayfish)	G2	VU B1+2c	GA, TN
Cambarus fasciatus Hobbs (Etowah Crayfish)	G3	VU B1+2c	GA
Cambarus friaufi Hobbs (Hairy Crayfish)	G4	LR lc	KY, TN
Cambarus gentryi Hobbs (Linear Cobalt Crayfish)	G4	LR lc	TN
Cambarus georgiae Hobbs (Little Tennessee Crayfish)	G2	VU B1+2c, D1	GA, NC
Cambarus girardianus Faxon (Tanback Crayfish)	G5	LR lc	AL, GA, MS, TN
Cambarus graysoni Faxon (Twospot Crayfish)	G5	LR lc	AL, KY, TN
Cambarus halli Hobbs (Slackwater Crayfish)	G3G4	LR lc	AL, GA
Cambarus hamulatus (Cope) (Prickly Cave Crayfish)	G3G4	LR lc	AL, TN
Cambarus harti Hobbs (Piedmont Blue Burrower)	G1	EN B1+2c	GA
Cambarus hiwasseensis Hobbs (Hiwassee Crayfish)	G3G4	VU B1+2c	GA, NC, TN
Cambarus hobbsorum Cooper (Rocky River Crayfish)	G3G4	LR lc	NC, SC
Cambarus howardi Hobbs and Hall (Chattahoochee Crayfish)	G3	VU B1+2c	AL, GA, NC
Cambarus hubbsi Creaser (Hubb's Crayfish)	G5	LR lc	AR
Cambarus hystricosus Cooper and Cooper (Sandhills Spiny Crayfish)	G2	VU B1+2c	NC
Cambarus jezerinaci Thoma (Powell River Crayfish)	G3	LR nt	TN, VA
Cambarus johni Cooper (Carolina Foothills Crayfish)	G3	VU B1+2C	NC
Cambarus jonesi Hobbs and Barr (Alabama Cave Crayfish)	G2	LR lc	AL
Cambarus laconensis Buhay and Crandall (Lacon Exit Cave Crayfish)	G1	VU B1+2C	AL
Cambarus latimanus (LeConte) (Variable Crayfish)	G5	LR lc	AL, FL, GA, NC, SC, TN
Cambarus lenati Cooper (Broad River Crayfish)	G2	VU B1+2c	NC
Cambarus longirostris Faxon (Longnose Crayfish)	G5	LR lc	AL, GA, NC, (SC), TN, VA
Cambarus longulus Girard (Atlantic Slope Crayfish)	G5	LR lc	NC, VA, WV
Cambarus ludovicianus Faxon (Painted Devil Crayfish)	G5	LR lc	AL, AR, KY, LA, MS, TN, TX

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Cambarus manningi Hobbs (Greensaddle Crayfish)	G4	LR lc	AL, GA, TN
Cambarus miltus Fitzpatrick (Rusty Gravedigger)	G2	VU B1+2c	AL, FL
Cambarus monongalensis Ortmann (Blue Crawfish)	G5	LR lc	VA, WV
Cambarus nerterius Hobbs (Greenbrier Cave Crayfish)	G2	VU B1+2c, D1	WV
Cambarus nodosus Bouchard and Hobbs (Knotty Burrowing Crayfish)	G4	LR lc	GA, NC, SC, TN
Cambarus obeyensis Hobbs and Shoup (Obey Crayfish)	G1	VU B1+2c	TN
Cambarus obstipus Hall (Sloped Crayfish)	G4	VU B1+2c	AL
Cambarus ortmanni Williamson (Ortmann's Mudbug)	G5	LR lc	KY
Cambarus parrishi Hobbs (Hiwassee Headwater Crayfish)	G1	VU B1+2c, D1	GA, NC
Cambarus parvoculus Hobbs and Shoup (Mountain Midget Crayfish)	G5	LR lc	AL, GA, KY, TN, VA
Cambarus pecki Hobbs (Phantom Cave Crayfish)	G1G2	VU B1+2c	AL
Cambarus polychromatus Thoma, Jezerinac, and Simon (Paintedhand Mudbug)	G5	LR lc	AL, KY, TN
Cambarus pristinus Hobbs (Pristine Crayfish)	G2	VU B1+2c, D1	TN
Cambarus pyronotus Bouchard (Fireback Crayfish)	G2	EN B1+2c	FL
Cambarus reburrus Prins (French Broad Crayfish)	G3	VU B1+2c	NC
Cambarus reduncus Hobbs (Sickle Crayfish)	G4G5	LR lc	NC, SC
Cambarus reflexus Hobbs (Pine Savannah Crayfish)	G4	LR lc	GA, SC
Cambarus robustus Girard (Big Water Crayfish)	G5	LR lc	KY, NC, TN, VA, WV
Cambarus rusticiformis Rhoades (Depression Crayfish)	G5	LR lc	(AL), KY, TN
Cambarus sciotensis Rhoades (Teays River Crayfish)	G5	LR lc	KY, VA, WV
Cambarus scotti Hobbs (Chatooga Crayfish)	G3	VU B1+2c	AL, GA
Cambarus setosus Faxon (Bristly Cave Crayfish)	G3	LR lc	AR, OK
Cambarus smilax Loughman, Simon, and Welch (Greenbrier Crayfish)	G4	LR lc	WV
Cambarus sp. 1 (Emory River Crayfish)	G1?	NE	TN
Cambarus speleocoopi Buhay and Crandall (Sweet Home Alabama Crayfish)	G1	VU B1+2C	AL

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Cambarus speciosus Hobbs (Beautiful Crayfish)	G2	VU B1+2c, D1	GA
Cambarus sphenoides Hobbs (Triangleclaw Crayfish)	G4	LR lc	KY, TN
Cambarus spicatus Hobbs (Broad River Spiny Crayfish)	G3	VU B1+2c	NC, SC
Cambarus striatus Hay (Hay Crayfish)	G5	LR lc	AL, FL, GA, KY, MS, SC, TN
Cambarus strigosus Hobbs (Lean Crayfish)	G2	EN B1+2c	GA
Cambarus subterraneus Hobbs (Delaware County Cave Crayfish)	G1	EN B1+2c	OK
Cambarus tartarus Hobbs and Cooper (Oklahoma Cave Crayfish)	G1	CR B1+2c	OK
Cambarus tenebrosus Hay (Cavespring Crayfish)	G5	LR lc	AL, GA, KY, TN
Cambarus thomai Jezerinac (Little Brown Mudbug)	G5	LR lc	KY, TN, WV
Cambarus trojanensis Simon and Morris (Spiny Collared Mudbug)	G5	LR lc	AL, FL, MS
Cambarus truncatus Hobbs (Oconee Burrowing Crayfish)	G2	EN B1+2c	GA
Cambarus tuckasegee Cooper and Schofield (Tuckasegee Stream Crayfish)	G1G2	VU B1+2c	NC
Cambarus unestami Hobbs and Hall (Blackbarred Crayfish)	G2	VU B1+2c	AL, GA
Cambarus veitchorum Cooper and Cooper (White Spring Cave Crayfish)	G1	EN A1, B1+2c	AL
Cambarus veteranus Faxon (Big Sandy Crayfish)	G2G3	VU B1+2c	KY, VA, WV
Cambarus williami Bouchard and Bouchard (Brawleys Fork Crayfish)	G2	EN B1+2c	TN
Cambarus zophonastes Hobbs and Bedinger (Hell Creek Cave Crayfish)	G1	CR B1+2c	AR
Distocambarus carlsoni Hobbs (Mimic Crayfish)	G2G3	VU B1+2c	SC
Distocambarus crockeri Hobbs and Carlson (Piedmont Prairie Burrowing Crayfish)	G3	VU B1+2c, D1	SC
Distocambarus devexus (Hobbs) (Broad River Burrowing Crayfish)	G1	VU B1+2c, D1	GA
Distocambarus hunteri Fitzpatrick and Eversole (Saluda Burrowing Crayfish)	G1	EN B1+2c	SC
Distocambarus youngineri Hobbs and Carlson (Newberry Burrowing Crayfish)	G1	VU B1+2c, D1	SC

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Fallicambarus burrisi Fitzpatrick (Burrowing Bog Crayfish)	G3	VU B1+2c	AL, MS
Fallicambarus byersi (Hobbs) (Lavender Burrowing Crayfish)	G4	LR lc	AL, FL, MS
Fallicambarus caesius Hobbs (Timberlands Burrowing Crayfish)	G4	LR lc	AR
Fallicambarus danielae Hobbs (Speckled Burrowing Crayfish)	G2	VU B1+2c	AL, MS
Fallicambarus devastator Hobbs and Whiteman (Texas Prairie Crayfish)	G3	VU B1+2c	TX
Fallicambarus dissitus (Penn) (Pine Hills Digger Crayfish)	G4	VU B1+2c	AR, LA
Fallicambarus fodiens (Cottle) (Digger Crayfish)	G5	LR lc	AL, AR, FL, GA, KY, LA, MD, MS, NC, OK, SC, TN, TX, VA, WV
Fallicambarus gilpini Hobbs and Robison (Jefferson County Crayfish)	G1	VU B1+2c, D1	AR
Fallicambarus gordoni Fitzpatrick (Camp Shelby Burrowing Crayfish)	G1	VU B1+2c, D1	MS
Fallicambarus harpi Hobbs and Robison (Ouachita Burrowing Crayfish)	G1	EN B1+2c	AR
Fallicambarus hortoni Hobbs and Fitzpatrick (Hatchie Burrowing Crayfish)	G1	EN B1+2c	TN
Fallicambarus houstonensis Johnson (Houston Burrowing Crayfish)	G3	VU B1+2c, D1	TX
Fallicambarus jeanae Hobbs (Daisy Burowing Crayfish)	G2	EN B1+2c	AR
Fallicambarus kountzeae Johnson (Big Thicket Burrowing Crayfish)	G3	VU B1+2c, D1	TX
Fallicambarus macneesei (Black) (Old Prairie Digger Crayfish)	G3	VU B1+2c, D1	LA, TX
Fallicambarus oryktes (Penn and Marlow) (Flatwoods Digger Crayfish)	G4	EN B1+2c, B2	AL, LA, MS
Fallicambarus petilicarpus Hobbs and Robison (Slenderwrist Burrowing Crayfish)	G1	CR B1+2c	AR
Fallicambarus strawni (Reimer) (Saline Burrowing Crayfish)	G1G2	VU B1+2c	AR
Faxonella beyeri (Penn) (Sabine Fencing Crayfish)	G4	LR lc	LA, TX
Faxonella blairi Hayes and Reimer (Blair's Fencing Crayfish)	G2	LR lc	AR, OK
Faxonella clypeata (Hay) (Ditch Fencing Crayfish)	G5	LR lc	AL, AR, FL, GA, LA, MS, OK, SC, TX

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Faxonella creaseri Walls (Ouachita Fencing Crayfish)	G2	VU B1+2c, D1	LA
Hobbseus attenuatus Black (Pearl Riverlet Crayfish)	G2	VU B1+2c, D1	MS
Hobbseus cristatus (Hobbs) (Crested Rivulet Crayfish)	G3	VU B1+2c	MS
Hobbseus orconectoides Fitzpatrick and Payne (Oktibbeha Riverlet Crayfish)	G3	VU B1+2c	MS
Hobbseus petilus Fitzpatrick (Tombigbee Riverlet Crayfish)	G2	VU B1+2c	MS
Hobbseus prominens (Hobbs) (Prominence Riverlet Crayfish)	G4G5	LR lc	AL, MS
Hobbseus valleculus (Fitzpatrick) (Choctaw Riverlet Crayfish)	G1	VU B1+2c	MS
Hobbseus yalobushensis Fitzpatrick and Busack (Yalobusha Riverlet Crayfish)	G3	VU B1+2c, D1	MS
Orconectes acares Fitzpatrick (Redspotted Stream Crayfish)	G4	LR lc	AR, OK
Orconectes alabamensis (Faxon) (Alabama Crayfish)	G5	LR lc	AL, MS, TN
Orconectes australis australis (Rhoades) (Southern Cave Crayfish)	G5T4	LR lc	AL, TN
Orconectes australis packardi Rhoades (Appalachian Cave Crayfish)	G2	EN B1+2c	KY
Orconectes barrenensis Rhoades (Barren River Crayfish)	G4	LR lc	KY, TN
Orconectes barri Buhay and Crandall (Cumberland Plateau Cave Crayfish)	G2	VU B1+2C	KY, TN
Orconectes bisectus Rhoades (Crittenden Crayfish)	G1	VU B1+2c, D1	KY
Orconectes blacki Walls (Calcasieu Crayfish)	G2	VU B1+2c, D1	LA
Orconectes burri Taylor and Sabaj (Burr Crayfish)	G1	EN B1+2c, D1	KY, TN
Orconectes carolinensis Cooper and Cooper (North Carolina Crayfish)	G3	LR lc	NC
Orconectes causeyi Jester (Western Plains Crayfish)	G4	LR lc	OK, TX
Orconectes chickasawae Cooper and Hobbs (Chickasaw Crayfish)	G5	LR lc	AL, MS
Orconectes compressus (Faxon) (Slender Crayfish)	G5	LR lc	AL, KY, MS, TN
Orconectes cooperi Cooper and Hobbs (Flint River Crayfish)	G1	VU B1+2c, D1	AL, TN
Orconectes cristavarius Taylor (Spiny Stream Crayfish)	G5	LR lc	KY, NC, TN, VA, WV
Orconectes deanae Reimer and Jester (Conchas Crayfish)	G4	VU B1+2c, D1	OK

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Orconectes difficilis (Faxon) (Painted Crayfish)	G3	LR lc	AR, OK
Orconectes durelli Bouchard and Bouchard (Saddle Crayfish)	G5	LR lc	AL, KY, TN
Orconectes erichsonianus (Faxon) (Reticulate Crayfish)	G5	LR lc	AL, GA, TN, VA
Orconectes etnieri Bouchard and Bouchard (Etnier's Crayfish)	G4	LR lc	MS, TN
Orconectes eupunctus Williams (Coldwater Crayfish)	G2	EN B1+2c, D2	AR, MO
Orconectes forceps (Faxon) (Surgeon Crayfish)	G5	LR lc	AL, GA, TN, VA
Orconectes hartfieldi Fitzpatrick and Suttkus (Yazoo Crayfish)	G2	VU B1+2c	MS
Orconectes hathawayi Penn (Teche Painted Crayfish)	G3	VU B1+2c	LA
Orconectes hobbsi Penn (Pontchartrain Painted Crayfish)	G4	LR lc	LA, MS
Orconectes holti Cooper and Hobbs (Bimaculate Crayfish)	G3	VU B1+2c	AL
Orconectes immunis (Hagen) (Calico Crayfish)	G5	LR lc	AL, KY, TN
Orconectes incomptus Hobbs and Barr (Tennessee Cave Crayfish)	G1	VU B1+2c, D1	TN
Orconectes inermis inermis Cope (Northern Cave Crayfish)	G5T4	LR lc	KY
Orconectes jeffersoni Rhoades (Louisville Crayfish)	G1	VU B1+2c, D1	KY
Orconectes jonesi Fitzpatrick (Sucarnoochee River Crayfish)	G3	VU B1+2c	AL, MS
Orconectes juvenilis (Hagen) (Kentucky River Crayfish)	G4	LR lc	KY
Orconectes kentuckiensis Rhoades (Kentucky Crayfish)	G4	VU B1+2c	KY
Orconectes lancifer (Hagen) (Shrimp Crayfish)	G5	LR lc	AL, AR, KY, LA, MS, OK, TN, TX
Orconectes leptogonopodus Hobbs (Little River Creek Crayfish)	G4	LR lc	AR, OK
Orconectes limosus (Rafinesque) (Spinycheek Crayfish)	G5	VU A1, B1	MD, WV
Orconectes longidigitus (Faxon) (Longpincered Crayfish)	G4	LR lc	AR
Orconectes luteus (Creaser) (Golden Crayfish)	G5	LR lc	AR
Orconectes macrus Williams (Neosho Midget Crayfish)	G4	LR lc	AR, OK
Orconectes maletae Walls (Kisatchie Painted Crayfish)	G2	VU B1+2c	LA, TX
Orconectes marchandi Hobbs (Sharp River Crayfish)	G2	VU B1+2c, D1	AR
Orconectes margorectus Taylor (Livingston Crayfish)	G2	EN B1+2c	KY

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Orconectes meeki brevis Williams (Meek's Short Pointed Crayfish)	G4T3	EN B1+2c	AR, OK
Orconectes meeki meeki (Faxon) (Meek's Crayfish)	G4T4	LR lc	AR
Orconectes menae (Creaser) (Mena Crayfish)	G3	VU B1+2c	AR, OK
Orconectes mirus (Ortmann) (Wonderful Crayfish)	G4	LR lc	AL, TN
Orconectes mississippiensis (Faxon) (Mississippi Crayfish)	G3	VU B1+2c	MS
Orconectes nais (Faxon) (Water Nymph Crayfish)	G5	LR lc	AR, OK, TX
Orconectes nana Williams (Midget Crayfish)	G3	VU B1+2c	AR, OK
Orconectes neglectus chaenodactylus Williams (Gaped Ringed Crayfish)	G5T3	VU B1+2c	AR
Orconectes neglectus neglectus (Faxon) (Ringed Crayfish)	G5T5	LR lc	AR, OK
Orconectes obscurus (Hagen) (Allegheny Crayfish)	G5	LR lc	MD, VA, WV
Orconectes ozarkae Williams (Ozark Crayfish)	G5	LR lc	AR
Orconectes pagei Taylor and Sabaj (Mottled Crayfish)	G4	LR lc	TN
Orconectes palmeri creolanus (Creaser) (Creole Painted Crayfish)	G5T4	LR lc	(GA), LA, MS
Orconectes palmeri longimanus (Faxon) (Western Painted Crayfish)	G5T5	LR lc	AR, OK, TX
Orconectes palmeri palmeri (Faxon) (Gray-speckled Crayfish)	G5T5	LR lc	AR, KY, MS, TN
Orconectes pardalotus Wetzel, Poly, Fetzner (Leopard Crayfish)	G1	VU B1+2C	KY
Orconectes pellucidus (Tellkampf) (Mammoth Cave Crayfish)	G4	LR lc	KY, TN
Orconectes perfectus Walls (Complete Crayfish)	G4G5	LR lc	AL, MS
Orconectes placidus (Hagen) (Placid Crayfish)	G5	LR lc	AL, KY, TN
Orconectes punctimanus (Creaser) (Spothand Crayfish)	G4G5	LR lc	AR
Orconectes putnami (Faxon) (Phallic Crayfish)	G5	LR lc	AL, KY, TN
Orconectes rafinesquei Rhoades (Rafinesque Crayfish)	G3	VU B1+2c, D1	KY
Orconectes rhoadesi Hobbs (Fishhook Crayfish)	G4	LR lc	TN
Orconectes ronaldi Taylor (Mud River Crayfish)	G3	LR lc	KY

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Orconectes rusticus (Girard) (Rusty Crayfish)	G5	LR lc	KY, (NC), (TN), (VA), (WV)
Orconectes sanbornii erismophorous Hobbs and Fitzpatrick	G4G5	LR lc	WV
Orconectes sanbornii sanbornii (Faxon) (Sanborn's Crayfish)	G4G5T4	LR lc	KY, WV
Orconectes saxatilis Bouchard and Bouchard (Kiamichi Crayfish)	G1	CR B1+2c	OK
Orconectes sheltae Cooper and Cooper (Shelta Cave Crayfish)	G1	EN B1+2c, D1	AL
Orconectes shoupi Hobbs (Nashville Crayfish)	G1G2	CR B1+2c	TN
Orconectes spinosus (Bundy) (Coosa River Spiny Crayfish)	G4	LR lc	AL, GA, TN
Orconectes taylori Schuster (Crescent Crayfish)	G2	VU B1+2C	TN
Orconectes tricuspis Rhoades (Rhode Crayfish)	G4	LR lc	KY
Orconectes validus (Faxon) (Powerful Crayfish)	G5	LR lc	AL, MS, TN
Orconectes virginiensis Hobbs (Chowanoke Crayfish)	G3	LR lc	NC, VA
Orconectes virilis Hagen (Virile Crayfish)	G5	LR lc	(AL), AR, (MD), MS, (NC), OK, (TN), TX, (VA), (WV)
Orconectes williamsi Fitzpatrick (Williams Crayfish)	G3G4	LR lc	AR
Orconectes wrighti Hobbs (Hardin Crayfish)	G2	VU B1+2c, D1	MS, TN
Procambarus ablusus Penn (Hatchie River Crayfish)	G4	LR lc	MS, TN
Procambarus acherontis (Lonnberg) (Orlando Cave Crayfish)	G1	EN B1+2c	FL
Procambarus acutissimus (Girard) (Sharpnose Crayfish)	G5	LR lc	AL, GA, MS
Procambarus acutus (Girard) (White River Crawfish)	G5	LR lc	AL, AR, DC, FL, GA, KY, LA, MD, MS, NC, OK, SC, TN, TX, VA, WV
Procambarus advena (LeConte) (Vidalia Crayfish)	G3	LR lc	GA
Procambarus alleni (Faxon) (Everglades Crayfish)	G4	LR lc	FL
Procambarus ancylus Hobbs (Coastal Plain Crayfish)	G4G5	LR lc	NC, SC
Procambarus angustatus (LeConte) (Sandhills Crayfish)	GX	CR B1+2c	GA
Procambarus apalachicolae Hobbs (Coastal Flatwoods Crayfish)	G2	VU B1+2c	FL
Procambarus attiguus Hobbs and Franz (Silver Glen Springs Crayfish)	G1G2	CR B1+2c	FL

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Procambarus barbatus (Faxon) (Wandering Crayfish)	G5	LR lc	GA, SC
Procambarus barbiger Fitzpatrick (Jackson Prairie Crayfish)	G2	VU B1+2c	MS
Procambarus bivittatus Hobbs (Ribbon Crayfish)	G5	LR lc	AL, FL, LA, MS
Procambarus blandingii (Harlan) (Santee Crayfish)	G4	LR lc	NC, SC
Procambarus braswelli Cooper (Waccamaw Crayfish)	G3	VU B1+2c	NC, SC
Procambarus brazoriensis Albaugh (Brazoria Crayfish)	G1	VU B1+2c, D1	TX
Procambarus capillatus Hobbs (Capillaceous Crayfish)	G3	VU B1+2c	AL, FL
Procambarus caritus Hobbs (Poor Crayfish)	G4	LR lc	GA
Procambarus ceruleus Fitzpatrick and Wicksten (Blueclaw Chimney Crayfish)	G1G3	EN B1+2c	TX
Procambarus chacei Hobbs (Cedar Creek Crayfish)	G4	LR lc	GA, SC
Procambarus clarkii (Girard) (Red Swamp Crawfish)	G5	LR lc	AL, AR, FL, (GA), KY, LA, (MD), MS, (NC), OK, (SC), TN, TX, (VA)
Procambarus clemmeri Hobbs (Cockscomb Crayfish)	G5	LR lc	AL, LA, MS
Procambarus cometes Fitzpatrick (Mississippi Flatwoods Crayfish)	G1	VU B1+2c, D1	MS
Procambarus connus Fitzpatrick (Carrollton Crayfish)	GH	VU B1+2c, D1	MS
Procambarus curdi Reimer (Red River Burrowing Crayfish)	G5	LR lc	AR, OK, TX
Procambarus delicatus Hobbs and Franz (Bigcheek Cave Crayfish)	G1	CR B1+2c	FL
Procambarus dupratzii Penn (Southwestern Creek Crayfish)	G5	LR lc	AR, OK, LA, TX
Procambarus echinatus Hobbs (Edisto Crayfish)	G3	VU B1+2c	SC
Procambarus econfinae Hobbs (Panama City Crayfish)	G1	EN B1+2c	FL
Procambarus elegans Hobbs (Elegant Creek Crayfish)	G4	LR lc	AR, LA, MS
Procambarus enoplosternum Hobbs (Black Mottled Crayfish)	G4G5	LR lc	GA, SC
Procambarus epicyrtus Hobbs (Humpback Crayfish)	G3	VU B1+2c	GA
Procambarus erythrops Relyea and Sutton (Santa Fe Cave Crayfish)	G1G2	EN B1+2c	FL
Procambarus escambiensis Hobbs (Escambia Crayfish)	G2	VU B1+2c, D1	AL, FL

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Procambarus evermanni (Faxon) (Panhandle Crayfish)	G4	LR lc	AL, FL, MS
Procambarus fallax (Hagen) (Slough Crayfish)	G5	LR lc	FL, GA
Procambarus ferrugineus Hobbs and Robison (Lonoke Crayfish)	G1	EN B1+2c	AR
Procambarus fitzpatricki Hobbs (Spinytail Crayfish)	G2	VU B1+2c	MS
Procambarus franzi Hobbs and Lee (Orange Lake Cave Crayfish)	G1G2	EN B1+2c	FL
Procambarus geminus Hobbs (Twin Crawfish)	G3G4	LR lc	AR, LA
Procambarus geodytes Hobbs (Muddiver Crayfish)	G4	LR lc	FL
Procambarus gibbus Hobbs (Muckalee Crayfish)	G3	VU B1+2c	GA
Procambarus gracilis (Bundy) (Prairie Crayfish)	G5	LR lc	AR, OK, TX
Procambarus hagenianus hagenianus (Faxon) (Southeastern Prairie Crayfish)	G4G5T4	LR lc	AL, MS
Procambarus hagenianus vesticeps Fitzpatrick (Egyptian Crayfish)	G4G5T3	VU B1+2c	MS
Procambarus hayi (Faxon) (Straightedge Crayfish)	G5	LR lc	AL, MS, TN
Procambarus hinei (Ortmann) (Marsh Crayfish)	G5	LR lc	LA, TX
Procambarus hirsutus Hobbs (Shaggy Crayfish)	G4	LR lc	SC
Procambarus horsti Hobbs and Means (Big Blue Springs Crayfish)	G1	EN B1+2c	FL
Procambarus howellae Hobbs (Ornate Crayfish)	G5	LR lc	GA
Procambarus hubbelli (Hobbs) (Jackknife Crayfish)	G4	LR lc	AL, FL
Procambarus hybus Hobbs and Walton (Smoothnose Crayfish)	G5	LR lc	AL, MS
Procambarus incilis Penn (Cut Crayfish)	G4	LR lc	TX
Procambarus jaculus Hobbs and Walton (Javelin Crayfish)	G4	LR lc	LA, MS
Procambarus kensleyi Hobbs (Free State Chimney Crayfish)	G4	LR lc	LA, TX
Procambarus kilbyi (Hobbs) (Hachet Crayfish)	G4	LR lc	FL
Procambarus lagniappe Black (Lagniappe Crayfish)	G2	VU B1+2c	AL, MS
Procambarus latipleurum Hobbs (Wingtail Crayfish)	G2	VU B1+2c, D1	FL
Procambarus lecontei (Hagen) (Mobile Crayfish)	G3G4	VU B2+3a	AL, MS

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Procambarus leitheuseri Franz and Hobbs (Coastal Lowland Cave Crayfish)	G1G2	VU B1+2c, D1	FL
Procambarus leonensis Hobbs (Blacknose Crayfish)	G4	LR lc	FL
Procambarus lepidodactylus Hobbs (Pee Dee Lotic Crayfish)	G4	LR lc	SC
Procambarus lewisi Hobbs and Walton (Spur Crayfish)	G4	VU B2+3a	AL
Procambarus liberorum Fitzpatrick (Osage Burrowing Crayfish)	G4	LR lc	AR, OK
Procambarus litosternum Hobbs (Blackwater Crayfish)	G4	LR lc	GA
Procambarus lophotus Hobbs and Walton (Mane Crayfish)	G5	LR lc	AL, GA, TN
Procambarus lucifugus alachua (Hobbs) (Alachua Light Fleeing Crayfish)	G2G3T2T3	VU B2+3a, D2	FL
Procambarus lucifugus lucifugus (Hobbs) (Florida Cave Crayfish)	G2G3T2	EN B1+2c	FL
Procambarus lunzi (Hobbs) (Hummock Crayfish)	G4	LR lc	GA, SC
Procambarus lylei Fitzpatrick and Hobbs (Shutispear Crayfish)	G2	VU B1+2c	MS
Procambarus machardyi Walls (Caddo Chimney Crawfish)	G1G2	LR lc	LA
Procambarus mancus Hobbs and Walton (Lame Crayfish)	G4	EN B1+2c	MS
Procambarus marthae Hobbs (Crisscross Crayfish)	G3	VU B1+2c	AL
Procambarus medialis Hobbs (Tar River Crayfish)	G3	VU B1+2c	NC
Procambarus milleri Hobbs (Miami Cave Crayfish)	G1	EN B1+2c	FL
Procambarus morrisi Hobbs and Franz (Putnum County Cave Crayfish)	G1	CR B1+2ae, C1+2b	FL
Procambarus natchitochae Penn (Red River Crayfish)	G5	LR lc	AR, LA, TX
Procambarus nechesae Hobbs (Neches Crayfish)	G2	VU B1+2c	TX
Procambarus nigrocinctus Hobbs (Blackbelted Crayfish)	G1G2	VU B1+2c, D1	TX
Procambarus nueces Hobbs and Hobbs (Nueces Crayfish)	G1	CR B1+2c	TX
Procambarus okaloosae Hobbs (Okaloosa Crayfish)	G4	LR lc	AL, FL
Procambarus orcinus Hobbs and Means (Woodville Karst Cave Crayfish)	G1	VU B1+2c, D1	FL
Procambarus ouachitae Penn (Ouachita River Crayfish)	G5	LR lc	AR, MS

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Procambarus paeninsulanus (Faxon) (Peninsula Crayfish)	G5	LR lc	AL, FL, GA
Procambarus pallidus (Hobbs) (Pallid Cave Crayfish)	G2G3	VU B1+2c, D1	FL
Procambarus parasimulans Hobbs and Robison (Bismark Burrowing Crayfish)	G4	LR lc	AR
Procambarus pearsei (Creaser) (Sandhills Crayfish)	G4	LR lc	NC, SC
Procambarus penni Hobbs (Pearl Blackwater Crayfish)	G3	VU B1+2c	AL, LA, MS
Procambarus pentastylus Walls and Black (Calcasieu Creek Crayfish)	G3	LR lc	LA
Procambarus petersi Hobbs (Ogeechee Crayfish)	G3	VU B1+2c	GA
Procambarus pictus (Hobbs) (Spotted Royal Crayfish)	G2	VU B1+2c	FL
Procambarus planirostris Penn (Flatnose Crayfish)	G4	LR lc	LA, MS
Procambarus plumimanus Hobbs and Walton (Croatan Crayfish)	G4	VU B1+2c	NC
Procambarus pogum Fitzpatrick (Bearded Red Crayfish)	G1	VU B1+2c, D1	MS
Procambarus pubescens (Faxon) (Brushnose Crayfish)	G4G5	LR lc	GA, SC
Procambarus pubischelae deficiens Hobbs (Hookless Crayfish)	G5T3	LR lc	GA
Procambarus pubischelae pubishelae Hobbs (Brushpalm Crayfish)	G5T5	LR lc	FL, GA
Procambarus pycnogonopodus Hobbs (Stud Crayfish)	G4G5	LR lc	FL
Procambarus pygmaeus Hobbs (Christmas Tree Crayfish)	G4	LR lc	FL, GA
Procambarus raneyi Hobbs (Disjunct Crayfish)	G4	LR lc	GA, SC
Procambarus rathbunae (Hobbs) (Comb Claw Crayfish)	G2	VU B1+2c	FL
Procambarus regalis Hobbs and Robison (Regal Burrowing Crayfish)	G2G3	VU B1+2c	AR
Procambarus reimeri Hobbs (Irons Fork Burrowing Crayfish)	G1	VU B1+2c, D1	AR
Procambarus rogersi campestris Hobbs (Field Crayfish)	G4T3	VU B1+2c, D1	FL
Procambarus rogersi expletus Hobbs and Hart (Perfect Crayfish)	G4T1	EN B1+2c	FL
Procambarus rogersi ochlocknensis Hobbs (Ochlockonee Crayfish)	G4T2T3	VU B1+2c	FL
Procambarus rogersi rogersi (Hobbs) (Seepage Crayfish)	G4T1T2	EN B1+2c	FL

Table 1.—Continued.

Species	Global Rank	Red List	Distribution
Procambarus seminolae Hobbs (Seminole Crayfish)	G5	LR lc	FL, GA
Procambarus shermani Hobbs (Gulf Crayfish)	G4	LR lc	AL, FL, LA, MS
Procambarus simulans (Faxon) (Southern Plains Crayfish)	G5	LR lc	AR, LA, OK, TX
Procambarus spiculifer (LeConte) (White Tubercled Crayfish)	G5	LR lc	AL, FL, GA, TN, SC
Procambarus steigmani Hobbs (Parkhill Prairie Crayfish)	G1G2	CR B1+2c	TX
Procambarus suttkusi Hobbs (Choctawhatchee Crayfish)	G3G4	LR lc	AL, FL
Procambarus talpoides Hobbs (Mole Crayfish)	G5	LR lc	FL, GA
Procambarus tenuis Hobbs (Ouachita Mountain Crayfish)	G3	VU B1+2c	AR, OK
Procambarus texanus Hobbs (Bastrop Crayfish)	G1	CR B1+2c	TX
Procambarus troglodytes (LeConte) (Eastern Red Swamp Crayfish)	G5	LR lc	GA, SC
Procambarus truculentus Hobbs (Bog Crayfish)	G3	LR lc	GA
Procambarus tulanei Penn (Giant Bearded Crayfish)	G5	LR lc	AR, LA
Procambarus verrucosus Hobbs (Grainy Crayfish)	G4	LR lc	AL, GA
Procambarus versutus (Hagen) (Sly Crayfish)	G5	LR lc	AL, FL, GA
Procambarus viaeviridis (Faxon) (Vernal Crayfish)	G5	LR lc	AL, AR, KY, LA, MS, TN
Procambarus vioscai paynei Fitzpatrick (Payne's Creek Crayfish)	G5T4	LR lc	AL, MS, TN
Procambarus vioscai vioscai Penn (Percy's Creek Crayfish)	G5T4	LR lc	AR, LA
Procambarus youngi Hobbs (Florida Longbeak Crayfish)	G2	EN B1+2c	FL
Procambarus zonangulus Hobbs and Hobbs (Southern White River Crawfish)	G5	LR lc	AL, LA, (MD), MS, TX, VA, (WV)
Troglocambarus maclanei Hobbs (Spider Cave Crayfish)	G2	VU B1+2c	FL
Troglocambarus sp. 1 (Orlando Spider Cave Crayfish)	G1	VU B1+2c, D1	FL

by Master (1991) as species in need of conservation attention.

The causes of loss of aquatic species and population declines have been attributed to three major categories: (1) habitat fragmentation due to loss, degradation, or alteration of habitat; (2) chemical pollution from contaminants and disturbance from anthropogenic use; and (3) introduction of nonindigenous organisms and overexploitation (Allan & Flecker 1993; Richter et al. 1997; Wilcove et al. 2000). The imperilment of some crayfish taxa is due to limited natural range (e.g., one locality or one drainage system). The lack of recent distributional information is problematic; however, significant progress has been made mostly as a result of the publication of numerous state field guides. Life history, ecological, and current distribution information

Figure 1.—Distribution of states included in the conservation status assessment of southeastern crayfishes. State inclusion is based on membership in the American Fisheries Society Southern Division. States in gray are included in the conservation status, while those in white are excluded.

are still lacking for about 60% of the North American fauna (Flinders & Magoulick 2005). For crayfishes, land use change can affect habitat resources (Burskey & Simon 2010) by degrading habitat and homogenization of substrate surfaces. Loss of such habitat components through dredging and channelization can drastically affect crayfish populations by making them more susceptible to predation. Crayfish depend on instream cover including gravel and boulder substrates, woody debris, and vegetation for refuge from predators (Stein 1977; Burskey & Simon 2010). These factors increase fish predation by impounding lotic habitat, which affects relative abundance of crayfish by increasing concentrations of major predators on crayfish, such as black basses and sunfish, and altering both the physical and chemical structure of streams (Williams et al. 1993).

Crayfish are among the most sensitive aquatic organisms and exhibit specific differences in tolerance to contaminants (Simon & Morris 2009). Crayfish show extreme sensitivity when exposed to pesticides and metals (Mayer & Ellersieck 1986; Jarvinen & Ankley 1999; Besser et al. 2006) and data show significant variability among genera, species, and life stages (Berrill et al. 1985; Peake et al. 2004; Wigginton & Birge 2007; Simon & Morris 2009). These observations suggest that crayfish may be important indicators of habitat degradation due to pollutants (Burskey & Simon 2010).

The introduction of nonindigenous organisms may be among the most serious threats to conservation of biodiversity of native crayfish (Lodge et al. 2000; Clavero & García-Berthou 2005). In North America, crayfishes are transported easily and can be inadvertently introduced into aquatic habitats when they are discarded as unused bait (Simon 2002). Such bait-bucket introductions have led to dramatic range extensions of several species, most notably *Orconectes rusticus* (Rusty Crayfish) and *Procambarus clarkii* (Red Swamp Crawfish). The Rusty Crayfish is native to the Greater Miami River in Ohio and Indiana, northern Kentucky, and the Maumee River drainage in Indiana, northwestern Ohio, and extreme southeastern Michigan. The species has been introduced across the upper midwestern United States and Canada (Page 1985; Lodge et al. 2000) by expanding its range and displacing native crayfishes (Taylor & Redmer 1996). Possible displacement mechanisms include faster individual growth rates (Hill et al. 1993), differential susceptibility to fish predation (DiDonato & Lodge 1993), and hybridization (Perry et al. 2001). Imperiled crayfishes also have been affected by nonindigenous species. The federally endangered *Pacifastacus fortis* has been displaced over large portions of its native range by the nonindigenous *P. leniusculus* (Erman et al. 1993).

Additional risk from non-indigenous crayfish is due to the escape of pets from the aquarium and aquaculture trade (Simon 2002); however, increased prevention requires bait regulations that reduce nonindigenous crayfish spread (Holdrich 1999). States have either banned the sale of crayfish as bait or created a list of prohibited species, restricting the sale by bait dealers of nonindigenous species including transport across state lines. While no known North American cases of overexploitation of crayfish have been documented, commercial harvest has the potential of being a serious contributing factor when harvesting from wild populations. Species most vulnerable to over-harvesting are those with small native ranges, long life spans prior to adult maturation, and low reproductive potential. Gulf Coast populations are perhaps the most vulnerable to over-harvesting if not managed properly because of exposure to hurricanes. However, the majority of states that possess a highly diverse crayfish fauna with high levels of endemism lack any

protective measures or adequate funding structures to monitor the status of their respective state faunas.

In conclusion, more than half of the southern crayfish species are stable and slightly less are in need of critical management intervention to protect remaining populations. More effort is needed to document distribution and habitat needs. Human encroachment causing habitat fragmentation, followed by contaminants and introductions of nonindigenous species, are among the most frequent reasons for imperilment. Wise land use development, protection of riparian corridors, and control of anthropogenic influences are needed to protect crayfish biodiversity in North America.

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APPENDIX 1.
THE INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES (IUCN) RED LIST CRITERIA ASSESSMENT PROCESS FOR CONSERVATION STATUS DETERMINATIONS.

CRITICALLY ENDANGERED (CR)

A) A taxon is Critically Endangered when it is facing an extremely high risk of extinction in the wild in the immediate future, as defined by any of the following criteria (A to E):

1) An observed, estimated, inferred or suspected reduction of at least 80% over the last 10 years or three generations, whichever is the longer, based on (and specifying) any of the following:

a) direct observation
b) an index of abundance appropriate for the taxon
c) a decline in area of occupancy, extent of occurrence and/or quality of habitat
d) actual or potential levels of exploitation
e) the effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.

2) A reduction of at least 80%, projected or suspected to be met within the next 10 years or three generations, whichever is the longer, based on (and specifying) any of (b), (c), (d) or (e) above.

B) Extent of occurrence estimated to be less than 100 km^2 or area of occupancy estimated to be less than 10 km^2, and estimates indicating any two of the following:

1) Severely fragmented or known to exist at only a single location.
2) Continuing decline, observed, inferred or projected, in any of the following:

a) extent of occurrence
b) area of occupancy
c) area, extent and/or quality of habitat
d) number of locations or subpopulations
e) number of mature individuals

3) Extreme fluctuations in any of the following:

a) extent of occurrence
b) area of occupancy
c) number of locations or subpopulations
d) number of mature individuals

C) Population estimated to number less than 250 mature individuals and either:

1) An estimated continuing decline of at least 25% within three years or one generation, whichever is longer or
2) A continuing decline, observed, projected, or inferred, in numbers of mature individuals and population structure in the form of either:

a) severely fragmented (i.e. no subpopulation estimated to contain more than 50 mature individuals)
b) all individuals are in a single subpopulation

D) Population estimated to number less than 50 mature individuals.

E) Quantitative analysis showing the probability of extinction in the wild is at least 50% within 10 years or three generations, whichever is the longer.

ENDANGERED (EN)

A taxon is Endangered when it is not Critically Endangered but is facing a very high risk of extinction in the wild in the near future, as defined by any of the following criteria (A to E):

A) Population reduction in the form of either of the following:

1) An observed, estimated, inferred or suspected reduction of at least 50% over the last 10 years or three generations, whichever is the longer, based on (and specifying) any of the following:

a) direct observation
b) an index of abundance appropriate for the taxon
c) a decline in area of occupancy, extent of occurrence and/or quality of habitat
d) actual or potential levels of exploitation
e) the effects of introduced taxa, hybridisation, pathogens, pollutants, competitors or parasites.

2) A reduction of at least 50%, projected or suspected to be met within the next 10 years or three generations, whichever is the longer, based on (and specifying) any of (b), (c), (d), or (e) above.

B) Extent of occurrence estimated to be less than 5000 km^2 or area of occupancy estimated to be less than 500 km^2, and estimates indicating any two of the following:

1) Severely fragmented or known to exist at no more than five locations.
2) Continuing decline, inferred, observed or projected, in any of the following:

a) extent of occurrence
b) area of occupancy
c) area, extent and/or quality of habitat
d) number of locations or subpopulations
e) number of mature individuals

3) Extreme fluctuations in any of the following:

a) extent of occurrence
b) area of occupancy
c) number of locations or subpopulations
d) number of mature individuals

C) Population estimated to number less than 2500 mature individuals and either:

1) An estimated continuing decline of at least 20% within five years or two generations, whichever is longer, or
2) A continuing decline, observed, projected, or inferred, in numbers of mature individuals and population structure in the form of either:

a) severely fragmented (i.e. no subpopulation estimated to contain more than 250 mature individuals)
b) all individuals are in a single subpopulation.

D) Population estimated to number less than 250 mature individuals.

E) Quantitative analysis showing the probability of extinction in the wild is at least 20% within 20 years or five generations, whichever is the longer.

VULNERABLE (VU)

A taxon is Vulnerable when it is not Critically Endangered or Endangered but is facing a high risk of extinction in the wild in the medium-term future, as defined by any of the following criteria (A to E):

A) Population reduction in the form of either of the following:

1) An observed, estimated, inferred or suspected reduction of at least 20% over the last 10 years or three generations, whichever is the longer, based on (and specifying) any of the following:

a) direct observation
b) an index of abundance appropriate for the taxon
c) a decline in area of occupancy, extent of occurrence and/or quality of habitat
d) actual or potential levels of exploitation
e) the effects of introduced taxa, hybridisation, pathogens, pollutants, competitors or parasites.

2) A reduction of at least 20%, projected or suspected to be met within the next ten years or three generations, whichever is the longer, based on (and specifying) any of (b), (c), (d) or (e) above.

B) Extent of occurrence estimated to be less than 20,000 km^2 or area of occupancy estimated to be less than 2000 km^2, and estimates indicating any two of the following:

1) Severely fragmented or known to exist at no more than ten locations.
2) Continuing decline, inferred, observed or projected, in any of the following:

a) extent of occurrence
b) area of occupancy
c) area, extent and/or quality of habitat
d) number of locations or subpopulations
e) number of mature individuals

3) Extreme fluctuations in any of the following:

a) extent of occurrence
b) area of occupancy
c) number of locations or subpopulations
d) number of mature individuals

C) Population estimated to number less than 10,000 mature individuals and either:

1) An estimated continuing decline of at least 10% within 10 years or three generations, whichever is longer, or
2) A continuing decline, observed, projected, or inferred, in numbers of mature individuals and population structure in the form of either:

a) severely fragmented (i.e. no subpopulation estimated to contain more than 1000 mature individuals)
b) all individuals are in a single subpopulation

D) Population very small or restricted in the form of either of the following:

1) Population estimated to number less than 1000 mature individuals.
2) Population is characterized by an acute restriction in its area of occupancy (typically less than 100 km^2) or in the number of locations (typically less than five). Such a taxon would thus be prone to the effects of human activities (or stochastic events whose impact is increased by human activities) within a very short period of time in an unforeseeable future, and is thus capable of becoming Critically Endangered or even Extinct in a very short period.

E) Quantitative analysis showing the probability of extinction in the wild is at least 10% within 100 years.

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MAMMALS OF THE INDIANAPOLIS INTERNATIONAL AIRPORT CONSERVATION PROPERTIES, HENDRICKS COUNTY, INDIANA, WITH COUNTY RECORDS

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ABSTRACT. Mammals at the Indianapolis International Airport conservation properties were studied from 2008 until 2010 in association with on-going studies of the bat community at the site. Results from a combination of mist-netting, small mammal trapping, and observational records are reported herein. Thirty-seven of the 59 species of mammals known to occur in Indiana were documented at the conservation properties owned by the Indianapolis International Airport. Species not previously captured in Hendricks County were the masked shrew (*Sorex cinereus*), deer mouse (*Peromyscus maniculatus*), and western harvest mouse (*Reithrodontomys megalotis*). These individuals of the western harvest mouse also represent the easternmost record of this species' known range.

Keywords: Mammals, *Reithrodontomys megalotis*, Indianapolis International Airport, Hendricks County

INTRODUCTION

Biological diversity surveys are strongly encouraged by the Indiana Biological Survey of the Indiana Academy of Science. These surveys provide a valuable service by documenting the flora and fauna of the different lands throughout Indiana, both public and private. Many areas at an urban/rural interface, such as those surrounding the Indianapolis International Airport (IND), have not had a comprehensive survey of mammals.

In 1991, IND and the U.S. Fish and Wildlife Service agreed to set aside mitigation land for bat conservation (Whitaker et al. 2004). In 1997, Indiana State University and the Indianapolis Airport Authority (IAA) became partners in a series of studies aimed at ascertaining the status and biology of the federally endangered Indiana myotis (*Myotis sodalis*) on properties near IND (Sparks et al. 1998). Mist netting since 1997 has produced much data on the bat species composition of the area (Whitaker et al. 2004), and in previous years fish and herptile surveys have also been conducted on these lands (Foster et al. 2005; Ritzi et al. 2005). The purpose of this paper is to summarize information on the mammal fauna, including the presence of three previously undocumented species in Hendricks County: the masked shrew (*Sorex cinereus*), and two species of cricetid rodent, the deer mouse (*Peromyscus maniculatus*) and the western harvest mouse (*Reithrodontomys megalotis*).

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METHODS

Study site.—The Indianapolis Airport Authority (IAA) conservation lands are made up of several small woodlots within a matrix of residential, industrial and agricultural areas. The area is bordered by US Highway 40 and Indiana Highway 67 to the north and south, respectively (Fig. 1), and Indiana Highway 267 borders at the west. To the east of the study area lies the Indianapolis airport. Interstate 70 runs from east to west through the center of the area. The southern areas consist of wetlands, and the East Fork of White Lick Creek runs from north to south through the site. Most of the creek is wooded on both sides, and the open areas are primarily cultivated for agriculture or hay fields.

Sampling.—Small mammal trapping was conducted during the summers of 2008 to 2010 for a total of 7120 trap-nights. All trapping occurred from 15 May–15 August with the exceptions of

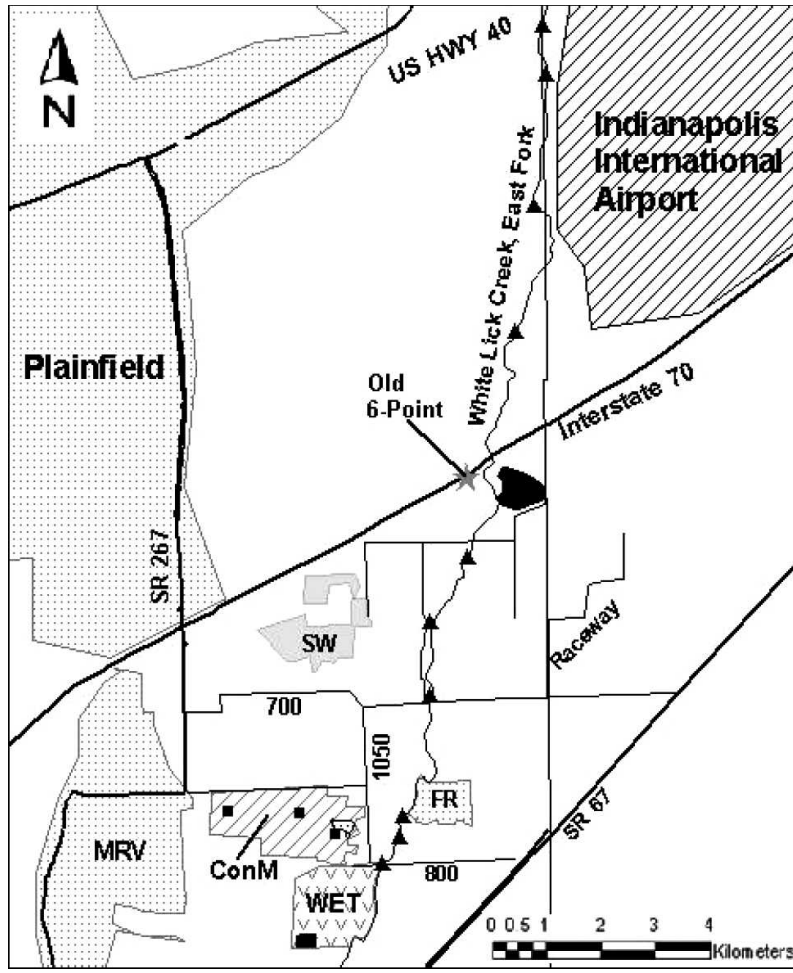

Figure 1.—Map of the Indianapolis Airport Authority (IAA) Conservation Lands. Abbreviations are as follows: ConM = Conservation Management and Red Squirrel Pond (stippled area), FR = Friendswood Golf Course, MRV = Mooresville, SW = Sodalis Woods and WET = wetlands. Black triangles represent net sites for bats along East Fork of White Lick Creek and black squares within Conservation Management denote net sites within. County roads are labeled for reference.

2009, in which trapping efforts extended until 26 August, and in 2010 occasional mole trapping began by 13 April. Most trapping was done with Sherman live traps. Snap traps were occasionally used in supplementary sampling efforts. In 2008, and to a lesser extent in 2009, one-liter pitfall traps were placed along fallen logs and other natural barriers in order to catch shrews. Mole traps were occasionally set in fresh tunnel systems, usually on the edges of small woodlots.

Bats were captured from 1997–1999 and 2002–2010 using two- and three- tier mist nets placed under adequate tree canopy over the East Fork of White Lick Creek (Fig. 1), or on trails and near roost trees in wooded areas. All netting began no earlier than 15 May and ended no later than 15 August, with the exception of 2010 when netting began 10 April. A more detailed account of protocols used for bats can be found in Whitaker et al. (2008, 2009, 2010).

Information on larger mammals was obtained through a combination of observation, field notes, and specimens in the Indiana State University Vertebrate Collections (ISUVC). Observations were defined as witnessing live individuals directly or dead via road mortalities, but also via indirect methods such as scat presence, track or vocalizations.

Table 1.—Summary of small mammals trapped on Indianapolis International Airport Conservation Lands, Hendricks County, during 2008–2010. Total trap-nights and success rates as percentage are listed below yearly and cumulative totals. A (*) denotes that an individual was found dead and was not captured in a trap.

	Year			
	2008	2009	2010	Total
Talpidae				
Scalopus aquaticus	1*	1	1	3
Soricidae				
Blarina brevicauda	14	5	0	19
Sorex cinereus	8	2	0	10
Cricetidae: Arvicolinae				
Microtus ochrogaster	0	48	14	62
Microtus pennsylvanicus	0	32	27	59
Cricetidae: Neotominae				
Peromyscus leucopus	7	54	4	65
Peromyscus maniculatus	0	5	26	31
Reithrodontomys megalotis	0	10	3	13
Muridae				
Mus musculus	0	2	2	4
Dipodidae				
Zapus hudsonius	1	1	21	23
Sciuridae				
Tamias striatus	1	0	0	1
Total	32	160	98	290
Trap-nights	1211	2924	2985	7120
Captures/trap-night	0.026	0.055	0.033	0.041

RESULTS

A total of 37 species of mammals were captured or observed at this study site. A total of 290 individuals representing 11 species of small mammals were captured over a total of 7120 trap-nights at the Indianapolis International Airport Conservation Properties from 2008 through 2010 (Table 1). From 1997 through 2010, ten species of bats have been captured in mist nets (Table 2). Results are listed in systematic order below.

DIDELPHIMORPHA

Didelphidae.—Virginia opossums (*Didelphis virginiana*) are relatively abundant in the area and were observed on many occasions, most often after nightfall. We have observed them living in hollowed out trees, as well as in a rocky, underground burrow. An adult with 5 juveniles was found near the junction of Stafford and Perry roads in 2002 (ISUVC catalog #7695, 7696, 7697, 7698, 7699, 7700).

INSECTIVORA

Talpidae.—The eastern mole (*Scalopus aquaticus*) occurs frequently based on observed tunneling. In addition, one individual was found dead in 2008 on a trail near Red Squirrel Pond. In 2009 and 2010, a total of 2 moles were captured in 28 trap-nights. Both of these individuals were captured within two meters of the east fork of White Lick Creek; one in 2009 in the wetlands, and one in 2010 near the wetlands along CR 800 (Fig. 1).

Soricidae.—Two species of shrews were captured on the IAA properties: the northern short-tailed shrew (*Blarina brevicauda*) and the masked shrew (*S. cinereus*; Table 1). The individuals of *S. cinereus* ($n = 10$) represent the first record of this species in Hendricks County. Most shrews were captured in wooded areas using pitfall traps; however 3 individuals of *B. brevicauda* were captured in open areas: one in a grassy field, one in a newly planted woodlot, and the third in a very disturbed location near

I-70. Most of the *S. cinereus* were only captured in a riparian area using pitfall traps. However, one individual was captured in a Sherman trap located in a grassy area near Conservation Management. An additional individual of *S. cinereus* (ISUVC #6421) was captured in 1998.

CHIROPTERA

Vespertilionidae.—From 1997–2010, 2384 bats representing 10 species were captured (Table 2). Seven of these were captured annually, while three were rare. The most common bat present is the big brown bat (*Eptesicus fuscus*), followed by (in order of abundance) the Indiana myotis (*Myotis sodalis*), red bat (*Lasiurus borealis*), tri-colored bat (*Perimyotis subflavus*), northern myotis (*Myotis septentrionalis*), little brown myotis (*Myotis lucifugus*), evening bat (*Nycticeius humeralis*), silver-haired bat (*Lasionycteris noctivagans*), hoary bat (*Lasiurus cinereus*), and the gray bat (*Myotis grisescens*).

Table 2.—Total mist net captures of 10 bat species on the IAA conservation lands between 1997 and 2010. No netting occurred in 2000 or 2001. Numbers in parentheses represent the percent total of each species.

	Cumulative Years Netting	
	1997–2010	
Eptesicus fuscus	1273	(53.4)
Myotis sodalis	240	(10.1)
Lasiurus borealis	238	(10.0)
Perimyotis subflavus	199	(8.3)
Myotis septentrionalis	161	(6.8)
Myotis lucifugus	154	(6.5)
Nycticeius humeralis	102	(4.3)
Lasionycteris noctivagans	8	(0.3)
Lasiurus cinereus	8	(0.3)
Myotis grisescens	1	(0.04)
Total	2384	

RODENTIA

Castoridae.—American beavers (*Castor canadensis*) were seen on many occasions in or near White Lick Creek. No beavers have been documented anywhere else on the project site.

Cricetidae: Arvicolinae.—At least 6 species of the family Cricetidae occur at the IAA study site, 3 in the Neotominae and 3 in the Arvicolinae. The meadow vole and the prairie vole (*Microtus pennsylvanicus* and *M. ochrogaster*, respectively) are abundant in the study area (Table 1). The muskrat (*Ondatra zibethicus*) is the only other arvicoline which occurs at the study site. Muskrats at the study area are largely limited to marshier parts of the area, as well as riparian corridor following WLC. One muskrat was taken from the IAA land in 2002 (ISUVC #7097). Two other arvicolines were not captured. Efforts have been made to capture pine voles (*Microtus pinetorum*) using pitfalls and Sherman traps sunken into soil, particularly near spots which looked ideal. The southern bog lemming (*Synaptomys cooperi*) has not been captured, despite trapping in various areas of suitable habitat.

Cricetidae: Neotominae.—The white-footed mouse (*Peromyscus leucopus*) occurs frequently, and is the only cricetid found in the wooded areas at the site. Many individuals of the deer mouse (*Peromyscus maniculatus*) have also been captured ($n = 31$), especially in areas adjacent to Interstate 70. Surprisingly, this is also a new record in Hendricks County, which is likely due to an absence of trapping efforts in agricultural fields and habitat with little to no vegetative cover. This species was rarely captured alongside any other species, especially in areas with little to no understory vegetation. *Peromyscus maniculatus* is the only species of small mammal which has been frequently seen to inhabit areas with much bare ground (Whitaker 1967, Whitaker & Mumford 2009). The two species of Peromyscus at the study area exhibited very little habitat overlap, with *P. maniculatus* captured only in open areas and *P. leucopus* most often captured in the woods; the only location where the two species were found together was a location adjacent to Interstate 70.

The first recorded occurrence of the western harvest mouse (*Reithrodontomys megalotis*) in Hendricks County was in 2009, in a field in the southwest portion of the wetlands (Fig. 1). Additional individuals were captured in a trenched area adjacent to Interstate 70. This species is a relatively recent immigrant into Indiana (Whitaker & Sly 1970). It exists in low numbers in the study area, with ten individuals captured in Sherman traps in 2009, and three more captured in 2010. These individuals represent a new county record for this species, as well as the eastern-most record of the species' known range (Leibacher & Whitaker 1998, Whitaker & Mumford 2009).

Muridae: Murinae.—The house mouse (*Mus musculus*) is the only Old World rodent that has been observed. No rats were found. In 2009, two

individuals were taken in snap traps from a barn. We have also seen them under piles of refuse which are frequently dumped by humans. In June 2010, two house mice were captured in Sherman traps: one reproductive male and one pregnant female.

Dipodidae: Zapodinae.—The meadow jumping mouse (*Zapus hudsonius*) was captured in all three years of the study. One male was captured in July 2008 at a north-facing slope just south of I-70 at the former six-points interchange. A reproductive female was captured in July 2009 at the same location. In 2010, 22 individuals of this species were captured, 12 female, nine male and one which was not checked. All 22 from 2010 were captured to the east of the six-points location. This spot was also trapped in 2009; however none were captured.

In 2010, 2 individuals were outfitted with radio-transmitters to find their nesting locations. The first, a female, was radio-tracked during the daytime to a nest located about halfway up a south-facing slope. The opening to the nest was about 3 centimeters in diameter and surrounded by tufts of grass and much thatch. The second individual, also a female, was tracked to a small drainage ditch at the edge of a wooded area to the south of the site. The area surrounding the opening had much bare ground and several tree roots, as well as some human trash.

Sciuridae.—We have observed 5 species of squirrels at the IND conservation lands. The fox squirrel (*Sciurus niger*) was seen most often, and the red squirrel (*Tamiasciurus hudsonicus*) was observed in more wooded areas by Red Squirrel pond and in the southern riparian areas. One individual was taken in July 2004 (ISUVC # 9036). Southern flying squirrels (*Glaucomys volans*) are seen often at night in the older growth woodlots and are occasionally captured in mist nets. Eastern chipmunks (*Tamias striatus*) are seen occasionally, and one male was captured in a Sherman trap in 2008 (Table 1). Woodchucks (*Marmota monax*) are uncommon, although they are seen relatively regularly in the riparian woodlots to the south of County road 700 (Fig. 1).

LAGOMORPHA

Leporidae.—The eastern cottontail (*Sylvilagus floridanus*) is the only species of rabbit present and is very common on the IAA-managed lands. It is not uncommon to see several at a time near roads and residential areas. In 2010, an injured European rabbit (*Oryctolagus cuniculus*) was observed near a barn owned by IAA. It appeared to have been released and was likely wounded while crossing the road.

CARNIVORA

Canidae.—The coyote (*Canis latrans*; ISUVC #8427) has been seen on several occasions. Although this species has been recorded south of I-70, it has been most often seen to the north in more developed areas. We observed the red fox (*Vulpes vulpes*) rarely in the southern part of the conservation lands, but not to the north of I-70.

Felidae.—The only felid recorded was the introduced feral cat (*Felis domesticus*). Cats were seen quite often, and are likely affiliated to a high degree with the residential areas. In 2010, a female with 6 kittens was found near Sodalis Woods.

Mustelidae.—The long-tailed weasel (*Mustela frenata*; ISUVC #8561) was seen occasionally in the areas south of I-70. The least weasel (*M. nivalis*), though likely present, has not been observed. The mink (*M. vison*) has been seen rarely, and one was found as a road mortality near the junction of Stafford and Six-Points Road in 1998 (ISUVC #6404). Badgers (*Taxidea taxus*), scarce in Indiana, have not been seen at this location.

Mephitidae.—Striped skunks (*Mephitis mephitis*) have been rarely observed in the area, mostly dead on the road or indirectly by odor. In 2008, the senior author observed one crossing a road (CR-1050) after nightfall.

Procyonidae.—Raccoons (*Procyon lotor*) are the most abundant carnivore at IAA, and were seen in all habitat types. On several occasions, we have witnessed local residents hunting them, and they have been often found dead along roadsides.

ARTIODACTYLA

Cervidae.—White-tailed deer (*Odocoileus virginianus*) are abundant and were most often observed in the agricultural and hay fields and near the borders of open and wooded areas.

DISCUSSION

The conservation lands at the Indianapolis International Airport contain a wide variety of habitats and at least 37 of the 59 non-domesticated species of mammals known to exist in Indiana (Gikas et al. 2009; Whitaker & Mumford 2009). Three of the 37 species are newly reported records

for Hendricks County: the masked shrew (*Sorex cinereus*), deer mouse (*Peromyscus maniculatus*), and western harvest mouse (*Reithrodontomys megalotis*). Our records of *R. megalotis* at this study area represent the eastern-most record for this species.

The western harvest mouse may have arrived in Hendricks County using road right-of-ways as a dispersal path, and it is likely that they represent an eastward expansion of the individuals which Leibacher & Whitaker (1998) captured to the east of the Wabash River in Parke County. Given the small number of individuals captured, and the absence of this species in 2008, it is possible that this species arrived at the site in 2009. The small southern population is separated from the northern population by 5.2 km., and attempts at trapping in suitable habitat between these two sites have not produced this species.

The bat community at the airport has been well studied (Whitaker et al. 2004), with 10 of the 13 Indiana species captured, and 7 of the 10 species being captured annually. The silver-haired bat (*Lasionycteris noctivagans*), hoary bat (*Lasiurus cinereus*), and the gray bat (*Myotis grisescens*) were only seen rarely (n = 8, 8, 1, respectively). The silver-haired bat is a spring and fall migrant through the area, so it is expected that we would capture them in low numbers during the summer trapping. The hoary bat is probably more abundant than is suggested by these eight captures (Gehrt & Chelsvig 2004; Sparks et al. 2005). This species probably flies high and thus is seldom caught in nets (Whitaker & Mumford 2009). In 2009, we outfitted 1 female hoary bat with a radio transmitter in an attempt to track the individual; however it had left the area by the following day. In 2003, Sparks et al. (2005) tracked a juvenile hoary bat for 8 days and found it using eastern cottonwood trees as day roosts.

The most unexpected bat species captured was the 2005 capture of a gray bat (*M. grisescens*). Several gray bats taken in Indiana prior to 1980 were considered to be of accidental occurrence. In 1978, Cope & Richter (1978) caught 8 pregnant females at Sellersburg, Clark Co. Brack et al. (1982), found the maternity colony at a flooded quarry in Sellersburg (Brack et al. 1984). This colony contained about 400 individuals in 1982, but the population had increased to over 6000 bats in 2008. Gray bats have also been captured at various spots along the Ohio River (Whitaker & Gummer 2001, Whitaker et al. 2001). This individual captured at IND appears to have been a vagrant (Tuttle et al. 2005), possibly from the Sellersburg colony.

Eight species of mammals that could reasonably occur by virtue of distribution and habitat preferences have not been recorded on the study site. The least shrew (*Cryptotis parva*) and the southeastern shrew (*Sorex longirostrus*) were not captured but could likely occur. It is possible that the least shrew exists in the grassy fields but does not get captured in Sherman traps due to its low body mass. We did see a shrew fleeing a trap in 2009; however we were unable to capture it. The only masked shrew (*S. cinereus*) captured in a Sherman was in the same area in the same summer. The Norway rat (*Rattus norvegicus*), southern bog lemming (*Synaptomys cooperi*), and the pine vole (*Microtus pinetorum*) are also probable residents. The Gray squirrel (*Sciurus carolinensis*) could occur by general range, but is probably not as likely by habitat as this species usually occurs in deeper, more mature woods than that found at the IAA areas (Whitaker & Mumford 2009).

The gray fox (*Urocyon cinereoargenteus*) is likely to have occurred in the past, but may have been locally extirpated. The red fox and especially the gray fox have been decreasing throughout the state (Whitaker & Mumford 2009). This decline in species abundance could be due to a mixture of increasing development near the study site (Ordenana et al. 2010) and competition with the coyote (Major & Sherburne 1987; Sargeant & Allen 1989). The thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) has been observed in other parts of Hendricks, Marion, and Morgan Counties (Whitaker & Mumford 2009), but none have been observed on the study site. This species is rather obvious, especially along roadsides, and would be readily observed on some of the more maintained northern parts of the study site. This species appears to not be present in the study area.

The present study was carried out on an area preserved and enhanced as a ''conservation area'' near the Indianapolis International Airport, particularly for the Indiana myotis. However, the area has attracted many other species, including at least 37 of the 59 mammal species known to currently occur in Indiana. This demonstrates the importance to wildlife of areas such as this. As more land becomes developed and urban sprawl continues, these areas of

conservation become increasingly important. Increased efforts should be given to preserve similar areas, and also to survey all public areas such as parks, fish and wildlife areas, etc., and also private lands in order to record presence of species. Additionally, the occurrence of three new mammal county records shows that these surveys enhance our knowledge of species ranges. Although this conservation area is at the edge of a heavily urbanized area, very little is known with regard to the effect that urban sprawl has on species composition and behavior. Much more research should be done in similar areas to determine how these ''urban parks'' contribute to species diversity.

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ON THE DETECTION OF DENDRITIC CURRENTS USING FUNCTIONAL MAGNETIC RESONANCE IMAGING

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ABSTRACT. The currents associated with neuronal activity generate their own magnetic fields which potentially cause a measurable phase change in a magnetic resonance (MR) signal. The feasibility of directly measuring neuronal currents is still under debate. In this paper we model individual dendrites as magnetic current dipoles on a variable lattice structure in order to calculate the magnetic fields and phase shifts generated by active neuronal tissue. Our results show the field produced by an ensemble of simultaneously active dendrites may produce effects measurable using current MRI technology.

Keywords: MRI, phase shift, dendrite, brain, dipole

INTRODUCTION

Magnetic resonance imaging.—Over the past several decades great strides have been made with magnetic resonance imaging (MRI). This powerful technology exploits the intrinsic magnetic properties of matter on the atomic scale to probe materials noninvasively. Although the use of MRI is not limited to the medical fields, that is where it is perhaps most well known. Armed with the excellent spatial resolution provided by MRI, researchers have been able to provide many key insights into the structure and functioning of the brain. In the pursuit of this goal, functional MRI (fMRI) was developed to detect brain activity itself instead of examining solely structural features. Functional magnetic resonance works by detecting a so-called blood oxygenation level-dependent (BOLD) signal (Ogawa 1990). The physical basis for this signal is the fact the hemoglobin contained in red blood cells possesses different magnetic properties depending on whether or not it is oxygenated. However, the BOLD signal does not directly measure neural activity; instead, it is an indirect marker of perfusion and the signature of the metabolic processes of the brain (Heuttel et al. 2009).

Direct detection of neuronal activity.—Direct detection of the magnetic fields from neuronal currents themselves would provide a signal that closely follows the spatial and especially the temporal distribution of neural activity. Direct neuronal mapping through fMRI would give a noninvasive method for mapping the brain's neural pathways (Kwong et al. 1992). This capability would offer a number of immediate benefits:

1) It would allow direct measurements of nerve conduction velocities, an invaluable parameter for neurosurgeons wishing to determine the severity of damaged nerve bundles noninvasively.
2) It could allow for the diagnosis of diseases of the brain when in their preliminary stages. Important examples of these include Multiple Sclerosis, Parkinson's disease, and Alzheimer's.
3) It could facilitate an improved understanding of the ways in which drugs are delivered to the brain as well as the rest of the body.
4) It could provide new insights into the study of chronic pain.
5) It could aid in studies of cognitive function.

Scientific work to date.—Many researchers have tried to detect neuronal currents using MRI (Bodurka and Bandettini 2002; Cassara et al. 2008; Hagberg et al. 2006; Johnston et al. 1996; Kamei et al. 1999; Paley et al. 2009; Troung and Song 2006). However, the feasibility of such studies is still under debate (Konn

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et al. 2003; Xue et al. 2009). Some studies in the past have attempted to calculate the magnetic fields from action currents and other neural activity from first principles (Cassara et al. 2008; Paley et al 2009). Furthermore, a large body of research exists in the literature in which the magnetic fields from nerves, muscles, and single axons have been evaluated numerically (Swinney & Wikswo 1980; van Egeraat & Wikswo 1993; Woosley et al. 1985). Experimental work has also measured these fields using ferrite-core, wound-wire toroids (Gielen et al. 1986, 1991; Roth & Wikswo 1985; van Egeraat et al. 1990, 1993; Wijesinghe et al. 1991; Wikswo et al. 1980, 1990, 1991). Various investigations also present the results obtained from water phantoms (a type of experimental model), bloodless turtle brains, humans, rat brains, and theoretical calculations (Bandettini et al. 1992; Bodurka et al. 1999; Cassara et al. 2008, Kraus et al. 2008). Some of these studies claim to find measurably large magnetic fields and MRI phase and signal changes generated within the brain (Bodurka & Bandettini 2002; Park & Lee 2007; Xue et al. 2009). Others maintain that the resultant field intensity from human brain activity is simply too weak to detect a meaningful signal in MRI experiments (Chu et al. 2004; Xue et al. 2009). A proposed threshold for the minimum detectable field is on the order of 0.1 nT, corresponding to a minimum phase shift of approximately 0.0002 radians over a voxel (Bandettini et al. 2005). Our goal in this paper is to use calculated magnetic signals generated by dendrite currents in order to estimate the changes in an MRI signal.

METHODS

The dipole model.—From the standpoint of physically modeling the magnetic fields of complex biological systems, one must choose some current distribution to generate the magnetic field. A popular approach (Kaufman et al. 1991; Konn et al. 2003), and one that we continue in this investigation, models magnetic field sources as current dipoles. Past studies have used this method to model large portions of the brain using tens to hundreds of dipoles. Our study expands on this by modeling individual dendrites as magnetic current dipoles. It is also worth noting that the magnetic field from an arbitrary current distribution may be represented as what is known as a multipole expansion (Jackson 1999). In this expansion, the dominating contribution generally comes from the leading dipole term. It is only in the case of special symmetries that higher-order terms deliver large effects, and these special symmetries are not expected to be present in the brain.

The mathematics of the model.—In this model we use the well-known quasi-static approximation of Maxwell's equations. Although the brain can hardly be called a static system, dynamic changes occur at frequencies well below those required for the use of the full machinery of Maxwell's dynamic theory. Generally speaking, the quasi-static approximation is appropriate whenever velocities are much less than the speed of light ($v/c << 1$), a condition satisfied by the brain. Therefore the magnetic field, **B**, generated by an arbitrary current distribution may be given by the Biot-Savart law:

$$\vec{B} = \int \frac{\mu}{4\pi} \frac{I \, d\overrightarrow{l'} \times \vec{r}}{r^3}. \quad (1)$$

In the above equation μ is the magnetic permeability, I is the current, ***dl'*** is the differential length element, and r is the vector distance from the source point to the field point; the primed length element indicates that the integration is to be carried out over the source distribution.

For the case of a magnetic current dipole, the integration may be evaluated to yield the following formula:

$$\vec{B} = \frac{\mu}{4\pi} \frac{\vec{p} \times \vec{R}}{R^3}. \quad (2)$$

Here **p** is the current dipole; **R** is the vector from the dipole at the source point to the field point. In the case of MRI, one is interested in only one Cartesian component of the magnetic field. Without loss of generality, we take this to be the component parallel to the z-axis. For simplicity, we take the field point also to be on the z-axis. These considerations allow us then to write Eq. 2 in the following form with explicit reference to the vector components:

$$B_z = \frac{\mu}{4\pi} \frac{\left(-p_x y' + p_y x'\right)}{\left(z^2 + (x')^2 + (y')^2 + (z')^2 - 2z \cdot z'\right)^{3/2}}. \quad (3)$$

The resultant field from a group of dipoles (i.e. dendrites) will then be a linear superposition of the individual fields given by Eq. 3. Although the brain is a highly nonlinear physical system, the phenomenon of magnetism is linear with

regards to the combination of fields. As such, nonlinearities must introduce themselves within the source distribution and then be transmitted linearly. The effect of nonlinearities is discussed again in our conclusions.

The total resultant field generated by neuronal activity will make a contribution to the phase of the precessing magnetic moments within the sample, which is what MRI actually detects. The phase contribution due to the neuronal magnetic field at a given observation (field) point (x,y,z) in an activated voxel or volume may be calculated via the following equation (Xue et al. 2009):

$$\varphi(x,y,z) = \int_0^{TE} \gamma \cdot B_z(x,y,z,t)dt.$$

In the above equation, TE is the echo time of the MRI echo sequence. γ is the gyromagnetic ratio of the proton, a known value from quantum mechanics of 2.7×10^8 $s^{-1}T^{-1}$ (Wijesinghe & Roth 2009). In our calculations, we approximate this integral by assuming that the dendrites are active for a time less than echo sequence of the MRI, which allows the magnetic field B_z to be taken outside the integral. Thus, the phase shift is approximately the product $\varphi \approx \gamma \cdot B_z \cdot t_{act.}$, where $t_{act.}$ is the activation time of the dendrites, taken to be approximately 0.01 seconds.

Generally speaking, the various magnetic moments in a sample will precess at the so-called Larmor frequency, which depends on the magnitude of the applied magnetic field (for example, by the MRI equipment). From the perspective of the precessing magnetic moments, the biomagnetic field generated by neuronal activity also contributes to the applied magnetic field, thereby altering their precessional frequencies. Thus in this case the phase shift essentially measures the change in the angle between the (precessing) net magnetization before and after neuronal activation.

Model-specific details.—Real macroscopic volumes of human brain tissue are overwhelmingly complex physical systems. Narrowing the focus to relatively small groups of cells or even single cells does not necessarily improve the situation; the complex arborization of dendritic trees have, for example, provided a continual barrier to researchers seeking to understand and model neurons. In this model we are chiefly interested in finding upper bounds on the magnetic field magnitudes and their corresponding phase shifts. The nature of this goal allows us to make a number of simplifying assumptions. We assume: 1.) All the dendrites in the voxel are arranged on a uniform variable lattice structure. 2.) All dendrites are synchronously active. 3.) All dendrites have the same physical properties. 4.) The surrounding medium is homogenous and isotropic.

Dendrite properties: The empirical value for dendrite density in human cortical tissue is on the order of $10^6/mm^3$(Nunez 2006). Our simulations consider the effects of this density as well as several others. The physical dimensions of dendrites vary widely. Apical dendrites may reach lengths of around 300 microns, while the shortest branching dendrites may have lengths of only around 10 microns. A given cortical volume will generally contain more dendrites on the shorter end of the spectrum, and our simulations assume that all dendrites have an average length of 30 microns. Under the assumption that an active dendrite possesses an intracellular current of $\mathbf{I} = 1$ nA (Cassara et al. 2008; Park & Lee 2007), one finds the equivalent current dipole of a dendrite to be:

$$\vec{\mathbf{p}} = \vec{\mathbf{I}} \cdot \mathrm{L} = 3 \times 10^{-5} \text{ nAm}.$$

Spatial properties: As mentioned above, the surrounding medium is taken to be homogenous and isotropic with a magnetic permeability of 1.257×10^{-6} N/A^2. Also, the dendrites are taken to be arranged uniformly on a lattice structure and all to face in a given direction. Although these are both somewhat crude assumptions, they are appropriate given the goal of finding upper bounds for different arrangements. Clearly, the largest fields will be generated by parallel, synchronously active dendrites. Other spatiotemporal distributions would only result in smaller fields. Although these reduced-magnitude fields are perhaps not without interest, they are only of peripheral importance at the moment. Our computer model also has the capability to consider the effects of random dendrite orientations; examples of this are included below.

Computational details.—Our modeling was done independently in Matlab and in Java, with both languages yielding comparable results. Large simulations were carried out on Ball State University's Beowulf supercomputing cluster, a 32-node computer with 64 2.8 GHz Xeon processors. Smaller simulations were carried out on desktop PCs.

Table 1.—Summary of maximum simulated magnetic fields and phase shifts.

	Dendrite Density (dipoles / mm^3)	Maximum B-Field (nT) (Threshold: 0.1 T)	Phase Shift (radians $\times 10^{-3}$) (Threshold: 0.2×10^{-3})
Line (100)	100	0.005	0.082
Layer (10000)	10000	0.24	0.400
Full Voxel	$10^3 = 1000$	0.01	0.021
Full Voxel	$20^3 = 8000$	0.06	0.200
Full Voxel	$30^3 = 9000$	0.19	0.670
Full Voxel	$40^3 = 64{,}000$	0.47	1.600
Full Voxel	$50^3 = 125{,}000$	0.92	3.200
Full Voxel	$60^3 = 216{,}000$	1.60	5.500
Full Voxel	$70^3 = 343{,}000$	2.54	8.800
Full Voxel	$80^3 = 512{,}000$	3.80	13.000
Full Voxel	$90^3 = 729{,}000$	5.41	19.000
Full Voxel	$100^3 = 1{,}000{,}000$	7.43	26.000
Full Voxel	$215^3 = 9\ 938\ 375$	62.1	125.000
Full Voxel (Randomized)	10,000	0.09	0.241
Full Voxel (Randomized)	50,000	0.13	0.348
Full Voxel (Randomized)	100,000	0.41	1.097
Full Voxel (Randomized)	$50^3 = 125{,}000$	0.056	0.150
Full Voxel (Randomized)	1,000,000	0.22	0.589

CALCULATIONS AND RESULTS

In our investigation we conducted a variety of simulations using the model described above. We began by calculating the fields generated by relatively small numbers of dipoles and working up to full-voxel simulations with realistic dendrite densities. The results may be seen in Table 1, with results for randomly oriented dendrites appearing at the bottom.

As one would anticipate, in the case of uniform orientation the maximum simulated magnetic field rose linearly with increasing dendrite density. Because the phase shift given by Eq. 3 is essentially proportional to the magnetic field, the phase shift also rose linearly with increasing dendrite density. Both of these behaviors may be seen clearly in Fig. 1 and Fig. 2, respectively. Fig. 1 and Fig. 2 also display the results of the simulations with random dendrite orientations, and one observes that the magnetic field magnitudes are dramatically smaller.

In order to provide an easier visualization of the field behavior with increasing dendrite density, a cross section of the field was taken from each full-voxel simulation, and they are plotted together in Fig. 3. The cross sections were selected such that they include the fields' extremal values. A three-dimensional representation of the results for two full-voxel simulations may also be seen in Fig. 4 and Fig. 5. Fig. 4 shows the magnetic field at the center of the voxel for the case of 10^6 dendrites. Similarly, Fig. 5 demonstrates the effects of 125,000 randomly oriented dendrites.

DISCUSSION

Taken as a whole, our results demonstrate the low magnitudes associated with the magnetic fields generated by neural activity. In the case of uniform dendrite orientation, the maximum field magnitude approaches and even exceeds the proposed threshold of 0.1 nT at high densities, achieving a value of roughly 7 nT with 10^6 dendrites (see Table 1). In the case of the simulations with randomly oriented dipoles, the results are dramatically smaller (see Table 1 and Fig. 1 & 2), but one observes that many of them are still on the order of the threshold of 0.1 nT proposed by Bandettini. The following section offers interpretation of these results.

CONCLUSIONS AND SUMMARY

The main goal of this project was to construct a theoretical model to describe the magnetic fields generated by active dendrites in human cortical tissue and to assess the feasibility of imaging such activity via MRI. From the results of the simulations above, we report that the magnetic fields generated by dendrites

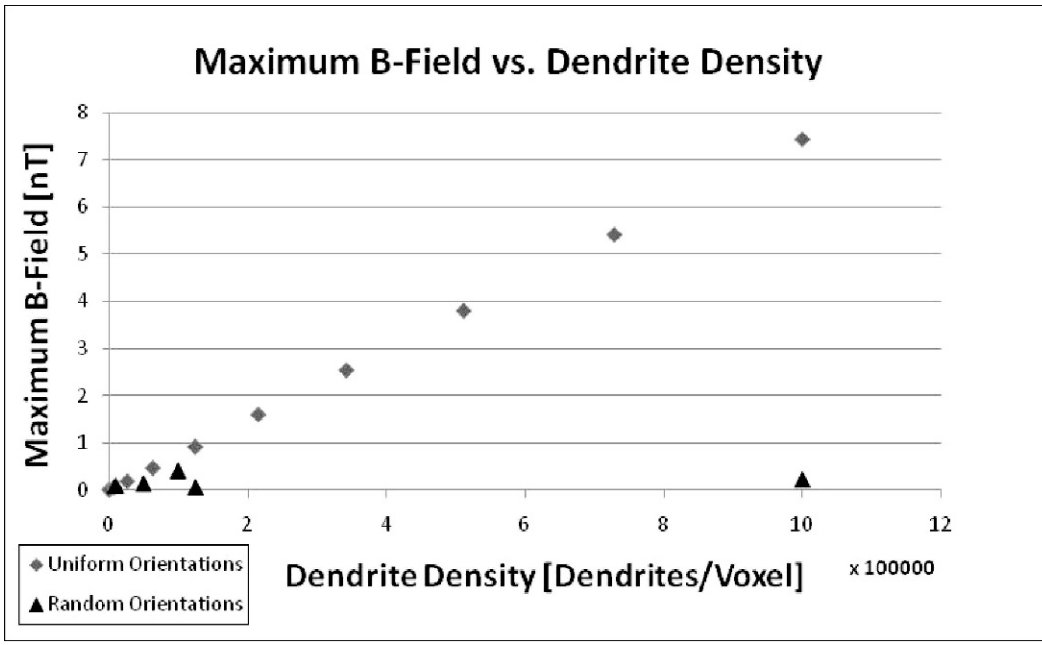

Figure 1.—A plot showing the maximum magnetic field in a simulation versus the dendrite density of the simulation.

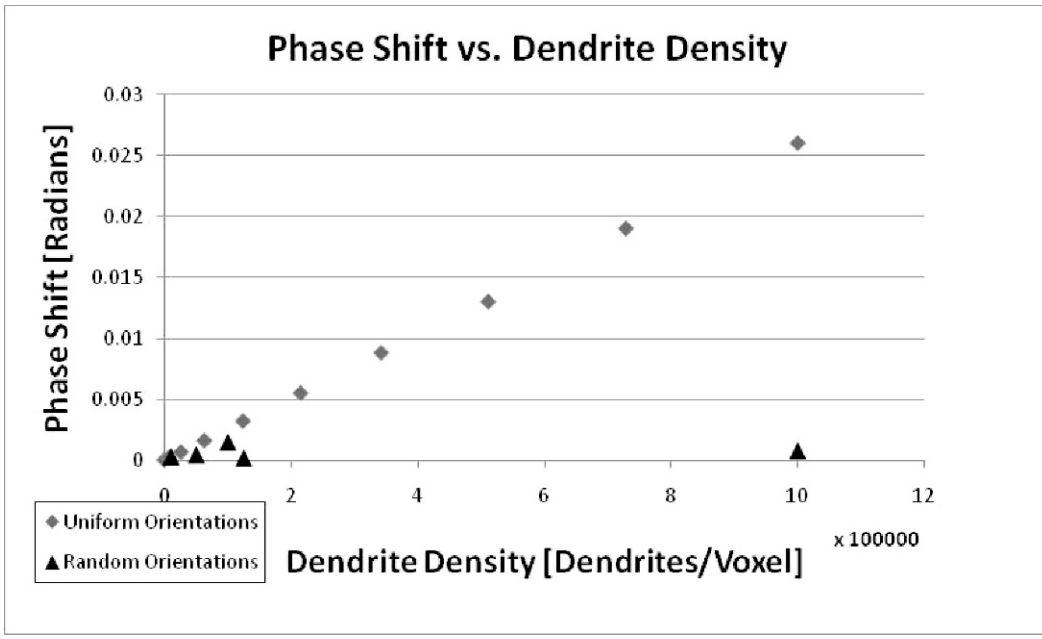

Figure 2.—A plot showing the calculated phase shift in an MR signal versus the dendrite density of the simulation.

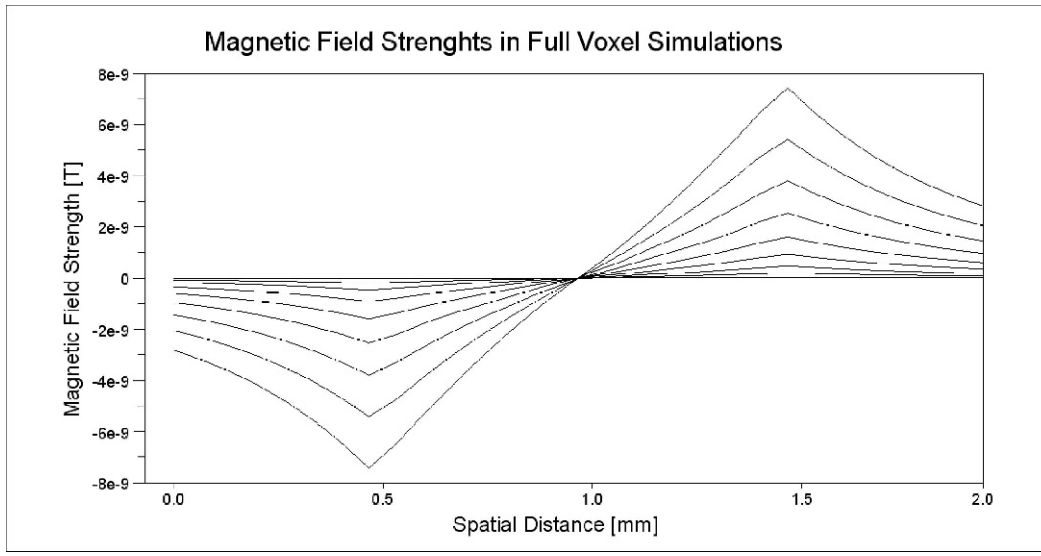

Figure 3.—A plot showing cross sections of the magnetic field in a voxel for the first ten full-voxel simulations given in Table 1. The sharp peaks correspond to the maxima and minima of the fields within the voxels. The sharply pointed top curve corresponds to a dendrite density of 10^6 / mm^3 while the flattest curve corresponds to a dendrite density of 1000 / mm^3.

are potentially measurable using MRI techniques in the near future. Nevertheless, we now address several possible sources of overestimation regarding the fields and phase shifts in our model. 1.) The magnetic field of a dendrite consists of a biphasic signal because of the physical depolarization and repolarization processes. Although the repolarization occurs more slowly than the initial depolarization, it also is weaker, such that the integrated phase shifts from the two nearly cancel. Unless very brief (~ms), precisely-timed pulse sequences are developed, this activity will be very difficult to detect. However, it is possible that such pulse sequences may be developed in the near future. 2.) This model considers the field just outside

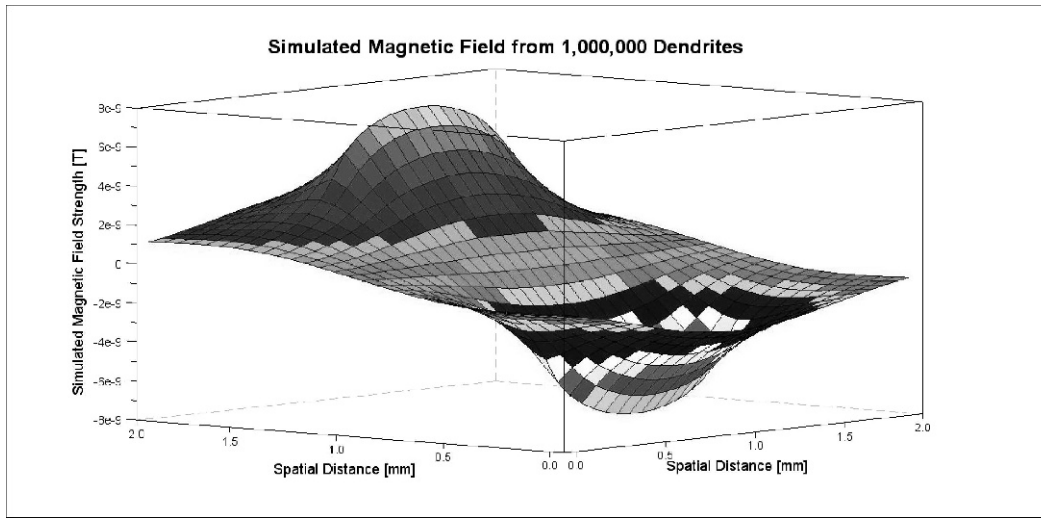

Figure 4.—A three-dimensional plot showing the behavior of the magnetic field at the vertical center of the voxel for the case of 10^6 dendrites / mm^3.

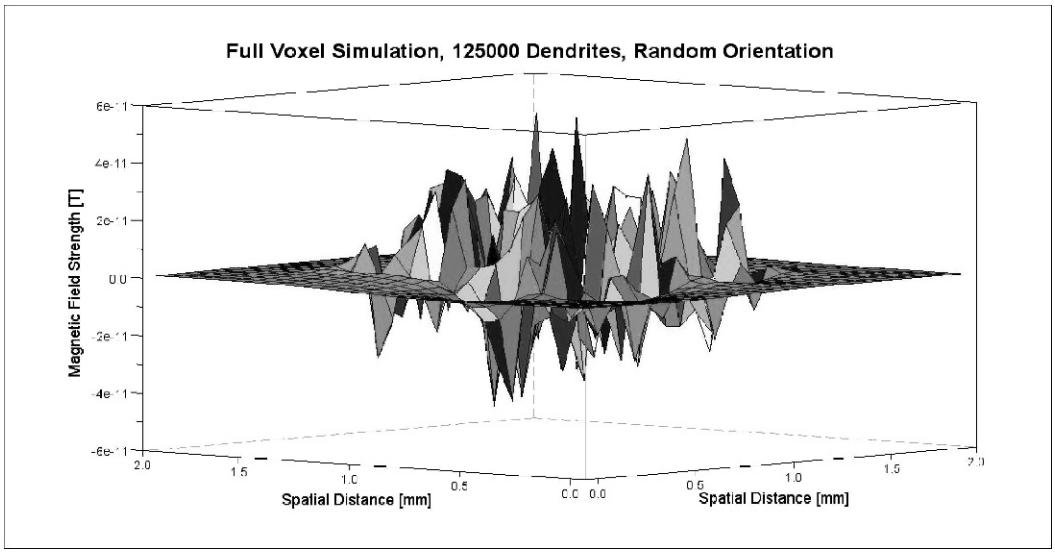

Figure 5.—A three-dimensional plot showing the behavior of the magnetic field at the vertical center of the voxel for the case of 125,000 randomly oriented dendrites. One observes that the maximum field strength is diminished in comparison with the case of parallel orientation given in Table 1.

the dendrites, where it is the largest. Generally speaking, the field will fall off sharply with increasing distance. 3.) Not all dendrites in a realistic voxel will be active simultaneously, suggesting that the lower-density simulations may provide a more accurate description of real tissue. 4.) The currents of the brain will generally not point in the same direction. This complication we have addressed in simulations using random dipole orientations (Table 1 & Fig. 5), which seem to suggest that the fields – in spite of being reduced by at least an order of magnitude – are still on the order of the proposed threshold of 0.1 nT.

Our analysis assumes that the magnetic resonance study is performed using a typical static magnetic field strength on the order of 1 T. As such, the fractional change in magnetic field strength brought about by the neuronal activity is seen to be extremely small, thereby making detection extremely difficult. However, these currents might more easily be measured using ultra-low field MRI systems (Cassara & Maraviglia 2008; Kraus et al. 2008). The capabilities of these systems have yet to be tested on biomagnetic signals. Perhaps the signal-to-noise ratio could be increased by applying a large field to polarize the spins; during the detection phase the large field could then be quickly reduced in order to maximize the fractional field change due to neuronal activity. Clearly, great ingenuity would be required in order to achieve this rapid switching without also introducing confounding effects.

In conclusion, based on our calculations using a dipole model, we are cautiously optimistic about the prospects for direct detection in the future. Researchers in magnetic resonance technology continually develop clever and imaginative new techniques that allow for the solution of previously intractable problems. We hope that our analysis will help clarify the roadblocks on the path to true functional imaging of dendritic and neuronal activity.

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Approved at 3-30-11 meeting

Minutes of the Meetings of the Indiana Academy of Science 2011

Academy Council Meeting
Friday, March 4, 2011
University Place Conference Center and Hotel

The meeting was called to order at 2:00pm
Members in attendance were:
Jim Curry, President
Rick Kjonaas, President-Elect
Ed Frazier, Treasurer
Paul Rothrock, Immediate Past President
Mike Foos, Webmaster
Dan Ruch, Newsletter Editor
Uwe Hansen, Proceedings Editor
Amanda Piegza, Academy Librarian
Horia Petache, Physics and Astronomy Section Chair

President's Report – Jim Curry
Emphasized to the Council the importance of "thinking big", and that IAS should continue to collaborate with the Indiana State Museum. He recommended three areas of possible collaboration with the Museum: the public (efforts to educate and entertain), Science education, and Basic Research.

Treasurer's Report – Ed Frazier
2010 was an unusual year because of the changes to the Academy structure that took place at the end of the year– Provided a detailed description as reflected in the handout.

Nominating /Elections Committee Report – Delores Brown
Nominating committee – Delores Brown, Terry West, and John Haddock. 5 positions open, President- elect, member at large (1 year), member at large (2 years), foundation committee and research grants committee.
118 Ballots returned. Results : Michael Finkel president-elect, Michael Foos member at large (one year), Ron Richards member at large (two years), Stan Burden, Foundation Committee, Melody Myers Kenzie, Research Grants Committee

Webmaster Report – Mike Foos
Walked through the particulars of the Website, describing the functionality of the web over the past few months.
1. Website recognizes a member but people can sign on anonymously. Emeritus issue = anyone can claim to be an emeritus or student – there is no mechanism to check. We can either hope that most members are honest, or develop a mechanism that requires documentation such as a co-signature. There was no desire among the members to engage in developing a mechanism to check on emeritus or student members. Question to the Council was posed about whether to ask for birthdates from the members. There was no desire to ask for members to give birthdates in their membership profile to help determine when one is eligible to become emeritus.
2. We have the capability of doing surveys online and instead we used 3x5 cards. Students were voting and they don't have voting privileges. Cut down the number of vote invites to people that you don't want to vote to curtail certain voters without blocking them
3. In December we voted to have the dues Oct-Sept as the dues collecting time for the annual membership. When website set up, we gave them three months to pay and meetings were in Oct. Because of that grace period people who were on the registration for 2010, are still on list until March 31st. Can change to Jan-Mar to Oct-Dec. We really have 503 members if they don't renew. You can pay dues any time you want. Only for first year membership – once a member, it is a rolling membership.
5. Publish meeting minutes in the newsletters? Many Council prefer reading hard copy than online. Older generation requests hard copies. Send out an email alert – let them know that it's online and available four times a year.
6. Discussion of making meeting minutes a link on the website.
7. One of the most common problems members have with the website is entering and changing their password.

Approved at 3-30-11 meeting

8. Dues increased on the 7th of March 2011. New dues price is 75.00 sustaining, members 50.00, students and emeritus 25.00, life members become 750.00. There is no club membership.

Proceedings Editor Report – Uwe Hansen
Appreciate Jim's (Berry) help during the transition-- signed the contract with Allen Press
Noted that the next issue should be fairly small; few entries.
Inquired about the availability of scanning back issues.

Academy Librarian Report – Amanda Piegza
As of July 1 2010, the Academy collection contained over 13,000 volumes. The site of the collection is approximately 4500 linear feet, including all volumes, maps and microfiche. Prior estimates of approx. 500 foreign and national exchange publications is actually only 284, as estimated between Aug 2010 – Feb 2011.

In December budget meeting, asked for 3000 for mailing Proceedings to our foreign exchange partners the last four issues worth. To keep it at 1200 we need to cut some of the partners. Recommend we cut the foreign language journals. Reluctant to cut those, but if they don't exchange with us, they get cut. 1/3 are not reciprocating currently. Recommended that the Academy not send to foreign language and those who are not reciprocating. Voted and seconded. Some foreign language journals also have English translation inside of them.

She sends out to companies that index the abstracts – can this be expanded? Not sure who originally set up, what company is being used? Not sure. Need to consult spreadsheet.

126th Annual Academy Meeting Report – Delores Brown
260 preregistered, including 94 students, 167 presentations including 50 posters, 14 sections participating, 3 exhibit tables : library, IAS, STEF. 22 volunteers, 9 hotel guests at the University Place Hotel, 17 hotel guests at the Marriott. Signage will be visible to help people move around.
Walked through the agenda for Saturday, noting expectations.
She also emphasized the objective and plan for the Section Business Meetings, the Section Chairs, and the Academy Business Meeting. 8 Sections met for a luncheon meeting at the Museum in February, to discuss the plan for the Annual Meeting. The Section Chairs have a Section Business Meeting agenda to follow for their meeting in the morning, and will report the discussion/recommendations of their Sections (including recommendations for 2012 Annual Meeting, recommendations for combining Sections, and the names of the 2011 Section Chair and Vice Chair), at the Annual Business Meeting at the end of the day, 3:45 pm .

Business
New

A. Operating Policies – Ed Frazier
Ed went through and made changes and amendments to the operating policy. Recommendation is to adopt it as printed and edited. Relatively easy to change 30 day notice 2/3 vote of the council, refine and rework if necessary. Move to adopt these policies as listed and edited. Voted and seconded.

b. Site for 2012 Annual Academy Meeting—Concern expressed about the cost of the University Place location---hidden cost, cost not a part of the contract, noting the money would have to come out of grants.

B. 2011 Fall Forum (audience, date, location)-Delores Brown
Delores asked for agreement on the site, day and time for the Fall Forum. It was agreed that the Fall Forum will be held on Saturday November 12, 2011 at the Indiana State Museum. Program to be worked out.

Meeting adjourned at 4:55pm Reception and dinner followed.

Recorded by Delores Brown in the absence of Heather Bruns

Edited minutes submitted by Heather A. Bruns, IAS Secretary

INDIANA ACADEMY OF SCIENCE
2011 Year End Financial Report

	Balance 1-Jan-11	Revenues	Expenses	Balance 31-Dec-11
OPERATING FUND				
Dues		21,135.00		
Interest		41.03		
Misc. Income		952.00		
Annual Meeting		21,492.00		
Foundation Support		173,527.70		
Officer's Expenses			112,660.70	
Operating Expenses			7,861.38	
Financial Expenses			3,509.83	
Newsletter Expenses			2,994.39	
Annual Meeting			53,856.45	
Web Site			19,328.74	
Operating Fund Total	22,642.25	217,147.73	200,211.49	39,578.49
RESTRICTED FUNDS				
Proceedings	17,035.11	11,315.99	11,214.06	17,137.04
Publications	(23,670.41)	22,053.10	18,236.58	(19,853.89)
* Research Grants	24,841.83	87,395.08	89,753.74	22,483.17
Lilly Library	6,578.44	0.00	0.00	6,578.44
Welch Fund	7,416.63	0.00	500.00	6,916.63
Life Members Fund	11,295.25	1,750.00	0.00	13,045.25
Past Presidents Fund	8,599.17	0.00	0.00	8,599.17
Special Projects	(5,887.16)	43,125.00	14,998.47	22,239.37
Transition Office	277.38	0.00	1,370.36	(1,092.98)
Total Restricted Funds	46,486.24	165,639.17	136,073.21	76,052.20
Prepaid Dues	2,885.00	5,885.00	2,885.00	5,885.00
TOTAL FUNDS	**72,013.49**	**388,671.90**	**339,169.70**	**121,515.69**
FUNDS ON DEPOSIT				
Checking Account	45,023.04	390,376.73	387,073.60	48,326.17
Money Market Savings	13,802.12	70,027.86	23,828.79	60,001.19
Cert. of Deposit	13,188.33	0.00	0.00	13,188.33
TOTAL FUNDS DEPOSITED	**72,013.49**	**460,404.59**	**410,902.39**	**121,515.69**

* Provided 28 senior member grants and 42 high school grants.

ACADEMY FOUNDATION FUNDS		
TOTAL FOUNDATION FUNDS	**7,635,006.00**	**7,428,367.00**

Foundation Funded Used For:

Proceedings	11,103.06
Research Grants	80,124.26
Publications	13,840.00
Special Projects	23,125.00
Operating Func	173,527.70
Total	301,720.02

Edward L. Frazier
Treasurer

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