

Prepared in cooperation with the Indiana Department of Environmental Management,  
Division of Water, Assessment Branch

# Relations of Principal Components Analysis Site Scores to Algal-Biomass, Habitat, Basin-Characteristics, Nutrient, and Biological-Community Data in the Whitewater River and East Fork White River Basins, Indiana, 2002



Scientific Investigations Report 2007–5229



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

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Scientific Investigations Report 2007–5229

**U.S. Department of the Interior  
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Suggested citation:  
Caskey, B.J., Frey, J.W., and Lowe, B. S., 2007, Relations of Principal Component Analysis site scores algal-biomass, habitat, basin-characteristics, nutrient, and biological community data in the Whitewater River and East Fork White River Basin, Indiana, 2002: U.S. Geological Survey Scientific Investigations Report 2007–5229, 31 p.

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## Conversion Factors and Datum

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

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

$$^{\circ}\text{F}=(1.8\times^{\circ}\text{C})+32$$

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<sup>2</sup>).

## 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 Whitewater River and East Fork White River Basins, Indiana, 2002

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

## Abstract

Data were gathered from May through September 2002 at 76 randomly selected sites in the Whitewater River and East Fork White River Basins, 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. 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.

The periphyton PC1 site score was not significantly related to the nine habitat or 12 nutrient variables examined. One land-use variable, drainage area, was negatively related to the periphyton PC1. Of the 43 fish-community attributes and metrics examined, the periphyton PC1 was negatively related to one attribute (large-river percent) and one metric score (carnivore percent metric score). It was positively related to three fish-community attributes (headwater percent, pioneer percent, and simple lithophil percent). The periphyton PC1 was not statistically related to any of the 21 invertebrate-community attributes or metric scores examined.

Of the 12 nutrient variables examined two were negatively related to the seston PC1 site score in two seasons: total Kjeldahl nitrogen (July and September), and TP (May and September). There were no statistically significant relations between the seston PC1 and the five basin-characteristics or nine habitat variables examined. Of the 43 fish-community attributes and metrics examined, the seston PC1 was positively related to one attribute (headwater percent) and negatively related to one metric score (large-river percent metric score). Of the 21 invertebrate-community attributes and metrics examined, the seston PC1 was negatively related to one metric score (number of individuals metric score).

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 sites selected in the Whitewater River Basin were generally small drainage basins; compared to Whitewater River Basin sites, the sites selected in the East Fork White River Basin were generally larger drainage basins. Although both basins were dominated by agricultural land use the Whitewater River Basin sites had more land in agriculture than the East Fork White River Basin sites.

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 IX and USEPA Level III Ecoregions 55 and 71. Several nutrient values were greater than the 25<sup>th</sup> percentile of published USEPA values. Chlorophyll *a* (periphyton and seston) values were either greater than the 25<sup>th</sup> percentile of published USEPA values or they extended data ranges in the Aggregate Nutrient and Level III Ecoregions. If the values for the 25<sup>th</sup> percentile as proposed by the USEPA were adopted as nutrient water-quality criteria, many samples in the Whitewater River and East Fork White River Basins 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, 2000 a,b).

The Clean Water Act (CWA) established a national goal of achieving water quality that provides for the protection and

## 2 Relations of PCA Site Scores in the Whitewater River and East Fork White River Basins, Indiana, 2002

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* (CHL<sub>a</sub>) 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, 2000 a,b). USEPA reviewed selected data and set the proposed nutrient water-quality criteria for nitrate, TKN, TN, TP, CHL<sub>a</sub> (periphyton and seston), and turbidity at the 25<sup>th</sup>-percentile value of all data for each variable.

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, 2000 a,b). An extension was given to Indiana and other states that adopted plans describing the data needs and the process to

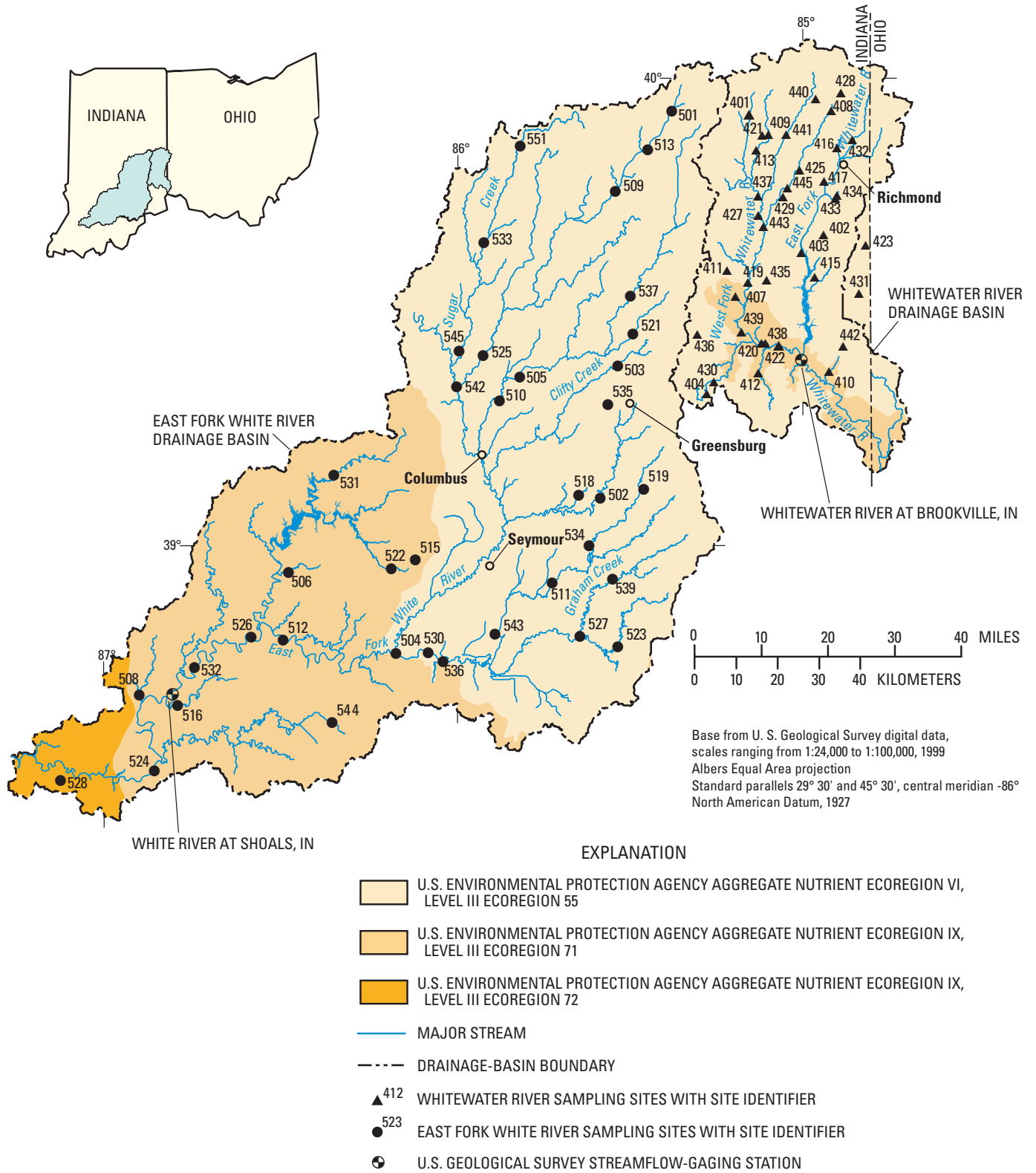
develop nutrient water-quality criteria. Beginning in 2001, the Indiana Department of Environmental Management (IDEM) and the U.S. Geological Survey (USGS) are cooperating on several studies that will assist the State of Indiana in developing nutrient water-quality criteria as mandated by the USEPA. 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), Upper Wabash River Basin (2003), 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 nutrient data from 2001 through 2003; to augment the IDEM WMP studies and better understand nutrient enrichment in streams, the USGS collected algal biomass during all years of the study and also collected the nutrient data 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 Whitewater River and East Fork White River Basins in 2002. In this report, the environmental variables included nutrients, habitat, and basin characteristics. 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 CHL<sub>a</sub> 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 76 randomly selected sites in 2002; 38 sites in the Whitewater River Basin and 38 sites in the East Fork White River Basin in 2002 (fig. 1). A discussion of the basin characteristics of the 76 sites describes how drainage area and land use affect the analysis. This report also compares nutrient values (nitrite plus nitrate as N, TKN



**Figure 1.** Map showing location of the 76 algal-biomass sampling sites in the Whitewater River and East Fork White River Basins, Indiana, May through September, 2002.

as N, TN as N, TP as P) and CHLa (periphyton and seston) collected by IDEM and USGS for the Whitewater River and East Fork White River Basins to values published by the USEPA for Aggregate Nutrient Ecoregion VI—Corn Belt and Northern Great Plains, for Aggregate Nutrient Ecoregion IX—Southeastern Temperate Forested Plains and Hills, for Level III Ecoregion 55—Eastern Corn Belt Plains, and for Level III Ecoregion 71—Interior Plateau. Aggregate Nutrient Ecoregions consist of one or more Level III Ecoregions (U.S. Environmental Protection Agency, 2000 a,b).

In the text, the Aggregate Nutrient Ecoregions are referred to as Ecoregion VI or IX; Level III Ecoregions are referred to as Ecoregion 55 or 71. The nutrients are described as concentrations of nitrate, TKN, TN, and TP. For this report periphyton CHLa, ash-free dry mass (AFDM), seston CHLa, and particulate organic carbon (POC) are measures of algal biomass.

## Description of the Whitewater River Basin

The Whitewater River Basin (fig. 1) drains more than 3,170 km<sup>2</sup> of southeastern Indiana and southwestern Ohio before draining into the Ohio River (Debrewer and others, 2000). The annual mean streamflow in the 2002 water year at the USGS streamflow-gaging station, Whitewater River at Brookville, IN (03276500) (fig. 1), was 66.6 m<sup>3</sup>/s (Stewart and others, 2003). Streamflow in the 2002 water year was above average; the annual mean long-term streamflow (1916–2002) was 37.2 m<sup>3</sup>/s at Brookville (Stewart and others, 2003).

The climate in the Whitewater River Basin is characterized as temperate continental with warm, occasionally hot summers and cold winters. The mean annual temperature within the Whitewater River Basin is 10.6°C, and average monthly temperatures range from -3.3°C in January to 22.8°C in July (Indiana Department of Natural Resources, 1988). Average annual precipitation is about 101.6 cm; however, annual totals range from 73.7 cm in very dry years to 127 cm in very wet years (Indiana Department of Natural Resources, 1988).

The dominant land use is row-crop agriculture (93 percent), primarily corn and soybean (Debrewer and others, 2000). Less than 4 percent of the basin is forest and urban. Typical of many streams in the state, streams in the Whitewater River Basin streams have low gradients and velocities. The largest urban area is Richmond, which has a population of about 37,900 (U.S. Census Bureau, 2000) (fig. 1).

## Description of the East Fork White River Basin

The East Fork White River Basin (fig. 1) drains more than 14,880 km<sup>2</sup> of central and southern Indiana and flows into the main branch of the White River (Hogatt, 1975). In water year 2002, the annual mean streamflow at the USGS streamflow-gaging station East Fork White River at Shoals, IN (03373500)(fig. 1), was 291.7 m<sup>3</sup>/s (Stewart and others, 2003).

Streamflow in the 2002 water year was above average; the annual mean long-term streamflow (1904–2002) was 158.3 m<sup>3</sup>/s at Shoals (Stewart and others, 2003).

The climate in the East Fork White River Basin is characterized as humid continental with well-defined winter and summer seasons. The mean monthly temperature at Columbus, IN ranged from -2.8°C in January to 23.8°C in July (Schnoebelen and others, 1999). The mean annual precipitation ranged from 96.5 cm in the northern part of the basin to 111.8 cm in the southern part (Schnoebelen and others, 1999).

The dominant land use is row-crop agriculture (69 percent), primarily corn and soybeans (Baker and Frey, 1997). Twenty-five percent of the basin area is forest and 5 percent is urban (Baker and Frey, 1997). Typical of many streams in the state, streams in the East Fork White River Basin have low gradients and velocities. The largest urban areas are Columbus (population about 32,500), Greensburg (population about 9,300), and Seymour (population about 15,600) (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 2002, the focus was the Whitewater River and East Fork White River Basins. 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 September 2002, a total of 76 sites (fig. 1, table 1) in the Whitewater River and the East Fork White River Basins (38 sites in each basin) were sampled three times for algal biomass and nutrients. The samples were collected three times to measure



**Table 1. Location and basin characteristics of the 76 algal-biomass sampling sites in the Whitewater River and East Fork White River Basins, Indiana, May through September, 2002.**—

[IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; ID, identification number; USEPA, U.S. Environmental Protection Agency; FT, feet; US, upstream; R, River; RD, Road; CR, County Road; 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; IN, Indiana; OH, Ohio; trib, tributary; USHWY, U.S. Highway; RMI, River mile]

Short IDEM site number <sup>1</sup>	USGS station ID	USGS station name	USEPA Aggregate Nutrient Ecoregion <sup>2</sup>	Level III Ecoregion <sup>3</sup>	Latitude (degrees)	Longitude (degrees)
Whitewater River Basin						
401	395620085090801	WHITewater R 400 FT DS HOOVER RD NR HAGERSTOWN, IN	VI	55	39.93886	-85.15209
402	394041084562001	RICHLAND CK 3800 FT US CR 100W NR CLIFTON, IN	VI	55	39.67814	-84.93903
403	393827085001401	E FK WHITEwater R 2700 FT US SR 44 NR LIBERTY, IN	VI	55	39.64075	-85.00390
404	392010085163801	SALT CK 1500 FT DS ENOCHSBURG RD AT ENOCHSBURG, IN	VI	55	39.33613	-85.27721
407	393249085113501	N BR GARRISON CK 200 FT DS CR 650S AT ALPINE, IN	IX	71	39.54706	-85.19307
408	395649084545201	FOUNTAIN CK AT WHITEwater RD AT FOUNTAIN CITY, IN	VI	55	39.94703	-84.91431
409	395350085054001	MORGAN CK 2000 FT DS SR 38 NR GREENS FK, IN	VI	55	39.89713	-85.09450
410	392254084554401	BIG CEDAR CK 9400 FT US USHWY52 NR CEDAR GROVE, IN	IX	71	39.38171	-84.92901
411	393609085155801	FALL CK 1000 FT DS CR 425W NR COLUMBIA, IN	VI	55	39.60240	-85.21611
412	392251085074801	PIPE CK 900 FT US PUMPHOUSE RD NR OAK FOREST, IN	IX	71	39.38089	-85.12989
413	395148085074801	MARTINDALE CK 3900 FT US I-70 NR JACKSONBURG, IN	VI	55	39.86319	-85.12989
415	393514084580001	UNNAMED TRIB 1800 FT DS CR 175W NR ROSEBURG, IN	VI	55	39.58728	-84.96669
416	395158084535701	W FK WHITEwater R 2000 FT DS I-70 NR RICHMOND, IN	VI	55	39.86605	-84.89922
417	394735084561001	LICK CK 2200 FT US ABINGTON PIKE NR RICHMOND, IN	VI	55	39.79307	-84.93633
419	393439085092401	WHITEwater R 1200 FT US CR 480S AT NULLTOWN, IN	VI	55	39.57748	-85.15678
420	392645085071101	WHITEwater R 8200 FT DS USHWY 52 AT METAMORA, IN	IX	71	39.44582	-85.11985
421	395344085064201	OSER CK 2700 FT DS SR 38 NR HAGERSTOWN, IN	VI	55	39.89553	-85.11174
422	392616085042301	WHITEwater R 4 MI DS USHWY 52 AT YELLOW BANK, IN	IX	71	39.43783	-85.07293
423	393913084491301	LITTLE FOUR MI CK 2 MI US SR 44 NR FAIRHAVEN, OH	VI	55	39.65362	-84.82024

**Table 1. Location and basin characteristics of the 76 algal-biomass sampling sites in the Whitewater River and East Fork White River Basins, Indiana, May through September, 2002.—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; R, River; RD, Road; CR, County Road; 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; IN, Indiana; OH, Ohio; trib, tributary; USHWY, U.S. Highway; RMI, River mile]

Short IDEM site number <sup>1</sup>	USGS station ID	USGS station name	USEPA Aggregate Nutrient Ecoregion <sup>2</sup>	Level III Ecoregion <sup>3</sup>	Latitude (degrees)	Longitude (degrees)
425	394904085002401	CROWN CK 400 FT DS MCMINN RD AT CENTERVILLE, IN	VI	55	39.81765	-85.00669
427	394318085073301	WHITEWATER R 7200 FT US CR 440N AT BEESONS, IN	VI	55	39.72157	-85.12593
428	395902084530801	FOUNTAIN CK 2800 FT US SEANEY RD NR BETHEL, IN	VI	55	39.98391	-84.88557
429	394649085023001	NOLANDS FK 2600 FT DS MCCONAHA RD NR PINHOOK, IN	VI	55	39.78034	-85.04153
430	392142085152501	SALT CK 2300 FT US CR 250W NR HAMBURG, IN	VI	55	39.36165	-85.25686
431	393259084502801	INDIAN CK 900 FT DS SAND RUN RD NR CONTRERAS, OH	VI	55	39.54973	-84.84098
432	395301084511501	M FK E FK WW R 2500 FT US SR 227 NR MIDDLEBORO, IN	VI	55	39.88351	-84.85426
433	394527084541701	ELKHORN CK 5000 FT US USHWY 27 NR LOCUST GROVE, IN	VI	55	39.75750	-84.90459
434	394545084540201	ELKHORN CK 6600 FT US USHWY 27 NR LOCUST GROVE, IN	VI	55	39.76244	-84.90065
435	393450085061401	WILSON CK 700 FT DS SR 1 NR EVERTON, IN	VI	55	39.58044	-85.10384
436	392755085181001	BULL FK 2 MI US STIPPS HILL RD NR BUENA VISTA, IN	VI	55	39.46538	-85.30271
437	394550085073301	WHITEWATER R 2 ML DS E MILTON RD NR MILTON, IN	VI	55	39.76390	-85.12593
438	392638085063101	WHITEWATER R 2 MI DS USHWY 52 NR METAMORA, IN	IX	71	39.44398	-85.10865
439	392809085103801	WHITEWATER R 2 MI DS LAUREL RD NR LAUREL, IN	IX	71	39.46913	-85.17668
440	395821084572801	MORGAN CK 2600 FT US MEYERS RD NR WILLIAMSBURG, IN	VI	55	39.97276	-84.95754
441	395348085023701	GREENS FK 1200 FT US SR 38 AT GREENS FK, IN	VI	55	39.89619	-85.04318
442	392610084531501	E FK BIG CEDAR CK 2 MI US SR 252 NR PALESTINE, IN	VI	55	39.43597	-84.88743
443	394147085064301	WHITEWATER R 2900 FT DS CR 440N AT WATERLOO, IN	VI	55	39.69634	-85.11201
445	394540085031701	CENTRAL RUN 7100 FT US CHAPEL RD NR PINHOOK, IN	VI	55	39.76121	-85.05469

**Table 1. Location and basin characteristics of the 76 algal-biomass sampling sites in the Whitewater River and East Fork White River Basins, Indiana, May through September, 2002.—Continued**

[IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; ID, identification number; USEPA, U.S. Environmental Protection Agency; FT, feet; U.S, upstream; R, River; RD, Road; CR, County Road; 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; IN, Indiana; OH, Ohio; trib, tributary; USHWY, U.S. Highway; RMI, River mile]

Short IDEM site number <sup>1</sup>	USGS station ID	USGS station name	USEPA Aggregate Nutrient Ecoregion <sup>2</sup>	Level III Ecoregion <sup>3</sup>	Latitude (degrees)	Longitude (degrees)
		East Fork White River Basin				
501	395651085222501	BIG BLUE R 2000 FT N NORTH RD AT NEW CASTLE, IN	VI	55	39.94761	-85.37384
502	390637085350201	SAND CK 1450 FT N CR 850N NR BREWERSVILLE, IN	VI	55	39.11014	-85.58388
503	392348085315501	CLIFTY CK 840 FT N CR 400N NR ADAMS, IN	VI	55	39.39670	-85.53197
504	384632086094201	E FK WHITE R 1 RMI US MUSCATATUCK R NR MEDORA, IN	VI	55	38.77558	-86.16173
505	392221085483701	FLATROCK R 300 FT DS CR 150W NR FLATROCK, IN	VI	55	39.37244	-85.81015
506	385657086275601	LITTLE SALT CK 400 FT DS CR 650N NR COVEYVILLE, IN	IX	71	38.94904	-86.46553
508	384054086530301	BOGGS CK 1600 FT DS CR 15 NR LOOGOOTE, IN	IX	71	38.68172	-86.88416
509	394628085321301	BIG BLUE R 1400 FT W CR 450W NR KNIGHTSTOWN, IN	VI	55	39.77432	-85.53700
510	391917085520901	FLATROCK R 900 FT N CR 800N AT ST LOUIS CROSSING, IN	VI	55	39.32146	-85.86906
511	385539085431501	VERNON FK MUSC R 2700 FT E CR 400 S NR HAYDEN, IN	VI	55	38.92737	-85.72081
512	384810086284601	E FK WHITE R 1 MI N CR 500 S NR YOCKEY, IN	IX	71	38.80266	-86.47955
513	395149085263701	BIG BLUE R 1200 FT N CR 500S NR GREENSBORO, IN	VI	55	39.86362	-85.44368
515	385836086062401	LITTLE SALT CK 2700 FT S SR 258 AT FREETOWN, IN	IX	71	38.97669	-86.10655
516	383934086463201	BEAVER CK 900 FT S CR 44 AT IRONTON, IN	IX	71	38.65946	-86.77551
518	390659085383801	WYALOOSING CK 200 FT S 900N NR SARDINIA, IN	VI	55	39.11632	-85.64397
519	390745085273801	SUGAR CK 1000 FT W CR 1000N NR ZENAS, IN	VI	55	39.12909	-85.46048
521	392751085291801	LITTLE FLATROCK R 2700 FT W SR 3 NR MILROY, IN	VI	55	39.46429	-85.48840
522	385730086102801	S FK SALT CK 1200 FT N SR 58 NR KURTZ, IN	IX	71	38.95841	-86.17455
523	384717085320901	BIG CK 1 MI W CR 700W AT VOLGA, IN	VI	55	38.78793	-85.53588

**Table 1. Location and basin characteristics of the 76 algal-biomass sampling sites in the Whitewater River and East Fork White River Basins, Indiana, May through September, 2002. —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; R, River; RD, Road; CR, County Road; 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; IN, Indiana; OH, Ohio; trib, tributary; USHWY, U.S. Highway; RMI, River mile]

Short IDEM site number <sup>1</sup>	USGS station ID	USGS station name	USEPA Aggregate Nutrient Ecoregion <sup>2</sup>	Level III Ecoregion <sup>3</sup>	Latitude (degrees)	Longitude (degrees)
524	383107086502501	E FK WHITE R 4000 FT W CR 400E NR THALES, IN	IX	71	38.51858	-86.84026
525	392508085545301	BIG BLUE R 2700 FT E CR 750W NR MT AUBURN, IN	VI	55	39.41886	-85.91475
526	384832086340901	E FK WHITE R 1300 FT W CR 450W NR COXTON, IN	IX	71	38.80884	-86.56921
527	384838085383401	BIG CK 900 FT E SR 3 NR DEPUTY, IN	VI	55	38.81067	-85.64281
528	382939087060901	BEAR CK 200 FT N CR 400N NR OTWELL, IN	IX	72	38.49412	-87.10251
530	384635086041401	MUSCATATUCK R 2600 FT N MT EDEN RD NR MILLPORT, IN	VI	55	38.77645	-86.07056
531	390939086202001	N FK SALT CK 2250 FT US SR 46 AT BELMONT, IN	IX	71	39.16073	-86.33886
532	384433086434501	E FK WHITE R 100 FT N WILLIAMS RD NR HURON, IN	IX	71	38.74239	-86.72917
533	393950085544601	SUGAR CK 1500 FT DS CR 1000N NR PLEASANT VIEW, IN	VI	55	39.66397	-85.91280
534	390028085365701	VERNON FK MUSC R 1150 FT US WWTF AT N VERNON, IN	VI	55	39.00767	-85.61584
535	391848085333801	FALL FK 650 FT N SR 3 AT EWINGTON, IN	VI	55	39.31332	-85.56055
536	384525086014401	MUSCATATUCK R 1200 FT E MT EDEN RD NR MILPORT, IN	VI	55	38.75708	-86.02883
537	393249085294401	FLATROCK R 500 FT DS CR 450S NR MILROY, IN	VI	55	39.54688	-85.49546
539	385607085325901	GRAHAM CK 300 FT S CR 350E AT WALNUT RIDGE, IN	VI	55	38.93529	-85.54985
542	392105085592401	BIG BLUE R 2400 FT W SR 31 AT EDINBURGH, IN	VI	55	39.35142	-85.99013
543	384858085525501	VERNON FK MUSC R 1800 FT E CR 800E NR RETREAT, IN	VI	55	38.81615	-85.88198
544	383726086202701	LOST R 100 FT N CR 450N NR ORLEANS, IN	IX	71	38.62390	-86.34069
545	392544085585801	SUGAR CK 2600 FT E CR 350S AT AMITY, IN	VI	55	39.42880	-85.98279
551	395221085483001	SUGAR CK 500 FT N CR 600N NR MAXWELL, IN	VI	55	39.87275	-85.80836

<sup>1</sup>The full IDEM site number has a prefix of INRB, for example INRB401.

<sup>2</sup>U.S. Environmental Protection Agency, 2000a,b.

<sup>3</sup>U.S. Environmental Protection Agency, 1997b.



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. Sampling at two sites in late spring was delayed until early July because of high water. The biological community (fish and invertebrate) and habitat were sampled one time by IDEM personnel, June through October 2002.

### **Algal-Biomass, Habitat, 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 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 one time from June through October 2002 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). A list of these habitat metrics are listed in appendix 1 (Dufour, 2002).

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 2002, 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 in the Whitewater River and East Fork White River Basins in 2002. 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. These approaches provided an exploratory analysis to identify which biological and environmental variables were significantly related to algal biomass. These approaches also were 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. 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 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, 2000 a,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. More than ninety percent of the ammonia data were censored below the reporting levels. Because most of the ammonia concentrations were below the reporting level, it was not included in the analysis. About eleven percent of the TP concentrations and two percent of the nitrate concentrations were below the reporting levels of 0.03 mg/L and 0.01 mg/L, respectively. For nitrate and TP concentrations, 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, July, and the September samplings were used; for the yearly seston PCA site scores the mean seston CHLa and POC data from the May, July, 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 2002. 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 25<sup>th</sup>-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, 2000 a,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 25<sup>th</sup>-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—10<sup>th</sup>, 25<sup>th</sup>, 50<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup>). 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, IX, 55, and 71 (U.S. Environmental Protection Agency, 2000 a,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 2002 IDEM/USGS data, the periphyton PC1 site score was constrained by AFDM (September); the PC2 site score was constrained by CHLa (May). The combination of PC1 (43.3 percent) and PC2 (21.9 percent) site scores accounted for 65.2 percent of the total variation in the data set. The seston PC1 site score was constrained by the POC (July); the PC2 site score was constrained by the POC (May). The combination of PC1 (52.4 percent) and PC2 (23.7 percent) site scores accounted for 76.1 percent of the variation in the data set. Because the PC1 site score accounted for a large amount of the total variation in both of the algal-biomass data sets, only PC1 (periphyton and seston) site score were related to the seasonal algal-biomass, habitat, basin-characteristics (drainage area and land use), seasonal nutrient, and biological-community (fish and invertebrates) attributes and metric score data.

The periphyton PC1 site score (table 2) was negatively related to one algal-biomass variable in three seasons: mean AFDM (May, July, and September). Although this result does not offer inferences about 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.

## 12 Relations of PCA Site Scores in the Whitewater River and East Fork White River Basins, Indiana, 2002

**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, 2002.

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

Category	Parameter and attribute/metric	$r_s$
Parameter		
Periphyton algal biomass (n=50)	Mean ash-free dry mass, May-June	-0.7289
	Mean ash-free dry mass, July-August	-.6469
	Mean ash-free dry mass, September	-.8203
Habitat (n=41)	ns	ns
Basin characteristics (n=52)	Drainage area	-.5191
Nutrients (n=41)	ns	ns
Attribute/metric		
Fish (n=51)	Carnivore percent metric score	-.4942
	Headwater percent	.5258
	Large-river percent	-.5697
	Pioneer percent	.4890
	Simple lithophil percent	.5519
Invertebrates (n=34)	ns	ns

One basin-characteristic variable, drainage area, was negatively related to the periphyton PC1 site score. This relation suggests that as the drainage area (a surrogate for stream order) decreases, the amount of periphyton CHLa and AFDM increase. This relation supports the River Continuum Concept (Vannote and others, 1980) that periphyton is more important than seston for primary production in small and medium-sized streams than in large streams.

The periphyton PC1 site score, which was most influenced by September AFDM (table 2), had no statistically significant relations between the periphyton PC1 and the nine habitat or 12 nutrient variables examined. 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. In similar studies conducted in 2001 in the West Fork White River Basin (Frey and others, 2007) and in 2003 in the Upper Wabash River Basin (Leer and others, 2007), no significant relations were found between the nutrients and the periphyton algal biomass. Algal biomass can be influenced by several natural factors that could account for this lack of relations. Scouring of algal growth by increased streamflow can cause 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).

Of the 21 fish-community attributes and metric scores examined, the periphyton PC1 site score was negatively related to one attribute (large-river percent) and one metric score (carnivore percent metric score). The periphyton PC1 site score was positively related to three attributes (headwater percent, pioneer percent, and simple lithophil percent). These findings suggest that as periphyton algal biomass increases, fish-community composition shifts from one dominated by carnivores and other niche-specific specialists to a community dominated by generalist-feeding and pioneer species. The central stoneroller (*Campostoma anomalum*), an algivore that is included in the pioneer species metric is commonly found in the nutrient and algal-rich streams in Indiana. It may be useful to create some nutrient-related metrics such as percentage central stoneroller, instead of grouping them into pioneer species that could mask possible relations. Petersen and Femmer (2002) found the percentage of central stonerollers was important to explain benthic chlorophyll levels in Ozark streams.

Of the 21 invertebrate-community attributes and metric scores examined, there were no statistically significant relations with the periphyton PC1 site score. The lack of significant findings with invertebrate communities may be the result of three possible factors: (1) sample size, (2) sample method, and (3) resolution of invertebrate data. In multivariate studies,



sample size has been shown to affect the inferences that can be drawn from a data set; this may be why no relations were noted between the PC1 (periphyton and seston) site scores and the invertebrate-community attributes and metric scores. In this study, invertebrates were collected only at sites with riffles; only 45 sites had invertebrate data, compared to 73 and 76 sites for fish-community and algal-biomass data, respectively. Another possible reason for the lack of invertebrate-community attributes and metric score relations could be the result of the collection methods. The invertebrate-community samples were collected from a specific habitat type (riffle) within the sampling reach; this targeted-habitat approach represents only the habitat sampled. If a multihabitat approach had been used for the collection of the invertebrate communities it may have revealed a community more representative of the entire sampling reach; this multihabitat approach may have increased the number of significant relations between the PC1 sites scores and the invertebrate-community attributes and metrics. Finally, the lack of invertebrate-community attribute/metric score relations also could be the result of invertebrate-identification resolution. IDEM identifies invertebrates to family level; therefore, it is possible that if identification were to the lower taxonomic level, a statistically significant rela-

tion between PC1 (periphyton and seston) site scores and invertebrate-community attributes and metric scores could be documented.

The seston PC1 site score (table 3) was negatively related to two algal-biomass variables in three seasons: mean seston CHLa (May, July, and September), and POC (May, July, and September). Although this result does not offer inferences about 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 periphyton PC1 site score represents an algal-biomass gradient that is largely defined by seston CHLa and POC.

The seston PC1 site score, which was most influenced by July POC (table 3), had no statistically significant relations with the basin-characteristics or habitat variables. The lack of a significant relation between the seston PC1 and drainage area was surprising. Vannote demonstrated in the River Continuum Concept, that as drainage area (surrogate for stream order) increases, the amount of primary production from seston CHLa and POC should increase (Vannote and others, 1980). On the basis of the River Continuum Concept, it was expected that seston CHLa would become more significant in this study compared to a similar study conducted by IDEM

**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, 2002.

[r<sub>s</sub>, Spearman’s rho statistic (probability <0.05); n, number of samples; ns, no statistically significant relations;N, nitrogen; P, Phosphorus]

Category	Parameter and attribute/metric	r <sub>s</sub>
Parameter		
Seston algal biomass (n=50)	Mean seston chlorophyll <i>a</i> , May-June	-0.4502
	Mean seston chlorophyll <i>a</i> , July-August	- .6886
	Mean seston chlorophyll <i>a</i> , September	- .6816
	Particulate organic carbon, May-June	- .4883
	Particulate organic carbon, July-August	- .7538
	Particulate organic carbon, September	- .7132
Habitat (n=51)	ns	ns
Basin characteristics (n=50)	ns	ns
Nutrients (n=39)	Total Kjeldahl nitrogen-N, July-August	- .5525
	Total Kjeldahl nitrogen-N, September	- .7010
	Total phosphorus-P, May-June	- .5814
	Total phosphorus-P, September	- .4846
Attribute/metric		
Fish (n=50)	Headwater percent	.5897
	Large-river percent metric score	- .5204
Invertebrates (n=33)	Number of individuals metric score	- .5024

and USGS in 2001. Basins with large drainage areas (boat sites) were sampled in 2002 and not in 2001 (Frey and others, 2007).

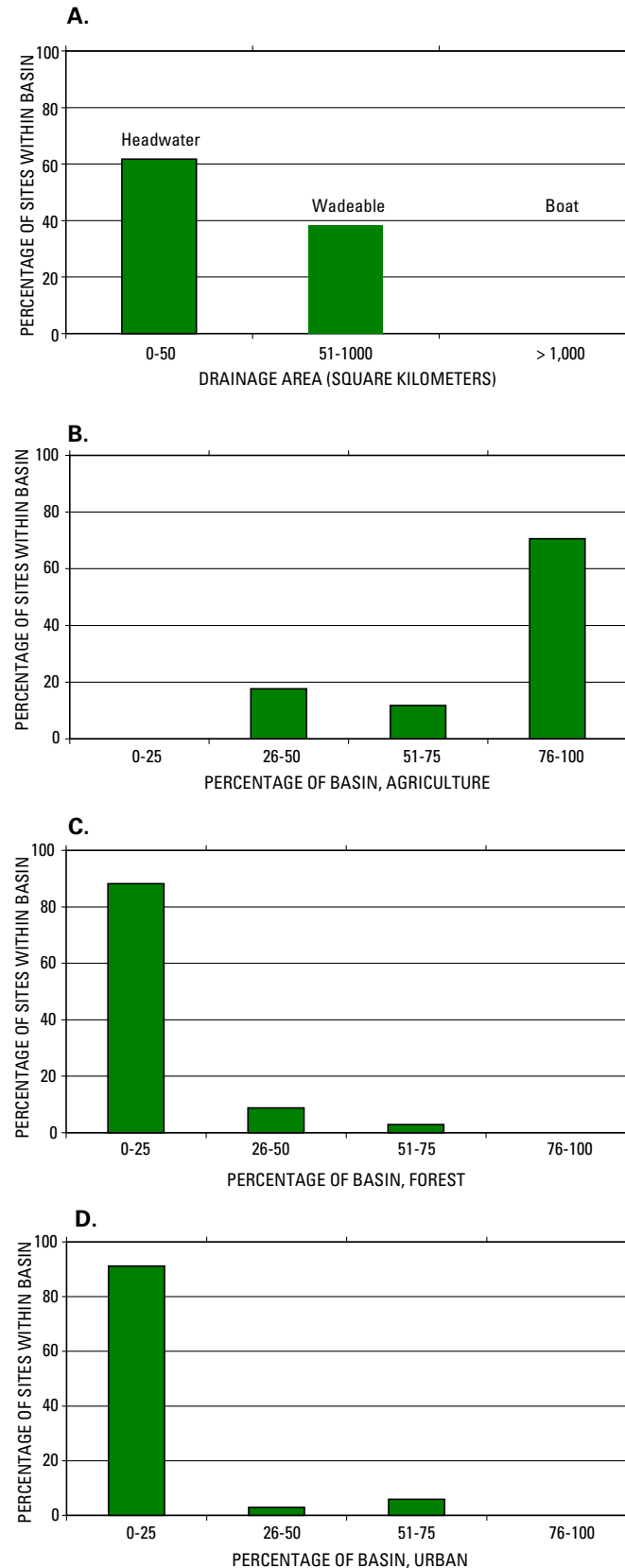
Of the 12 nutrient variables examined two were negatively related to the seston PC1 site score in two seasons: TKN (July and September), and TP (May and September). More significant relations were found in summer and fall samples than in spring samples which suggest that the time to see the strongest relations between nutrients and algal biomass may be in the late summer and fall. Nutrient concentrations may be uniformly high in these basins during the spring and early summer because of fertilizer application and subsequent runoff into streams. Also, higher algal growth tends to occur during periods of stable flow and warmer weather typically associated with summer and fall. Previous studies (Van Nieuwenhuysse and Jones, 1996; Soballe and Kimmel, 1987) found significant relations between seston CHLa and TP. However, seston algae (whether euplankters or dislodged periphyton) in streams and rivers can account for appreciable total nutrient concentrations in water samples because algal cells contain both phosphorus and organic nitrogen (Stephen D. Porter, Texas State University, written commun., August 8, 2007).

Of the 43 fish-community attributes and metric scores examined, the seston PC1 site score was positively related to one attribute (headwater percent) and negatively related to one metric score (large-river percent metric score). Of the 21 invertebrate-community attributes and metric scores examined, the seston PC1 site score was negatively related to one metric score (number of individuals metric score). This suggests that the impacts of nutrients may be reflected better by the fish than by invertebrates. In this study and in the 2001 West Fork White River Basin study, there were more significant relations between algal biomass and fish communities (9) than between invertebrates and algal biomass (Frey and others, 2007).

### 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 sites selected in the Whitewater River Basin were generally small drainage basins; compared to Whitewater River Basin sites, the sites selected in the East Fork White River Basin were generally larger drainage basins (fig. 2A). Of the 38 sites sampled in the Whitewater River Basin, about 55 percent of the sites were headwater streams (less than 50 km<sup>2</sup>); about 10 percent of the 38 sites sampled in East Fork White River Basin were headwater streams (fig. 2A). About 13 percent of sites sampled in the Whitewater River Basin were large streams (greater than 1,000 km<sup>2</sup>), and almost 29 percent of the sites sampled in East Fork White River Basin were large streams (fig. 2A). The drainage basins of the Whitewater River



**Figure 2.** Graphs showing the percentages of drainage area and agricultural, forest, and urban land use in the Whitewater River and East Fork White River Basins, Indiana, 2002.

Basin sites ranged from 3.3 km<sup>2</sup> to 2,134 km<sup>2</sup>, and the drainage basins of the East Fork White River Basin sites ranged from 9.3 km<sup>2</sup> to 14,362 km<sup>2</sup> (table 4).

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. This preliminary data analysis, however, indicates statistically significant relations between PC1 (periphyton) and drainage area (table 2).

Although both basins were dominated by agricultural land use the Whitewater River Basin sites had more land in agriculture than the East Fork White River Basin sites; about 90 percent of the sites in the Whitewater River Basin and about 50 percent of the sites in the East Fork White River Basin had basins that consisted of more than 75 percent agricultural land use (fig. 2B). Land use ranged from 51.5 to 98.2 percent agricultural, and in the East Fork White River Basin sites ranged from 14.5 to 98.4 percent (table 4). In a study of Midwest agriculturally dominated landscapes, Wang and others (1997) noted when land use within a drainage basin consists of 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, however, a significant negative relation can be seen with Index of Biological Integrity (IBI) scores. Because most basins in this study had more than 80 percent agricultural land use, especially in the Whitewater River Basin, an agricultural land-use gradient was not found. This lack of a 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.

Almost 95 percent of the sites in the Whitewater River Basin and about 55 percent of the sites in the East Fork White River Basin had basins that consisted of less than 25 percent forest land use (fig. 2C). The forested land use of the Whitewater River Basin sites ranged from 1.8 to 48.4 percent, and the forested land use of the East Fork White River Basin sites ranged from 0.2 to 83.6 percent (table 4). Studies have shown that forested landscapes are less likely to have elevated nutri-

ents, 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 Whitewater River Basin and in the East Fork White River Basin had basins that consisted of less than 25 percent urban land use (fig. 2D). The urban land use of the Whitewater River Basin sites ranged from 0 to 22.1 percent, and the urban land use of the East Fork White River Basin sites ranged from 0 to 8.6 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), the environmental variables, and biological-community (fish and invertebrates) attributes and metric scores.

## Comparison of the Data to Ecoregion Nutrient Criteria

The values for nutrients (nitrate, TKN, TN, and TP) and CHLa (periphyton and seston) were compared to published USEPA values for the respective ecoregions (U.S. Environmental Protection Agency, 2000 a,b). A comparison of the values from the IDEM/USGS and USEPA data sets was done to (1) determine whether USEPA data that 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 25<sup>th</sup>-percentile nutrient water-quality criteria for the ecoregions. Of the 76 sites sampled, 57 sites on 38 streams were in Ecoregion VI; 19 sites on 13 streams were in Ecoregion IX (fig. 1, table 1). All of the 57 sites on 38 streams in Ecoregion VI were also in Ecoregion 55. Of the 19 sites on 13 streams in Ecoregion IX, 18 sites on 12 streams were in Ecoregion 71. The one remaining stream sampled in Ecoregion IX was in USEPA Level III Ecoregion 72. Because only one stream was in Ecoregion 72, no comparison was made with published USEPA values.

The IDEM/USGS values for TKN fell within the range of the published values for Ecoregions VI, IX, 55, and 71 (table 5). The IDEM/USGS 25<sup>th</sup>-percentile TKN values were greater than published values for Ecoregion IX, 55, and 71. If the proposed USEPA TKN water-quality criteria for Ecoregions VI and IX were enacted, 46.3 percent of the individual samples collected in Ecoregion VI and 89.1 percent of the samples in Ecoregion IX would have exceeded those criteria.

The IDEM/USGS values for nitrate fell within or were close to the range of the published values for Ecoregions VI, IX, 55, and 71 (table 6). The IDEM/USGS 25<sup>th</sup>-percentile nitrate values were greater than or equal to the published values for Ecoregions VI, IX, and 55. If the proposed USEPA nitrate water-quality criteria for Ecoregions VI and IX were enacted, 63.4 percent of the individual samples collected in Ecoregion VI and 88.0 percent of the samples in Ecoregion IX would have exceeded those criteria.

**Table 4.** Basin characteristics of the 76 algal-biomass sampling sites in the Whitewater River and East Fork White River Basins, Indiana, May through September 2002.

Short IDEM site number <sup>1</sup>	USGS station name	Drainage area (km <sup>2</sup> )	Landuse (percentage) <sup>2</sup>			
			Ag	Forest	Urban	Other
Whitewater River Basin						
401	WHITEWATER R 400 FT DS HOOVER RD NR HAGERSTOWN, IN	64.2	86.9	12.5	0.2	0.4
402	RICHLAND CK 3800 FT US CR 100W NR CLIFTON, IN	17.8	98.2	1.8	0	.1
403	E FK WHITEWATER R 2700 FT US SR 44 NR LIBERTY, IN	607	78.2	15.2	5.5	1.1
404	SALT CK 1500 FT DS ENOCHSBURG RD AT ENOCHSBURG, IN	71.5	79.8	19.5	.5	.2
407	N BR GARRISON CK 200 FT DS CR 650S AT ALPINE, IN	29.2	51.5	48.4	.1	.1
408	FOUNTAIN CK AT WHITEWATER RD AT FOUNTAIN CITY, IN	17.6	92.6	7.1	0	.3
409	MORGAN CK 2000 FT DS SR 38 NR GREENS FK, IN	38.6	95.3	4.6	0	.1
410	BIG CEDAR CK 9400 FT US USHWY52 NR CEDAR GROVE, IN	57.7	86.9	12.7	0	.3
411	FALL CK 1000 FT DS CR 425W NR COLUMBIA, IN	8.4	88.3	11.7	0	0
412	PIPE CK 900 FT US PUMPHOUSE RD NR OAK FOREST, IN	93.2	66.8	31.5	1.0	.8
413	MARTINDALE CK 3900 FT US I-70 NR JACKSONBURG, IN	139	92.4	6.7	.2	.7
415	UNNAMED TRIB 1800 FT DS CR 175W NR ROSEBURG, IN	4.0	94.4	5.3	0	.3
416	W FK WHITEWATER R 2000 FT DS I-70 NR RICHMOND, IN	46.7	90.2	7.0	2.2	.6
417	LICK CK 2200 FT US ABINGTON PIKE NR RICHMOND, IN	40.4	68.2	8.9	22.1	.8
419	WHITEWATER R 1200 FT US CR 480S AT NULLTOWN, IN	1,352	88.0	9.3	1.7	1.0
420	WHITEWATER R 8200 FT DS USHWY 52 AT METAMORA, IN	1,944	79.2	18.5	1.4	.9
421	OSER CK 2700 FT DS SR 38 NR HAGERSTOWN, IN	4.5	95.9	3.0	.2	.9
422	WHITEWATER R 4 MI DS USHWY 52 AT YELLOW BANK, IN	2,134	77.5	20.3	1.3	.9
423	LITTLE FOUR MI CK 2 MI US SR 44 NR FAIRHAVEN, OH	49.0	96.4	3.1	.3	.3
425	CROWN CK 400 FT DS MCMINN RD AT CENTERVILLE, IN	6.9	81.7	4.1	14.2	.1
427	WHITEWATER R 7200 FT US CR 440N AT BEESONS, IN	798	89.7	8.2	1.1	1.0
428	FOUNTAIN CK 2800 FT US SEANEY RD NR BETHEL, IN	5.3	89.3	10.3	0	.3

[IDEM, Indiana Department of Environmental Management; USGS, U.S. Geological Survey; ID, identification number; USEPA, U.S. Environmental Protection Agency; FT, feet; US, upstream; R, River; RD, Road; CR, County Road; 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; IN, Indiana; OH, Ohio; trib, tributary; USHWY, U.S. Highway; RMI, River mile]



**Table 4.** Basin characteristics of the 76 algal-biomass sampling sites in the Whitewater River and East Fork White River Basins, Indiana, May through September 2002.—  
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; R, River; RD, Road; CR, County Road; 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; IN, Indiana; OH, Ohio; trib, tributary; USHWY, U.S. Highway; RMI, River mile]

Short IDEM site number <sup>1</sup>	USGS station name	Drainage area (km <sup>2</sup> )	Landuse (percentage) <sup>2</sup>			
			Ag	Forest	Urban	Other
429	NOLANDS FK 2600 FT DS MCCONAHA RD NR PINHOOK, IN	187	90.6	7.0	1.6	.8
430	SALT CK 2300 FT US CR 250W NR HAMBURG, IN	102	73.6	23.8	1.5	1.1
431	INDIAN CK 900 FT DS SAND RUN RD NR CONTRERAS, OH	45.4	96.7	2.5	.4	.4
432	M FK E FK WW R 2500 FT US SR 227 NR MIDDLEBORO, IN	107	91.4	7.6	.3	.7
433	ELKHORN CK 5000 FT US USHWY 27 NR LOCUST GROVE, IN	50.8	87.0	11.1	.3	1.6
434	ELKHORN CK 6600 FT US USHWY 27 NR LOCUST GROVE, IN	50.4	87.3	10.9	.3	1.5
435	WILSON CK 700 FT DS SR 1 NR EVERTON, IN	3.3	82.7	15.2	2.1	.1
436	BULL FK 2 MI US STIPPS HILL RD NR BUENA VISTA, IN	7.1	97.5	2.5	0	0
437	WHITEWATER R 2 ML DS E MILTON RD NR MILTON, IN	520	89.1	8.6	1.3	1.0
438	WHITEWATER R 2 MI DS USHWY 52 NR METAMORA, IN	1,949	79.1	18.7	1.4	1.0
439	WHITEWATER R 2 MI DS LAUREL RD NR LAUREL, IN	1,557	83.6	13.9	1.5	1.0
440	MORGAN CK 2600 FT US MEYERS RD NR WILLIAMSBURG, IN	4.2	95.7	4.1	0	.2
441	GREENS FK 1200 FT US SR 38 AT GREENS FK, IN	172	92.8	5.7	.7	.8
442	E FK BIG CEDAR CK 2 MI US SR 252 NR PALESTINE, IN	13.6	94.4	5.1	0	.5
443	WHITEWATER R 2900 FT DS CR 440N AT WATERLOO, IN	805	89.7	8.2	1.1	1.1
445	CENTRAL RUN 7100 FT US CHAPEL RD NR PINHOOK, IN	7.4	96.4	3.4	0	.2
	Minimum	3.3	51.5	1.8	0	0
	Mean	345.0	86.7	11.0	1.7	.6
	Median	50.6	89.2	8.4	.3	.6
	Maximum	2,134	98.2	48.4	22.1	1.6
	Standard deviation	613.5	9.9	9.1	4.2	.4

**Table 4.** Basin characteristics of the 76 algal-biomass sampling sites in the Whitewater River and East Fork White River Basins, Indiana, May through September 2002.—  
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; R, River; RD, Road; CR, County Road; 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; IN, Indiana; OH, Ohio; trib, tributary; USHWY, U.S. Highway; RMI, River mile]

Short IDEM site number <sup>1</sup>	USGS station name	Drainage area (km <sup>2</sup> )	Landuse (percentage) <sup>2</sup>				
			Ag	Forest	Urban	Other	
East Fork White River Basin							
501	BIG BLUE R 2000 FT N NORTH RD AT NEW CASTLE, IN	113	85.7	9.7	1.6	3.0	
502	SAND CK 1450 FT N CR 850N NR BREWERSVILLE, IN	34	81.0	16.0	2.5	.5	
503	CLIFTY CK 840 FT N CR 400N NR ADAMS, IN	145	96.0	3.4	.1	.4	
504	E FK WHITE R 1 RMI US MUSCATATUCK R NR MEDORA, IN	6,662	85.3	10.5	2.8	1.3	
505	FLATROCK R 300 FT DS CR 150W NR FLATROCK, IN	1,037	93.4	4.6	1.1	1.0	
506	LITTLE SALT CK 400 FT DS CR 650N NR COVEYVILLE, IN	113	21.7	77.9	.3	.1	
508	BOGGS CK 1600 FT DS CR 15 NR LOOGOOTEE, IN	224	33.2	57.1	.6	9.2	
509	BIG BLUE R 1400 FT W CR 450W NR KNIGHTSTOWN, IN	414	84.6	8.9	4.9	1.5	
510	FLATROCK R 900 FT N CR 800N AT ST LOUIS CROSSING, IN	1,280	93.4	4.2	1.4	1.0	
511	VERNON FK MUSC R 2700 FT E CR 400 S NR HAYDEN, IN	595	61.0	36.5	1.3	1.2	
512	E FK WHITE R 1 MI N CR 500 S NR YOCKEY, IN	10,355	76.2	19.5	2.3	2.0	
513	BIG BLUE R 1200 FT N CR 500S NR GREENSBORO, IN	176	78.3	10.5	8.6	2.6	
515	LITTLE SALT CK 2700 FT S SR 258 AT FREETOWN, IN	28.8	39.1	58.9	.2	1.7	
516	BEAVER CK 900 FT S CR 44 AT IRONTON, IN	179	22.4	76.6	.2	.8	
518	WYALOOSING CK 200 FT S 900N NR SARDINIA, IN	62.1	89.1	9.9	.6	.4	
519	SUGAR CK 1000 FT W CR 1000N NR ZENAS, IN	18.4	66.5	33.2	0	.3	
521	LITTLE FLATROCK R 2700 FT W SR 3 NR MILROY, IN	120	96.5	2.5	.6	.4	
522	S FK SALT CK 1200 FT N SR 58 NR KURTZ, IN	75.8	40.8	58.0	.5	.8	
523	BIG CK I MI W CR 700W AT VOLGA, IN	215	43.8	44.3	1.2	10.7	
524	E FK WHITE R 4000 FT W CR 400E NR THALES, IN	14,362	65.8	29.9	2.2	2.1	
525	BIG BLUE R 2700 FT E CR 750W NR MT AUBURN, IN	1,480	89.6	6.1	3.0	1.3	
526	E FK WHITE R 1300 FT W CR 450W NR COXTON, IN	12,196	69.3	26.0	2.5	2.2	

**Table 4.** Basin characteristics of the 76 algal-biomass sampling sites in the Whitewater River and East Fork White River Basins, Indiana, May through September 2002. — 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; R, River; RD, Road; CR, County Road; 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; IN, Indiana; OH, Ohio; trib, tributary; USHWY, U.S. Highway; RMI, River mile]

Short IDEM site number <sup>1</sup>	USGS station name	Drainage area (km <sup>2</sup> )	Landuse (percentage) <sup>2</sup>			
			Ag	Forest	Urban	Other
527	BIG CK 900 FT E SR 3 NR DEPUTY, IN	415	54.2	37.6	1.4	6.8
528	BEAR CK 200 FT N CR 400N NR OTWELL, IN	12.1	93.7	6.3	0	0
530	MUSCATATUCK R 2600 FT N MT EDEN RD NR MILLPORT, IN	2,898	60.4	34.5	1.5	3.5
531	N FK SALT CK 2250 FT US SR 46 AT BELMONT, IN	278	14.5	83.6	.5	1.3
532	E FK WHITE R 100 FT N WILLIAMS RD NR HURON, IN	12,311	69.1	26.2	2.5	2.2
533	SUGAR CK 1500 FT DS CR 1000N NR PLEASANT VIEW, IN	327	92.5	4.4	2.2	.8
534	VERNON FK MUSC R 1150 FT US WWTF AT N VERNON, IN	284	70.5	28.0	.7	.8
535	FALL FK 650 FT N SR 3 AT EWINGTON, IN	9.3	98.4	.2	1.3	.1
536	MUSCATATUCK R 1200 FT E MT EDEN RD NR MILPORT, IN	2,799	61.4	33.4	1.6	3.6
537	FLATROCK R 500 FT DS CR 450S NR MILROY, IN	505	93.9	3.5	1.6	1.0
539	GRAHAM CK 300 FT S CR 350E AT WALNUT RIDGE, IN	195	48.2	44.7	.3	6.8
542	BIG BLUE R 2400 FT W SR 31 AT EDINBURGH, IN	1,520	89.5	6.2	2.9	1.4
543	VERNON FK MUSC R 1800 FT E CR 800E NR RETREAT, IN	993	63.9	32.2	1.9	2.0
544	LOST R 100 FT N CR 450N NR ORLEANS, IN	86.8	91.7	7.7	.1	.4
545	SUGAR CK 2600 FT E CR 350S AT AMITY, IN	891	91.3	4.0	4.0	.7
551	SUGAR CK 500 FT N CR 600N NR MAXWELL, IN	147	94.1	4.4	.6	.9
	Minimum	9.3	14.5	.2	0	0
	Mean	1,943.8	71.1	25.3	1.6	2.0
	Median	305.8	77.3	17.7	1.4	1.2
	Maximum	14,362	98.4	83.6	8.6	10.7
	Standard deviation	3,825.5	23.8	23.4	1.6	2.4

<sup>1</sup>The full IDEM site number has a prefix of INRB, for example INRB02-401.

<sup>2</sup>Land use percentages were determined by the USGS using the National Land Cover Dataset (U.S. Geological Survey, 2000).

20 Relations of PCA Site Scores in the Whitewater River and East Fork White River Basins, Indiana, 2002

**Table 5.** Total Kjeldahl nitrogen as N values collected in 2002 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 IX and Level III Ecoregions 55 and 71.

[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; shading indicates value exceeds published U.S. Environmental Protection Agency value for Aggregate Nutrient Ecoregions VI and IX, all seasons; **bold** text indicates value exceeds published U.S. Environmental Protection Agency value for Level III Ecoregions 55 and 71 reference conditions, all seasons; nc, not calculated in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/ USGS values	USEPA values, all seasons <sup>1</sup>		IDEM/ USGS values	USEPA values, all seasons		
		Aggregate Nutrient Ecoregion VI <sup>2</sup>	Level III Ecoregion 55 <sup>2</sup>		Aggregate Nutrient Ecoregion IX <sup>3</sup>	Level III Ecoregion 71 <sup>3</sup>	
Number of streams	38	628	198	13	1,609	12	65
Minimum value	.240	.025	.050	.265	.000	.265	.050
10 <sup>th</sup> percentile	.300	nc	nc	.350	nc	.350	nc
25 <sup>th</sup> percentile	<b>.430</b>	.591	.400	<b>.490</b>	.300	<b>.465</b>	.284
50 <sup>th</sup> percentile	.578	nc	nc	.710	nc	.683	nc
75 <sup>th</sup> percentile	.650	nc	nc	.790	nc	.820	nc
90 <sup>th</sup> percentile	.820	nc	nc	.880	nc	.880	nc
Maximum value	1.05	4.50	3.50	1.01	4.83	1.01	2.05

<sup>1</sup>Values are the median of all seasons for all samples collected from a single stream.

<sup>2</sup>U.S. Environmental Protection Agency, 2000a.

<sup>3</sup>U.S. Environmental Protection Agency, 2000b.

**Table 6.** Nitrite plus nitrate as N values collected in 2002 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 IX and Level III Ecoregions 55 and 71.

[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 equals published U.S. Environmental Protection Agency value for Aggregate Nutrient Ecoregions VI and IX, all seasons; all seasons; nc, not calculated in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/ USGS values	USEPA values, all seasons <sup>1</sup>		IDEM/ USGS values	USEPA values, all seasons		
		Aggregate Nutrient Ecoregion VI <sup>2</sup>	Level III Ecoregion 55 <sup>2</sup>		Aggregate Nutrient Ecoregion IX <sup>3</sup>	Level III Ecoregion 71 <sup>3</sup>	
Number of streams	38	717	219	13	1,671	12	109
Minimum value	.125	.010	.025	.060	.000	.060	.008
10 <sup>th</sup> percentile	.380	nc	nc	.072	nc	.072	nc
25 <sup>th</sup> percentile	1.60	.633	1.60	.395	.125	.274	.345
50 <sup>th</sup> percentile	3.00	nc	nc	.915	nc	.778	nc
75 <sup>th</sup> percentile	4.20	nc	nc	2.50	nc	2.03	nc
90 <sup>th</sup> percentile	6.40	nc	nc	4.10	nc	2.97	nc
Maximum value	7.85	10.7	8.13	5.00	9.78	5.00	5.37

<sup>1</sup>Values are the median of all seasons for all samples collected from a single stream.

<sup>2</sup>U.S. Environmental Protection Agency, 2000a.

<sup>3</sup>U.S. Environmental Protection Agency, 2000b.

**Table 7.** Total nitrogen as N values collected in 2002 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 IX and Level III Ecoregions 55 and 71.

[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; shading indicates a new minimum value or exceeds the published U.S. Environmental Protection Agency value for Aggregate Nutrient Ecoregions VI and IX, all seasons; **bold** text indicates a new minimum value or exceeds the published U.S. Environmental Protection Agency value for Level III Ecoregions 55 and 71 reference conditions, all seasons; nc, not calculated in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/ USGS values	USEPA values, all seasons <sup>1</sup>		IDEM/ USGS values	USEPA values, all seasons		USEPA values, all seasons
		Aggregate Nutrient Ecoregion VI <sup>2</sup>	Level III Ecoregion 55 <sup>2</sup>		Aggregate Nutrient Ecoregion IX <sup>3</sup>	Level III Ecoregion 71 <sup>3</sup>	
Number of streams	38	77	2	13	274	12	10
Minimum value	<b>.560</b>	.885	3.63	.758	.240	.758	.625
10th percentile	1.37	nc	nc	.950	nc	.950	nc
25th percentile	2.19	2.18	3.63	1.04	.692	<b>1.02</b>	.800
50th percentile	3.58	nc	nc	1.45	nc	1.44	nc
75th percentile	4.69	nc	nc	2.97	nc	2.44	nc
90th percentile	7.04	nc	nc	4.75	nc	3.46	nc
Maximum value	<b>8.23</b>	10.1	3.78	5.51	12.4	<b>5.51</b>	4.35

<sup>1</sup>Values are the median of all seasons for all samples collected from a single stream.

<sup>2</sup>U.S. Environmental Protection Agency, 2000a.

<sup>3</sup>U.S. Environmental Protection Agency, 2000b.

The IDEM/USGS values for TN fell within or were close to the range of the published values for Ecoregions VI, IX, 55, and 71 (table 7). The IDEM/USGS 25<sup>th</sup>-percentile TN values were greater than published values for Ecoregions VI, IX and 71. New minimum concentrations were measured for Ecoregions VI and 55 and new maximum concentrations were measured for Ecoregions 55 and 71. If the proposed USEPA TN water-quality criteria for Ecoregions VI and IX were enacted, 70.5 percent of the individual samples collected in Ecoregion VI and 91.3 percent of the samples in Ecoregion IX would have exceeded those criteria.

The IDEM/USGS values for TP fell within the range of the published values for Ecoregions VI, IX, 55, and 71 (table 8). The IDEM/USGS 25<sup>th</sup>-percentile TP values were less than values for Ecoregions VI, IX, 55, and 71. The IDEM/USGS maximum concentrations were about 10 times lower than for Ecoregions VI, IX, 55, and 71. If the proposed USEPA TP water-quality criteria for Ecoregions VI and IX were enacted,

46.0 percent of the individual samples collected in Ecoregion VI and 72.5 percent of the samples in Ecoregion IX would have exceeded those criteria.

The IDEM/USGS values for mean periphyton CHL<sub>a</sub> provided a new range for Ecoregions VI, 55, and 71 (table 9). The IDEM/USGS 25<sup>th</sup>-percentile mean periphyton CHL<sub>a</sub> value was greater than the published value for Ecoregion IX. The IDEM/USGS maximum concentration was about two times higher than that for Ecoregion IX. No criteria were set for Ecoregions VI, 55, or 71. If the proposed USEPA mean periphyton CHL<sub>a</sub> water-quality criterion for Ecoregion IX were enacted, 72.5 percent of the individual samples collected in Ecoregion IX would have exceeded that criterion.

The IDEM/USGS values for mean seston CHL<sub>a</sub> fell within or were close to the range of the published values for Ecoregions VI, IX, and 71 and provided a new range for Ecoregion 55 (table 10). The IDEM/USGS 25<sup>th</sup>-percentile mean seston CHL<sub>a</sub> values were less than published values for

**Table 8.** Total phosphorus as P values collected in 2002 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 IX and Level III Ecoregions 55 and 71.

[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; nc, not calculated in U.S. Environmental Protection Agency Nutrient Criteria documents]

Statistic	IDEM/ USGS values	USEPA values, all seasons <sup>1</sup>		IDEM/ USGS values	USEPA values, all seasons		
		Aggregate Nutrient Ecoregion VI <sup>2</sup>	Level III Ecoregion 55 <sup>2</sup>		Aggregate Nutrient Ecoregion IX <sup>3</sup>	Level III Ecoregion 71 <sup>3</sup>	
Number of streams	38	815	225	13	2,104	12	117
Minimum value	.015	.005	.010	.015	.000	.015	.003
10th percentile	.032	nc	nc	.015	nc	.015	nc
25th percentile	.043	.076	.063	.033	.037	.029	.030
50th percentile	.063	nc	nc	.055	nc	.057	nc
75th percentile	.084	nc	nc	.062	nc	.068	nc
90th percentile	.150	nc	nc	.105	nc	.105	nc
Maximum value	.210	2.23	1.82	.130	2.40	.130	1.28

<sup>1</sup>Values are the median of all seasons for all samples collected from a single stream.

<sup>2</sup>U.S. Environmental Protection Agency, 2000a.

<sup>3</sup>U.S. Environmental Protection Agency, 2000b.

Ecoregions VI, IX, and 71. A new minimum concentration for Ecoregion IX and a new maximum concentration for Ecoregion 71 were established. No criteria were set for Ecoregion 55. If the proposed USEPA mean seston water-quality criteria for Ecoregions VI and IX were enacted, 56.9 percent of the individual samples collected in Ecoregion VI and 70.6 percent of the samples in Ecoregion IX would have exceeded those criteria.

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**Table 9.** Mean periphyton chlorophyll a values collected in 2002 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 IX and Level III Ecoregions 55 and 71.

[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; shading indicates value exceeds published U.S. Environmental Protection Agency value for Level II Ecoregions VI and IX, all seasons; 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 <sup>1</sup>		IDEM/ USGS values	USEPA values, all seasons		
		Aggregate Nutrient Ecoregion VI <sup>2</sup>	Level III Ecoregion 55 <sup>2</sup>		Aggregate Nutrient Ecoregion IX <sup>3</sup>	Level III Ecoregion 71 <sup>3</sup>	
Number of streams	38	nd	nd	13	6	12	nd
Minimum value	2.89	nd	nd	11.6	11.0	11.6	nd
10 <sup>th</sup> percentile	12.3	nd	nd	17.6	nc	17.6	nd
25 <sup>th</sup> percentile	35.1	nd	nd	26.4	20.4	29.1	nd
50 <sup>th</sup> percentile	55.5	nd	nd	47.4	nc	47.8	nd
75 <sup>th</sup> percentile	70.9	nd	nd	80.6	nc	80.9	nd
90 <sup>th</sup> percentile	97.7	nd	nd	81.4	nc	81.4	nd
Maximum value	288	nd	nd	113	62.0	113	nd

<sup>1</sup>Values are the median of all seasons for all samples collected from a single stream.

<sup>2</sup>U.S. Environmental Protection Agency, 2000a.

<sup>3</sup>U.S. Environmental Protection Agency, 2000b.



**Table 10.** Mean seston chlorophyll *a* values collected in 2002 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 IX and Level III Ecoregions 55 and 71.

[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; **bold** text indicates a new minimum value or exceeds the published U.S. Environmental Protection Agency value for Level III Ecoregions 55 and 71 reference conditions, all seasons; 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 <sup>1</sup>		IDEM/ USGS values	USEPA values, all seasons		
		Aggregate Nutrient Ecoregion VI <sup>2</sup>	Level III Ecoregion 55 <sup>2</sup>		Aggregate Nutrient Ecoregion IX <sup>3</sup>	Level III Ecoregion 71 <sup>3</sup>	
Number of streams	38	63	nd	13	71	12	9
Minimum value	.993	.250	nd	.807	.225	<b>.807</b>	2.60
10 <sup>th</sup> percentile	1.30	nd	nd	.910	nd	.910	nd
25 <sup>th</sup> percentile	2.10	2.70	nd	2.02	2.25	2.00	3.85
50 <sup>th</sup> percentile	2.95	nc	nd	3.48	nc	3.91	nc
75 <sup>th</sup> percentile	4.22	nc	nd	7.28	nc	7.45	nc
90 <sup>th</sup> percentile	6.07	nc	nd	8.20	nc	8.20	nc
Maximum value	20.8	47.6	nd	19.4	36.7	<b>19.4</b>	15.4

<sup>1</sup>Values are the median of all seasons for all samples collected from a single stream.

<sup>2</sup>U.S. Environmental Protection Agency, 2000a.

<sup>3</sup>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 at 38 sites in both the Whitewater River and East Fork White River Basins, Indiana from May through September 2002, on 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 algal-biomass, habitat, basin-characteristics, nutrient, biological-community attribute and metric score data.

On the basis of this initial analysis in the Whitewater River and East Fork White River Basins, periphyton PC1 does not relate to nutrient variables; no significant relations were found between nutrients and periphyton PC1 in similar studies conducted in 2001 in the West Fork White River and in 2003 in the Upper Wabash River Basin. Significant relations between nitrogen variables and seston algal biomass were found in late summer (July) and fall (September) but not in the spring. Significant relations between seston algal biomass and TP were found throughout the growing season. Based on the preliminary analysis in this report, fish-community attributes and metric scores may reflect the effects of algal biomass better than invertebrate-community attributes and metric scores. There were significant relations between periphyton and seston algal biomass and fish-community attributes and metrics. As periphyton algal biomass increases, fish-community composition shifts from one dominated by carnivores and other niche-specific specialists to one dominated by generalist-feeding and pioneer species.

There was one significant relation between periphyton or seston algal biomass and invertebrate communities. This lack of relations may be the result of three possible factors: (1) sample size, (2) sample method, and (3) resolution of invertebrate data. Additionally, previous studies and results from this analysis suggest that drainage-basin size and land use are factors affecting the relations between algal biomass, nutrients, and biological communities.

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<sup>th</sup>-percentile published USEPA values or extended data ranges in Aggregate Nutrient and Level III Ecoregions. If the values for the 25<sup>th</sup> percentile proposed by the USEPA were adopted as nutrient

water-quality criteria, the percentage of samples in the Whitewater River and East Fork White River Basins that would have exceeded these criteria ranged from 46.0 percent in Ecoregion VI for TP to 91.3 percent in Ecoregion IX for TN.

## Acknowledgments

The authors thank Indiana Department of Environmental Management and U.S. Geological Survey personnel for collecting and processing the data. We thank Patricia H. Long and Donald R. Leer of the USGS for editorial support and Dennis Clark (IDEM) and Stephen D. Porter (USGS) for their technical reviews of the manuscript. Projects of this type are a team effort and could not have been completed without the efforts of the IDEM and USGS personnel, which are sincerely appreciated.

## 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](http://www.epa.gov/nerlcwww/m445_0.pdf)
- Baker, N.T., and Frey, J.W., 1997, Fish community and habitat data at selected sites in the White River Basin, Indiana, 1993-95: U.S. Geological Survey Open-File Report 96-653A, 44 p.
- 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 programs (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 Assessment*, 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.
- 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.
- Debrewer, L.M., Rowe, G.L., Reutter, D.C., Moore, R.C., Hambrook, J.A., and Baker, N.T., 2000, Environmental setting and effects on water quality in the Great and Little Miami River Basins, Ohio and Indiana: U.S. Geological Survey Water-Resources Investigations Report 99-4201, 98 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 2007-5222, 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.
- Indiana Department of Natural Resources, 1988, Water resource availability in the Whitewater River Basin, Indiana: Indiana Department of Natural Resources, Division of Water, Water Resources Assessment 88-2, 126 p.
- 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.
- Leer, D.R., Caskey, B.J., and Frey, J.W., 2007, 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: U.S. Geological Survey Scientific Investigations Report 2007-5231, 25 p.
- 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.
- 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 initiated by the U.S. Geological Survey-Effects of nutrient enrichment on stream ecosystems: U.S. Geological Survey Fact Sheet FS-118-03, 4 p.
- National Research Council, 1978, *Nitrates—An environmental assessment*: Washington, D.C., National Academy of Sciences, 723 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.
- Petersen, J.C., and Femmer, S.R., 2002, Periphyton communities in streams of the Ozark Plateaus and their relations to selected environmental factors: U.S. Geological Survey Water-Investigation Report 02-4210, 77 p.
- Primer-E Ltd, 2006, *Primer User Manual / Tutorial* (version 6): Plymouth Marine Laboratory, United Kingdom, 111-119 p.
- 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.
- Schnoebelen, D.J., Fenelon, J.M., Baker, N.T., Martin, J.D., Bayless, E.R., Jacques, D.V., and Crawford, C.G., 1999, Environmental setting and natural factors and human influences affecting water quality in the White River Basin, Indiana: U.S. Geological Survey Water-Resources Investigations Report 97-4260, 66 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.
- Stewart, J.A., Keeton, C.R., Hammil, L.E., Nguyen, H.T., and Majors, D.K., 2003, *Water resources data, Indiana, water year 2002*: U.S. Geological Survey Water-Data Report IN-02-1, 647 p.
- U.S. Census Bureau, 2000, Cartographic boundary files: accessed it September 6, 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 IX: U.S. Environmental Protection Agency, Office of Water, EPA 822-B-00-019.
- 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 Nieuwenhuysse, 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 <sup>1</sup>	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 <sup>2</sup>	
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).



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.

<sup>1</sup>Each of the fish- and invertebrate community metrics also has a corresponding attribute with the same name but consists of the raw data.

<sup>2</sup> Specific fish species associated with each metric can be found in Dufour, 2002.





Caskey, Frey, and Lowe—**Relations of Principal Components Analysis Site Scores to Algal-Biomass, Habitat, Basin-Characteristics, Nutrient, and Biological-Community in the Whitewater River and East Fork White River Basins, Indiana, 2002**—Scientific Investigations Report 2007–5229