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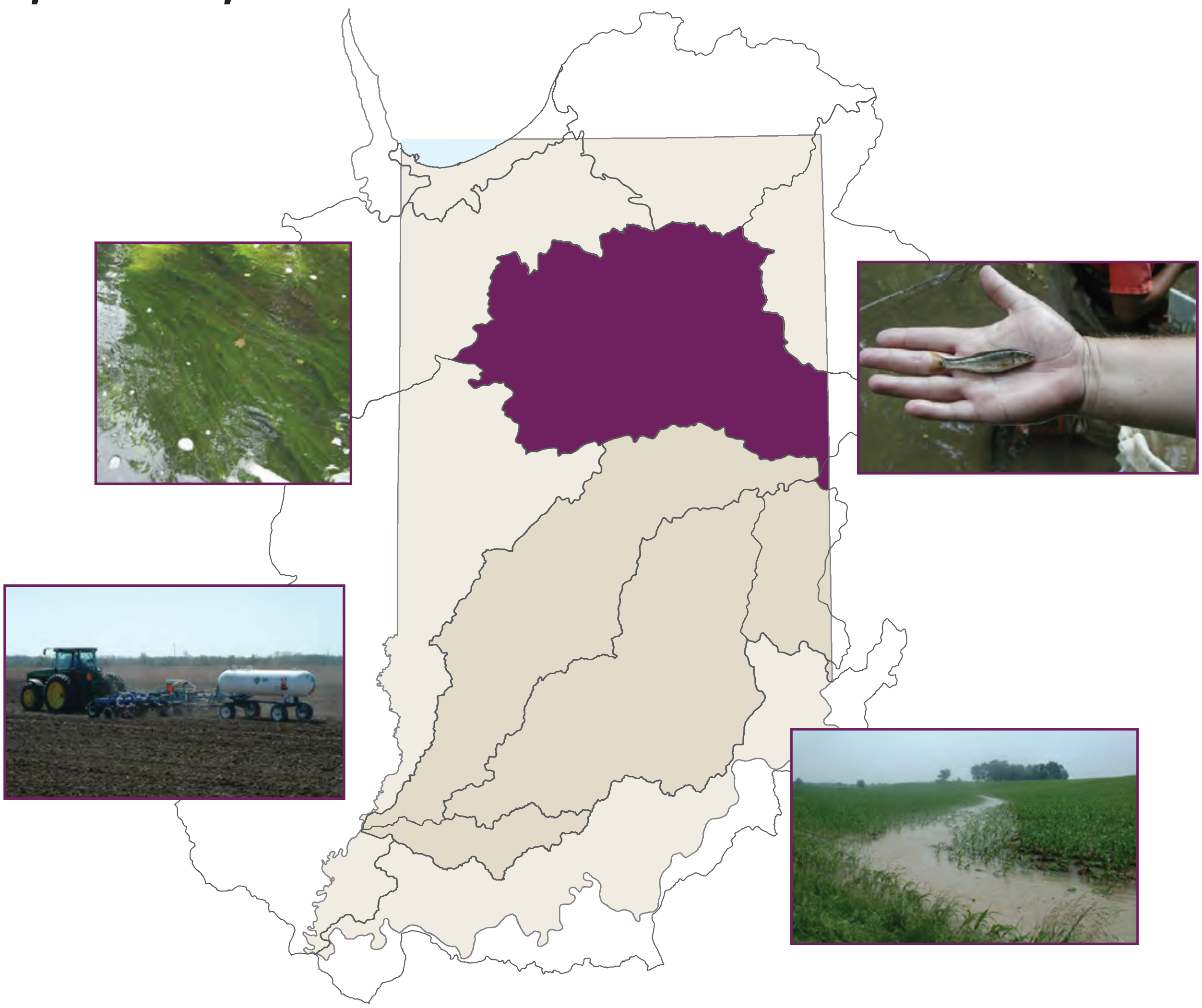
Relations of Principal Components Analysis Site Scores to Algal-Biomass, Habitat, Basin-Characteristics, Nutrient, and Biological-Community Data in the Upper Wabash River Basin, Indiana, 2003



Scientific Investigations Report 2007–5231

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By Donald R. Leer, Brian J. Caskey, Jeffrey W. Frey, and B. Scott Lowe

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Contents

Figures

Tables

Conversion Factors and Datum

Multiply	By	To obtain
centimeter (cm)	0.3937	inch (in.)
square kilometer (km^2)	0.3861	square mile (mi^2)
cubic meter per second (m^3/s)	35.31	cubic foot per second (ft^3/s)
liter (L)	0.2642	gallon (gal)

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

$$°F=(1.8\times°C)+32$$

Horizontal coordinate information used in the map is referenced to the North American Datum of 1927 (NAD 27). Sampling-site coordinate information used a Global Positioning System (GPS) referenced to North American Datum of 1983 (NAD 83), which then was converted to NAD 27.

Concentrations of chemical and algal-biomass constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L). Concentrations of periphyton algal biomass are given in milligrams per meter squared (mg/m^2).

Abbreviations

Abbreviations and acronyms used in this report:

PC1	Principal components analysis first axis site score
EPT	Ephemeroptera, Plecoptera, and Trichoptera
USEPA	U.S. Environmental Protection Agency
N	nitrogen
P	phosphorus
CWA	Clean Water Act
IBI	Index of Biotic Integrity
TN	total nitrogen
TP	total phosphorus
CHLa	chlorophyll *a*
TKN	total Kjeldahl nitrogen
IDEM	Indiana Department of Environmental Management
USGS	U.S. Geological Survey
WMP	Watershed Monitoring Program
PCA	Principal components analysis
AFDM	ash-free dry mass
POC	particulate organic carbon
IN	Indiana
NAWQA	National Water-Quality Assessment Program
NWQL	National Water Quality Laboratory
QHEI	Qualitative Habitat Evaluation Index
NLCD	National Land Cover Dataset
r_s	Spearman’s rho statistic

Relations of Principal Components Analysis Site Scores to Algal-Biomass, Habitat, Basin-Characteristics, Nutrient, and Biological-Community Data in the Upper Wabash River Basin, Indiana, 2003

By Donald R. Leer, Brian J. Caskey, Jeffrey W. Frey, and B. Scott Lowe

Abstract

Data were gathered from May through October 2003 at 38 randomly selected sites in the Upper Wabash River Basin, Indiana, for algal biomass, habitat, nutrients, and biological communities (fish and invertebrates). Basin characteristics (drainage area and land use) and biological-community attributes and metric scores were determined for each sampling site's basin. Yearly Principal Components Analysis site scores were calculated for algal biomass (periphyton and seston). The yearly Principal Components Analysis site scores for the first axis (PC1) were related, using Spearman's rho, to the seasonal algal-biomass, basin-characteristics, habitat, seasonal nutrient, biological-community attribute and metric score data.

The periphyton PC1 site score, which was most influenced by September chlorophyll *a*, was not related to the five basin-characteristics, nine habitat, or 12 nutrient variables examined. Of the 43 fish-community attributes and metric scores examined, the periphyton PC1 was negatively related to one attribute (tolerant percent) and positively related to one metric score (insectivore percent metric score). Of the 43 invertebrate-community attributes and metric scores examined, the periphyton PC1 was negatively related to three attributes (Ephemeroptera, Plecoptera, and Trichoptera (EPT) count, EPT-to-total ratio, and number of taxa) and four metric scores (EPT count metric score, EPT to total ratio metric score, macroinvertebrate Index of Biotic Integrity metric score, and number of taxa metric score). The seston PC1 was not related to the five basin-characteristics, nine habitat, or 12 nutrient variables or to the 43 fish- and 21 invertebrate-community attributes or metric scores examined.

To understand how the choice of sampling sites might have affected the results, an analysis of the drainage area and land use was done. The random site selection in 2003 in the Upper Wabash River Basin was skewed to small streams. The lack of large streams in the Upper Wabash River Basin could account for the few relations among the seston and the biological-community data.

The values for nutrients (nitrate, total Kjeldahl nitrogen, total nitrogen, and total phosphorus) and chlorophyll *a* (periphyton and seston) were compared to published U.S. Environmental Protection Agency (USEPA) values for Aggregate Nutrient Ecoregions VI and VII and USEPA Level III Ecoregions 55 and 56. Several nutrient values were greater than the 25th percentile of the published USEPA values. Chlorophyll *a* (periphyton and seston) values either were greater than the 25th percentile of published USEPA values or extended data ranges in the Aggregate Nutrient and Level III Ecoregions. If the proposed values for the 25th percentile were adopted as nutrient water-quality criteria, many samples in the Upper Wabash River Basin would have exceeded the criteria.

Introduction

Excessive inputs of nutrients into streams have human-health, economic, and ecological consequences. Excess amounts of nutrients—nitrogen (N) and phosphorus (P)—have been shown to be a source of eutrophication in aquatic ecosystems, which sometimes has been linked to fish kills, shifts in species composition, taste and odor in drinking-water supplies, and blooms of harmful algae in freshwater and estuaries (Munn and Hamilton, 2003; U.S. Environmental Protection Agency, 2000a,b).

The Clean Water Act (CWA) established a national goal of achieving water quality that provides for the protection and propagation of aquatic organisms and wildlife, and recreation in and on the water. In 1996, the U.S. Environmental Protection Agency's (USEPA) National Water Quality Inventory identified excess amounts of nutrients as the second leading cause of impairment in rivers and streams across the United States (the first cause was siltation) (U.S. Environmental Protection Agency, 1997a). The excess amounts of nutrients that have been documented in many rivers and streams have resulted in streams that do not meet the goal of the CWA in Indiana and the nation.

USEPA drinking-water criteria (maximum contaminant levels) are 10 mg/L for nitrate as N and 1 mg/L nitrite as N (U.S. Environmental Protection Agency, 2005). In addition, aquatic-life criteria established to protect aquatic organisms have been set for ammonia as N (the ammonia as N aquatic-life criteria varies with pH, temperature, and life-stage) (U.S. Environmental Protection Agency, 2005). These criteria do not address the effects on the biological communities resulting from increased nutrients in rivers and streams. Typically, nutrient concentrations must be extremely high to be toxic to biological communities; such concentrations rarely are found in the environment (Ohio Environmental Protection Agency, 1999). For example, nitrate as N concentrations below 90 mg/L would not have direct effects on warmwater fish. Exceptions are concentrations of ammonia after accidental discharges from wastewater-treatment facilities, combined-sewer overflows, or confined-animal feedlots (Ohio Environmental Protection Agency, 1999). Previous analysis of the effects of nutrients on biological communities in Ohio found few relations between nutrients and fish and invertebrate-community data (Miltner and Rankin, 1998). Only total phosphorus was significantly correlated with any of the fish or invertebrate attributes or metrics (fish Index of Biotic Integrity [IBI] scores in headwater streams).

Many streams have been placed by the USEPA on the CWA Section 303(d) list of impaired water bodies because of excess amounts of nutrients. In 2000, the USEPA proposed nutrient water-quality criteria for causal variables—total nitrogen (TN) and total phosphorus (TP)—and for response variables—periphyton and seston chlorophyll *a* (CHLa) and turbidity. Criteria also have been proposed for nitrate and total Kjeldahl nitrogen (TKN) because TN is the sum of nitrate and TKN. These proposed nutrient water-quality criteria are based on Aggregate Nutrient Ecoregions, areas with similar geographic features (topography, soils, geology, land use, and biogeography) (U.S. Environmental Protection Agency, 2000a,b). USEPA reviewed selected data and set the proposed nutrient water-quality criteria for nitrate, TKN, TN, TP, CHLa (periphyton and seston), and turbidity at the 25th-percentile value of all data for each parameter.

USEPA mandated that by 2004 states either accept the proposed nutrient water-quality criteria or provide their own set of criteria that are more appropriate to the waters within each state (U.S. Environmental Protection Agency, 2000a,b). An extension was given to Indiana and other states that adopted plans describing the data needs and the process to develop nutrient water-quality criteria. Beginning in 2001, the Indiana Department of Environmental Management (IDEM) and the U.S. Geological Survey (USGS) are cooperating on several studies that will assist the State of Indiana in developing nutrient water-quality criteria as mandated by the USEPA. The multivariate approach used in this report should allow the results to be used in similar ecoregions in Illinois and Ohio.

Purpose and Scope

Data in this report were collected as part of an ongoing cooperative effort between IDEM and the USGS in which similar studies have been conducted as part of the IDEM probabilistic Watershed Monitoring Program (WMP) in the West Fork White River Basin (2001), Whitewater River and East Fork White River Basins (2002), Lower Wabash River and Kankakee River Basins (2004), and Ohio River and Great Lakes Basins (2005) (Indiana Department of Environmental Management, 2001). In all of these yearly WMP studies, IDEM collected habitat and biological community data and the nutrients from 2001 through 2003; to augment the IDEM WMP studies the USGS collected algal biomass during all years of the study and also collected nutrients in 2004 and 2005. The long-term goal of these studies is to provide data and analysis to aid IDEM in the development of nutrient water-quality criteria. An objective of this report was to develop a preliminary understanding of how algal biomass relates to biological community and environmental variables in the Upper Wabash River Basin in 2003. An additional purpose of this report was to compile a list of the most statistically significant relations between algal biomass, nutrients, and biological metrics that may be helpful in future investigations.

Two approaches were used for the preliminary analysis of the data sets. The first approach included ordination and regression analyses of the algal biomass, nutrient, and environmental data. The second approach compared the CHLa and nutrient values collected by IDEM and USGS personnel to USEPA published values. The purpose of this preliminary analysis was to investigate all potential relations and identify those relations that were the strongest and warrant further investigation.

This report discusses the relations of yearly Principal Components Analysis (PCA) site axis scores, which represent algal biomass (periphyton and seston), to habitat, basin-characteristics, nutrient, and biological-community (fish and invertebrates) attributes and metric scores (appendix 1; Dufour, 2002). Data were collected at 38 randomly selected sites in the Upper Wabash River Basin in 2003 (fig. 1). A discussion of the basin characteristics of the 38 sites describes how drainage area and land use affect the analysis. This report also compares nutrient values (nitrite plus nitrate as N, TKN as N, TN as N, TP as P) and CHLa (periphyton and seston) collected by IDEM and USGS for the Upper Wabash River Basin to values published by the USEPA for Aggregate Nutrient Ecoregion VI—Corn Belt and Northern Great Plains, for Aggregate Nutrient Ecoregion VII—Mostly Glaciated Dairy Region, for Level III Ecoregion 55—Eastern Corn Belt Plains, and for Level III Ecoregion 56—Southern Michigan/Northern Indiana Drift Plains. Aggregate Nutrient Ecoregions consist of

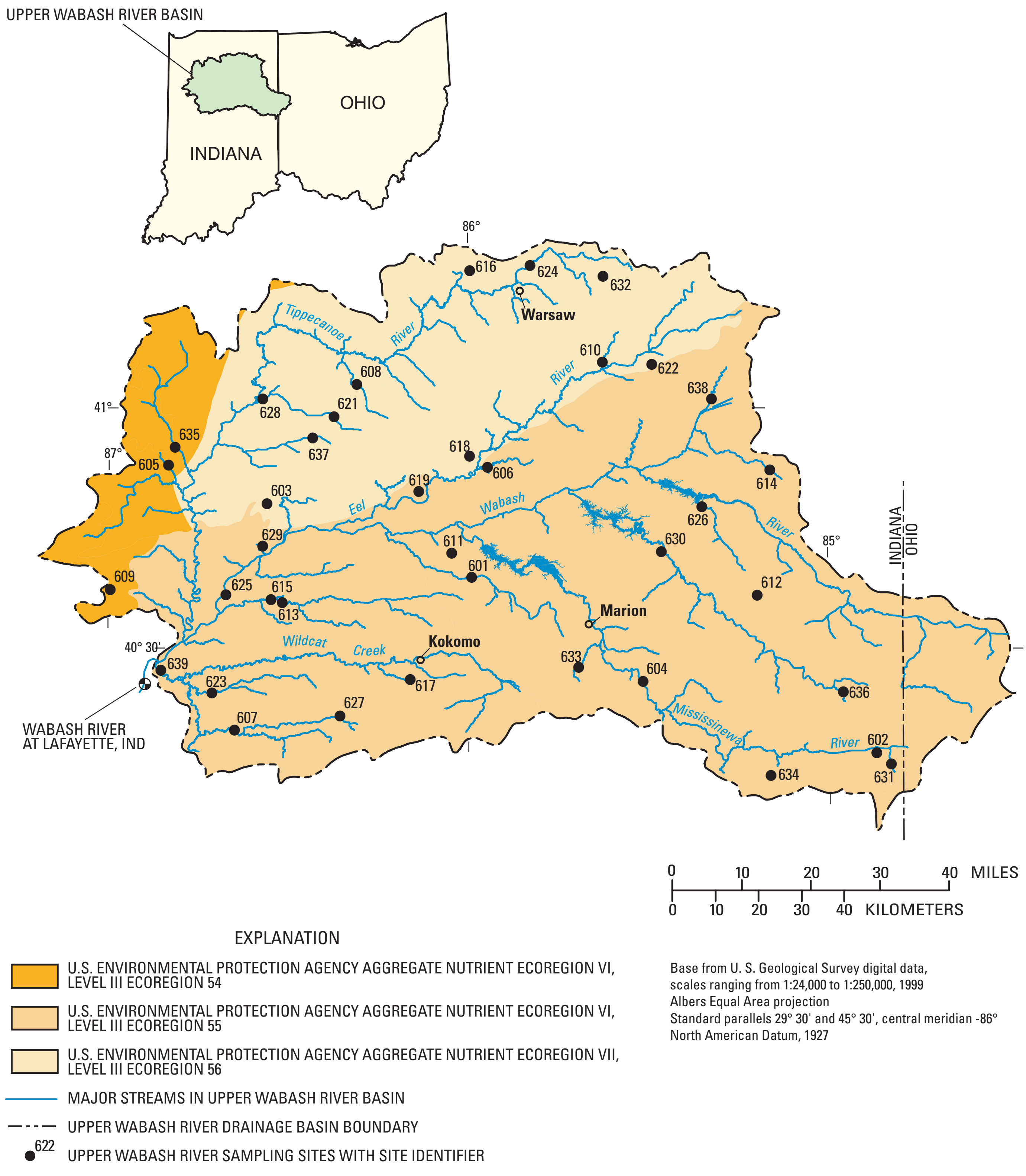

Figure 1. Map showing location of the 38 algal-bilmass sampling sites in the Upper Wabash River Basin, Indiana, 2003.

one or more Level III Ecoregions (U.S. Environmental Protection Agency, 2000a,b).

In the text, the Aggregate Nutrient Ecoregions are referred to as Ecoregion VI or VII; Level III Ecoregions are referred to as Ecoregion 55 or 56. The nutrients are described as concentrations of nitrate, TKN, TN, and TP. In this report, periphyton CHLa, ash-free dry mass (AFDM), seston CHLa, and particulate organic carbon (POC) are measures of algal biomass.

Description of the Upper Wabash River Basin

The Upper Wabash River Basin (fig. 1) drains more than 18,655 km^2 of central Indiana and parts of western Ohio before draining into the middle branch of the Wabash River (Hogatt, 1975). The annual mean streamflow in the 2003 water year at the USGS streamflow-gaging station Wabash River at Lafayette, IN (03335500) (fig. 1) was 234.3 m^3/s (Morlock and others, 2004). Streamflow in the 2003 water year was above average; the annual mean long-term streamflow (1924-2003) was 189.7 m^3/s at Lafayette (Morlock and others, 2004). The gaging station at Lafayette is 8.2 km downstream from the basin boundary of the Upper Wabash River and is the gaging station closest to the basin boundary.

The climate in the Upper Wabash River Basin is characterized as humid continental with mild to warm summers and cold winters. The mean monthly temperature ranges from -4.3°C in winter to 22.9°C in summer (Purdue Applied Meteorology Group, 2005). The mean annual precipitation ranges from 94.5 cm to 104 cm (Purdue Applied Meteorology Group, 2005).

The dominant land use of the Upper Wabash Basin is agriculture (92 percent), primarily corn and soybeans. Approximately 5 percent of the basin area is forested, 1 percent is urban, and 2 percent is other land-use types. Typical of many streams in the State, streams in the Upper Wabash River Basin have low gradients and velocities. The total population of the 97 cities and towns in the basin is 294,680; the largest urban areas are Kokomo (population of 46,113) and Marion (population of 31,320) (U.S. Census Bureau, 2000) (fig. 1).

Study Methods

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

Site Selection and Sampling Strategies

Sampling sites were selected randomly by the USEPA as part of the IDEM probabilistic Watershed Monitoring Program (WMP) (Indiana Department of Environmental Management, 1999, 2001). Each selected sampling site (table 1) represents a specific stream order; therefore, statistically valid extrapolations can be made from the randomly sampled streams to the entire class of streams in a particular basin. The IDEM WMP works on a 5-year rotating basin schedule, focusing on 1-2 selected basins each year, with a complete assessment of the state at the end of each 5-year cycle. In 2003, the focus was the Upper Wabash River Basin. After the sampling sites were selected and prior to collection of field data, IDEM personnel completed a visual assessment of the potential sampling sites and determined the area to be sampled at each site. At each of the USEPA randomly selected sites, the latitude and longitude was used as the middle point of the reach, with half of the reach upstream and half downstream of the middle point.

During periods of stable flow from May through October 2003 in the Upper Wabash River Basin, 38 sites (fig. 1, table 1) were sampled three times for algal biomass and nutrients. The samples were collected three times to measure seasonal changes in the algal biomass and nutrients. Round one sampling was done in May and June (spring); round two sampling was done in July and August (summer); and round three sampling was done in September (fall). In this report, round one sampling will be referred to as May or spring, round two as July or summer, and round three as September or fall. The algal-biomass and nutrient samples were collected on the same day at about the same time. The biological community (fish and invertebrate) and habitat were sampled one time by IDEM personnel from June through October 2003.

Algal-Biomass, Habitat, Basin-Characteristics, Nutrient, and Biological-Community Data-Collection and Processing Methods

Algal-biomass samples were collected and processed for periphyton and seston, as described in USGS protocols, with several modifications. The National Water-Quality Assessment (NAWQA) Program algal protocols for periphyton (Moulton and others, 2002) are a reach-based sampling methodology in which five periphyton subsamples were collected from five different locations within the sampling reach. At each location, the stream depth, velocity, shading, and substrate were recorded. The subsamples were composited and marked as a single sample. At each site periphyton samples were collected using the same substrate type—epilithic (rocks), epidendric (sticks), or epipsammic (sand)—during the sampling period. One modification to this study from the NAWQA protocols was that ten periphyton subsamples were collected from the same substrate as close to the center of the reach as possible. Then five subsamples were selected that best represented the

Table 1. Location and basin characteristics of the 38 algal-biomass sampling sites in the Upper Wabash River Basin, Indiana, May through October 2003.

[IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; ID, identification number; USEPA, U.S. Environmental Protection Agency; FT, feet; US, upstream; trib, tributary; CR, county road; R, River; SR, state route; DS, downstream; CK, Creek; FK, Fork; DCH, Ditch; NR, near; AV, Avenue; ST, Street; N, North; S,South; E, East; W, West; USHWY, U.S. Highway; IN, Indiana; OH, Ohio]

Short IDEM site number[1]	USGS station ID	USGS station name	USEPA Aggregate Nutrient Ecoregion[2]	Level III Ecoregion[3]	Latitude (degrees)	Longitude (degrees)
601	403857085593501	PIPE CK 100 FT US PIPE CK AT SANTA FE, IN	VI	55	40.64950	-85.99200
602	401643084521501	HARSHMAN CK 0.9 MI US MISSISSINEWA R NR SALEM, IN	VI	55	40.27870	-84.87020
603	404805086333701	GALBREATH DITCH 0.5 MI DS CR 200N NR LK CICOTT, IN	VII	56	40.80107	-86.56016
604	402554085305901	MISSISSINEWA R 0.8 MI US SR 26 NR FOWLERTON, IN	VI	55	40.43180	-85.51480
605	405236086495201	BIG MONON CK 0.5 MI US SR 16 NR MONON, IN	VI	54	40.87680	-86.83150
606	405243085564701	PAW PAW CK 0.3 MI US CR 800N NR ROANN, IN	VI	55	40.87859	-85.94648
607	401943086385101	S FK WILDCAT CK 0.6 MI US CR 800W NR MULBERRY, IN	VI	55	40.32850	-86.64740
608	410301086184501	MUD CK 0.3 MI US GRUBE DITCH NR PERSHING, IN	VII	56	41.04981	-86.31161
609	403712086593901	MEYERS DITCH 820 FT US I-65 NR BADGER GROVE, IN	VI	54	40.61974	-86.99414
610	410543085373101	CLEAR CK 980 FT US SPRING CK AT SOUTH WHITLEY, IN	VII	56	41.09500	-85.62430
611	404156086024501	LITTLE PIPE CK 900 FT DS CR 150E NR SOUTH PERU, IN	VI	55	40.69930	-86.04680
612	403633085115401	ROCK CK 770 FT US CR 900S NR PETROLEUM, IN	VI	55	40.60936	-85.19817
613	403542086305901	DEER CK 0.7 MI US MERIDIAN LINE RD NR CAMDEN, IN	VI	55	40.59506	-86.51631
614	405208085093701	EIGHTMILE CK 0.4 MI US SR 1 AT OSSIAN, IN	VI	55	40.86910	-85.16019
615	403603086325101	DEER CK 0.6 MI DS SR 75 AT CAMDEN, IN	VI	55	40.60070	-86.54740
616	411707085593601	EASTERDAY DITCH 0.3 MI DS CR 400N NR ATWOOD, IN	VII	56	41.28654	-85.99596
617	402612086094301	E FK LITTLE WILDCAT CK 880 FT US CR200W AT ALTO, IN	VI	55	40.43653	-86.16117
618	405346085595201	WILSON RHODES DITCH 0.6 MI US CR850N NR ROANN, IN	VII	56	40.89640	-85.99760
619	404912086080401	UNNAMED TRIB 1.2 MI US EEL R AT MEXICO, IN	VI	55	40.82060	-86.13430
621	405856086223001	MILL CK 600 FT US HINES DITCH NR MARSHTOWN, IN	VII	56	40.98213	-86.37508

Table 1. Location and basin characteristics of the 38 algal-biomass sampling sites in the Upper Wabash River Basin, Indiana, May through October 2003.—Continued

[IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; ID, identification number; USEPA, U.S. Environmental Protection Agency; FT, feet; US, upstream; trib, tributary; CR, county road; R, River; SR, state route; DS, downstream; CK, Creek; FK, Fork; DCH, Ditch; NR, near; AV, Avenue; ST, Street; N, North; S,South; E, East; W, West; USHWY, U.S. Highway; IN, Indiana; OH, Ohio]

Short IDEM site number[1]	USGS station ID	USGS station name	USEPA Aggregate Nutrient Ecoregion[2]	Level III Ecoregion[3]	Latitude (degrees)	Longitude (degrees)
622	410526085291501	STONY CK 720 FT US MERIDIAN RD AT PEABODY, IN	VII	56	41.09090	-85.48870
623	402423086423501	MID FK WILDCAT CK 0.4 MI DS HOG RUN AT PETTIT, IN	VI	55	40.40630	-86.71070
624	411753085493801	TIPPECANOE R 880 FT DS CR 100E NR ISLAND PARK, IN	VII	56	41.29765	-85.82738
625	403639086402301	WABASH R 1.9 MI US SR 39 NR DELPHI, IN	VI	55	40.61088	-86.67318
626	404739085210101	ROCK CK 0.4 MI US SR 3 NR MARKLE, IN	VI	55	40.79580	-85.35150
627	402135086211701	KILMORE CK 500 FT US CR 800E NR FOREST, IN	VI	55	40.35993	-86.35473
628	410048086350101	MILL CK 0.6 MI US TIPPECANOE R NR WINAMAC, IN	VI	55	41.01320	-86.58340
629	404248086341901	UNNAMED TRIB 0.8 MI US BURNETTS CK AT LOCKPORT, IN	VI	55	40.71320	-86.57180
630	404203085274901	DETAMORE DITCH 700 FT DS I-69 NR WARREN, IN	VI	55	40.70118	-85.46385
631	401517084495201	LITTLE MISSISSINEWA R 2.8 MI DS SR28 NR COSMOS, OH	VI	55	40.25505	-84.83130
632	411625085372301	UNNAMED TRIB 450 FT US GAFF DITCH NR ETNA, IN	VII	56	41.27390	-85.62304
633	402739085413701	DEER CK 0.25 MI US CR 100W NR WEAVER, IN	VI	55	40.46050	-85.69430
634	401401085413701	ELKHORN CK 0.25 MI US CR 500N NR FARMLAND, IN	VI	55	40.23280	-85.16620
635	405501086490201	BIG MONON CK 0.2 MI US BIG MONON DITCH NR MONON, IN	VI	54	40.91790	-86.81700
636	402425084574101	LITTLE SALAMONIE R 0.4 MI US CR 120S AT LIBER, IN	VI	55	40.40696	-84.96156
637	405617086260401	TRAVERS DITCH 560 FT DS CR1000W NR GRASS CREEK, IN	VII	56	40.93800	-86.43380
638	410106085191601	ABOITE CK 0.3 MI US USHWY 24 AT TIMBERCREST, IN	VI	55	41.01850	-85.32080
639	402710086510501	WILDCAT CK 150 FT US SR 25 AT LAFAYETTE, IN	VI	55	40.45283	-86.85122

[1]The full IDEM site number has a prefix of INRB, for example INRB03-601.

[2]U.S. Environmental Agency, 2000a,b.

[3]U.S. Environmental Protection Agency, 1997b.

average algal cover at the sampled reach and these subsamples were composited into a single sample (Charles and others, 2000).

Seston samples were collected in the center of the sampling reach along a line that extended from the left edge to the right edge of the streambank (transect). The wetted channel width and water depths (one-quarter, one-half, and three-quarters points) along the chosen transect were recorded. The seston samples were collected with a 3-L bottle and a 0.476-cm nozzle; either a grab sample or a multiple vertical method was used (Shelton, 1994).

Algal-biomass samples were collected, homogenized, and filtered onto glass-fiber filters in the field by USGS personnel. All filters collected by the USGS were placed on dry ice and transported to the USGS Indiana Water Science Center laboratory for analysis. The CHLa and AFDM filters were analyzed at the USGS Indiana Water Science Center laboratory; all the POC filters were analyzed at the USGS National Water Quality Laboratory (NWQL) in Lakewood, Colorado.

Concentrations of CHLa were determined, following USEPA method 445, with a Turner Designs TD-700 fluorometer outfitted for CHLa analysis (Arar and Collins, 1997). There were two exceptions to method 445; filters were ground in Nalgene centrifuge tubes instead of glass to counter the problem of tube breakage, and samples were centrifuged at 1,500 revolutions per minute (approximately 320 g) for 15 minutes. At the modified centrifuge rate, the filter residue and acetone solution separated well. If samples did not separate well, they were placed in the centrifuge a second time or care was taken not to decant the solute. For consistency, all samples were allowed to steep for 2.5 hours. Concentrations of AFDM were determined, following USGS method B–3520–85 (Britton and Greeson, 1988) with two exceptions: the samples were filtered in the field, and the filters were not baked and weighed in the crucibles before use.

Quality-assurance methods for algal biomass samples included triplicate filters from the same sample to measure variability and a blank filter collected at each sampling reach to measure bias. Additionally, a fifth filter was collected at each site and 10 percent of these fifth CHLa filters were analyzed at the NWQL to measure laboratory variability.

Habitat assessments were collected from June through October 2003 at each site by IDEM personnel following standard IDEM methods (Indiana Department of Environmental Management, 1992, 2002). Habitat assessments were made at the same time fish communities were sampled. Habitat assessments include in-stream and riparian measurements that are incorporated into the Qualitative Habitat Evaluation Index (QHEI).

Nutrient samples (ammonia, nitrate, TKN, TN, and TP) were collected by IDEM personnel following approved IDEM methods (Beckman, 2000). Nutrient quality-assurance methods followed approved IDEM methods (Bowren and Ghiasuddin, 1999). The nutrient samples were preserved by IDEM personnel, placed on wet ice, and taken to an independent laboratory (Test America, Indianapolis) for analysis.

Biological-community (fish and invertebrates) samples were collected one time, June through October 2003, at each site by IDEM personnel following standard IDEM methods (Indiana Department of Environmental Management, 1992, 2002; Barbour and others, 1999). IDEM personnel calculated the biological-community attributes and metric scores for this study. Attributes are the raw data and metric scores are rankings of the data from poor (a score of one), fair to good (a score of three), and excellent (a score of 5). The metric scores are ranked using a large data set that includes unimpaired reference sites to impaired sites. Each attribute has a corresponding metric score (Dufour, 2002). A list of the fish and invertebrate attributes and metrics are listed in appendix 1.

Data sets for sampling dates, laboratory-analysis dates, and algal-biomass (periphyton and seston) are available at: *http://in.water.usgs.gov/NAWQAWHMI/neet.php*

Data Analysis

In large environmental datasets, natural variability often masks the relations among variables. An objective of this report was to develop a preliminary understanding of how algal biomass relates to biological community and environmental variables collected in the Upper Wabash Basin in 2003. In this report, the environmental variables included nutrients, habitat, and basin characteristics. Two approaches were used for the preliminary analysis of the data sets. The first approach included ordination and regression analyses of the algal biomass, nutrient, and environmental data. The second approach compared the CHLa and nutrient values collected by IDEM and USGS personnel to USEPA published values.

The purpose of this preliminary analysis was to investigate all potential relations and identify those relations that were the strongest and warrant further investigation. In this report, for a relation to be considered statistically significant using PCA, the Spearman's rho statistic (r_S) was required to be greater than the absolute value of 0.45 and have at least a 95 percent significance level based on the sample size. Although an r_S of 0.45 is considered significant, it has a possibility of introducing a Type I error. The methods used provided an exploratory analysis to identify which biological and environmental variables were significantly related to algal biomass. This technique also was used as a data censoring tool and allowed researchers to determine the relations of interest to use as a starting point in future studies.

The ordination approach consisted of determining yearly site scores for the periphyton and seston data using PCA. The ordination approach consisted of determining site scores for the periphyton and seston data using PCA. In each PCA two measures of algal biomass for periphyton (CHLa and AFDM) and seston (CHLa and POC) were used. The site scores are considered yearly because all of the seasonal algal biomass data are included in the PCA site score determination. The regression approach related the periphyton and seston PCA site scores from the first axis to five basin characteristics, nine habitat, 12 nutrient variables, and 43 fish and 21 invertebrate attribute and metric scores. All data were normalized to a z-score prior to use in the data analyses, allowing for comparison of variables that were recorded in different units.

Basin-Characteristics Data

The basin characteristics used in this analysis were drainage area and land use (percentage of agriculture, forest, other, and urban) and these were determined by the USGS for this study. Drainage area was derived from the basin boundaries. Basin boundaries for each site were generated following the method outlined by Ries, III, and others (2004). This method combines the National Elevation Dataset, Digital Elevation Model data, and the National Hydrography Dataset, which is a comprehensive set of digital surface-water features. The basin boundaries were used to extract land-use and soil information from the National Land Cover Dataset (NLCD) (U.S. Geological Survey, 2000). This conversion allowed the land-use data to be compared among and between basins. Each sampling site was assessed to determine in which Aggregate Nutrient Ecoregion (U.S. Environmental Protection Agency, 2000a,b) and Level III Ecoregion (U.S. Environmental Protection Agency, 1997b) it was located.

Nutrient Data

The nutrients used in this analysis were dissolved ammonia as N, dissolved nitrate plus nitrite as N, total Kjeldahl nitrogen as N, total nitrogen as N, and total phosphorus as P. Because concentrations of nitrate typically are two orders of magnitude greater than nitrite and because nitrite usually does not exceed 0.5 mg/L in surface water (National Research Council, 1978), concentrations of nitrite plus nitrate are referred to as nitrate in this report. Concentrations of total nitrogen were calculated as the sum of nitrate and TKN. Because almost ninety percent of the ammonia concentrations were below the reporting level, it was not included in the analysis. About thirteen percent of the TP concentrations were below the reporting level, 0.03 mg/L, and one-half of the reporting level was used in the analysis. For nutrient analyses, the scope of this report is narrowed to nitrate, TKN, TN, and TP. Nutrient data was analyzed separately for the spring (May), summer (July), and the fall (September) samples; the seasonal nutrient and the seasonal algal-biomass data were then compared to the PC1 sites scores using Spearman's rho to determine the most significant seasonal relations.

Principal Components Analysis

Principal Components Analysis, an ordination technique, was used to calculate individual yearly algal-biomass site scores for the periphyton and seston samples. PCA site scores (Gauch, 1982; Jongman and others, 1995; McCune and Grace, 2002) are theoretical variables that minimize the total residual sum of squares after fitting straight lines to the algal-biomass data. Mean CHLa (periphyton and seston) and AFDM values were calculated from the three filters for each sample. Mean algal-biomass (periphyton and seston) and other data were normalized prior to running the ordination analysis. To calculate the yearly periphyton PCA site scores, the mean periphyton CHLa and mean AFDM data from the May and June, July and August, and the September samplings were used; for the yearly seston PCA site scores the mean seston CHLa and POC data from the May and June, July and August, and the September samplings were used.

In this report, a positive site score indicates an increase in algal-biomass along the axis and a negative site score indicates a decrease in algal-biomass along the axis. Only the principal components (PC) site scores from the first axis (PC1) are presented in this preliminary analysis, because the PC1 axis best explains the algal-biomass data (McCune and Grace, 2002). In theory, the PC1 site scores should be related to the variables that were used in the calculations, and the variable with the highest loading accounts for the majority of the variation, which should also have the highest correlation coefficient. As a validation step, the yearly periphyton and yearly seston PC1 site scores were related to the algal biomass-values, using Spearman's rho. The purpose of this preliminary analysis was to investigate all potential relations and identify those relations that were the strongest and warrant further investigation. In this report, for a relation to be considered statistically significant using PCA, the Spearman's rho statistic (r_S) was required to be greater than the absolute value of 0.45 and have at least a 95 percent significance level based on the sample size. Although an r_S of 0.45 is considered significant, it has a possibility of introducing a Type I error.

Several procedures – such as the Bonferroni correction – are available for adjusting the significance level when performing a large number (or "family") of tests simultaneously (Van Sickle, 2003). This adjustment reduces the chances of a Type I error (the relation is declared present when the relation is not present) at a specific alpha level. Although useful in reducing Type I error, this technique increases the chance of producing a Type II error (no relation declared when a relation is present). In this study, the goal was to investigate all potential relations and identify which relations were the strongest. Because this was a preliminary analysis and there were a limited number of significant relations, no corrections were applied.

The yearly PC1 site scores spatially represent the (periphyton and seston) algal-biomass and were related, using Primer V.6.1.5 (Primer-E Ltd, 2006), to the habitat, basin-characteristics (drainage area and land use), seasonal nutrient (spring (May), summer (July), and fall (September) nitrate, TKN, TN, and TP), seasonal algal biomass (spring (May), summer (July), and fall (September) CHLa, AFDM, and POC), and biological-community (fish and invertebrates) attributes and metric score data collected in 2003. Spearman's rank order (rho) correlations are the preferred method when determining relations among environmental data because (1) the initial sample size was greater than 30 for most variables and (2) environmental data typically are not normally distributed. Although the data from this study were normalized prior to analysis, all variables were not normally distributed, requiring a non-parametric statistic. PC site scores were related in a correlation matrix to determine the strongest relations. The variables with the strongest relations could help in the development of nutrient criteria.

Comparison With U.S. Environmental Protection Agency Data

The USEPA proposed criteria in 2000 for nitrate, TKN, TN, TP, CHLa (periphyton and seston), and turbidity at the 25th-percentile value of all data for each variable for each Aggregate Nutrient and Level III Ecoregions. Consequently, the second analytical approach was to determine how the data collected by IDEM and USGS for CHLa and nutrients compared to USEPA published values (U.S. Environmental Protection Agency, 2000a,b). For the USEPA method, the median value for each variable for each stream was calculated. Then, percentiles were determined for each of the four seasons in each ecoregion and the 25th-percentile of the combined four seasons was used as the proposed criteria. In this report, median nutrient (nitrate, TKN, TN, and TP) and CHLa values were calculated for all streams sampled within the same Aggregate Nutrient and Level III Ecoregions. However, seasonal statistics were not calculated because IDEM and USGS did not collect winter samples and spring samples were limited—USEPA considers May as part of spring—which left a small number of samples in the USEPA "spring" season. In this report, the median values for all the streams within a specific ecoregion were used to calculate descriptive statistics (ranges—minimum and maximum—and percentiles—10th, 25th, 50^{th,} 75th, and 90th). For CHLa (periphyton and seston), a mean of the three filters collected at each site was calculated and then a median of the means for all streams within each ecoregion was used to calculate the descriptive statistics. The descriptive statistics then were compared to published USEPA values for Ecoregions VI, VII, 55, and 56 (U.S. Environmental Protection Agency, 2000a,b).

Relations of the Principal Components Analysis Site Scores to Algal-Biomass, Habitat, Basin-Characteristics, Nutrient, and Biological-Community Data

PCA was used to calculate PC1 site scores from the algal-biomass (periphyton and seston) data for each site (all seasons). In the preliminary analysis of the 2003 IDEM/USGS data, the periphyton PC1 site score, which represents yearly algal biomass was constrained by CHLa (September); the PC2 site score was constrained by AFDM (July). The combination of PC1 (38.2 percent) and PC2 (18.8 percent) site scores accounted for 57.0 percent of the total variation in the data set. The seston PC1 site score was constrained by CHLa (May); the PC2 site score was constrained by POC (September). The combination of PC1 (42.3 percent) and PC2 (26.2 percent) site scores accounted for 68.5 percent of the variation in the data set. Because the PC1 accounted for a large amount of the total variation in both of the algal-biomass data sets, only PC1 (periphyton and seston) site scores were related to the seasonal algal-biomass, basin-characteristics (drainage area and land use), habitat, seasonal nutrient, and biological-community (fish and invertebrates) attributes and metric score data.

The periphyton PC1 site score (table 2) was positively related to two algal-biomass variables in three seasons: mean AFDM (May and September) and mean periphyton CHLa (May, July, and September). Although these relations do not offer inferences to the relations of the periphyton PC1 site score to the environmental or biological-community (fish and invertebrate attributes and metric scores) data, these relations demonstrate that the periphyton PC1 site score represents an algal-biomass gradient that is largely defined by AFDM and CHLa.

There were no statistically significant relations between the periphyton PC1 site score and the five basin-characteristics, nine habitat, and 12 nutrient variables. The lack of relations between nutrients and periphyton algal biomass is surprising. Biggs (2000) listed several studies that found significant relations between periphyton chlorophyll and nutrients. However, in similar studies conducted by IDEM and USGS in 2001 in the West Fork White River Basin (Frey and others, 2007) and in 2002 in the East Fork White River and Whitewater River Basins (Caskey and others, 2007) no significant relations were found between the nutrients and the periphyton algal biomass. Several factors could account for this lack of relations between the nutrients and the periphyton algal biomass. Scouring of algal growth by increased streamflow that causes algal growth to restart in the stream (Biggs and others, 1999) and annual and seasonal differences in storm events can allow for "wet" and "dry" years with high levels of algal biomass corresponding to high and low nutrients depending upon the season. Other factors include shading from canopy cover and turbidity (Wehr, 2003) and grazing by snails, invertebrates, and fish (Lamberti and others, 1987).

The periphyton PC1 site score was most influenced by September CHLa. Of the 43 fish-community attributes and metric scores examined, the periphyton PC1 was negatively related to one attribute (tolerant percent) and positively related to one metric score (insectivore percent metric score). These relations suggest that as periphyton algal biomass increases, the amount of tolerant species decreases and the number of insectivores increases. However, an opposite relation was seen in the 2001 data analysis for the West Fork White River Basin,

Table 2. Significant Spearman's rho relations of the yearly periphyton Principal Components Analysis axis 1 site scores (PC1) to algal-biomass, habitat, basin-characteristics, and nutrient parameters and fish- and invertebrate-community attributes and metric scores, 2003.

[r_s, Spearman's rho statistic (probability <0.05); n, number of samples; ns, no statistically significant relations; EPT, ephemeroptera, plecoptera, trichoptera; MIBI, macroinvertebrate Index of Biotic Integrity]

Category	Parameter and attribute/metric	r_s
	Parameter	
Periphyton algal biomass (n=35)	Mean ash-free dry mass, May-June	0.7952
	Mean ash-free dry mass, September	.5940
	Mean periphyton chlorophyll *a*, May-June	.7689
	Mean periphyton chlorophyll *a*, July-August	.7022
	Mean periphyton chlorophyll *a*, September	.8644
Habitat (n=35)	ns	ns
Basin characteristics (n=35)	ns	ns
Nutrients (n=35)	ns	ns
	Attribute/metric	
Fish (n=35)	Tolerant percent	- .4840
	Insectivore percent metric score	.4503
Invertebrates (n=21)	EPT count	- .5271
	EPT count metric score	- .5755
	EPT to total ratio	- .5377
	EPT to total ratio metric score	- .4747
	MIBI metric score	- .4865
	Number of taxa	- .6062
	Number of taxa metric score	- .4963

as periphyton algal-biomass increased, tolerant species also increased (Frey and others, 2007).

Of the 21 invertebrate-community attributes examined, the periphyton PC1 site score was negatively related to three attributes (EPT count, EPT-to-total ratio, and number of taxa) and four metric scores (EPT count metric score, EPT-to-total ratio metric score, macroinvertebrate Index of Biotic Integrity (MIBI) metric score, and number of taxa metric score). These relations suggest that as periphyton algal biomass increases, the diversity and quality of the invertebrate community decreases. This decrease in the invertebrate community also could affect the community of insectivore fish previously mentioned. This is the first time in the IDEM/USGS 2001 through 2003 studies that significant relations between invertebrates and algal biomass were more numerous than those between fish and algal biomass; there were in total four significant relations with invertebrates compared to nine for fish in the 2001 study in the West Fork White River Basin (Frey and others, 2007) and in 2002 in the Whitewater River and East Fork White River Basins (Caskey and others, 2007).

The seston PC1 site score, which was most influenced by July CHLa (table 3), was positively related to two algal-biomass variables in three seasons: mean seston CHLa (May, July, and September) and POC (May, July, and September). Although these relations do not offer inferences to the relations of the seston PC1 site score to the environmental or biological-community (fish and invertebrate attributes and metric scores) data, these relations demonstrate that the seston PC1 site score represents an algal-biomass gradient that is largely defined by CHLa and POC. There were no statistically significant relations between the seston PC1 score and the nine habitat, five basin characteristics, or 12 nutrient variables examined or the 43 fish- and 21 invertebrate-community attributes and metric scores examined.

The lack of a significant relation between the seston PC1 site score and drainage area was surprising. Vannote demonstrated in the River Continuum Concept, that as drainage area (surrogate for stream order) increases, the amount of seston CHLa and POC should increase (Vannote and others, 1980). Because of this concept, it was expected that seston CHLa

Table 3. Significant Spearman's rho relations of the yearly seston Principal Components Analysis axis 1 site scores (PC1) to algal-biomass, habitat, basin-characteristics, and nutrient parameters and fish- and invertebrate-community attributes and metric scores, 2003.

[r_s, Spearman's rho statistic (probability <0.05); n, number of samples; ns, no statistically significant relations]

Category	Parameter and attribute/metric	r_s
	Parameter	
Seston algal biomass (n=38)	Mean seston chlorophyll *a*, May-June	0.5465
	Mean seston chlorophyll *a*, July-August	.7387
	Mean seston chlorophyll *a*, September	.5779
	Particulate organic carbon, May-June	.6266
	Particulate organic carbon, July-August	.6759
	Particulate organic carbon, September	.4619
Habitat (n=38)	ns	ns
Basin characteristics (n=38)	ns	ns
Nutrients (n=38)	ns	ns
	Attribute/metric	
Fish (n=38)	ns	ns
Invertebrates (n=21)	ns	ns

would become more significant in this study in comparison to a similar study conducted by IDEM and USGS in 2001 because basins with large drainage areas (boat sites) were sampled in 2003 and not in 2001 (Frey and others, 2007). There were also no significant relations between seston PC1 and drainage area in 2001 (Frey and others, 2007) or 2002 (Caskey and others, 2007).

The USEPA proposed nutrient criteria in 2000 based on previous studies that had found significant relations between nutrients and seston and periphyton CHLa. The lack of relations in this study between nutrients and seston and periphyton algal biomass suggests that biological communities may be useful in measuring nutrient enrichment in streams. In a previous study in Ohio streams, the only significant relation found between nutrients and biological communities was the fish community (Miltner and Rankin, 1998). No relations between nutrients and the invertebrate community were found. In the 2001 (Frey and others, 2007) and 2002 (Caskey and others, 2007) IDEM and USGS studies, fish and invertebrate community variables had statistically significant relations to algal biomass, indicating both communities may be a useful measure of nutrient enrichment in streams. Additionally, in the IDEM and USGS studies conducted in 2001, 2002, and 2003 it appears that periphyton algae may be more important than seston algae in relating algal biomass with biological communities. The periphyton algae (17) had more significant relations with fish and invertebrate attributes and metric scores than seston algae (5).

Drainage Area and Land-Use Analysis

Basin characteristics such as drainage area and land use can affect the relations between nutrients, algal-biomass, and biological-community data. To understand how the sampling sites used in this analysis might have affected the results, an analysis of the drainage area and land use was done.

The random site selection in 2003 in the Upper Wabash River Basin was skewed to small streams. Forty-seven percent of the basins were less than 50 km^2 (headwater streams); 45 percent ranged between 501 and 1,000 km^2 (wading streams); and 8 percent ranged from greater than 1,000 km^2 to 11,000 km^2 (boat streams) (fig. 2A). Of the three boat sites, the two largest sites were about 2,000 km^2 and 11,000 km^2 (table 4). The lack of large streams in the Upper Wabash River Basin could account for the few relations among the seston and the biological-community data. The River Continuum Concept (Vannote and others, 1980) suggests that in smaller streams, periphyton would dominate primary production with more-suitable substrate (rock, sticks, sand), shallower water, and less turbidity than in larger streams. As streams get larger and deeper, seston would dominate the primary production.

Although there were few large streams sampled in the Upper Wabash, there was still a gradient from headwater to boat sites (fig. 2A). The large range of basin drainage areas that was sampled could account for or influence the significant relations documented by the PC1 (periphyton and seston) and the environmental and biological-community data. Previous studies suggest that drainage-basin size (stream order) could mask relations of environmental variables to biological communities. Stream size was significant in explaining relations between algal-community assemblages (Carpenter and Waite, 2000) and fish-community assemblages (Caskey, 2003) and environmental variables. Different fish and algal communities were found in small streams than in large streams. Additionally, stronger relations were found between nutrients and fish- and invertebrate-community attributes and metric scores when data was analyzed by basin size (Frey and Caskey, 2007). Miltner and Rankin (1998) found the only significant relations between nutrients (TP and total inorganic nitrogen) and biological communities (fish) were in headwater streams. For this report, to keep the sample size large, analysis was done on all sites combined.

Every site except one in the Upper Wabash River Basin had basins that consisted of more than 75 percent agricultural land use (fig. 2B). The agricultural land use of the Upper Wabash River Basin sites ranged from 73.5 to 97.9 percent (table 4). In a study of Midwest agriculturally-dominated landscapes, Wang and others (1997) noted when land use within a drainage basin is less than 50 percent agriculture, biological-community relations are complex and difficult to identify. As the percentage of agricultural land use increases to more than 50 percent, a significant negative relation can be seen with IBI scores. Because nearly all basins in this study had more than 80 percent agricultural land use, an agricultural land-use gradient was not found. This lack of an agricultural-land use gradient potentially could mask relations between the PC1 (periphyton and seston) and the environmental variables and biological-community (fish and invertebrates) attributes and metric scores.

Every site except one in the Upper Wabash River Basin had basins that consisted of less than 25 percent forest land use (fig. 2C). The forested land use of the Upper Wabash River Basin sites ranged from 0.3 to 25.2 percent (table 4). Studies have shown that forested landscapes are less likely to have elevated nutrients, in part, because an established riparian zone acts as a buffer and filters surface-water runoff (Jordan and others, 1993).

All of the sites in the Upper Wabash River Basin had basins that consisted of less than 25 percent urban land use (fig. 2D). The urban land use of the Upper Wabash River Basin sites ranged from 0 to 9.4 percent (table 4). Studies from across the U.S. have shown that agricultural and urban landscapes can have elevated levels of nutrients (Mueller and Helsel, 1996). If nutrient concentrations were elevated at all the sites and a nutrient gradient was not detected, it potentially could mask relations between the PC1 (periphyton and seston) and the environmental variables and biological-community (fish and invertebrates) attributes and metric scores.

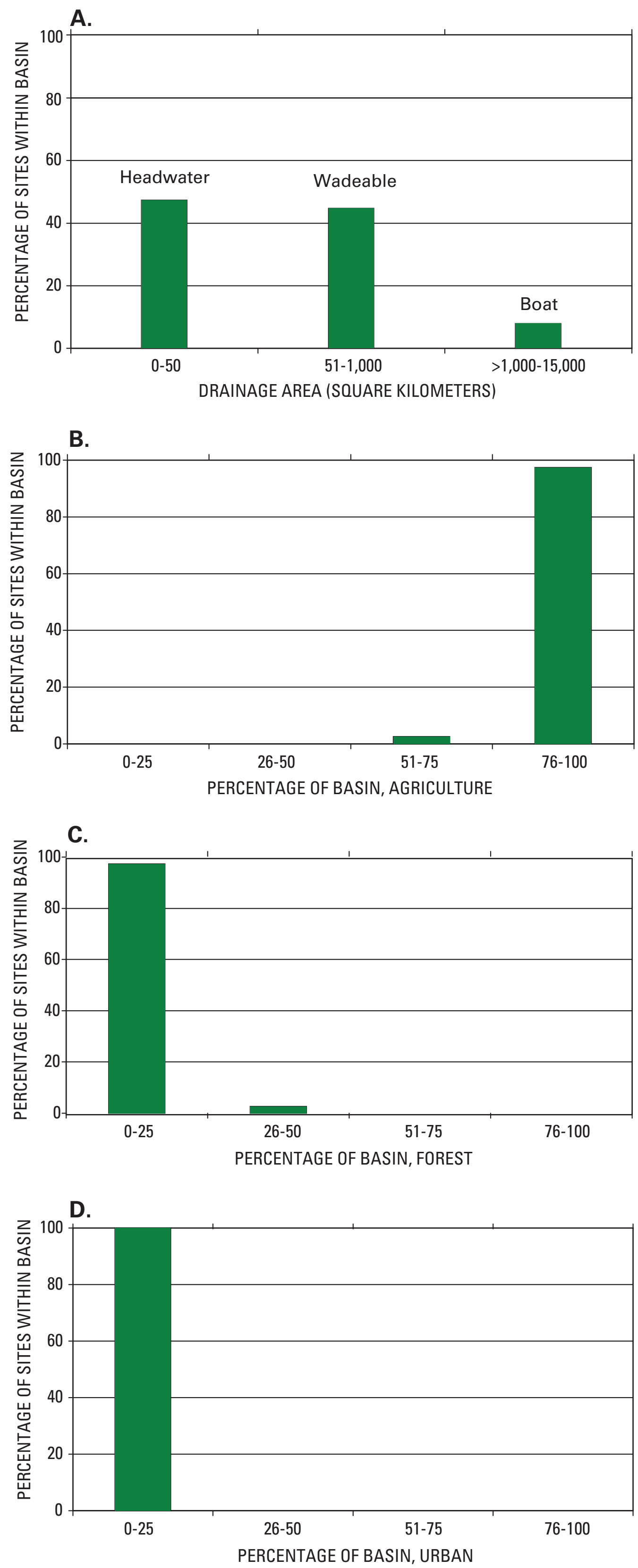

Figure 2. Graphs showing the percentage of drainage area and agricultural, forest, and urban land use in the Upper Wabash River Basin, Indiana, 2003.

Table 4. Basin characteristics of the 38 algal-biomass sampling sites in the Upper Wabash River Basin, Indiana, May through October 2003.

IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; ID, identification number; USEPA, U.S. Environmental Protection Agency; FT, feet; US, upstream; trib, tributary; CR, county road; R, River; SR, state route; DS, downstream; CK, Creek; FK, Fork; DCH, Ditch; NR, near; AV, Avenue; ST, Street; N, North; S,South; E, East; W, West; USHWY, U.S. Highway; IN, Indiana; OH, Ohio]

Short IDEM site number[1]	USGS station name	Drainage area (km^2)	Land use (percentage)[2]			
			Ag	Forest	Urban	Other
601	UNNAMED TRIB 100 FT US PIPE CK AT SANTA FE, IN	359.7	96.2	2.3	1.1	0.4
602	HARSHMAN CK 0.9 MI US MISSISSINEWA R NR SALEM, IN	33.6	95.7	2.8	.5	1.0
603	GALBREATH DITCH 0.5 MI DS CR 200N NR LK CICOTT, IN	6.1	73.5	25.2	.1	1.2
604	MISSISSINEWA R 0.8 MI US SR 26 NR FOWLERTON, IN	1,251.2	91.2	5.8	1.5	1.5
605	BIG MONON CK 0.5 MI US SR 16 NR MONON, IN	87.4	91.4	6.3	0	2.3
606	PAW PAW CK 0.3 MI US CR 800N NR ROANN, IN	93.8	94.7	4.2	.4	.7
607	S FK WILDCAT CK 0.6 MI US CR 800W NR MULBERRY, IN	460.1	94.8	1.6	2.5	1.1
608	MUD CK 0.3 MI US GRUBE DITCH NR PERSHING, IN	131.2	94.6	3.1	.6	1.7
609	MEYERS DITCH 820 FT US I-65 NR BADGER GROVE, IN	1.4	96.5	.4	3.0	.1
610	CLEAR CK 980 FT US SPRING CK AT SOUTH WHITLEY, IN	24.9	82.0	14.7	0	3.3
611	LITTLE PIPE CK 900 FT DS CR 150E NR SOUTH PERU, IN	28.4	93.9	5.2	0	.9
612	ROCK CK 770 FT US CR 900S NR PETROLEUM, IN	7.6	96.0	3.8	0	.2
613	DEER CK 0.7 MI US MERIDIAN LINE RD NR CAMDEN, IN	524.6	95.8	2.6	.7	.9
614	EIGHTMILE CK 0.4 MI US SR 1 AT OSSIAN, IN	64.2	96.1	3.2	.1	.6
615	DEER CK 0.6 MI DS SR 75 AT CAMDEN, IN	583.0	95.9	2.5	.7	.9
616	EASTERDAY DITCH 0.3 MI DS CR 400N NR ATWOOD, IN	14.7	88.4	8.7	.1	2.8
617	E FK LITTLE WILDCAT CK 880 FT US CR200W AT ALTO, IN	24.0	86.0	2.7	9.4	1.9
618	WILSON RHODES DITCH 0.6 MI US CR850N NR ROANN, IN	12.1	91.3	8.7	0	0
619	UNNAMED TRIB 1.2 MI US EEL R AT MEXICO, IN	4.2	92.2	6.8	0	1.0
621	MILL CK 600 FT US HINES DITCH NR MARSHTOWN, IN	77.8	95.5	2.5	.5	1.5
622	STONY CK 720 FT US MERIDIAN RD AT PEABODY, IN	24.9	94.4	3.9	.6	1.2
623	MID FK WILDCAT CK 0.4 MI DS HOG RUN AT PETTIT, IN	289.1	97.1	1.7	.5	.7
624	TIPPECANOE R 880 FT DS CR 100E NR ISLAND PARK, IN	317.3	76.2	11.3	1.6	10.9
625	WABASH R 1.9 MI US SR 39 NR DELPHI, IN	10,652.1	88.2	7.7	1.9	2.2
626	ROCK CK 0.4 MI US SR 3 NR MARKLE, IN	240.8	96.3	3.1	.1	.5

Table 4. Basin characteristics of the 38 algal-biomass sampling sites in the Upper Wabash River Basin, Indiana, May through October 2003.—Continued

IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; ID, identification number; USEPA, U.S. Environmental Protection Agency; FT, feet; US, upstream; trib, tributary; CR, county road; R, River; SR, state route; DS, downstream; CK, Creek; FK, Fork; DCH, Ditch; NR, near; AV, Avenue; ST, Street; N, North; S,South; E, East; W, West; USHWY, U.S. Highway; IN, Indiana; OH, Ohio]

Short IDEM site number[1]	USGS station name	Drainage area (km^2)	Land use (percentage)[2] Ag	Forest	Urban	Other
627	KILMORE CK 500 FT US CR 800E NR FOREST, IN	120.2	97.9	0.7	0.6	0.8
628	MILL CK 0.6 MI US TIPPECANOE R NR WINAMAC, IN	233.2	96.0	2.4	.4	1.2
629	UNNAMED TRIB 0.8 MI US BURNETTS CK AT LOCKPORT, IN	8.0	87.7	10.6	0	1.7
630	DETAMORE DITCH 700 FT DS I-69 NR WARREN, IN	12.9	96.2	2.3	1.0	.5
631	LITTLE MISSISSINEWA R 2.8 MI DS SR28 NR COSMOS, OH	44.0	92.7	2.3	4.2	.8
632	UNNAMED TRIB 450 FT US GAFF DITCH NR ETNA, IN	3.2	96.2	1.0	0	2.8
633	DEER CK 0.25 MI US CR 100W NR WEAVER, IN	76.0	97.6	1.1	.2	1.1
634	ELKHORN CK 0.25 MI US CR 500N NR FARMLAND, IN	9.1	95.8	3.1	.2	.9
635	BIG MONON CK 0.2 MI US BIG MONON DITCH NR MONON, IN	8.7	96.0	3.4	0	.6
636	LITTLE SALAMONIE R 0.4 MI US CR 120S AT LIBER, IN	50.6	88.2	9.8	.1	1.9
637	TRAVERS DITCH 560 FT DS CR1000W NR GRASS CREEK, IN	3.5	88.5	8.6	0	2.9
638	ABOITE CK 0.3 MI US USHWY 24 AT TIMBERCREST, IN	132.6	86.1	8.9	2.1	2.9
639	WILDCAT CK 150 FT US SR 25 AT LAFAYETTE, IN	2,064.8	91.9	3.2	3.4	1.5
	Minimum	1.4	73.5	.3	0	.1
	Maximum	10,652.1	97.9	25.2	9.4	10.9
	Mean	475.8	92.2	5.2	1.0	1.6
	Median	57.4	94.7	3.2	.4	1.1
	Standard deviation	1,740.1	5.7	4.8	1.8	1.8

[1]The full IDEM site number has a prefix of INRB, for example INRB03-601.

[2]Land-use percentages were determined by the U.S. Geological Survey using the National Land Cover Dataset (U.S. Geological Survey, 2000).

Comparison of the Data to Ecoregion Nutrient Criteria

The values for nutrients (TKN, nitrate, TN, and TP) and CHLa (periphyton and seston) were compared to published USEPA values for the respective ecoregions. A comparison of the values from the IDEM/USGS and USEPA data sets was done to (1) determine whether USEPA data, which were used to set the nutrient water-quality criteria, and the IDEM/USGS data had similar ranges of values and (2) determine if the streams in Indiana would exceed the proposed USEPA 25th-percentile nutrient water-quality criteria for the ecoregions. Of the 38 sites sampled, 28 sites on 24 streams were in Ecoregion VI; 10 sites on 10 streams were in Ecoregion VII (fig. 1, table 1). Of the 24 streams in Ecoregion VI, 22 were in Level III Ecoregion 55; the 3 remaining streams were in Level III Ecoregion 54. Because only three streams were in Ecoregion 54, no comparison was made with published USEPA values. The 10 streams sampled in Ecoregion VII were in Level III Ecoregion 56.

The IDEM/USGS values for TKN fell within or were close to the range of the published values for Ecoregions VI, VII, 55, and 56 (table 5). The IDEM/USGS 25th-percentile TKN values were greater than the published values for Ecoregions VI, VII, 55, and 56. New maximum concentrations were measured for Ecoregions VII and 56 and were about 5 and 10 times the published USEPA values, respectively. If the proposed USEPA TKN water-quality criteria for Ecoregions VI and VII were enacted, 69.6 percent of the individual samples collected in Ecoregion VI and 100 percent in Ecoregion VII would have exceeded those criteria.

The IDEM/USGS values for nitrate fell within, or were close to, the range of published values for Ecoregions VI, VII, 55, and 56 (table 6). The IDEM/USGS 25th-percentile nitrate values were greater than the published values for Ecoregions VI, VII, 55, and 56. A new maximum concentration was measured for Ecoregion 56. If the proposed USEPA nitrate water-quality criteria for Ecoregions VI and VII were enacted, 93.9 percent of the individual samples collected in Ecoregion VI and 93.1 percent in Ecoregion VII would have exceeded those criteria.

Table 5. Total Kjeldahl nitrogen as N values collected in 2003 from the Indiana Department of Environmental Management/U.S. Geological Survey study and the published U.S. Environmental Protection Agency values for Aggregate Nutrient Ecoregions VI and VII and Level III Ecoregions 55 and 56.

[All data except number of streams are total Kjeldahl nitrogen as nitrogen values in milligrams per liter; N, nitrogen; IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; **bold** text indicates value exceeds published U.S. Environmental Protection Agency value for Level III Ecoregions 55 and 56 reference conditions, all seasons; shading indicates value exceeds published U.S. Environmental Protection Agency value for Aggregate Nutrient Ecoregions VI and VII, all seasons; nc, not calculated in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/USGS values	USEPA values, all seasons[1]	IDEM/USGS values	USEPA values, all seasons	IDEM/USGS values	USEPA values, all seasons	
		Aggregate Nutrient Ecoregion VI[2]		Level III Ecoregion 55[2]		Aggregate Nutrient Ecoregion VII[3]	Level III Ecoregion 56[3]
Number of streams	25	628	23	199	10	705	50
Minimum value	.330	.025	.330	.050	.420	.000	.110
10th percentile	.380	nc	.480	nc	.600	nc	nc
25th percentile	.600	.591	.600	.400	**.830**	.240	.580
50th percentile	.690	nc	.690	nc	.925	nc	nc
75th percentile	.895	nc	.920	nc	1.80	nc	nc
90th percentile	1.10	nc	1.10	nc	12.7	nc	nc
Maximum value	1.60	4.50	1.60	3.50	**23.0**	4.70	2.06

[1]Values are the median of all seasons for all samples collected from a single stream.

[2]U.S. Environmental Protection Agency, 2000a.

[3]U.S. Environmental Protection Agency, 2000b.

Table 6. Nitrite plus nitrate as N values collected in 2003 from the Indiana Department of Environmental Management/U.S. Geological Survey study and the published U.S. Environmental Protection Agency values for Aggregate Nutrient Ecoregions VI and VII and Level III Ecoregions 55 and 56.

[All data except number of streams are nitrite plus nitrate as nitrogen values in milligrams per liter; N, nitrogen; IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; shading indicates value exceeds or equaled published U.S. Environmental Protection Agency value for Aggregate Nutrient Ecoregions VI and VII, all seasons; **bold** text indicates value exceeds or equals published U.S. Environmental Protection Agency value for Level III Ecoregions 55 and 56 reference conditions, all seasons; nc, not calculated in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/USGS values	USEPA values, all seasons[1]	IDEM/USGS values	USEPA values, all seasons	IDEM/USGS values	USEPA values, all seasons	
		Aggregate Nutrient Ecoregion VI[2]		Level III Ecoregion 55[2]		Aggregate Nutrient Ecoregion VII[3]	Level III Ecoregion 56[3]
Number of streams	25	717	23	219	10	435	73
Minimum value	.920	.010	.920	.025	.050	.003	.050
10th percentile	1.20	nc	1.20	nc	.625	nc	nc
25th percentile	2.00	.633	**1.90**	1.60	**1.50**	.300	.410
50th percentile	3.10	nc	3.10	nc	2.25	nc	nc
75th percentile	4.10	nc	4.30	nc	3.60	nc	nc
90th percentile	6.00	nc	6.00	nc	5.55	nc	nc
Maximum value	8.00	10.7	8.00	8.13	**6.60**	9.54	6.45

[1]Values are the median of all seasons for all samples collected from a single stream.

[2]U.S. Environmental Protection Agency, 2000a.

[3]U.S. Environmental Protection Agency, 2000b.

The IDEM/USGS values for TN fell within, or were close to, the range of published values for Ecoregions VI, VII, 55, and 56 (table 7). The IDEM/USGS 25th-percentile TN values were greater than the published values for Ecoregions VI, VII, and 56. New maximum concentrations were measured for Ecoregions VII, 55, and 56 and new minimum values were measured for Ecoregions 55 and 56. If the proposed USEPA TN water-quality criteria for Ecoregions VI and VII were enacted, 82.3 percent of the individual samples collected in Ecoregion VI and 100 percent in Ecoregion VII would have exceeded those criteria.

The IDEM/USGS values for TP fell within, or were close to, the range of published values for Ecoregions VI, VII, 55, and 56 (table 8). The IDEM/USGS 25th-percentile TP value was greater than the published values for Ecoregion 55. New maximum concentrations were measured for Ecoregions VII and 56. If the proposed USEPA TP water-quality criteria for Ecoregions VI and VII were enacted, 52.4 percent of the individual samples collected in Ecoregion VI and 72.4 percent in Ecoregion VII would have exceeded those criteria.

The IDEM/USGS values for mean periphyton CHLa provide a new range for Ecoregions VI, VII, 55, and 56 (table 9). No comparison was made for Ecoregions VI or VII because of the lack of published values in the USEPA nutrient-criteria document (Environmental Protection Agency, 2000a,b).

The IDEM/USGS values for mean seston CHLa fell within the range of the published values for Ecoregions VI, VII, and 56 (table 10). The IDEM/USGS median seston CHLa values provide a new range of values for Ecoregion 55. The IDEM/USGS 25th-percentile median seston CHLa values were less than published values for Ecoregions VI, VII, and 56. If the proposed USEPA mean seston CHLa water-quality criteria for Ecoregions VI and VII were enacted, 37.8 percent of the individual samples collected in Ecoregion VI and 51.7 percent in Ecoregion VII would have exceeded those criteria.

Table 7. Total nitrogen as N values collected in 2003 from the Indiana Department of Environmental Management/U.S. Geological Survey study and the published U.S. Environmental Protection Agency values for Aggregate Nutrient Ecoregions VI and VII and Level III Ecoregions 55 and 56.

[All data except number of streams are total nitrogen as nitrogen values in milligrams per liter; N, nitrogen; IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; **bold** text indicates a new minimum value or exceeds the published U.S. Environmental Protection Agency value for Level III Ecoregions 55 and 56 reference conditions, all seasons; shading indicates a new minimum value or exceeds the published U.S. Environmental Protection Agency value for Aggregate Nutrient Ecoregions VI and VII, all seasons; nc, not calculated in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/USGS values	USEPA values, all seasons[1]	IDEM/USGS values	USEPA values, all seasons	IDEM/USGS values	USEPA values, all seasons	
		Aggregate Nutrient Ecoregion VI[2]		Level III Ecoregion 55[2]		Aggregate Nutrient Ecoregion VII[3]	Level III Ecoregion 56[3]
Number of streams	25	77	23	2	10	125	5
Minimum value	1.70	.885	1.70	3.63	**.828**	.100	.900
10th percentile	2.09	nc	2.09	nc	1.43	nc	nc
25th percentile	2.64	2.18	2.48	3.63	2.18	.540	1.10
50th percentile	3.74	nc	3.71	nc	2.92	nc	nc
75th percentile	5.27	nc	5.44	nc	5.80	nc	nc
90th percentile	6.64	nc	6.64	nc	17.9	nc	nc
Maximum value	7.60	10.1	7.60	3.78	26.7	7.94	2.55

[1]Values are the median of all seasons for all samples collected from a single stream.

[2]U.S. Environmental Protection Agency, 2000a.

[3]U.S. Environmental Protection Agency, 2000b.

Table 8. Total phosphorus as P values collected in 2003 from the Indiana Department of Environmental Management/U.S. Geological Survey study and the published U.S. Environmental Protection Agency values for Aggregate Nutrient Ecoregions VI and VII and Level III Ecoregions 55 and 56.

[All data except number of streams are total phosphorus as phosphorus values in milligrams per liter; P, phosphorus; IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; **bold** text indicates value exceeds published U.S. Environmental Protection Agency value for Level III Ecoregions 55 and 56 reference conditions, all seasons; shading indicates value exceeds published U.S. Environmental Protection Agency value for Aggregate Nutrient Ecoregions VI and VII, all seasons; nc, not calculated in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/USGS values	USEPA values, all seasons[1]	IDEM/USGS values	USEPA values, all seasons	IDEM/USGS values	USEPA values, all seasons	
		Aggregate Nutrient Ecoregion VI[2]		Level III Ecoregion 55[2]		Aggregate Nutrient Ecoregion VII[3]	Level III Ecoregion 56[3]
Number of streams	25	815	23	225	10	910	64
Minimum value	.015	.005	.015	.010	.015	.001	.004
10th percentile	.041	nc	.043	nc	.015	nc	nc
25th percentile	.069	.076	**.069**	.063	.015	.033	.031
50th percentile	.082	nc	.085	nc	.070	nc	nc
75th percentile	.110	nc	.130	nc	.082	nc	nc
90th percentile	.275	nc	.275	nc	1.55	nc	nc
Maximum value	.390	2.23	.390	1.82	**2.00**	1.72	.300

[1]Values are the median of all seasons for all samples collected from a single stream.

[2]U.S. Environmental Protection Agency, 2000a.

[3]U.S. Environmental Protection Agency, 2000b.

Table 9. Mean periphyton chlorophyll *a* values collected in 2003 from the Indiana Department of Environmental Management/U.S. Geological Survey study and the published U.S. Environmental Protection Agency values for Aggregate Nutrient Ecoregions VI and VII and Level III Ecoregions 55 and 56.

[All data except number of streams are mean periphyton chlorophyll *a* values in milligrams per square meter; IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; nd, no data collected or published in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/ USGS values	USEPA values, all seasons[1]	IDEM/ USGS values	USEPA values, all seasons	IDEM/ USGS values	USEPA values, all seasons	
		Aggregate Nutrient Ecoregion VI[2]		Level III Ecoregion 55[2]		Aggregate Nutrient Ecoregion VII[3]	Level III Ecoregion 56[3]
Number of streams	25	nd	23	nd	10	nd	nd
Minimum value	3.28	nd	3.28	nd	2.69	nd	nd
10th percentile	9.10	nd	9.10	nd	3.08	nd	nd
25th percentile	28.5	nd	22.7	nd	4.69	nd	nd
50th percentile	46.4	nd	46.4	nd	23.1	nd	nd
75th percentile	60.0	nd	60.0	nd	41.2	nd	nd
90th percentile	89.2	nd	89.2	nd	92.9	nd	nd
Maximum value	143	nd	143	nd	107	nd	nd

[1]Values are the median of all seasons for all samples collected from a single stream.

[2]U.S. Environmental Protection Agency, 2000a.

[3]U.S. Environmental Protection Agency, 2000b.

Table 10. Mean seston chlorophyll *a* values collected in 2003 from the Indiana Department of Environmental Management/U.S. Geological Survey study and the published U.S. Environmental Protection Agency values for Aggregate Nutrient Ecoregions VI and VII and Level III Ecoregions 55 and 56.

[All data except number of streams are mean seston chlorophyll *a* values in micrograms per liter; IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; nd, no data collected or published in U.S. Environmental Protection Agency Nutrient Criteria documents; nc, not calculated in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/USGS values	USEPA values, all seasons[1]	IDEM/USGS values	USEPA values, all seasons	IDEM/USGS values	USEPA values, all seasons	
		Aggregate Nutrient Ecoregion VI[2]		Level III Ecoregion 55[2]		Aggregate Nutrient Ecoregion VII[3]	Level III Ecoregion 56[3]
Number of streams	25	63	23	nd	10	55	21
Minimum value	.403	.250	.403	nd	.630	.330	.330
10th percentile	.543	nc	.543	nc	.651	nc	nc
25th percentile	1.01	2.7	.677	nd	.767	1.54	3.50
50th percentile	2.02	nc	2.19	nd	1.68	nc	nc
75th percentile	3.97	nc	4.55	nd	2.65	nc	nc
90th percentile	10.3	nc	10.3	nd	4.45	nc	nc
Maximum value	35.7	47.6	35.7	nd	6.24	36.4	36.4

[1]Values are the median of all seasons for all samples collected from a single stream.

[2]U.S. Environmental Protection Agency, 2000a.

[3]U.S. Environmental Protection Agency, 2000b.

Conclusions

Excessive inputs of nutrients into streams have human-health, economic, and ecological consequences. In 2000, the USEPA proposed nutrient water-quality criteria to protect streams from excess nutrients. This report is one of several reports done as a cooperative effort between IDEM and the USGS to collect new data to assist IDEM in determination of nutrient criteria. Data were gathered from May through October 2003 at 38 randomly selected sites in the Upper Wabash River Basin, Indiana, for algal biomass, habitat, nutrients, and biological communities (fish and invertebrates). Basin characteristics (land use and drainage area) and biological-community attributes and metric scores were determined for the basin of each sampling site. The yearly Principal Components Analysis site scores were calculated for algal biomass (periphyton and seston). The yearly Principal Components Analysis site scores for the first axis (PC1) were related using Spearman's rho to the seasonal algal-biomass, basin-characteristics, habitat, seasonal nutrient, and biological-community attribute and metric score data.

Of the 43 fish-community attributes and metrics examined, the periphyton PC1 was negatively related to one fish-community attribute (tolerant percent) and positively related to one fish-community metric score (insectivore percent metric score). Of the 21 invertebrate-community attributes and metrics examined the periphyton PC1 site score was negatively related to three attributes (EPT count, EPT-to-total ratio, and number of taxa) and four metric scores (EPT count metric score, EPT-to-total ratio metric score, macroinvertebrate Index of Biotic Integrity metric score, and number of taxa metric score). This study in the Upper Wabash River in 2003 was the first time that invertebrates were found to be significantly related to the periphyton PC1 site scores when compared with similar studies conducted by IDEM and the USGS in the West Fork White River in 2001 and the Whitewater and East Fork White River Basins in 2002.

There were no statistically significant relations between the periphyton PC1 site score and the five basin characteristics, nine habitat, and 12 nutrient variables examined. The lack of relations between nutrients and periphyton algal biomass can possibly be explained by several factors including scouring of algal growth by increased streamflow that causes algal growth to restart in the stream, annual and seasonal differences in storm events that can allow for "wet" and "dry" years with high levels of algal biomass corresponding to high and low nutrients depending upon the season, shading from canopy cover and turbidity, and grazing by snails, invertebrates, and fish.

The seston PC1 site score, which was most influenced by July CHLa, had no significant relations among the five basin characteristics, nine habitat, and 12 nutrient variables examined, or the 43 fish- and 21 invertebrate-community attributes and metric scores examined. The lack of relations among seston and all environmental and biological-community attributes and metric scores may be attributed to the lack of large streams in this study. Additionally, the lack of relations between nutrients and seston and periphyton algal biomass in this study and similar studies conducted by IDEM and USGS in 2001 and 2002 suggests that biological communities may be useful in measuring nutrient enrichment in streams. Periphyton algae may be more important than seston algae in relating algal biomass with biological communities because the periphyton algae had more significant relations with fish and invertebrate attributes and metric scores than seston algae.

To understand how the choice of sampling sites might have affected the results, an analysis of the drainage area and land use was done. The random site selection in 2003 in the Upper Wabash River Basin was skewed to small streams. Forty-seven percent of the basins were less than 50 km^2 (headwater streams); 45 percent ranged between 501 and 1,000 km^2 (wading streams). The lack of large streams in the Upper Wabash River Basin could account for the few relations among the seston and the biological-community data. Every site except one in the Upper Wabash River Basin had basins that consisted of more than 75 percent agricultural land use. This lack of an agricultural-land use gradient potentially could mask relations between the PC1 (periphyton and seston) and the environmental variables and biological-community (fish and invertebrates) attributes and metric scores.

The values for nutrients (nitrate, TKN, TN, and TP) and CHLa (periphyton and seston) were compared to published USEPA values for the respective ecoregions. CHLa (periphyton and seston) values either were greater than 25^{th}-percentile published USEPA values or extended data ranges in Aggregate Nutrient and Level III Ecoregions. If the values for the 25^{th} percentile proposed by the USEPA were adopted as nutrient water-quality criteria, the percentage of samples in the Upper Wabash River Basin that would have exceeded these criteria ranged from 37.8 percent in Ecoregion VI for seston CHLa to 100 percent in Ecoregion VII for TKN and TN.

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References

Arar, E.J., and Collins, G.B., 1997, *In vitro* determination of chlorophyll *a* and phaeophytin *a* in marine and freshwater algae by fluorescence (revision 1.2): U.S. Environmental Protection Agency, National Exposure Research Laboratory, Office of Research and Development, Cincinnati, 22 p., accessed April 15, 2001, at *www.epa.gov/nerlcwww/m445_0.pdf*

Barbour, M.T., Gerritsen, Jeroen, Snyder, B.D., and Stribling, J.B., 1999, Rapid bioassessment protocols for use in streams and wadeable rivers—Periphyton, benthic macroinvertebrates and fish (2d ed.): EPA 841–B–99–002, U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Beckman, Timothy, ed., 2000, Surveys Section field procedure manual (revised April 2000): Indiana Department of Environmental Management, Office of Water Management, Assessment Branch, Surveys Section, IDEM 032/02/006/2000, 70 p.

Biggs, B.J.F., Smith, R.A., and Duncan, M.J., 1999, Velocity and sediment disturbance of periphyton in headwater streams: biomass and metabolism: Journal of North American Benthological Society, vol. 18, No. 2, p. 222–241

Biggs, B.J.F., 2000, Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae: Journal of North American Benthological Society, vol. 19, No. 1, p. 17–31

Bowren, Timothy, and Ghiasuddin, Syed, 1999, Quality assurance project plan for Indiana surface water monitoring plans (revision 2), June 1999: Indiana Department of Environmental Management, IDEM/32/01/1442/1999, 70 p.

Britton, L.J., ed., and Greeson, P.E., 1988, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A4, 685 p.

Carpenter, K.D., and Waite, I.R., 2000, Relations of habitat-specific algal assemblages to land use and water chemistry in the Willamette Basin, Oregon: Environmental Monitoring and Assesment v. 64, p. 247–257.

Caskey, B.J. 2003. Relations of Stream Fish Communities to Physical and Chemical Parameters and Land Use in the Mississippi Alluvial Plain Ecoregion. U.S. Geological Survey Open-File Report OFR–03–31, 31 p.

Caskey, B.J., Frey, J.W., and Lowe, B.S., 2007, Relations of principal components analysis site scores to algal-biomass, habitat, basin-characteristics, nutrient, and biological-community data in the Whitewater and East Fork White River Basins, Indiana, 2002: U.S. Geological Survey Scientific Investigations Report 200X–5229, p. 31.

Charles, Donald, Winter, Diane, and Hoffman, Michael, 2000, Field sampling procedures for the New Jersey Algae Indicators Project (revision 0[9/00]): Academy of Natural Sciences, Patrick Center for Environmental Research, Procedure No. P–13–64, 9 p.

Dufour, R.L., 2002, Guide to appropriate metric selection for calculating the Index of Biotic Integrity (IBI) for Indiana rivers and streams: Indiana Department of Environmental Management, Office of Water Quality, Assessment Branch, Biological Studies Section, accessed September 15, 2005, at: *http://www.in.gov/idem/water/assessbr/biostud/ibiflowchrt.pdf*

Frey, J.W., and Caskey, B.J., 2007, Nutrient, habitat, and basin-characteristics data and relations with fish- and invertebrate communities in Indiana streams, 1998–2000: U.S. Geological Survey Scientific Investigations Report 2007–5076, 40 p.

Frey, J.W., Caskey, B.J., and Lowe, B.S., 2007, Relations of principal components analysis site scores to algal-biomass, habitat, basin-characteristics, nutrient, and biological-community data in the West Fork White River Basin, Indiana, 2001: U.S. Geological Survey Scientific Investigations Report 2007–5231, 25 p.

Gauch, H.G., 1982, Multivariate Analysis in Community Ecology, Cambridge University Press, United Kingdom, 298 p.

Hogatt, R.E., 1975, Drainage areas of Indiana streams: U.S. Geological Survey, Water Resources Division, 231 p.

Indiana Department of Environmental Management, 1992, Standard operating procedures—Appendices of operational equipment manuals and procedures (revision 1): Indiana Department of Environmental Management, Office of Water Management, Biological Studies Section, Surveillance and Standards Branch, IDEM/OWM/WQSSB/BSS–SOP [various pagination].

Indiana Department of Environmental Management, 1999, Summary of protocols: Probability based site assessment for Watershed Program field activities (revision 6): Indiana Department of Environmental Management, Office of Water Quality, Assessment Branch, IDEM 32/03/002/1999, 32 p.

Indiana Department of Environmental Management, 2001, Surface water quality monitoring strategy (revision September 2001): Indiana Department of Environmental Management, Office of Water Quality, Assessment Branch, IDEM 32/01/016/1998, 3 p.

Indiana Department of Environmental Management, 2002, How to complete the Qualitative Habitat Evaluation Index (QHEI) modified from (OHIO EPA 1989) (revision 2): Indiana Department of Environmental Management, Office of Water Quality, Assessment Branch, Biological Studies Section, IDEM/OWQ/Assessment Branch/BSS–SOP.

Jongman, R.H.G., Ter Braak, C.J.F., and Van Tongren, O.F.R., 1995, Data Analysis in Community and Landscape Ecology, Cambridge University Press, United Kingdom, 299 p.

Jordan, T.E., Correll, D.L., and Weller, D.E., 1993, Nutrient interception by a riparian forest receiving inputs from adjacent cropland: Journal of Environmental Quality, v. 22, p. 467–473.

Lamberti, G.A., Ashkenas, L.R., Gregory, L.V., Steinman, A.D., 1987, Effects of three herbivores on periphyton communities in laboratory streams: Journal of North American Benthological Society, v. 6, p.92–104.

McCune, B. and Grace, J.B., 2002, Analysis of Ecological Communities, MjM Software Design, Gleneden Beach, Oregon, USA, 300 p.

Miltner, R.J., and Rankin, E.T., 1998, Primary nutrients and the biotic integrity of rivers and streams: Freshwater Biology, v. 40, p. 143–158.

Morlock, S.E., Nguyen, H.T., and Majors, D.K., 2004, Water Resources Data, Indiana, Water Year 2003: U.S. Geological Survey Water-Data Report IN–03–1, 610 p.

Moulton, II, S.R., Kennen, J.G., Goldstein, R.M., and Hambrook, J.A., 2002, Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 02–150, 75 p.

Mueller, D. K., and Helsel, D.R., 1996, Nutrients in the water—Too much of a good thing?: U.S. Geological Survey Circular 1136, 24 p.

Munn, M.D., and Hamilton, P.A., 2003, New studies intiated by the U.S. Geological Survey—Effects of nutrient enrichment on stream ecosystems: U.S. Geological Survey Fact-Sheet FS–118–03, 4 p.

Ohio Environmental Protection Agency, 1999, Association between nutrients, habitat, and the aquatic biota in Ohio rivers and streams: Ohio Environmental Protection Agency Technical Bulletin MAS/1999–1–1, 78 p.

Primer-E Ltd, 2006, Primer User Manual / Tutorial (version 6): Plymouth Marine Laboratory, United Kingdom, 111–119 p.

Purdue Applied Meteorology Group, Indiana Climate Page, accessed February 22, 2005, at *http://shadow.agry.purdue.edu/sc.norm-geog.html*

Ries, K.G., III, Steeves, P.A., Coles, K.D., Rea, A.H., and Stewart, D.W., 2004, Stream Stats: A U.S. Geological Survey web application for stream information: U.S. Geological Survey Fact Sheet, FS2004–3115, 4 p.

Shelton, L.R., 1994, Field guide for collecting and processing stream-water samples for the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 94–455, 646 p.

Soballe, D.M., and Kimmel, B.L., 1987, A large-scale comparison of factors influencing phytoplankton abundance in rivers, lakes, and impoundments: Ecology, v. 68, p. 1943–1954.

U.S. Census Bureau, 2000, Cartographic boundary files: accessed February 16, 2005, at *http://www.census.gov/geo/www/cob/ua2000.html*

U.S. Environmental Protection Agency, 1997a, National water quality inventory 1996—Report to Congress: U.S. Environmental Protection Agency, Office of Water, EPA 841–R–97–008, 527 p.

U.S. Environmental Protection Agency, 1997b, Level III ecoregions of the conterminous United States (revision of Omernik, 1987): Corvallis, Oreg., U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory Map M–1, various scales.

U.S. Environmental Protection Agency, 2000a, Ambient water quality criteria recommendations—Information supporting the development of state and tribal nutrient criteria—Rivers and streams in Nutrient Ecoregion VI: U.S. Environmental Protection Agency, Office of Water, EPA 822–B–00–017.

U.S. Environmental Protection Agency, 2000b, Ambient water quality criteria recommendations—Information supporting the development of state and tribal nutrient criteria—Rivers and streams in Nutrient Ecoregion VII: U.S. Environmental Protection Agency, Office of Water, EPA 822–B–00–018.

U.S. Environmental Protection Agency, 2005, Water quality criteria—Current national recommended water quality criteria: accessed September 15, 2005, at *http://www.epa.gov/waterscience/criteria/wqcriteria.html*

U.S. Geological Survey, 2000, The national land cover dataset 1992 (NLCD): accessed August 27, 2005, at http://seamless.usgs.gov

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., and Cushing, C.E., 1980, The River Continuum Concept, Canadian Journal of Fisheries and Aquatic Sciences, v. 37, p. 130–137.

Van Nieuwenhuyse, E.E., and Jones, J.R., 1996, Phosphorus-Chlorophyll relationship in temperate streams and its variation with stream catchment area: Canadian Journal of Fisheries and Aquatic Sciences, v. 53, p. 99–105.

Van Sickle, John, 2003, Analyzing correlations between stream and watershed attributes: Journal of American Water Resources Association, v. 39, p. 717–726.

Wang, Lizhu, Lyons, John, Kanehl, Paul, and Gatti, Ronald, 1997, Influences of watershed land use on habitat quality and biotic integrity in Wisconsin streams: Fisheries 22:6–12.

Wehr, J.D., 2003, Freshwater habitats of algae, in Freshwater Algae of North America: Ecology and Classification, Wehr, J.D. and Sheath, R.G., eds., p. 11–57.

Appendix 1. Metrics used by Indiana Department of Environmental Management for habitat, fish, and invertebrates (Dufour, 2002).

Metrics[1]	Definition
	Habitat
SubstrateScore	A metric to evaluate substrate type, origin, silt cover, and embeddedness.
InstreamCoverScore	Instream cover types and the amount (availability) of instream cover.
ChannelMorphologyScore	Quality of the stream channel related to the creation and stability of instream habitat (channel sinuosity, channel development, channelization, stability, and modifications).
RiparianZoneandBankErosionScore	Quality of the riparian buffer zone and flood-plain vegetation, looking at riparian width, predominant surrounding land uses, and bank-erosion status.
PoolGlideQualityScore	Quality of pool/glide taking into account maximum pool depth, morphology, and velocity.
RiffleRunQualityScore	Quality of riffle/run, taking into account riffle/run depth, substrate, and embeddedness.
GradientScore	A measure of the influence of gradient and stream size on the biological community and available habitat.
	Fish[2]
SpeciesCount	Number of species, excluding hybrid species (exclude gizzard shad if in the Wabash River mainstem and drainage area is greater than 5,180 square kilometers).
DMS_SpeciesCount	Number of darter, madtom, and sculpin species, excluding hybrid species.
Darter_SpeciesCount	Number of darter species, excluding hybrid species.
Headwater_Percent	Percent of headwater individuals.
LargeRiver_Percent	Percent of large river individuals (exclude gizzard shad if in the Wabash River mainstem and drainage area greater than 5,180 square kilometers).
Sunfish_SpeciesCount	Number of sunfish species, excluding hybrid species.
Centrarchid_SpeciesCount	Number of centrarchidae species, excluding hybrid species.
Minnow_SpeciesCount	Number of minnow species, excluding hybrid species.
Sucker_SpeciesCount	Number of sucker species, excluding hybrid species.
RoundBodySucker_SpeciesCount	Number of round-body sucker species, excluding hybrid species.
Salmonid_SpeciesCount	Number of salmonid species, excluding hybrid species.
Sensitive_SpeciesCount	Number of sensitive species, excluding hybrid species.
Tolerant_Percent	Percent of tolerant individuals (exclude gizzard shad if in the Wabash River mainstem and drainage area greater than 5,180 square kilometers).
Omnivore_Percent	Percent of omnivore individuals (exclude gizzard shad if in the Wabash River mainstem and drainage area greater than 5,180 square kilometers).
Insectivore_Percent	Percent of insectivore or invertivore individuals (exclude gizzard shad if in the Wabash River mainstem and drainage area greater than 5,180 square kilometers).
Pioneer_Percent	Percent of pioneer individuals.
Carnivore_Percent	Percent of carnivore or piscivore individuals (exclude gizzard shad if in the Wabash River mainstem and drainage area greater than 5,180 square kilometers).
CatchPerUnitEffort	Catch per unit effort (CPUE) or total number of individuals.
CPUElessShads	Catch per Unit Effort (CPUE), excluding the number of gizzard shad individuals if in the Wabash River mainstem and drainage area greater than 5,180 square kilometers).
SimpleLithophil_Percent	Percent of simple lithophilic species (exclude gizzard shad if in the Wabash River mainstem and drainage area greater than 5,180 square kilometers).
DELT_Percent	Percent of individuals with deformities, eroded fins, lesions, and tumors (DELT), including multiple DELTs (exclude gizzard shad if in the Wabash River mainstem and drainage area greater than 5,180 square kilometers).

Appendix 1. Metrics used by Indiana Department of Environmental Management for habitat, fish, andinvertebrates (Dufour, 2002).—Continued

Metrics[1]	Definition
	Invertebrates
Family Level HBI	Summation of the tolerance value times the number of individuals for a specific family divided by the total count of individuals for families with a tolerance value.
Number of Taxa	Number of families identified in the subsample.
Number of Individuals	Total number of individuals for all families identified in the subsample.
Percent Dominant Taxa	Highest number of individuals for a given family divided by the total number of individuals in the subsample.
EPT Index	Total number of families represented in the orders Ephemeroptera, Plecoptera, and Trichoptera.
EPT Count	Total number of individuals for orders Ephemeroptera, Plecoptera, and Trichoptera.
EPT Count to Total Number of Individuals	Total number of individuals for orders Ephemeroptera, Plecoptera, and Trichoptera divided by the total number of individuals in the subsample.
EPT Count to Chironomid Count	Total number of individuals for orders Ephemeroptera, Plecoptera, and Trichoptera divided by the total number of chironomidae.
Chironomid Count	Total number of chironomids in the subsample.
Total Number of Individuals to Number of Squares Sorted	Total number of individuals in the subsample divided by the number of squares needed to reach the total number of individuals.

[1]Each of the fish- and invertebrate community metrics also has a corresponding attribute with the same name but consists of the raw data.

[2] Specific fish species associated with each metric can be found in Dufour, 2002.

Leer, Frey, Caskey, and Lowe—**Relations of Principal Components Analysis Site Scores to Algal-Biomass, Habitat, Basin-Characteristics, Nutrient, and Biological-Community Data in the Upper Wabash River Basin, Indiana, 2003**—Scientific Investigations Report 2007–5231