National Water-Quality Assessment Program

Biological-Community Composition in Small Streams and its Relations to Habitat, Nutrients, and Land Use in Agriculturally Dominated Landscapes in Indiana and Ohio, 2004, and Implications for Assessing Nutrient Conditions in Midwest Streams

Scientific Investigations Report 2009–5055

U.S. Department of the Interior
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Cover: ***Upper left***–Cows drinking from stream in the Mad River watershed, August 25, 2003. (Photograph by Stephanie D. Janosy, U.S. Geological Survey (USGS).)

Upper right–Tractor applying fertilizer to a farm field in the Sugar Creek watershed, June 7, 2007. (Photograph by John T. Wilson, USGS.)

Bottom left–Field crew electrofishing using barge equipment to collect a fish community sample at Sugar Creek, (USGS STAID 394340085524601), August 15, 2008. (Photograph by David S. Nail, USGS.)

Bottom right–USGS employee collecting a benthic invertebrate sample at Mud Creek (USGS STAID 393659085340301), August 11, 2004. (Photograph by Brian J. Caskey, USGS.)

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By Brian J. Caskey and Jeffrey W. Frey

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KEN SALAZAR, Secretary

U.S. Geological Survey
Suzette M. Kimball, Acting Director

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Foreword

The U.S. Geological Survey (USGS) is committed to providing the Nation with accurate and timely scientific information that helps enhance and protect the overall quality of life and that facilitates effective management of water, biological, energy, and mineral resources (*http://www.usgs.gov/*). Information on the Nation's water resources is critical to ensuring long-term availability of water that is safe for drinking and recreation and suitable for industry, irrigation, and habitat for fish and wildlife. Population growth and increasing demands for water make the availability of that water, now measured in terms of quantity and quality, even more essential to the long-term sustainability of our communities and ecosystems.

The USGS implemented the National Water-Quality Assessment (NAWQA) Program in 1991 to support national, regional, State, and local information needs and decisions related to water-quality management and policy (*http://water.usgs.gov/nawqa*). The NAWQA Program is designed to answer: What is the condition of our Nation's streams and ground water? How are conditions changing over time? How do natural features and human activities affect the quality of streams and ground water, and where are those effects most pronounced? By combining information on water chemistry, physical characteristics, stream habitat, and aquatic life, the NAWQA Program aims to provide science-based insights for current and emerging water issues and priorities. From 1991-2001, the NAWQA Program completed interdisciplinary assessments and established a baseline understanding of water-quality conditions in 51 of the Nation's river basins and aquifers, referred to as Study Units (*http://water.usgs.gov/nawqa/studyu.html*).

In the second decade of the Program (2002–2012), a major focus is on regional assessments of water-quality conditions and trends. These regional assessments extend the findings in the Study Units by filling critical gaps in characterizing the flow and quality of surface water and ground water, and by determining trends at sites that have been consistently monitored for more than a decade. In addition, the regional assessments continue to build an understanding of how natural features and human activities affect water quality, and establish links between *sources* of contaminants, the *transport* of those contaminants through the hydrologic system, and the potential *effects* of contaminants on humans and aquatic ecosystems. Many of the regional assessments employ modeling and other scientific tools, developed on the basis of data collected at individual sites, to help extrapolate and forecast conditions in unmonitored, yet comparable areas within the regions. The models thereby enhance the value of our existing data and our understanding of the hydrologic system. In addition, the models are useful in evaluating various resource-management scenarios and to predict how our actions, such as by adjusting nonpoint and point sources of contamination, converting land use, and altering flow and (or) pumping regimes, are likely to affect water conditions within a region.

Other activities planned during the second decade include continuing national syntheses of information on pesticides, volatile organic compounds (VOCs), nutrients, and selected trace elements; and continuing national topical studies on the fate of agricultural chemicals, effects of urbanization on stream ecosystems, nutrient enrichment, bioaccumulation of mercury in aquatic organisms, and transport of contaminants to public-supply wells.

The USGS aims to disseminate credible, timely, and relevant science information to inform practical and effective water-resource management and strategies that protect and restore water quality. We hope this NAWQA publication will provide you with insights and information to meet your needs, and will foster increased citizen awareness and involvement in the protection and restoration of our Nation's waters.

The USGS recognizes that a national assessment by a single program cannot address all water-resource issues of interest. External coordination at all levels is critical for cost-effective management, regulation, and conservation of our Nation's water resources. The NAWQA Program, therefore, depends on advice and information from other agencies—Federal, State, regional, interstate, Tribal, and local—as well as nongovernmental organizations, industry, academia, and other stakeholder groups. Your assistance and suggestions are greatly appreciated.

Robert M. Hirsch
Associate Director for Water

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Conversion Factors

Multiply	By	To obtain
	Length	
meter (m)	3.281	foot (ft)
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
micrometer (µm)	0.001	millimeter (mm)
	Area	
square kilometer (km^2)	247.1	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
milliliter (mL)	0.03381	ounce, U.S. liquid (oz)
	Mass	
kilogram (kg)	2.205	pound, avoirdupois (lb)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8\times°C)+32$$

Concentrations of chemical constituents are given in milligrams per liter (mg/L).

Biological-Community Composition in Small Streams and its Relations to Habitat, Nutrients, and Land Use in Agriculturally Dominated Landscapes in Indiana and Ohio, 2004, and Implications for Assessing Nutrient Conditions in Midwest Streams

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Abstract

The objective of this study was to relate algal-, invertebrate-, and fish-community composition to habitat, nutrients, and land-use variables in small streams in agriculturally dominated landscapes of the Midwest in Indiana and Ohio. Thirty sample locations were selected from a single ecoregion; all were small wadable streams within agriculturally dominated landscapes with similar substrate and canopy. Biological and nutrient samples were collected during stable flow conditions in August 2004. Canonical correspondence analysis was used to determine which variables most influenced each community. Total phosphorus concentrations significantly influenced the depositional-targeted habitat algal-diatom community and the richest-targeted habitat invertebrate community. Multivariate statistical analysis showed that habitat variables were more influential to the richest-targeted habitat algal-diatom and fish communities than nutrient concentrations. Although the nutrient concentrations measured during this study indicate that most streams were not eutrophic, the biological communities were dominated by eutrophic species, suggesting streams sampled were eutrophic. Consequently, it was concluded that biological relations to nutrients in agriculturally-dominated landscapes are complex and habitat variables should be included in biological assessments of nutrient conditions in agriculturally-dominated landscapes.

Introduction

The U.S. Environmental Protection Agency (USEPA) National Water-Quality Inventory identified excess nutrients (nitrogen and phosphorus) as the second leading cause of river and stream impairment within the United States. The inventory also identified agricultural activities as major nonpoint sources of nutrient enrichment to surface waters (U.S. Environmental Protection Agency, 1996). Because excess concentrations of nutrients have ecological and economic consequences, the USEPA proposed regional ambient nutrient criteria for streams across the United States. The proposed USEPA nutrient criteria have prompted additional studies to assess the ecological effects of nutrients on streams (U.S. Environmental Protection Agency, 2000a).

To assess the response of biological communities to anthropogenic nutrient concentrations, study sites are often selected along a range of environmental conditions commonly called an environmental gradient. In regional-gradient studies of biological communities, it is important to minimize the effects of natural variables that can mask the relations between the variables of interest and their effects on the biological community. These natural variables can include geology, soils, land use, and physical habitat (Wang and others, 1997; Caskey, 2003; Taylor and others, 2006). An underlying premise in gradient analysis is that species reside along an environmental gradient with the highest species abundances at their environmental optima (Gauch, 1982; ter Braak, 1994). Traditionally, biological attributes, metrics, and indices were commonly used to measure biological communities because they have been shown to respond to anthropogenic and other variables along a gradient (Wang and others, 1997; Brightbill and Bilger, 1998; Carpenter and Waite, 2000; Caskey, 2003; Frey and Caskey, 2007). Recent studies have used biological-community composition and structure to assess nutrient enrichment in streams (Bowman and others, 2005; Dodds and others, 2002; Munn and others, 2002; Petersen and Femmer, 2002). Several studies have used multiple biological communities (primarily fish and invertebrate communities) to measure stream condition (Cuffney and others, 2000; Miltner and Rankin, 1998; Robertson and others, 2006). These studies rely less on an index approach and instead use multivariate statistics to evaluate multiple biological communities in a gradient approach (Cuffney and others, 1997).

Purpose and Scope

The current study was one of eight regional studies that assessed the effects of nutrient enrichment in streams as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Program (Munn and Hamilton, 2003). The analysis in this report relates algal-, invertebrate-, and fish-community composition to habitat, nutrients, and land-use variables along a previously determined nutrient-concentration gradient. The study was conducted in 30 agriculturally dominated watersheds within the USEPA Level III Eastern Corn Belt Plains (Ecoregion 55) part of the White River and Great and Little Miami River Basins (WHMI) NAWQA Study Unit in Indiana and Ohio, hereafter termed Midwest. Information from this study may help determine which biological-community-composition group or groups are most appropriate in nutrient-assessment studies and nutrient-criteria development.

Description of the Study Area

The study area covers 48,400 km^2 of central to southern Indiana and central to southwestern Ohio, and is in the Ecoregion 55 part of the WHMI NAWQA Study Unit (fig. 1). The landscape is dominated by row-crop agriculture, primarily corn and soybeans (Debrewer and others, 2000; Schnoebelen and others, 1999). The climate is characterized as humid with well-defined winters and summers. The mean annual precipitation is 72.4 cm, and the mean annual temperature is 12.2°C. The study area was mostly glaciated, and the soils are nutrient-rich and fertile. In addition, soils are poorly drained and often require tile drains for row-crop agriculture. If present, the riparian zones along the streams, generally consist of narrow deciduous forest and woody wetlands adjacent to row-crop fields.

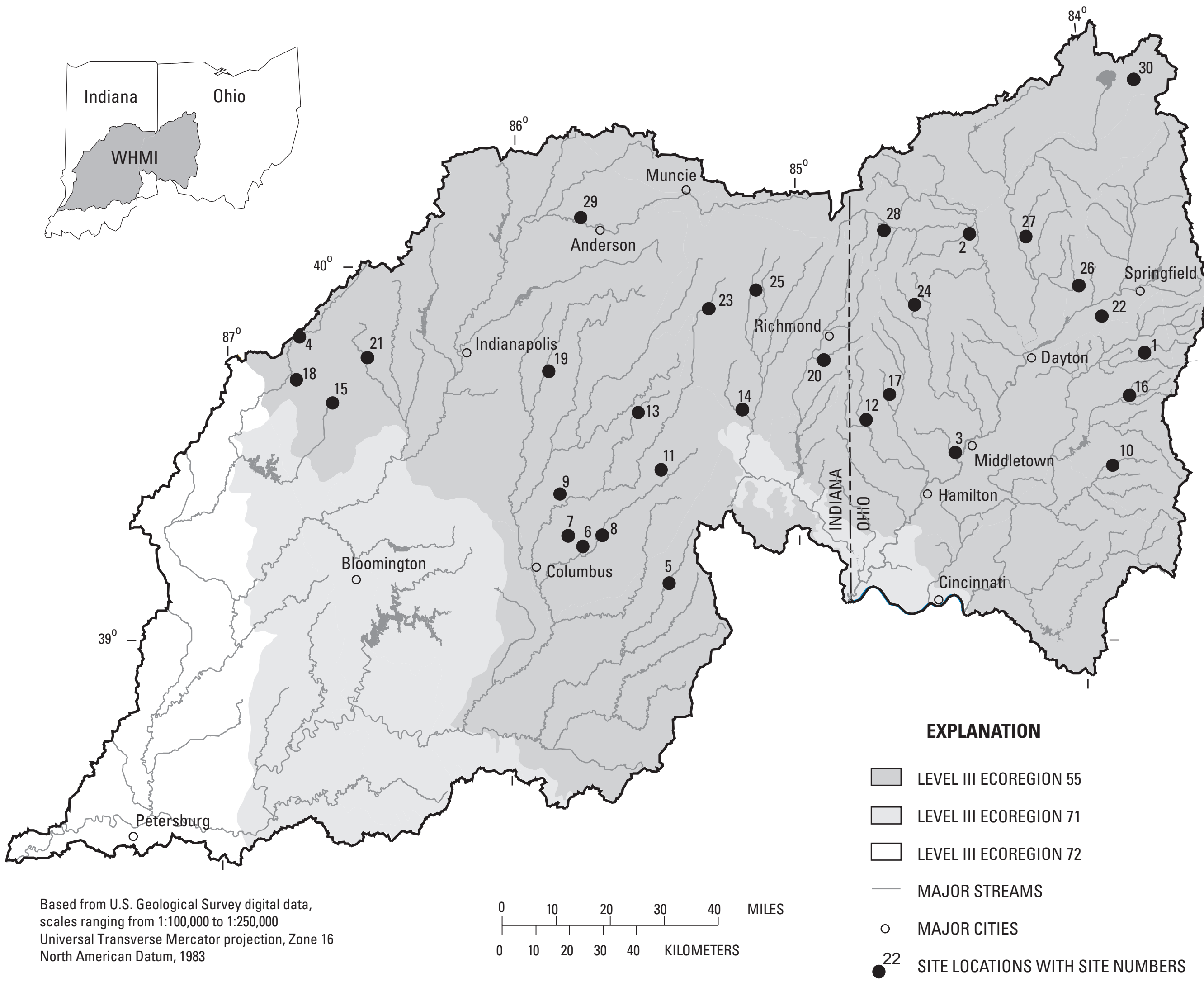

Figure 1. Location of study sites and Level III Ecoregions in the White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program, 2004. (Site descriptions are in table 1.)

Study Methods

This study used field and analytical methods from the USGS. The following sections describe the site selection and data collection; field and laboratory methods used in collecting, processing, and analyzing biological-community, basin and riparian characteristics, habitat, and nutrients data; and data analysis used in this report.

Site Selection

To minimize natural variables that could mask relations between the biological community composition and nutrients, potential study sites had to be within small agricultural basins (50 to 500 km^2) with similar substrates (cobble/gravel riffles) that did not have point-source discharges. Sites were selected to have similar physical variables when possible, including substrates (cobble/gravel riffles) and canopy cover. Also, sites were selected to capture a range (gradient) in nutrient concentrations (nitrogen and phosphorus) on the basis of existing data and sampling during a reconnaissance survey. The final list consisted of 30 sites within Ecoregion 55 that had relatively homogenous habitat (table 1).

Data Collection

Biological communities (algal, invertebrate, and fish) were assessed at the 30 sites in August 2004 using standard NAWQA protocols (Moulton and others, 2002). Two algal-community samples were collected from each sampled reach: a depositional-targeted habitat (DTH) sample and a richest-targeted habitat (RTH) sample. The DTH algal-community samples were a composite of five petri-dish subsamples collected in sand/silt substrates. Each DTH subsample was collected by pushing the lid of a 47-mm petri dish into the sand/silt substrate and then sliding a spatula under the petri dish. The material trapped in the petri dish was washed into a 500-mL wide-mouth bottle. The RTH algal-community samples were collected from riffle habitats within the reach that were dominated by gravel/cobble substrates. The sample was a composite of five subsamples. The collected subsamples represented the average overall density of algal cover within the site. The algae from a measured area were scraped off the top of the substrate and collected into a 500-mL wide-mouth bottle. At the subsample-collection locations for the DTH and RTH samples, field personnel measured stream velocity, substrate type, and canopy closure. Algal-community samples (DTH and RTH) were preserved with sufficient formaldehyde to obtain a 5-percent buffered formalin (Moulton and others, 2002) and sent to the Academy of Natural Sciences in Philadelphia, Pa., and were identified to the lowest possible taxonomic level.

The invertebrate RTH community samples were a composite of five samples collected from riffle habitats with gravel/cobble substrates and moderate to high stream velocities (Moulton and others, 2002). Each RTH invertebrate community subsample was collected using a Slack sampler with a 500-µm dolphin bucket, field processed, and preserved with 10-percent formalin. The RTH invertebrate community samples were sent to the USGS National Water Quality Laboratory in Lakewood, Colo., and identified to the lowest possible taxonomic level.

Fish communities were sampled from a stream-reach length that was approximately 20 times the mean wetted-channel width at each of the 30 sites sampled; the lengths ranged from 150 to 420 m (table 2). Methods for assessing the fish community consisted of two-pass electrofishing techniques and seining (Moulton and others, 2002). Fish were identified to species, measured, and weighed in the field.

Physical-habitat assessments of the fish-community stream reaches followed protocols developed by the USGS NAWQA Program (Fitzpatrick and others, 1998). These reach-based habitat protocols measure physical stream variables such as wetted-channel depth and width, velocity, and substrate along 11 equally spaced transects (Brightbill and Munn, 2008). The mean depth, width, and velocity of the wetted channel from the 11 transects are presented in table 2.

Basin and riparian land-use variables were determined using Environmental Systems Research Institute's (ESRI) ArcView Geographical Information System (Environmental Systems Research Institute, 2005). Basins and land-use variables were determined from digital topographic and hydrologic maps ranging from 1:24,000 to 1:250,000 scale, depending on the size of the basin. The source of the basin data was an enhanced version of the USGS National Land-Cover Data 1992 (U.S. Geological Survey, 1999). The amount of nitrogen (N) and phosphorus (P) that was applied within the basin were based on combined 2001 estimates of atmospheric data, animal populations, and fertilizer sales within each basin (Ruddy and others, 2006; Brightbill and Munn, 2008). Basin land-use variables used in the analysis included drainage basin area, percent agriculture land-use, percent forest land-use, percent urban land-use, and estimates of N and P applied within the basin. Additionally, the percent of agriculture land-use in the basin was refined to include only row crops and the percent of forest within the basin was refined to include only deciduous forest (table 3).

Riparian land-use data were determined in accordance with protocols developed by Johnson and Zelt (2005). Riparian land-use variables used in the analysis included percent of riparian zone evergreen forest, and percent of riparian zone row crops (table 3), although numerous variables were calculated for the study (Brightbill and Munn, 2008). The variables for each basin classification were converted from vector to raster format at 30-m resolution, then the 30-m resolution for each basin classification was used to determine the area in which each classification occurred, and these values were then converted to percentages. The conversion of basin variables allowed for normalization of the data and comparisons among basins. Brightbill and Munn (2008) published the complete environmental, algae, and benthic invertebrate datasets for the Nutrient Enrichment Effect on Stream Ecosystems studies from 2003–04.

Table 1. Descriptions of the 30 sites in the White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program, 2004. (Site descriptions are in figure 1.)

[USGS, U.S. Geological Survey; STAID, station identification; OH, Ohio; IN, Indiana; FK, Fork; R, River; CR, County Road; N, North; E, East; NR, Near; CO, County; RD, Road; FT, feet; US, Upstream; S, South; W, West]

Site number	USGS STAID	USGS station name	Latitude (decimal degrees)	Longitude (decimal degrees)
1	03240500	NORTH FORK MASSIE CREEK AT CEDARVILLE, OH	39.75637	-83.79130
2	03264900	PAINTER CREEK NEAR SUGAR GROVE, OH	40.08353	-84.39552
3	03272200	ELK CREEK AT MILTONVILLE, OH	39.50192	-84.45972
4	03357330	BIG WALNUT CREEK NEAR ROACHDALE, IN	39.81640	-86.75183
5	390948085274301	VERNON FK MUSCATATUCK R AT CR 1220N NEAR ZENAS, IN	39.16440	-85.46230
6	391545085454301	DUCK CREEK AT CR 850E NR NEWBERN, IN	39.26188	-85.76147
7	391726085485101	HAW CREEK AT CR 600N NR NORTONBURG, IN	39.28890	-85.81608
8	391732085414401	CLIFTY CREEK AT CO RD 1150 E NEAR HARTSVILLE, IN	39.29223	-85.69510
9	392402085503001	SLASH CREEK AT CR 850S NR LEWIS CREEK, IN	39.81487	-86.63770
10	392735083544101	TODD FORK CREEK AT HALE RD NR WILMINGTON, OH	39.46008	-83.91093
11	392751085291801	LITTLE FLATROCK RIVER 700 FT US CR 1000S NEAR MILROY, IN	39.46313	-85.48943
12	393619084461200	FOURMILE CREEK AT CAMDEN COLLEGE CORNER RD NEAR COVINGTON, OH	39.59335	-84.77042
13	393659085340301	MUD CREEK AT 650W NEAR ARLINGTON, IN	39.61498	-85.56932
14	393723085120201	WILLIAMS CREEK AT SNYDER RD NR CONNERSVILLE, IN	39.62313	-85.20473
15	393828086381301	MILL CREEK AT CR 625W NEAR STILESVILLE, IN	39.64113	-86.63600
16	393837083505401	CAESAR CREEK AT HOOP RD NEAR XENIA, OH	39.64512	-83.84583
17	393930084410901	PAINT CREEK AT CAMDEN SUGAR VALLEY RD NEAR CAMDEN, OH	39.65927	-84.68645
18	394211086454801	CLEAR CREEK AT CR 300N NEAR FILLMORE, IN	39.70322	-86.76352
19	394340085524601	SUGAR CREEK AT CO RD 400 S AT NEW PALESTINE, IN	39.73728	-85.88186
20	394510084545801	ELKHORN CREEK AT ESTEB RD NEAR ABINGTON, IN	39.75337	-84.91508
21	394544086305601	WEST FORK WHITE LICK CREEK AT ELLIS PARK AT DANVILLE, IN	39.76272	-86.51507
22	395121083561701	MUD RUN CREEK AT HUNTER RD NEAR ENON, OH	39.85608	-83.93745
23	395327085190801	FLATROCK RIVER AT CR 350E NEAR NEW CASTLE, IN	39.89098	-85.31833
24	395350084353800	TWIN CREEK AT EUPHEMIA-CASTINE RD NEAR WEST MANCHESTER, OH	39.89635	-84.59500
25	395623085090401	WEST FORK WHITEWATER RIVER AT HOOVER RD NEAR HAGERSTOWN, IN	39.94053	-85.15090
26	395625084010101	HONEY CREEK AT NEW CARLISLE PIKE NEAR NEW CARLISLE, OH	39.94097	-84.01703
27	400421084115601	SPRING CREEK AT PIQUA TROY RD NEAR TROY, OH	40.07348	-84.19552
28	400540084415601	WEST BRANCH GREENVILLE CREEK AT NASHVILLE RD NEAR GRANVILLE, OH	40.09632	-84.69997
29	400806085455601	INDIAN CREEK AT CR 200N NEAR HAMILTON, IN	40.13560	-85.76635
30	402901083482601	SOUTH FORK GREAT MIAMI RIVER AT CR 96 NEAR BELLE CENTER, OH	40.48482	-83.80682

Table 2. Habitat variable values of the 30 sites in the White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program, 2004.

[m, meter; cm/s, centimeter per second; m², square meter: Std. dev., standard deviation]

Site number	Reach length (m)	Mean wetted channel width (m)	Mean bankfull width (m)	Mean depth (m)	Mean bankfull depth (m)	Mean instantaneous point velocity (cm/s)	Reach surface water area (m²)	Reach surface-water gradient (unitless)	Mean canopy closure (percent)	Mean shape index (percent)	Boulder habitat cover (percent)	Clay dominant substrate (percent)	Sand dominant substrate (percent)	Very coarse gravel dominant habitat (percent)	Riffle habitat (percent)	Ratio percent pool to percent riffle
1	150	7.0	9.8	0.21	1.03	2.13	1,050	0.0012	51.8	19.8	0	29.1	30.9	0	10.8	0
2	174	9.2	14.4	.25	1.11	1.22	1,600	.0014	86.8	21.1	7.8	5.6	31.5	14.8	16.1	.31
3	300	12.0	21.4	.11	.96	3.05	3,600	.0044	66.8	52.4	25.5	1.8	7.3	16.4	59.4	.04
4	420	19.0	23.6	.53	1.75	11.00	7,980	.0009	86.4	19.7	18.9	0	39.4	3	9.7	.33
5	300	13.4	20.6	.35	1.78	.31	4,020	.0012	57.8	6.7	1.8	36.5	11.5	3.8	6.8	6.53
6	168	7.8	11.1	.20	1.45	0	1,310	.0001	91.2	6.7	0	40.0	45.5	7.3	3.1	30.70
7	150	4.6	12.6	.11	1.36	.61	690	.0016	76.2	6.5	0	20.0	58.2	0	11.2	1.02
8	250	14.0	23.5	.12	1.30	1.52	3,500	.0026	89.6	11.5	3.9	0	38.9	7.4	15.6	.35
9	150	5.2	11.5	.21	1.46	.61	780	.0014	77.8	5.7	0	1.8	83.6	0	14.2	.53
10	206	8.4	14.7	.24	1.27	2.13	1,730	.0026	79.4	15.2	1.9	3.6	34.5	14.5	26.8	.53
11	181	11.3	16.1	.18	1.27	1.22	2,050	.0022	83.7	8.2	3.8	14.5	21.8	0	15.6	.71
12	300	11.2	21.6	.16	1.07	1.83	3,360	.0098	73.8	22.0	74.5	0	20.0	9.1	67.1	.05
13	150	5.3	8.0	.14	1.29	1.52	800	.0009	96.0	5.9	1.8	27.3	70.9	0	10.3	.94
14	200	10.2	17.6	.06	1.18	3.05	2,040	.0081	69.2	9.7	10.9	0	12.7	7.3	46.1	.05
15	251	12.0	16.2	.12	1.65	1.88	3,010	.0007	88.8	10.1	1.8	0	85.5	0	6.7	.67
16	186	7.4	14.1	.38	1.38	.61	1,380	.0017	69.5	7.5	10.9	9.1	32.7	10.9	10.0	4.05
17	150	6.6	11.9	.14	.95	.61	990	.0037	69.3	17.6	5.5	1.8	40.0	20.0	8.5	.46
18	155	9.5	16.8	.11	1.12	1.52	1,470	.0019	66.3	8.4	5.5	0	61.8	0	10.4	.69
19	218	12.1	18.0	.36	1.63	4.88	2,640	.0009	94.1	14.4	5.7	0	33.3	3.0	3.1	6.43
20	162	9.2	16.1	.09	1.12	2.44	1,490	.0084	97.1	39.3	27.3	0	10.9	23.6	49.2	0
21	154	8.2	13.8	.17	1.23	.91	1,260	.0006	78.9	6.2	0	0	80.0	10.9	5.5	1.69
22	165	6.9	9.3	.12	.96	3.66	1,140	.0010	96.8	25.4	0	5.5	5.5	0	21.0	0
23	156	9.1	10.9	.20	1.19	1.52	1,420	.0007	69.3	6.5	0	3.6	83.6	0	3.4	2.38
24	220	10.4	14.0	.29	1.13	.31	2,290	.0003	91.6	14.9	0	30.9	30.9	3.6	29.4	.28
25	167	8.8	11.8	.18	1.22	2.13	1,470	.0018	83.4	7.5	0	5.5	69.1	0	13.5	1.48
26	200	9.6	14.9	.18	1.25	2.44	1,920	.0041	92.0	29.3	18.9	1.8	23.6	32.7	34.4	.42
27	154	7.6	12.8	.21	.97	.31	1,170	.0032	85.8	40.4	11.3	5.6	1.9	27.8	18.8	.45

Table 2. Habitat variable values of the 30 sites in the White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program, 2004. —Continued

[m, meter; cm/s, centimeter per second; m^2, square meter: Std. dev., standard deviation]

Site number	Reach length (m)	Mean wetted channel width (m)	Mean bankfull width (m)	Mean depth (m)	Mean bankfull depth (m)	Mean instanta-neous point velocity (cm/s)	Reach surface water area (m^2)	Reach surface-water gradient (unitless)	Mean canopy closure (percent)	Mean shape index (percent)	Boulder habitat cover (percent)	Clay dominant substrate (percent)	Sand dominant substrate (percent)	Very coarse gravel dominant habitat (percent)	Riffle habitat (percent)	Ratio percent pool to percent riffle
28	150	7.1	9.0	0.37	1.55	3.05	1,070	0.0003	95.0	12.7	4.0	23.6	58.2	0	11.9	0.35
29	150	6.7	8.5	.17	1.17	.31	1,010	.0009	94.1	7.7	1.8	9.1	63.6	16.4	2.3	5.49
30	177	10.4	13.6	.34	1.51	1.83	1,840	.0004	97.6	15.6	12.7	12.7	32.7	18.2	3.9	0
Minimum	150	4.6	8.0	.06	.95	0	690	.0001	51.8	5.7	0	0	1.9	0	2.3	0
Mean	197	9.3	14.6	.21	1.28	1.95	2,000	.0023	81.9	15.8	8.5	9.7	40.7	8.4	18.2	2.23
Median	171	9.2	14.1	.18	1.24	1.52	1,480	.0014	84.8	12.1	3.9	4.6	33.9	5.6	11.6	.50
Maximum	420	19.0	23.6	.53	1.78	11.00	7,980	.0098	97.6	52.4	74.5	40.0	85.5	32.7	67.1	30.70
Std. dev.	63	3.0	4.3	.11	.23	2.05	1,450	.0025	12.5	11.6	14.6	12.2	25.3	9.3	17.1	5.69

Table 3. Nutrient concentration (August), basin, and land-use variable values of the 30 sites in the White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program, 2004.

[NO_2 + NO_3, Nitrate plus Nitrite as Nitrogen; mg/L, milligram per liter; TN, Total Nitrogen; OP, Orthophosphate as Phosphorus; TP, Total Phosphorus; km^2, square kilometer; N, Nitrogen; kg, kilogram; P, Phosphours; m, meter; Std. dev., standard deviation; Estimated N and P applied to a basin in 2001 (Ruddy and others, 2006)]

Site number	NO_3+NO_2 (mg/L)	TN (mg/L)	OP (mg/L)	TP (mg/L)	Drainage basin area (km^2)	Agriculture land use within basin (percent)	Forest land use within basin (percent)	Urban land use within basin (percent)	Estimate N applied to a basin in 2001 (kg)	Estimate P applied to a basin in 2001 (kg)	Riparian zone (75 to 105 m) evergreen forest within basin (percent)	Riparian zone (75 to 105 m) row crops within basin (percent)	Row crop agriculture land-use within basin (percent) [1]	Deciduous forest land-use within basin (percent) [2]
1	1.25	1.52	0.015	0.034	74.3	97.3	2.4	0.1	901,000	153,000	0	84.7	86.3	2.4
2	.35	1.04	.054	.099	117.0	96.4	2.2	1.1	1,850,000	401,000	0	81.5	88.6	2.2
3	.37	.58	.005	.017	120.0	80.9	16.9	1.8	987,000	170,000	1.3	27.2	38.7	15.2
4	.44	.89	.019	.064	340.0	95.3	3.7	.7	2,570,000	446,000	0	66.9	82.0	3.7
5	.52	1.05	.034	.079	95.6	83.8	15.7	.1	891,000	173,000	.7	19.2	41.2	15.4
6	.14	1.47	.055	.200	51.4	93.9	4.4	.5	480,000	85,000	.1	43.1	69.0	4.4
7	.58	1.00	.098	.148	58.5	93.5	3.9	2.0	516,000	88,000	0	37.1	63.5	3.8
8	1.69	2.11	.060	.098	228.0	95.0	4.2	.3	2,650,000	524,000	.1	34.5	75.8	4.1
9	2.38	2.46	.012	.027	54.6	95.1	2.0	2.7	467,000	82,000	0	91.1	79.5	2.0
10	1.11	1.35	.012	.029	86.0	91.4	7.7	.6	1,110,000	186,000	.2	59.5	73.9	7.5
11	2.02	2.35	.059	.086	120.0	96.5	2.5	.6	1,280,000	240,000	0	45.8	78.5	2.5
12	2.08	2.41	.015	.032	99.7	91.5	8.3	.1	1,180,000	210,000	.1	58.4	79.5	8.2
13	3.94	3.98	.052	.007	43.2	98.4	1.2	.1	468,000	88,000	0	69.1	88.5	1.3
14	.88	1.14	.011	.030	73.9	84.6	14.7	.3	568,000	103,000	1.1	23.7	65.2	14.4
15	.65	.89	.015	.028	136.0	92.8	6.2	.8	928,000	156,000	0	51.6	66.3	6.2
16	1.52	2.09	.011	.064	106.0	92.0	4.7	2.5	1,160,000	198,000	.2	56.0	76.4	4.6
17	.80	1.19	.514	.557	36.6	80.6	10.2	7.4	370,000	65,000	.1	32.7	64.3	9.0
18	1.02	1.28	.080	.119	75.1	86.8	10.6	.5	496,000	86,000	0	34.9	65.9	10.5
19	.19	.49	.036	.063	246.0	92.2	5.0	1.9	2,070,000	364,000	0	43.6	76.0	4.6
20	1.63	1.84	.016	.026	58.1	85.1	12.7	.3	505,000	88,000	.4	44.6	69.0	12.3
21	1.38	1.63	.018	.030	74.5	94.3	3.6	1.6	516,000	87,000	0	59.1	75.7	3.5
22	5.07	5.28	.015	.030	49.4	86.2	9.1	4.5	525,000	87,000	.1	47.8	55.6	8.3
23	3.45	3.70	.019	.039	61.6	95.2	3.9	.3	505,000	83,000	0	72.3	80.8	3.9
24	.31	.75	.055	.083	72.8	96.9	2.5	.4	1,120,000	238,000	0	81.0	89.2	2.5
25	1.64	1.83	.006	.013	64.3	86.9	12.5	.2	457,000	79,000	0	60.8	67.8	12.5
26	2.92	3.06	.012	.052	92.3	89.3	8.8	1.5	1,030,000	172,000	0	50.4	71.8	8.6

Table 3. Nutrient concentration (August), basin, and land-use variable values of the 30 sites in the White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program, 2004. —Continued

[$NO_2 + NO_3$, Nitrate plus Nitrite as Nitrogen; mg/L, milligram per liter; TN, Total Nitrogen; OP, Orthophosphate as Phosphorus; TP, Total Phosphorus; km^2, square kilometer; N, Nitrogen; kg, kilogram; P, Phosphours; m, meter; Std. dev., standard deviation; Estimated N and P applied to a basin in 2001 (Ruddy and others, 2006)]

Site number	NO_3+NO_2 (mg/L)	TN (mg/L)	OP (mg/L)	TP (mg/L)	Drainage basin area (km^2)	Agriculture land use within basin (percent)	Forest land use within basin (percent)	Urban land use within basin (percent)	Estimate N applied to a basin in 2001 (kg)	Estimate P applied to a basin in 2001 (kg)	Riparian zone (75 to 105 m) evergreen forest within basin (percent)	Riparian zone (75 to 105 m) row crops within basin (percent)	Row crop agriculture land-use within basin (percent) [1]	Deciduous forest land-use within basin (percent) [2]
27	1.64	1.93	0.008	0.019	64.5	94.0	5.7	0.2	678,000	115,000	0.1	57.4	82.4	5.7
28	1.08	1.44	.005	.024	59.4	92.4	7.1	.2	931,000	204,000	0	83.3	85.5	7.0
29	.06	.34	.009	.044	46.3	95.4	2.2	1.5	430,000	71,000	0	49.1	76.8	2.2
30	.52	.81	.017	.040	122.0	87.0	12.1	.5	1,030,000	173,000	0	64.5	74.2	11.9
Minimum	.06	.34	.005	.007	36.6	80.6	1.2	.1	370,000	65,000	0	19.2	38.7	1.3
Mean	1.39	1.73	.045	.073	97.6	91.4	6.9	1.2	956,000	174,000	.2	54.4	72.9	6.7
Median	1.10	1.46	.017	.040	74.4	92.6	5.4	.6	896,000	155,000	0	53.8	75.8	5.2
Maximum	5.07	5.28	.514	.557	340.0	98.4	16.9	7.4	2,650,000	524,000	1.3	91.1	89.2	15.4
Std. dev.	1.19	1.11	.092	.101	66.4	5.0	4.5	1.5	609,000	118,000	.3	19.0	12.2	4.3

[1] Variable is a refinement of agriculture land-use variable.

[2] Variable is a refinement of forest land-use variable.

Water chemistry was sampled for nutrients in August 2004 in accordance with protocols developed by the USGS NAWQA Program (Shelton, 1994). Table 3 lists water samples collected and analyzed for nitrate + nitrite (NO_3+NO_2) as nitrogen, total nitrogen (TN), orthophosphate (OP) as phosphorus, and total phosphorus (TP), during low-flow periods in August 2004. Nutrient concentrations were analyzed at the USGS National Water Quality Laboratory in Lakewood, Colo., using colorimetric methods; ammonia (NH_3) plus organic nitrogen, and TP by microkjeldahl digestion (Patton and Truitt, 1992), NH_3 by salicylate hypochlorite (Fishman, 1993); NO_2 by diazotization (Patton and Truitt, 1992); NO_3+NO_2 by cadmium reduction (Patton and Truitt, 1992); and OP by phosphomolybdate (Patton and Truitt, 1992).

Data Analysis

The distribution of nutrient concentrations (NO_3+NO_2, TN, OP, and TP) for the August sample period was compared to the USEPA Aggregate Nutrient Ecoregion VI median 25th percentile for the summer values, because these values were more representative of our sampling period. The USEPA developed the proposed 25th percentile annual nutrient criteria that incorporated the median values for each stream by season. The median of the four seasonal values was used to calculate the proposed annual criteria using the 25th percentile for each nutrient constituent by ecoregion.

Canonical correspondence analysis (CCA) was used to relate the biological communities to selected environmental variables. Prior to analysis, the algal community data were processed to remove all non-diatom data; next, both the algal-diatom and invertebrate-community data were processed to remove ambiguous taxa. Finally, the DTH algal diatom-, RTH algal diatom-, RTH invertebrate-, and fish-community data were imported into CANOCO v. 4.54 (ter Braak and Smilauer, 2002) for CCA unimodel analysis. In each analysis, the raw abundance data were square-root transformed, and rare species (taxa) were downweighted.

Data on more than 400 environmental variables, including 4 nutrients, were collected in this study; consequently, statistical procedures were required to reduce the number of environmental variables for each biological dataset. A principal component analysis and regression analysis was run on each category (habitat, nutrient, and basin) of environmental data to show which variables within a category data type were related to one another and to identify possible collinearity, covariablity, and outliers within the datasets. This procedure reduced the original list of more than 400 variables to 30 variables, including the 4 nutrients. The selected variables were normalized because the majority of the variables were not normally distributed. The manual forward selection procedure in CANOCO 4.54 was used to further decrease the number of variables. This procedure used a Monte Carlo Permutation Test ($p \leq 0.05$) with unrestricted permutations to determine which of the selected variables were important to describe most of the variation within each biological community. The p-value is the probability of obtaining the computed test statistic, or one even less likely, when the null hypothesis is true (Helsel and Hirsch, 1992). Table 4 lists the significant variables for each biological community and their respective data distributions.

Description of the Sampled Basins

The land use of the drainage basin and habitat within the sample locations were relatively homogenous. Streamflow for many streams in the study area was controlled by tile drains and surface runoff during much of the year and by ground water during low-flow periods. Drainage basin areas ranged in size from 36.6 to 340 km^2, with a mean of 97.6 km^2 (table 3), and the mean land use within the drainage basin areas was dominated by agriculture (91.4 percent), followed by forest (6.9 percent), and urban area (1.2 percent).

Streams sampled within Ecoregion 55 have some of the highest nutrient loadings in the United States (Mueller and Spahr, 2006). Although streams within the ecoregion are nutrient enriched, there was a nutrient gradient for August samples (table 3). In the 30 basins that were studied, nutrient concentrations ranged as follows: NO_3+NO_2 from 0.060 to 5.07 mg/L as N; TN, from 0.34 to 5.28 mg/L; OP, from 0.005 to 0.514 mg/L as P; and TP, from 0.007 to 0.557 mg/L (table 3). Ruddy and others (2006) noted the estimated N applied to a basin in 2001 ranged from 370,000 to 2,650,000 kg, and estimated P applied to a basin in 2001 ranged from 65,000 to 524,000 kg (table 3). When nutrient concentrations were compared to USEPA Aggregate Nutrient Ecoregion VI median seasonal (summer) concentration values, 67 percent of NO_3+NO_2, 23 percent of TN (fig. 2), 27 percent of OP, and 20 percent of TP (fig. 3) samples collected equaled or exceeded the published USEPA values for proposed 25th percentile nutrient criteria (U.S. Environmental Protection Agency 2000b), suggesting eutrophication at some of the sampling locations.

Biological-Community Composition within the Sampled Basins

The DTH algal-diatom community consisted of 36,397 individuals, representing 211 taxa and ranged from 51 to 87 diatom taxa per site (appendix 1). Three genera (*Amphora*, *Navicula*, and *Nitzschia*) accounted for 65.9 percent of the total number of individuals collected. The most abundant taxa collected were *Amphora pediculus* (17.2 percent relative abundance), and *Navicula minima* (6.6 percent relative abundance).

The RTH algal-diatom community consisted of 18,859 individuals, representing 157 taxa and ranged from 24 to 72 diatom taxa per site (appendix 2). Three genera (*Amphora,* *Navicula*, and *Nitzschia*) accounted for 66.2 percent of the total number of individuals collected. The most abundant taxa collected were *Amphora pediculus* (23.2 percent relative abundance), and *Navicula minima* (9.2 percent relative abundance).

Table 4. Significant environmental variables and data ranges used in the canonical correspondence analysis, White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program, 2004.

[Min, minimum; Max, maximum; m, meter; m^2, square meter; mg/L, milligram per liter; kg, kilogram]

Category	Variable code	Variable name	Mean	(Min - Max)
Habitat	BOPct	Boulder habitat cover (percent) [4]	8.5	(0 - 74.5)
	Pct Clay	Clay substrate (percent) [4]	9.7	(0 - 40)
	CanClosr	Mean canopy closure (percent) [2]	81.9	(51.8 - 97.6)
	BFDepthA	Mean bankfull depth (m) [3,4]	1.28	(.95 - 1.78)
	BFWidthA	Mean bankfull width (m) [4]	14.6	(8.0 - 23.6)
	ShapeAvg	Mean shape index (percent) [1]	15.8	(5.7 - 52.4)
	Pool/Rif	Ratio percent pool to percent riffle [1,2]	2.23	(0 - 30.7)
	RchArea	Reach surface-water area (m^2) [3]	2,000	(690 - 7,980)
	Grad	Reach surface-water gradient (unitless) [4]	.0023	(.0001 - .0098)
	Pct Riff	Riffle habitat present (percent) [4]	18.2	(2.3 - 67.1)
	Pct Sand	Sand substrate (percent) [3]	40.7	(1.9 - 85.5)
	P vcGrav	Very coarse gravel (percent) [2]	8.4	(0 - 32.7)
Nutrient concentration	TP	Total phosphorus-August (mg/L) [1,3]	.073	(.007 - .557)
Land-use	Basinp-d	Deciduous forest within basin (percent) [1,4]	6.7	(1.3 - 15.4)
	PA_01	Estimated Phosphorus applied to a basin in 2001 (kg) [4]	174,000	(65,000 - 524,000)
	Rip-eve	Riparian zone (75 to 105 m) evergreen forest within basin (percent) [2]	.2	(0 - 1.3)
	Rip-rowc	Riparian zone (75 to 105 m) row crops within basin (percent) [2]	54.4	(19.2 - 91.1)

[1] Variable used in Depositional Targeted Habitat (DTH) algal diatom community data analysis.

[2] Variable used in Richest Targeted Habitat (RTH) algal diatom community data analysis.

[3] Variable used in Richest Targeted Habitat (RTH) invertebrate community data analysis.

[4] Variable used in fish community data analysis.

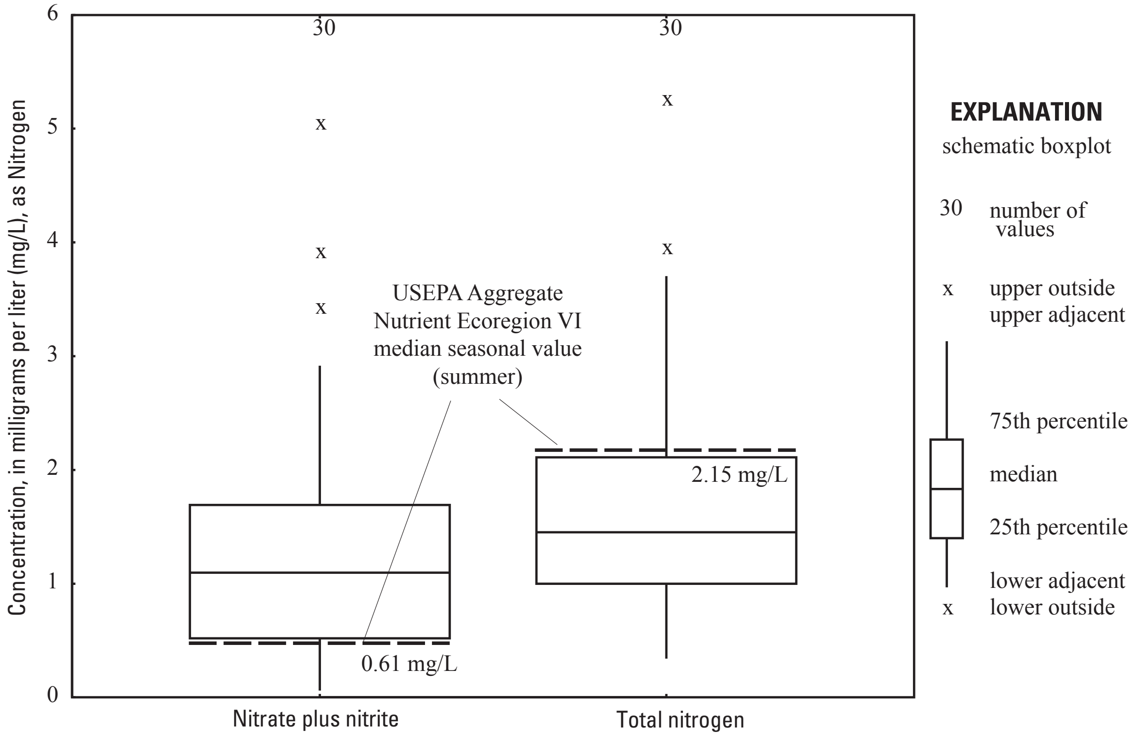

Figure 2. Distribution of concentrations of nitrogen constituents in samples collected in August 2004, compared to the U.S. Environmental Protection Agency (USEPA) Aggregate Nutrient Ecoregion VI median seasonal values (summer) (U.S. Environmental Protection Agency, 2002a), in the White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program.

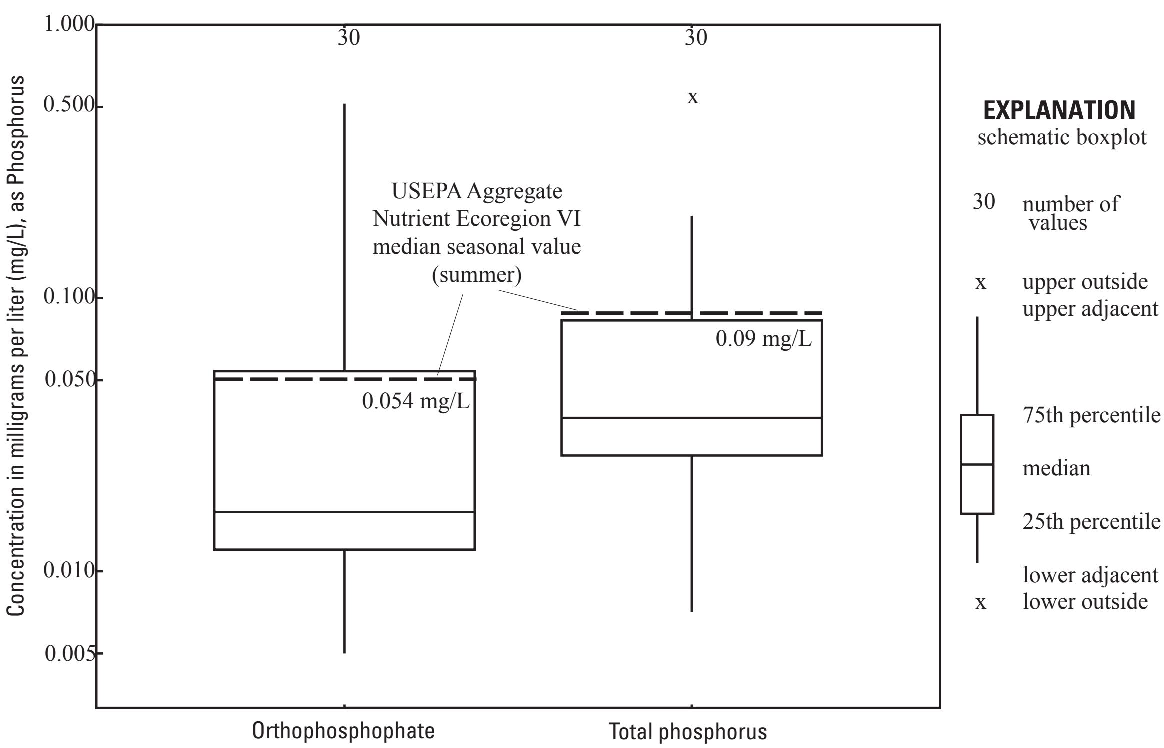

Figure 3. Distribution of concentrations of phosphorus constituents in samples collected in August 2004, compared to the U.S. Environmental Protection Agency (USEPA) Aggregate Nutrient Ecoregion VI median seasonal values (summer) (U.S. Environmental Protection Agency, 2002a), in the White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program.

The invertebrate community consisted of 165,336 individuals, representing 76 taxa and ranged from 20 to 32 taxa per site (appendix 3). Communities were dominated by the orders Ephemeroptera and Diptera**,** which accounted for 46.2 percent of the total number of individuals collected. The most abundant taxa collected were *Cheumatopsyche* species (15.8 percent relative abundance). The second most dominant taxa collected was *Ceratopsyche bronta* (12.8 percent relative abundance).

The fish community consisted of 21,103 individuals, representing 62 taxa and ranged from 9 to 32 taxa per site (appendix 4). Four species—central stoneroller (*Campostoma anomalum*), bluntnose minnow (*Pimephales notatus*), creek chubsucker (*Erimyzon oblongus*), and rainbow darter (*Etheostoma caeruleum*)—accounted for 52.2 percent of the number of individuals collected. The most abundant species collected were central stoneroller (25.7 percent relative abundance) and bluntnose minnow (11.1 percent relative abundance).

Variables Affecting Biological-Community Composition within the Sampled Basins

A CCA analysis was conducted to determine which variable most influenced each of the four biological communities. Eigenvalues (λ) for each CCA axis determine the relative importance of each axis to explain the data set; the sum of all eigenvalues and the cumulative percent variation (CPV) measure the percentage of data dispersion on a particular axis (Jongman and others, 1995; McCune and Grace, 2002). Higher eigenvalues and/or CPV equate to greater diversity within a dataset (meaning that data among the sites are dissimilar and distributed along a wide range on an axis). Lower eigenvalues and/or CPV indicate that the majority of the biological communities at each site are similar along the first CCA axis, as is the case with the data from this study. Sites with similar biological communities are placed in the center of the CCA plot(s) or near one another, whereas sites with dissimilar biological communities are placed on extreme ends of the CCA plot(s). Because many of the sites were placed close together on the CCA plots, the interpretation of the analysis focused on differences between sites observed at the extreme ends of the axes where differences between community compositions are greatest. Table 4 lists the significant variables used in the CCA along with their respective data ranges.

DTH algal-diatom community.—Four variables (one nutrient, two habitat, and one land-use) explained the variability among the sites in the DTH algal-diatom community (fig. 4A, table 5). A nutrient variable (TP, intersite correlation = 0.5509) and a habitat variable (ratio percent pool-to-percent riffle, intersite correlation = 0.7364) were dominant on the first and second CCA axes, respectively (table 5). The first CCA axis accounted for 6.9 percent of the variation in the distribution of the DTH algal diatom community, and the second axis explained 6.2 percent. Low eigenvalues ($\lambda 1 = 0.069$ and $\lambda 2 = 0.064$) and CPV (6.9 and 13.1) for the first and second CCA axes, respectively, suggest that overall the DTH algal diatom communities were similar among the 30 sites (table 5).

Although TP explained the most variability, this relation might also be associated with where the DTH samples were collected (areas with clay sediments). The DTH sample locations are mostly composed of fine clay particles that tend to have high phosphorus concentrations, so the significant relations may be a function of sampling method. Differences between the sites on the extreme ends (sites 8 and 17) along the first CCA axis show the influences of TP on the physical variables and the species composition of the DTH algal-diatom community (fig. 4A). Site 8 had higher nitrogen constituent (NO_3+NO_2 and TN) concentrations, estimated N and P applied to a basin in 2001, agriculture land-use within the basin, mean wetted channel width, mean instantaneous point velocity, and drainage basin area (table 2 and table 3). Site 17 had higher phosphorus constituents (TP and OP) concentrations, forest land-use within the basin, and mean wetted channel depth (table 2 and table 3). The diversity at the sites ranged from 65 taxa (site 17) to 76 taxa (site 8) (appendix 1), which suggests that as TP increased the number of taxa decreased .

Both sites 8 and 17 were dominated by the same genera (*Amphora* and *Navicula*), but the order of dominant species differed slightly. Site 8 was dominated by *Amphora pediculus* (17.7 percent relative abundance), *Navicula minima* (17.1 percent relative abundance), and *Navicula cryptotenella* (7.5 percent relative abundance); in contrast, site 17 was dominated by *Nitzschia inconspicua* (26.9 percent relative abundance), *Amphora pediculus* (12.2 percent relative abundance), and *Navicula minima* (7.0 percent relative abundance). Of the 65 taxa at site 17, 22 taxa were not found at site 8; these 22 taxa accounted for 9.3 percent relative abundance of individuals collected at site 17. Of the 76 taxa at site 8, 33 taxa were not documented at site 17; these 33 taxa accounted for 11.5 percent relative abundance of individuals collected at site 8. The five most dominant taxa for each site (8 and 17) were present at both locations (appendix 1).

RTH algal-diatom community. —Five variables (three habitat and two land-use) explained the variability among the sites in the RTH algal communities (fig 4B, table 4). A habitat variable (ratio percent pool-to-percent riffle, intersite correlation = 0.7561) and a basin variable (riparian-zone evergreen forest within the basin, intersite correlation = 0.4737) were dominant on first and second CCA axes, respectively (table 5). The first CCA axis accounted for 8.7 percent of the variation in the distribution of the RTH algal diatom community, and the second CCA axis explained 6.8 percent. Low eigenvalues ($\lambda 1 = 0.103$ and $\lambda 2 = 0.081$) and CPV (8.7 and 15.5) for the first and second CCA axes suggest the RTH algal-diatom community were similar among the 30 sites (table 5).

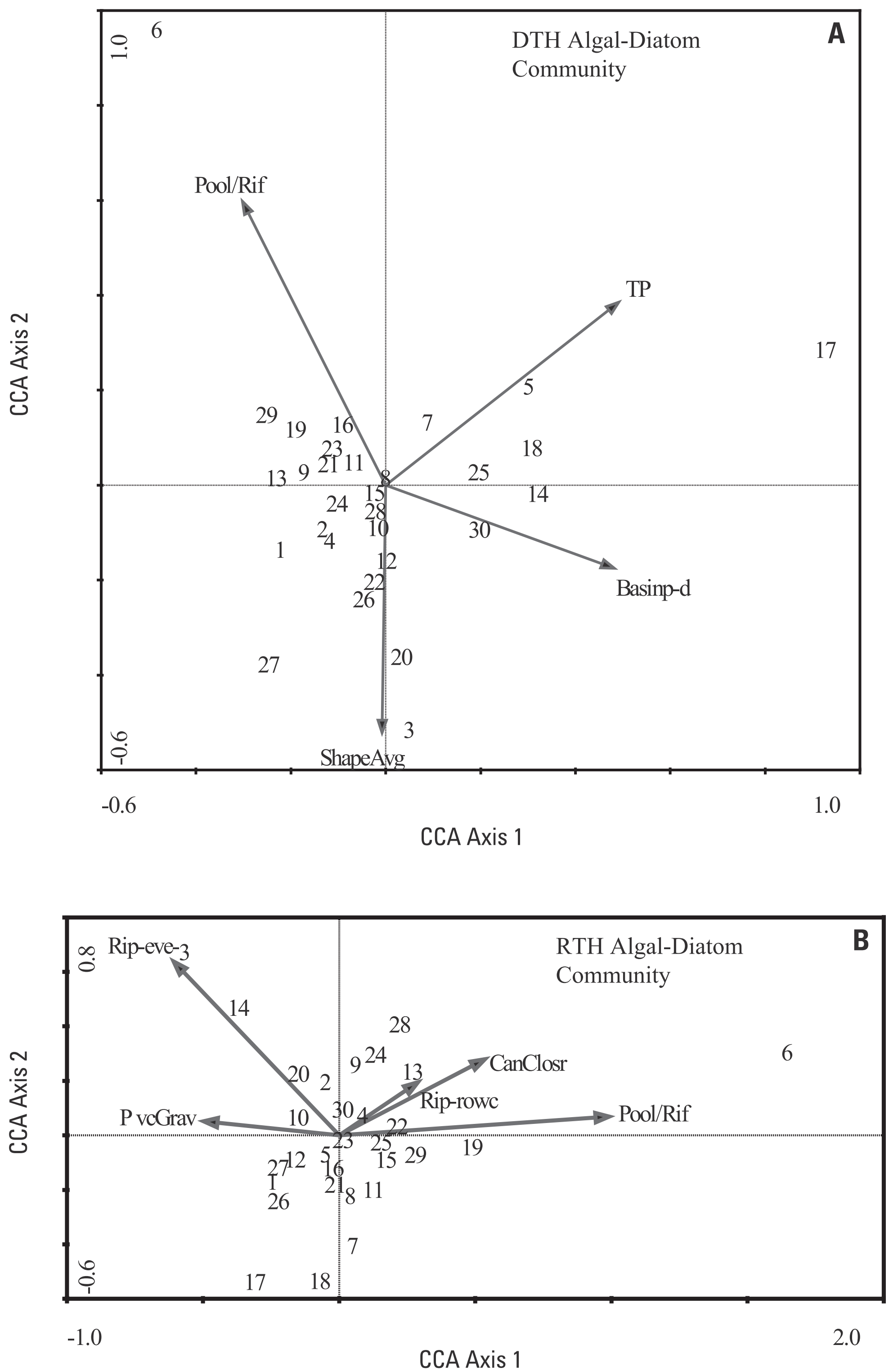

Figure 4. Canonical correspondence analysis (CCA) ordination plots of the (**A**) Depositional-Targeted Habitat (DTH) algal-diatom community; and the (**B**) Richest-Targeted Habitat (RTH) algal-diatom community, White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program (site numbers as listed in table 1; variable codes as listed in table 4).

Table 5. Summary of the canonical correspondence analysis (n=30), White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program, 2004.

[DTH, Depositional Targeted Habitat; RTH, Richest Targeted Habitat; m, meter; m^2, square meter; mg/L, milligram per liter; kg, kilogram; BOLD text, variable most influences the axis]

Variable name	DTH algal diatom community		RTH algal diatom community		RTH invertebrate community		Fish community	
	Axis 1	**Axis 2**	**Axis 1**	**Axis 2**	**Axis 1**	**Axis 2**	**Axis 1**	**Axis 2**
Habitat								
Boulder habitat cover (percent) [4]							0.3077	0.3279
Clay substrate (percent) [4]							-.4410	-.3859
Mean canopy closure (percent) [2]			0.4081	0.2073				
Mean bankfull depth (m) [3, 4]					0.1585	**0.8821**	**-.7674**	.0060
Mean bankfull width (m) [4]							-.2284	**.7157**
Mean shape index (percent) [1]	-0.0091	-0.6432						
Ratio percent pool to percent riffle [1, 2]	-.3388	**.7364**	**.7561**	.0513				
Reach surface-water area (m^2) [3]					-.0725	.7027		
Reach surface-water gradient (unitless) [4]							.6077	.3892
Riffle habitat present (percent) [4]							.5488	.4443
Sand substrate (percent) [3]					-.5394	-.3741		
Very coarse gravel (percent) [2]			-.3793	.0387				
Nutrient concentration								
Total phosphorus-August (mg/L) [1, 3]	**.5509**	.4751			**.5735**	.3286		
Land-use								
Deciduous forest within basin (percent) [1, 4]	.5403	-.2153					.4291	.0961
Estimated Phosphorus applied to a basin in 2001 (kg) [4]							-.4274	.6984
Riparian zone (75 to 105 m) evergreen forest within basin (percent) [2]			-.4634	**.4737**				
Riparian zone (75 to 105 m) row crops within basin (percent) [2]			.2201	.1444				
Eigenvalue	.069	.064	.103	.081	.127	.099	.201	.102
Species-environment correlations	.886	.931	.945	.816	.906	.911	.962	.909
Cumulative percentage variance (CPV) of								
species data	6.9	13.1	8.7	15.5	7.7	13.6	15.2	22.9
species-environment relation	30.1	57.6	31.3	55.9	36.1	64.4	33.3	50.2
Total inertia—Sum of unconstrained eigenvalues	1.012		1.179		1.653		1.324	
Sum of canonical eigenvalues	.231		.328		.351		.604	

[1] Variable used in Depositional Targeted Habitat (DTH) algal diatom community data analysis.

[2] Variable used in Richest Targeted Habitat (RTH) algal diatom community data analysis.

[3] Variable used in Richest Targeted Habitat (RTH) invertebrate community data analysis.

[4] Variable used in fish community data analysis.

Differences between the sites on the extreme ends (sites 6 and 23) along the first CCA axis show the influences of ratio percent pool-to-percent riffle on the composition of the RTH algal-diatom community (fig. 4B). Site 6 had a higher ratio percent pool-to-percent riffle, phosphorus constituents (OP and TP), mean canopy closure, and forest land-use within the basin (table 2 and table 3). Site 23 had a larger drainage basin area and higher nitrogen constituents (NO_3+NO_2 and TN) concentration, mean instantaneous point velocity, reach surface-water gradient, and agriculture land-use within the basin (table 2 and table 3). The diversity at the sites ranged from 60 taxa (site 6) to 43 taxa (site 23), which suggests that as the ratio percent pool-to-percent riffle increased the number of taxa increased (appendix 2).

Both sites were dominated by the same genus (*Amphora* and *Navicula)*. Site 6 was dominated by *Aulacoseira muzzanensis* (24.6 percent relative abundance) *Navicula minima* (10.4 percent relative abundance), and *Amphora pediculus* (9.8 percent); site 23 was dominated by *Amphora pediculus* (18.0 percent relative abundance), *Navicula minima* (10.8 percent relative abundance), and *Navicula cryptotenella* (10.2 percent relative abundance). During the sampling period, site 6 had low water levels and stream velocities because of beaver dams; this resulted in lower reach surface-water gradient. Consequently, these natural variables led to a stream reach with high pool-to-riffle ratios. Of the 60 taxa at site 6, 32 were not at site 23; these 32 taxa accounted for 48.2 percent relative abundance of the individuals collected at site 6, including the most dominant species (*Aulacoseira muzzanensis)* found at site 6 (appendix 2). Of the 43 taxa at site 23, 15 were not collected at site 6; these 15 taxa accounted for 18.2 percent relative abundance of individuals collected at site 23, including the third most dominant species (*Navicula cryptotenella*) found at site 23 (appendix 2).

RTH invertebrate community. —Four variables (three habitat and one nutrient) explained the variability among the sites in the RTH invertebrate community (fig. 5A, table 5). A basin-calculated nutrient variable (TP, intersite correlation = 0.5735) and a habitat variable (mean bankfull depth, intersite correlation = 0.8821) were dominant on the first and second CCA axes, respectively (table 5). The first CCA axis accounted for 7.7 percent of the variation in the distribution of the invertebrate species, and the second axis explained 5.9 percent. The low eigenvalues ($\lambda1 = 0.127$ and $\lambda2 = 0.099$) and CPV (7.7 and 13.6) for the first and second CCA axes, respectively, suggest overall that RTH invertebrate communities were similar (table 5).

Total phosphorus accounted for the majority of the variation along the first CCA axes, (table 5). The effects of the TP along the first CCA axis can be observed by examining the extreme ends (sites 8 and 30) of the TP vector along the first CCA axis (fig. 5A). Site 8 had higher estimated N and P applied to a basin in 2001, nutrient constituents (NO_3+NO_2, TN, OP, and TP) concentrations, drainage basin area, agriculture land-use within the basin, reach length, and reach surface-water gradient (table 2 and table 3). Site 30 had higher mean depths, mean instantaneous point velocities, and forested land-use within the basin (table 2 and table 3). The diversity at the sites ranged from 20 taxa (site 8) to 29 taxa (site 30), which suggests that as TP increased the number of taxa decreased.

The two most dominant taxa at site 8 were not found at site 30; the third most dominant taxa found at site 8 was the most dominant taxa at site 30. The most dominant taxa at site 8 were *Stempellinella* species (21.8 percent relative abundance), *Psephenus herricki* (19.8 percent relative abundance), and *Ceratopsyche bronta* (14.7 percent relative abundance). The three most dominant taxa found at site 30 were also found at site 8; including *Ceratopsyche bronta* (21.9 percent relative abundance), *Baetis intercalaris* (11.0 percent relative abundance), and *Cheumatopsyche* species (8.9 percent relative abundance). Of the 20 taxa found at site 8, 12 were not found at site 30; these 12 taxa accounted for almost 69.0 percent relative abundance of individuals collected at site 8 and includes the two most dominant taxa (*Stempellinella* species and *Psephenus herricki*). Of the 29 taxa found at site 30, 21 were not found at site 8; these 21 taxa accounted for 49.5 percent relative abundance of individuals collected at site 30 (appendix 3).

Fish community. —Eight variables (six habitat and two land-use) explained the variability among the sites in the fish community (fig. 5B, table 5). Two habitat variables, mean bankfull depth (intersite correlation = -0.7674) and mean bankfull width (correlation = 0.7157), were dominant on the first and second CCA axes, respectively (table 4). The first CCA axis accounted for 15.2 percent of the variation in the distribution of the fish species, and the second CCA axis accounted for 7.7 percent. The eigenvalues ($\lambda1 = 0.201$ and $\lambda2 = 0.102$) and the CPV (15.2 and 22.9) for the first and second CCA axes, respectively, were the highest among the four community datasets, which suggests that the fish communities differ across the first CCA axis more than the other three biological communities.

Differences between the sites on the extreme ends (sites 4 and 17) along the first CCA axis show the influences of mean bankfull depth on the fish community (fig. 5B). Site 4 had higher mean bankfull depth and width, agricultural land-use within basin, mean wetted channel width, mean instantaneous point velocity, and drainage basin area (table 2 and table 3). Site 17 had higher nutrient constituent (NO_3+NO_2, OP, TN, and TP) concentrations, urban and forested land–use within basin, and reach surface-water gradient (table 2 and table 3). The diversity at the sites ranged from 32 (site 4) to 13 (site 17) taxa, which suggests that as the mean bankfull depth increased the number of taxa increased.

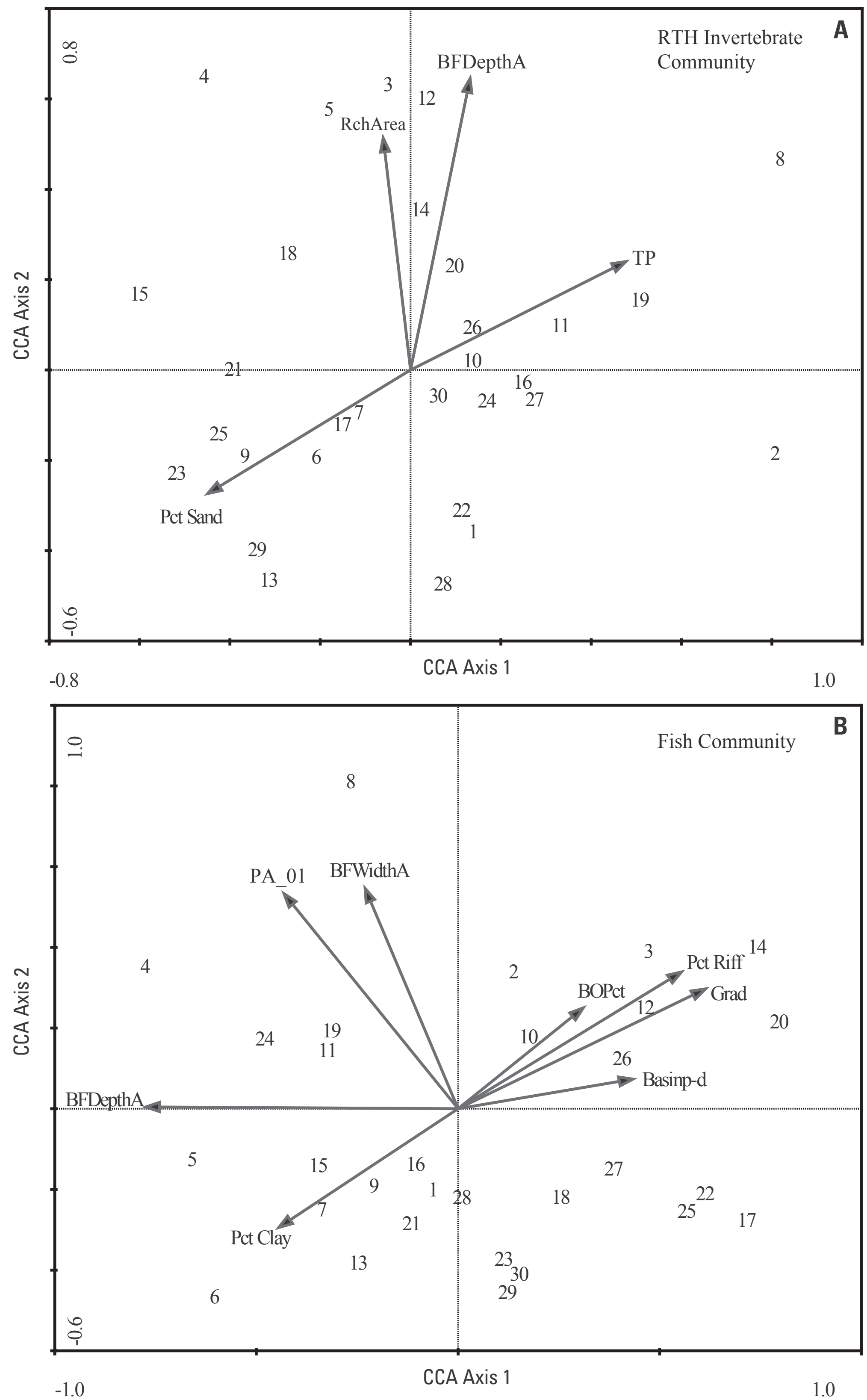

Figure 5. Canonical correspondence analysis (CCA) ordination plots of the (**A**) Richest-Targeted Habitat (RTH) invertebrate community; and the (**B**) fish community, White River and Great and Little Miami River Basins (WHMI) Study Unit of the National Water-Quality Assessment (NAWQA) Program (site numbers as listed in table 1; variable codes as listed in table 4).

The fish community composition among sites differed slightly, and the greatest differences between sites is evident at the extreme ends (sites 4 and 17) of the first CCA axis. Site 4 was dominated by bluntnose minnow (*Pimephales notatus,* 20.6 percent relative abundance), central stoneroller (*Campostoma anomalum,* 12.9 percent relative abundance), and longear sunfish (*Lepomis megalotis,* 12.7 percent relative abundance); site 17 was dominated by central stoneroller (*Campostoma anomalum,* 43.6 percent relative abundance), blackside darter (*Percina maculata,* 30.1 percent relative abundance) and bluntnose minnow (*Pimephales notatus,* 10.2 percent relative abundance). The large numbers of herbivores (central stoneroller) and omnivores (bluntnose minnow) at these sites suggests they are nutrient rich. Of the 32 taxa found at site 4, 22 taxa were not collected at site 17; these 22 taxa accounted for 51.2 percent relative abundance of individuals collected at site 4, including the third most dominant species (*Lepomis megalotis*) at site 4. Of the 13 taxa found at site 17, 3 taxa were not collected at site 4; these 3 taxa accounted for 30.5 percent relative abundance of individuals collected at site 17, including the second most dominant species (blackside darter, *Percina maculata)* found at site 17 (appendix 4).

Implications for Assessing Nutrient Conditions in Midwest Streams

A common sampling design used by states for estimating the percentage of impaired miles of streams for many stressors (including nutrients) is to randomly select sampling sites so that the findings at a limited number of sites can be extrapolated to all sites. This method works well to meet the objectives of the Clean Water Act in assessing the amount of impaired miles of streams. However, by randomly selecting sites, variables such as ecoregion, basin size, substrate, and canopy cover that could mask the relations between the stressor and the biological community cannot be controlled. Studies in Indiana used the randomly selected site design to assess the amount of impaired streams; however, results indicated that there were weak or no significant relations between nutrients and algal biomass (periphyton chlorophyll *a* or ash free dry mass) due to confounding variables (Caskey and others, 2007; Frey and others, 2007; Leer and others, 2007; Lowe and others, 2008).

In this study, the algal diatom- (DTH and RTH), invertebrate-, and fish-community data that were collected at 30 sites within the Ecoregion 55 portion of the NAWQA WHMI Study Unit were examined to determine whether the response among biological communities reflected differences along a nutrient concentration gradient from low to high. Study sites were selected to minimize physical habitat differences while maximizing nutrient concentration differences along a nutrient (TP and TN) gradient. The biological community structure was related to nutrient and physical variables to determine the significant environmental variables for each community. Next, the significant nutrient gradients were examined to determine whether specific species could be identified as biological indicators of nutrient enrichment.

Environmental Gradients

Several studies have shown that streams within Ecoregion 55 have elevated nutrient concentrations. Martin and others (1996), the USEPA (2000b), Reutter (2003), Caskey and others (2007), Frey and others (2007), and Leer and others (2007) have reported concentration ranges of total nitrogen from 0.56 to 9.26 mg/L and concentrations of total phosphorus from 0.015 to 0.285 mg/L throughout this region. Although many nutrient concentrations documented within the study area are elevated and many streams could be classified as nutrient-rich systems, it was thought there might be seasonal periods (such as August–September) during which evapotranspiration and algal uptake of nutrients would reduce nutrient concentrations to low levels, thereby permitting accurate definition of biological thresholds (Baker and others, 2006; Caskey and others, 2007; Frey and others, 2007; Leer and others, 2007; Lowe and others, 2008).

Because a nutrient gradient was found at the 30 sites, the biological community data were related to the nutrient and physical data using a CCA. The results from the CCA show that two nutrient and two physical variables accounted for the most variability on the first CCA axis. Nutrients, specifically TP, accounted for the majority of the variation in the DTH algal-diatom community and the RTH invertebrate community, and habitat accounted for the most variability in the RTH algal-diatom community and the fish community. This relation suggests that the DTH algal-diatom and RTH invertebrate communities might best be used to assess the eutrophication of streams. However, the DTH samples are mostly composed of fine clay particles that tend to have high phosphorus concentrations, so the significant relations may be a function of sampling method. Additionally, all eigenvalues were low, suggesting the biological community composition and structure were similar among the low to high nutrient sites. The composition of the different biological communities were dominated by species found in nutrient-rich waters. Despite the low-end nutrient gradient reflected by the August samples, the biological communities reflect higher nutrient conditions found throughout the year. This finding suggests that a single nutrient sample collected during the low-nutrient period of July–September may not accurately reflect the nutrient condition of a stream and could be misleading. A better measure of the nutrient condition within a stream would be one that encompasses the amount of nutrients that affect the biological communities throughout the year, such as annual mean or median concentrations. It also suggests that even though a low-end nutrient gradient was found, the annual nutrient concentrations may not be low enough to cause changes in the biological communities; that is, fewer algivores and omnivores. Dodds and others (2002) found significant breakpoints for TP (0.03 mg/L) and TN (0.04 mg/L) with periphyton chlorophyll *a*. Almost all of the TP data were higher than 0.03 mg/L, and all of the TN data were well above the 0.04 mg/L threshold. Additionally, the CCA results indicate that habitat is important when assessing streams for all four biological communities.

Biological Indicators

Findings from the gradient analysis showed that the biological communities were similar (as indicated by the low eigenvalues. The observed changes in the biological community composition were often in response to non-nutrient gradients. Consequently, the most significant gradients from each biological community were examined to determine whether changes could be detected in species composition and structure along each gradient. In all cases, the biological communities were dominated by taxa found in nutrient-rich systems (Ward, 1992; Feminella and Hawkins, 1995; Petersen and Femmer, 2002; Lowe, 2003). For example, in this study more than 65 percent of the DTH algal-diatom communities and more than 66 percent of the RTH algal-diatom communities were dominated by three diatom genera (*Amphora*, *Navicula*, and *Nitzschia*), the invertebrate communities were dominated by the order of Ephemeroptera (almost 50 percent), and the fish communities were dominated by *Campostoma* and *Pimephales* species (almost 37 percent). Mueller and Spahr (2006) showed that streams within Ecoregion 55, which includes a part of the study area, have some of the highest N and P loadings within the United States. Within these streams, about 25 percent of the TN and TP concentrations were greater than the USEPA proposed criteria, so it was not surprising to find communities dominated by eutrophic species. It was surprising that corresponding shifts in community composition and structure along the observed nutrient gradient were not apparent, these findings support the idea that streams sampled in the study were nutrient-saturated, resulting in communities dominated by eutrophic species. Furthermore, even though nutrient and biological samples were collected in periods known to have lower nutrients, the biological community reflected the periods of higher nutrient concentrations often associated with runoff from spring and winter periods within the study area.

Because there were no differences in the biological communities along the nutrient gradient, biological communities were assessed along the physical habitat gradients to see whether those gradients could account for the differences in the community composition and structure. The changes in the invertebrate communities were related to changes within instream (channel) substrate types, and changes in the fish community were related to stream/basin size. One dominant invertebrate group was Dipterans, which are often found in freshwater habitats with eutrophic conditions; consequently, Dipterans have been widely used as a pollution indicator (Ward, 1992). In the current study, as the pool-to-riffle ratio increased, the number of Dipterans decreased. Because the invertebrate samples were collected from riffle habitats with gravel/cobble substrates, it may be that as the pool-to-riffle ratio increases, restricting substrate availability, the number of Dipteran taxa would decrease because, in part, of the loss of substrate variability (Ward, 1992). The fish communities, as mentioned earlier, were also dominated by eutrophic species. In a study of Ozark streams, Petersen and Femmer (2002) found that *Campostoma* species, which are common in eutrophic environments, can be the dominant species in nutrient-rich streams because they are able to make use of the increased algal growth associated with increased nutrients. However, if nutrients are at saturation levels, other species requirements such as substrate type and water clarity (all shown to be associated with stream/basin size) can account for species abundances. No specific species were identified that suggest nutrient enrichment because the biological communities were similar and dominated by eutrophic species. Changes in composition and structure were most often the response of physical habitat changes.

Assessing Nutrient Rich Midwest Streams

The sites in this study, which are typical of Midwest streams, are in agriculturally dominated landscapes with elevated concentrations of nutrients (Mueller and Spahr, 2006). Understanding the complex interrelations among nutrients, environmental variables, and biological communities is difficult for these streams because these waters often are nutrient saturated, resulting in communities that are dominated by similar eutrophic species. Because all the streams in this study are dominated by eutrophic species, the small differences in community composition and structure tended to be a function of physical habitat rather than nutrients.

In this study, the ranges of nutrients found during the low-nutrient August period resulted in a gradient that should have been suitable for a nutrient gradient study. However, the biological communities reflected the higher nutrient conditions present throughout the year. Multiple nutrient samples at a site would more accurately assess the annual nutrient condition of a stream. Single nutrient samples collected during the low-nutrient period of June–September could be misleading. For example, in this study, if the biological community data had not been included in the study design, the nutrient data alone would have suggested that many streams are not nutrient-rich. However, biological communities reflect the long-term effects within an aquatic ecosystem, and the biological community data are therefore useful in assessing potential nutrient enrichment in the nutrient-rich Midwest.

For gradient studies to work effectively in the nutrient-rich Midwest, reference sites with low-annual nutrient concentrations need to be found. In the Midwest, reference sites are difficult to find because most of the land is flat, has fertile soils, and is conducive to agriculture. This results in application of fertilizer to fields that subsequently is transported into streams through several pathways. A possible target for low-nutrient conditions may be the 0.03 mg/L for TP and 0.04 mg/L for TN found by Dodds and others (2002). Although these low-end nutrient levels were not found at the 30 sites in this study, they have been found in other areas of the Midwest.

It was noteworthy that the most influential nutrients were also significantly related to physical-habitat characteristics. Caskey (2003) found that in agriculturally-dominated landscapes, relations among physical habitat can mask or affect the response of anthropogenic variables that could influence biological conditions. Often, habitat drives which species can live in a stream. The intent of this study, however, was to select sites that were as physically similar as possible to minimize the effects of habitat along a nutrient gradient. Despite the physical similarity between sites, the physical variables still explained the differences among the biological communities of streams better than the nutrient concentrations because the study sites were nutrient saturated.

Whether increases in nutrients have a negative impact on biological-community dynamics depends, in part, on the environmental setting. For example, in a headwater stream with elevated nutrient concentrations and a closed canopy, light attenuation may be limited to the extent that changes in algal-diatom community composition may not occur regardless of nutrient concentrations. This study demonstrates that many Midwestern agricultural streams are more habitat limited than nutrient limited and that the biological communities contain numerous eutrophic indicators that could be important for tracking future changes in nutrient conditions. It also suggests that if habitat is not included as part of stream assessments for nutrient criteria, even if nutrient reductions are made, habitat limitations could preclude improvements in the biological communities.

Conclusions

The objective of this study was to relate algal-, invertebrate-, and fish-community composition in small streams to habitat, nutrients, and land-use variables in agriculturally-dominated landscapes of the Midwest in Indiana and Ohio. To minimize the variability associated with non-nutrient variables, thirty sample locations were selected from a single ecoregion (Ecoregion 55-USEPA Level III Eastern Corn Belt Plains) in wadable streams, and when possible, with similar substrate and canopy cover, along a previously determined nutrient-concentration gradient. Biological and nutrient samples were collected during stable flow conditions in August 2004. Canonical correspondence analysis was used to determine which variables most influenced each community. Total phosphorus concentrations significantly influenced the depositional-targeted habitat (DTH) algal-diatom community and the richest-targeted habitat (RTH) invertebrate community, although the significant relations with the DTH algal-diatom community may be related to sampling the fine sediments as part of the DTH sampling methods. Multivariate statistical analysis showed that habitat variables were more influential to the richest-targeted habitat algal-diatom and fish communities than nutrient concentrations. Although the nutrient concentrations measured during the stable low flow period of August indicate that most streams were not eutrophic, the biological communities were dominated by eutrophic species, suggesting streams sampled were eutrophic. This also suggests that the biological community reflected the periods of higher nutrient concentrations often associated with runoff from spring and winter periods within the study area. This study demonstrates that many Midwestern agricultural streams are more habitat limited than nutrient limited. Furthermore, if habitat is not included as part of stream assessments for nutrient criteria, even if nutrient reductions are made, habitat limitations could preclude improvements in the biological communities.

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