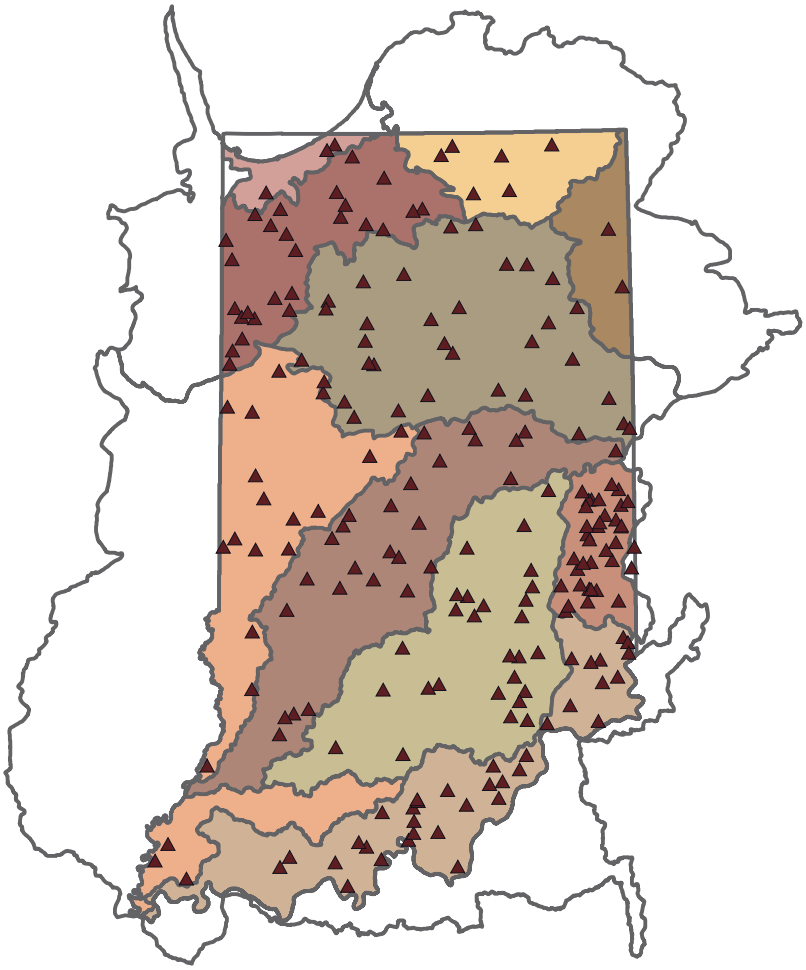
Prepared in cooperation with the Indiana Department of Environmental Management, Division of Water, Assessment Branch

Breakpoint Analysis and Assessment of Selected Stressor Variables on Benthic Macroinvertebrate and Fish Communities in Indiana Streams: Implications for Developing Nutrient Criteria



Scientific Investigations Report 2010–5026

U.S. Department of the Interior
U.S. Geological Survey

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By Brian J. Caskey, Jeffrey W. Frey, and Shivi Selvaratnam

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Conversion Factors and Abbreviations/Acronyms

Multiply	By	To obtain
	Length	
meter (m)	3.281	foot (ft)
millimeter (mm)	0.03937	inch (in.)
micrometer (µm)	0.001	millimeter (mm)
kilometer (km)	0.6214	mile (mi)
	Area	
square meter (m^2)	10.76	square foot (ft^2)
square kilometer (km^2)	247.1	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
milliliter (mL)	0.03381	ounce, U.S. liquid (oz)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound, avoirdupois (lb)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
°F=(1.8×°C)+32

Concentrations of nutrients and seston chlorophyll *a* in water are given in milligrams per liter (mg/L), concentrations of periphyton chlorophyll *a* are given in milligrams per square meter (mg/m^2), and concentrations of turbidity are given in Nephelometric turbidity units (NTU).

Abbreviations and acronyms used in this report

CHIR	Chironomidae
CHL*a*	chlorophyll *a*
CWA	Clean Water Act
DELT	deformity
EPT	Ephemeroptera, Plecoptera, and Trichoptera
GIS	Geographic Information System
HBI	Hilsenhoff's Biotic Index
IDEM	Indiana Department of Environmental Management
MDS	Multiple-dimensional scaling
MIBI	Macroinvertebrate Index of Biotic Integrity
N	nitrogen
NAWQA	National Water-Quality Assessment Program
NTU	Nephelometric turbidity units
NWQL	National Water Quality Laboratory
P	phosphorus
PCT	percentage
QHEI	Qualitative Habitat Evaluation Index
r^2	coefficient of determination
r_s	Spearman's rho statistics
TKN	Total Kjeldahl nitrogen
TN	total nitrogen
TP	total phosphorus
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WMP	Watershed Monitoring Program

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Breakpoint Analysis and Assessment of Selected Stressor Variables on Benthic Macroinvertebrate and Fish Communities in Indiana Streams: Implications for Developing Nutrient Criteria

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Abstract

Water chemistry, periphyton and seston chlorophyll *a* (CHL*a*), and biological community data were collected from 321 sites from 2001 through 2005 to (1) determine statistically and ecologically significant relations among the stressor (total nitrogen, total phosphorus, periphyton and seston CHL*a*, and turbidity) variables and response (biological community) variables; and, (2) determine the breakpoint of biological community attributes and metrics in response to changes in stressor variables. Because of the typically weak relations among the stressor and response variables, methods were developed to reduce the effects of non-nutrient biological stressors that could mask the effect of nutrients. Stressor variable concentrations ranged from 0.30 to 11.0 milligrams per liter (mg/L) for total nitrogen, 0.025 to 1.33 mg/L for total phosphorus, 2.9 to 768 milligrams per square meter (mg/m^2) for periphyton CHL*a*, and 0.37 to 42 micrograms per liter (µg/L) for seston CHL*a*. Turbidity, another stressor variable, ranged from 0.8 to 65.4 Nephelometric turbidity units (NTUs). When the nutrient and CHL*a* data were compared to Dodds' trophic classifications, 75.0 percent of the values for total nitrogen, 46.6 percent of the values for total phosphorus, 35.8 percent of the values for periphyton CHL*a*, and 3.5 percent of the values for seston CHL*a*, were eutrophic. The invertebrate communities were dominated by families considered highly nutrient tolerant, Chironimidae, (41.7 percent relative abundance), Hydropsychidae, (17.3 percent relative abundance), and Baetidae, (10.2 percent relative abundance). Fish communities were dominated by algivores and nutrient-tolerant species, specifically central stonerollers (13.3 percent relative abundance), creek chubs (9.9 percent relative abundance), and bluntnose minnows (9.3 percent relative abundance). Although not the dominant taxa, white sucker, spotted sucker, green sunfish, and bluegill species were correlated ($p < 0.05$) with the stressor variables. The median breakpoints ranged from 2.4 to 3.3 mg/L for total nitrogen, from 0.042 to 0.129 mg/L for total phosphorus, from 54 to 68 mg/m^2 for periphyton CHL*a*, from 4.5 to 7.5 µg/L for seston CHL*a*, and from 14.1 to 16.1 NTU for turbidity. The breakpoints determined in this study, in addition to Dodds' trophic classifications, were used as multiple lines of evidence to show changes in fish and invertebrate community and attributes based on annual exposure to nutrients.

Introduction

Although nutrients are essential to the development, health, and diversity of plants and animals in surface waters, excessive inputs of nutrients into streams have potential human-health, economic, and ecological consequences. Excess amounts of nitrogen (N) and phosphorus (P) have been shown to cause eutrophication in aquatic ecosystems, and this sometimes has been linked to fish kills, shifts in species composition, taste and odor in drinking-water, and blooms of harmful algae in freshwater and estuaries (Munn and Hamilton, 2003; U.S. Environmental Protection Agency, 2000a, b, c).

The Clean Water Act (CWA), as amended in 1976, established a national goal of achieving water quality that provides for the protection and propagation of aquatic organisms, 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 and as the primary cause of impairments in lakes and reservoirs (U.S. Environmental Protection Agency, 1997). 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.

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The USEPA drinking-water criteria (maximum contaminant levels) are 10 mg/L for nitrate as N and 1 mg/L for nitrite as N (U.S. Environmental Protection Agency, 2005), to protect human health. The nitrate criteria were set mainly to protect against methemoglobinemia or "blue baby syndrome." However, other studies have linked excessive nitrate and nitrite levels to adverse reproductive and developmental outcomes in animals and humans (Manassaram and others, 2006). In addition, aquatic-life criteria for the protection of aquatic organisms have been developed for ammonia as N. These criteria vary with pH, temperature, and life-stage (U.S. Environmental Protection Agency, 2005). Current criteria do not address the effects of increased nutrients in rivers and streams on the biological communities. 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). Exceptions are concentrations of ammonia associated with accidental discharges from wastewater-treatment facilities, combined-sewer overflows, or concentrated-animal feeding operations (Ohio Environmental Protection Agency, 1999).

Many streams within Indiana, as well as the United States, have been placed by the USEPA on the CWA Section 303(d) list of impaired water bodies because of excess nutrients (Indiana Department of Environmental Management, 2009). To address these impairments, the USEPA in 2000 developed nutrient criteria for the causal nutrients total nitrogen (TN) and total phosphorus (TP) and for the response variables periphyton and seston chlorophyll a (CHL*a*), and turbidity. These criteria are based on USEPA Aggregate Nutrient Ecoregions, which are areas with similar geographic features such as topography, soils, geology, land use, and biogeography (U.S. Environmental Protection Agency, 2000a, b, c). The USEPA reviewed selected data and utilized the frequency distribution approach to develop criteria for nitrate as N, total Kjeldahl nitrogen as N (TKN), TN, TP, CHL*a* (periphyton and seston), and turbidity based on the 25th percentile value for all data or the 75th percentile from reference sites for each causal variable. However, few of the available data from Indiana were included in the criteria development (Dennis Clark, Indiana Department of Environmental Management, oral commun., 2008; U.S. Environmental Protection Agency, 2000a, b, c) or, as was the case for CHL*a*, little or no data existed for many of the ecoregions, including those in Indiana (U.S. Environmental Protection Agency, 2000a, b, c). Therefore, the proposed nutrient, CHL*a*, and turbidity criteria may not accurately reflect existing local conditions.

In a typical stressor-response relation, a change in a stressor (causal) variable (TN and TP, in the case of nutrients) results in a corresponding change in a response variable. Several studies found significant relations between nutrients and periphyton CHL*a* for temperate streams (Van Nieuwenhuyse and Jones, 1996; Dodds and others, 1997). Robertson and others (2006) found significant relations between nutrients (nitrate, TN, dissolved P, and TP) and periphyton CHL*a* in Wisconsin. Biggs (2000) listed several studies that found significant relations between nutrients and periphyton CHL*a*, primarily in studies that analyzed a small number of streams. When a large number of streams were analyzed using a national dataset, Dodds and others (1997) found few and very weak significant relations between nutrients and algal biomass. In previous studies of nutrient-rich streams in Illinois (Figueroa-Nieves and others, 2006; Royer and others, 2008) and in Indiana (Frey and others, 2007; Caskey and others, 2007; Leer and others, 2007; Lowe and others, 2008), there were limited weak or no significant relations between nutrients and periphyton or seston CHL*a* variables. Algal biomass can be influenced by several natural factors that could account for a lack of significant relations with nutrients. 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 both high and low concentrations of nutrients, depending upon the season or year or both. Biggs (2000) found the strongest nutrient and algal biomass relations when a streamflow variable was incorporated into the analysis. Other factors affecting algal growth include shading from canopy cover, turbidity (Wehr, 2003), and grazing by snails, invertebrates, and fish (Lamberti and others, 1987).

Nutrients at concentrations found typically in streams are nontoxic to biological organisms (Miltner and Rankin, 1998), which indicates that the typical stressor-response relations found in toxicological based approaches would not be appropriate. Previous nutrient and algal biomass studies have shown mixed results because linear techniques are applied to nonlinear data. Instead, nonlinear techniques (that is, breakpoint analysis) may be better at discerning the relations between nontoxic stressor and response variables and identifying breakpoints (Qian and others, 2003). In small rivers and streams in Ohio, Miltner (2010) observed clear associations between nutrients, secondary response indicators, and biological communities by utilizing these techniques. Development of defensible nutrient criteria for rivers and streams in nutrient-rich areas will be complex and require intensive data analysis.

Indiana is part of the nutrient-rich Midwest that leads the nation in corn and soybean production. Production of these crops requires the use of significant amounts of nutrients—primarily from fertilizer and manure additions—which has increased dramatically since the 1940s (Goolsby and Battaglin, 2000). Mueller and Spahr (2006) noted that streams sampled within the Midwest have some of the highest nutrient loading in the United States. A model of the Mississippi River Basin found that Indiana, Illinois, and Ohio were leading contributors of N and P to the Gulf of Mexico, resulting in hypoxia of those waters (Alexander and others, 2008).

Given that Indiana is in a nutrient-rich area with observed weak relations between nutrients and algal biomass, other indicators such as biological species, community attributes and metrics, could be helpful in developing nutrient criteria

for streams and rivers in such regions (Lowe and others, 2008). Aquatic organisms and related community composition and attributes are frequently used in ecological analysis of communities because of the response of these organisms to anthropogenic inputs and other variables (Karr, 1981; Wang and others, 1997; Brightbill and Bilger, 1998; Carpenter and Waite, 2000; Caskey, 2003). For example, several recent studies used biological organisms and attributes to assess nutrients in streams by using a causal and response approach (Petersen and Femmer, 2002; Heatherly and others, 2007; Mabe, 2007; Wang and others, 2007; Weigel and Robertson, 2007). In water-quality studies, the covariation of physical and chemical variables can conceal the stressor-response relations. Because of the multiple and complex interactions between nutrients, algal biomass, and biological communities, almost all relations tend to be weak. One method that improves the strength of these relations is to remove anthropogenic and natural variations that conceal the nutrient, algal biomass, and biological community relations. By removing covariable stressors, such as basin size or ecoregion, the number and strength of statistically significant stressor-response relations increase (Miltner and Rankin, 1998; Frey and Caskey, 2007; Frey and others, 2007; Caskey and others, 2007; Leer and others, 2007). In this study, this resulted in more statistically significant stressor-response relations, leading to more breakpoint analyses. However, in a study conducted in Indiana and Ohio that accounted for variability of stream size, ecoregion, substrate, and canopy cover along a nutrient gradient, the biological communities reflected eutrophic conditions along the entire nutrient gradient, indicating that parts of the Midwest may be so nutrient rich that finding significant relations between nutrients, algal biomass, and biological communities may be difficult (Caskey and Frey, 2009).

The USEPA mandated that by 2004, states either adopt USEPA's nutrient criteria or develop criteria that are more appropriate to waters within each state (U.S. Environmental Protection Agency, 2000a, b, c). An extension was given to Indiana and other states that adopted plans describing the data needs, processes, and the approaches that would be considered in developing nutrient water-quality criteria. Beginning in 2001, the Indiana Department of Environmental Management (IDEM) and the U.S. Geological Survey (USGS) collaborated on several studies that will provide the technical background to assist in the development of nutrient criteria for surface waters for the State of Indiana. The results presented in this report are representative of similar nutrient-rich ecoregions in the Midwest.

Purpose and Scope

This is the final report in a series of six reports that previously presented the occurrence and distribution of, and relations among, total nitrogen as N, total phosphorus as P, periphyton and seston CHL*a*, turbidity, and biological community attributes and metrics in Indiana streams. The study has been a cooperative project between the Assessment Branch of the Office of Water at the IDEM and the USGS. In this report, stressor variables (TN, TP, periphyton and seston CHL*a*, turbidity) are related to biological response variables (benthic macroinvertebrate-, and fish-species, and community attributes and metrics) to determine statistically significant relations. To improve the strength of the relations, sites with water-quality standard violations for metals and other select variables were removed, leaving 74 sites with invertebrate community data and 232 sites with fish community data. Breakpoint analysis was used to detect the concentration of the stressor variables where there was a significant change in the response variables. Results of the breakpoint analysis will provide IDEM with relevant information that could be useful in the development of nutrient criteria using multiple lines of evidence, such as the incorporation of nutrient concentrations that show a specific biological response.

Description of Study Area

With approximately 6.1 million citizens (U.S. Census Bureau, 2000), the State of Indiana is comprised of 94,617 km^2 (Clark, 1980). The climate within Indiana is characterized as humid continental with well defined winter and summer seasons. Temperature and precipitation amounts vary between northern and southern Indiana. The mean monthly temperature in northern Indiana ranges from -5.17°C in winter to 22.8°C in summer. In southern Indiana, temperatures ranges from -1.29°C in winter to 24.5°C in summer (Purdue Applied Meteorology Group, 2008). The mean annual precipitation in Indiana ranges from 94.5 cm in the north to 119 cm in the south (Purdue Applied Meteorology Group, 2008). Samples were collected from all major river basins within Indiana, except the Patoka River Basin (fig. 1, table 1 at back of report).

Basin characteristics were determined for each of the sampling sites in the 5-year study. Typical of Midwestern states, the landscape in the study basins is relatively flat; the land is used mainly for the production of agricultural row crops, primarily corn and soybeans. Many agricultural fields use subterranean tiles to improve drainage, and a majority of the agriculturally influenced streams in the study area can be characterized as having low relief and velocities. All of the major river basins in which samples were collected during the study were predominantly agricultural; agricultural land use comprised 77 percent of all major basins in the study area. Within the study area, 16 percent of the land use was classified as forest and 4 percent was classified as urban. Other land uses including mining and wetlands constituted 3 percent of the study area (Lowe and others, 2008).

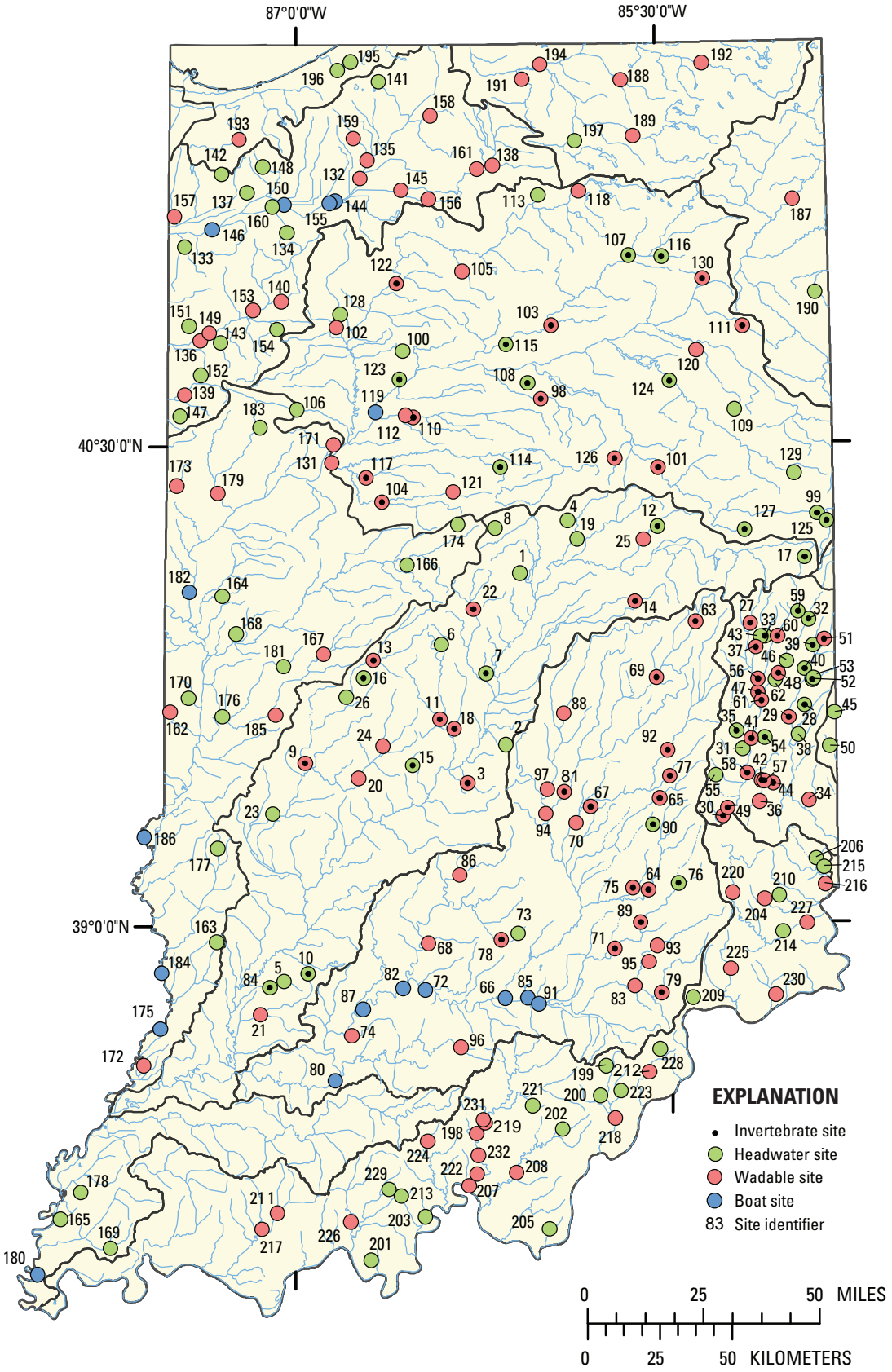

Figure 1. Location of the Indiana Department of Environmental Management Watershed Monitoring Program sampling sites used in the breakpoint analysis study, 2001–05.

The percentage of land use by classification varied among the major river basins, although agriculture was the dominant land use in all basins. The Upper Wabash River Basin had the most agricultural land use (92 percent) while the West Fork White River Basin, Whitewater River Basin, Kankakee River Basin, Lower Wabash River Basin, and Great Lakes Tributaries were heavily agricultural (averaging 82 percent). The East Fork White River Basin and the Ohio River Tributaries were less agricultural (71 percent and 52 percent, respectively), and had a higher percentage of forested areas than the other basins. Urbanization was most apparent within the West Fork White River Basin, where 8 percent of the land use was urban and includes the cities of Indianapolis, Muncie, and Anderson. Other land-use influences within the Kankakee River Basin and Great Lakes Tributaries were largely attributed to natural marshes and wetlands that are typical of the lowland, poorly drained areas of northern Indiana (Lowe and others, 2008).

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 and processing biological community, nutrient, algal biomass, and basin characteristics; and the approaches used in data analysis.

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 represents a specific stream order; therefore, statistically valid extrapolations can be made from the randomly sampled streams to streams not sampled in a particular basin (Indiana Department of Environmental Management, 1999, 2001). The IDEM WMP works on a 5-year rotating basin schedule, focusing on one or two selected basins each year, with a complete assessment of the state at the end of each 5-year cycle. In 2001, the focus was the West Fork White River Basin; in 2002, the Whitewater River and East Fork White River Basins; 2003, the Upper Wabash River Basin; 2004, the Kankakee and Lower Wabash River Basins; and in 2005, the Great Lakes and Ohio River Basins. The Patoka River Basin was not included in this study because the initial proposal in 2001 called for method development in the West Fork White River Basin only. Prior to collection of field data, IDEM personnel completed a visual assessment of selected sampling sites and determined the stream reach. At each site, the latitude and longitude provided by USEPA was used as the center point of the sampling reach, so that half of the reach was upstream and half of the reach was downstream of the midpoint. The reach length was determined by measuring the wetted channel width at the center point, and then the width was multiplied by 15.

During periods of stable low flow, a total of 321 sites were sampled from May through October 2001–2005 (Lowe and others, 2008). Within each major basin, 34 to 45 sites were sampled for nutrients and CHL*a*. The samples were collected multiple times in each basin to measure seasonal changes in nutrients and CHL*a*, with the exception of the West Fork White River Basin, which was sampled only two times during 2001 (rounds 2 and 3). Round 1 samples were collected in May and June, round 2 samples were collected in July and August, and round 3 samples were collected in September and October. At each site, the nutrient and CHL*a* samples were collected on the same day at about the same time.

Data Collection and Processing Methods

The following sections describe the field and laboratory methods used in collecting and processing samples of biological communities, nutrients, water-quality characteristics, and algal biomass, and in the determination of habitat and basin characteristics.

Collection of Biological Communities

Invertebrate and fish-community assessments followed IDEM methods (Indiana Department of Environmental Management, 1992, 1999). Invertebrates were collected from riffle habitats and identified to the family level; whereas, fish were collected within the entire reach and identified to the species level. Biological communities (invertebrate and fish) were assessed one time from midsummer to early fall (June through October) as part of the WMP. Approximately 10 percent of the sites were sampled a second time during the same year as part of the quality-assurance plan. After the assessments were completed, IDEM personnel calculated 20 invertebrate and 43 fish community attributes and metric scores (Simon, 1999; Dufour, 2002, Goldstein and Simon, 1999). In addition, USGS personnel calculated 89 invertebrate and 651 fish community attributes thought to be of interest or previously published in the literature. The community attributes and metric scores describing the invertebrate- and fish-community data are listed in appendixes 1 and 2.

Calculation of Biological-Community Attributes and Metrics

Invertebrate community attributes and metrics were calculated using USGS software, the Invertebrate Data Analysis System (IDAS) v 4.2.0.10 (Cuffney, 2003). Only 89 attributes and metrics could be calculated using the IDAS software because the invertebrate samples used in this analysis were

identified to the family level. The standard IDEM invertebrate metrics were also included, resulting in a list of 109 invertebrate response variables. The percentage of relative abundance was calculated for each invertebrate family that was represented in each sample. The final invertebrate attributes, metrics, counts, and percentage of relative abundance were used as response variables in the invertebrate breakpoint analysis.

Attributes found in an extensive literature review were used to calculate 651 fish community attributes. These attributes were based on individual counts, percentage of individual counts, species counts, percentage of species counts, biomass, and percentage of biomass. In addition, 43 attributes and metrics calculated by IDEM were included as response variables, resulting in a final list of 694 fish attributes and metrics. As with the invertebrate community, the fish community percentage of relative abundance was determined for each species that was represented with each sample.

Collection of Nutrients and Water-Quality Samples

Nutrient (TN and TP) and water-chemistry samples were collected using a grab method by IDEM and USGS personnel following approved IDEM sample collection (Beckman, 2000) and quality-assurance methods (Bowren and Ghiasuddin, 1999). After collection, samples analyzed for general chemistry were placed directly on wet ice, samples analyzed for nutrients were preserved with 2 mL sulfuric acid (H_2SO_4), samples analyzed for metals were preserved with 5 mL of concentrated nitric acid (HNO_3), and samples analyzed for cyanide were preserved with sodium hydroxide (NaOH). Samples were then analyzed by independent laboratories (Test America, Indianapolis; Heritage Environmental, Indianapolis; or Underwriters Laboratories, South Bend). The independent laboratories used USEPA test method 353.2 for nitrate plus nitrite analysis by copper-cadmium reduction; USEPA test methods 351.2 and 351.4 for Kjeldahl analysis by persulfate digestion and colorimetry; and USEPA test methods 365.1, 365.2, and SM4500P–E for total phosphorus by persulfate digestion and colorimetry. For the analysis in this report, total nitrogen is the sum of nitrate plus nitrite as nitrogen and Kjeldahl as nitrogen.

Collection of Periphyton and Seston Chlorophyll *a* Samples

Samples were collected and processed to estimate algal biomass, based on periphyton and seston CHL*a* concentrations. CHL*a* is a measure of the pigment found in algal cells that are attached to a substrate (periphyton) or floating in the water column (seston). The term “seston” is used instead of “phytoplankton,” because periphytic algae scoured from substrate can be included in the seston-algal samples.

Samples were collected and processed using modified USGS protocols. The National Water-Quality Assessment (NAWQA) Program algal protocols for periphyton (Moulton and others, 2002; U.S. Geological Survey, variously dated) collect five periphyton subsamples from five different locations within the sampling reach. At each location, the stream depth, velocity, shading, and substrate are recorded. The samples are composited and marked as a single sample. Periphyton samples were collected from the same substrate type—epilithic (rocks), epidendric (sticks), or epipsammic (sand), at each site during the sampling period, when possible. If available, epilithic substrates were the priority substrate for periphyton collection in smaller streams with less than 2,590 km^2 drainage area. The primary means of periphyton sampling during 2001 through 2003 was the top-rock scrape, which consisted of scraping the algae from the rock surface and determining the area with a foil template (Moulton and others, 2002). During 2004 and 2005, epilithic substrate was sampled using a SG-92 modified syringe sampling device or by completing an area scrape using the SG-92 to determine the sampled area (Moulton and others, 2002). One modification to this study from the NAWQA protocols was that 10 periphyton subsamples were collected from similar substrate as close to the center point of the reach as possible. Then five subsamples from rocks or sticks that best visually represented the average algal cover within the reach were collected subsamples, and these subsamples were composited into a single sample (Charles and others, 2000). The sample volume and total area sampled were recorded. A second modification to this study from the NAWQA protocols was that stream discharge, the point velocities at sample-collection sites, and light availability were not measured. Water-quality field measurements for each sample included water temperature, pH, specific conductance, turbidity, and dissolved oxygen.

The preferred method for collecting seston samples was the multiple vertical method described by Shelton (1994), in which a 3-L bottle with a 0.476-cm nozzle is used. In cases where water depths were a limiting factor, a grab method was used (Shelton, 1994). The seston samples were collected as close as possible to the center of the sampling reach, along a line that extended from the left edge of water to the right edge of water (transect). Along the transect, the wetted channel width and water depths (one-quarter, one-half, and three-quarters points) were recorded.

Filtered periphyton and seston volumes, needed for the CHL*a* analysis, were determined by examining the color density of the filter following USGS National Water Quality Laboratory (NWQL) guidelines for particulate carbon sampling and analysis (Patton and others, 2000). Each sample was homogenized and filtered onto glass-fiber filters in the field by USGS personnel. The filtered volume of each algal-biomass sample was recorded. The filters were then placed on dry ice and transported to the USGS Indiana Algal Biomass Laboratory for analysis. Filters were processed within 30 days of collection. Concentrations of CHL*a* were determined, following USEPA method 445, with a Turner Designs TD–700 fluorometer outfitted for CHL*a* analysis (Arar and Collins, 1997). There were two exceptions to method 445: filters were ground in high-density polyethylene centrifuge tubes rather than

glass to counter the problem of tube breakage, and samples were centrifuged at 1,500 to 2,000 revolutions per minute (approximately 320 to 569 g) for 15 minutes (Freeman, 1968). At the modified centrifuge rate, the filter residue and 90-percent acetone solution usually separated well. If samples did not separate well, they were placed in the centrifuge a second time. All samples were allowed to steep in 90 percent acetone for 2.5 hours at 4ºC.

Quality-assurance methods for periphyton and seston CHL*a* samples included collecting triplicate filters from the same sample to measure variability and a blank filter from each sampling reach to evaluate equipment decontamination procedures and possible sample processing contamination (Bowren and Ghiasuddin, 1999). In addition, a fifth filter was collected at each site and 10 to 15 percent of these filters were analyzed for CHL*a* at the NWQL to measure interlaboratory variability. Results from the quality-assurance assessment showed no significant bias or contamination, nor was there a significant difference between replicate filters analyzed at the NWQL (Lowe and others, 2008).

Physical Habitat

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

Basin Characteristics

Basin characteristics were determined by USGS scientists and included drainage area and land use, expressed as the percentage of agriculture, forest, and urban area. Drainage area (size) was derived from the basin boundaries by using a Geographic Information System (GIS). Drainage area was used to categorize streams as either headwater streams (0 to 51 km^2), wadable streams (52 to 2,590 km^2), or boatable streams (2,591 to 38,900 km^2). 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 formed a polygon that was used to extract land-use information from the National Land Cover Dataset (U.S. Geological Survey, 2000). Each sampling site was assessed to determine in which Aggregate Nutrient Ecoregion (U.S. Environmental Protection Agency, 2000a, b, c) and Level III Ecoregion (U.S. Environmental Protection Agency, 1998) it was located.

Data Analysis

Data analysis was steered by two main objectives. The first objective was to improve the strength of the relations among the stressor (TN, TP, periphyton and seston CHL*a*, and turbidity) and response variables (biological community attributes), because typically the relations between nutrients, algal biomass, and biological communities have been weak (Figueroa-Nieves and others, 2006; Frey and others, 2007; Caskey and others, 2007; Leer and others, 2007; Royer and others, 2008; Lowe and others, 2008). The first objective was accomplished by: (1) removing sites that were biologically impaired by other known factors; (2) examining the possible covariability of fish and invertebrate communities with non-nutrient variables; and (3) removing sites from the biological datasets when a species of interest was not collected because it could not be confirmed that the species was missing from the site in response to a stressor variable of interest. The second objective was to identify the most statistically and ecologically significant relations among the stressor and response variables and to determine the breakpoints for these significant relations. The second objective was accomplished by: (1) reducing the number of response variables to a list of candidate response variables for three datasets to be used in the breakpoint analysis; (2) selecting the most significant response variables from the candidate list; and (3) determining the concentration of the stressor variables where the greatest change (breakpoint) was seen for each attribute from the final list of significant response variables.

Improvement of the Relations among the Stressor and Response Variables

Sites were initially selected for this analysis if the data included the stressor variables (TN, TP, periphyton CHL*a*, seston CHL*a*, and turbidity) and response variables (invertebrate- and fish-community data). These initial sites included 77 sites with sufficient data for the invertebrate-community and 287 sites for fish-community analyses.

This study used multiple laboratories across multiple years, and thus the resolution of multiple reporting limits was required. Stressor variables, specifically TN (nitrate plus nitrite plus TKN) and TP, with multiple reporting limits were censored to one-half the highest reporting limit for each variable. For TKN there were 5 detection limits (0.1, 0.25, 0.3, 0.5, and 1 mg/L), and 129 values (about 17.7 percent of the samples) had values less than a reporting limit. For TP there were two detection limits, 0.03 and 0.05 mg/L, and about 21 percent of the samples had values less than those detection limits. Variables with a single reporting limit and values less than the reporting limit were increased to one-half of the reporting

limit. Variables with values greater than one-half of the highest reporting limits, but less than the highest reporting limit, were retained at the reported value. The means for each for the five stressor variables were calculated from the two to three samples collected at each site between May through October. To assess the trophic level of each stream, the mean stressor concentrations for TN, TP, periphyton and seston CHL*a* were compared to Dodds' trophic classification. Dodds and others (1998) classified the oligotrophic-mesotrophic boundaries of Midwest streams as 0.70 mg/L for TN, 0.025 mg/L for TP, 20 mg/m^2 for periphyton CHL*a*, and 10 µg/L for seston CHL*a* and the mesotrophic-eutrophic boundaries as 1.5 mg/L for TN, 0.075 mg/L for TP, 70 mg/m^2 for periphyton CHL*a*, and 30 µg/L for seston CHL*a*. The mean turbidity measurements were compared to 50 Nephelometric turbidity units (NTU), a turbidity biological impact classification (Nelson, 1993).

Sites with nonnutrient water-quality exceedances were removed (Indiana Department of Environmental Management, 2000); two sites were removed from the fish dataset, one for chloride and one for ammonia impairment. No sites were removed from the invertebrate community dataset based on water-quality exceedances. Next, sites where the concentrations of nonnutrient water-quality variables were greater than two standard deviations of the mean were removed to increase the probability that a nutrient was the primary stressor. During this step 52 sites from the fish datasets, including 3 sites from the invertebrate dataset, were removed, and most of the sites were removed based on high temperature, sulfate, arsenic, chemical oxygen demand (COD), and dissolved oxygen (greater than 16 mg/L) values. Of the 52 sites removed, 36 had multiple nonnutrient-related values greater than two standard deviations. After the 52 sites were removed, the final list of sites (table 1) used in the analysis consisted of 74 sites where invertebrate samples were collected from riffle habitats and identified to the family level and 232 sites where fish community were collected and identified to species level.

In lotic ecosystems, multiple habitat and water quality-related variables influence biological communities at any given time from their headwaters to tailwaters (Vannote and others, 1980; Simon, 1999; Goldstein and Meador, 2004). To examine the possible covariability associated with nonnutrient variables, the invertebrate and fish community datasets were analyzed using Multi-Dimensional Scaling (MDS) in the PRIMER software (version 6, Plymouth, United Kingdom). An MDS analysis is a similarity or dissimilarity matrix that can be used to examine relevant questions within a dataset (Clarke and Warwick, 2001). In this study, the MDS analysis was used to determine if differences among the invertebrate and fish communities were explained by variables such as basin size, IDEM's major river basins, Omernik's Level III ecoregions, and USEPA Nutrient Ecoregions. Of the variables examined, the only variable that strongly explained the differences in the fish community was basin size. None of the variables examined strongly explained the differences in the invertebrate community. The Index of Biotic Integrity (IBI) used in Indiana is based on three distinct stream basin sizes: headwater sites (drainage basins ≤ 51 km2), wadable sites (drainage basins > 51 km2 to < 2,590 km2), and boat sites (drainage basins ≥ 2,591 km2). Therefore, the MDS and breakpoint analyses were based on the Indiana IBI classification of stream basin size.

In ecological or biocriteria assessments, the lack of an organism at a specific site does not necessarily indicate the organism was absent. The organism may not naturally occur within a certain region or stream, or the organism cannot be collected due to sampling methods, habitat, or stream conditions. In dose-response studies, the absence of an organism provides important toxicological information and is included in further analysis. In this study, sites were removed from this analysis if an organism was absent because (1) nutrient values found in samples from a site typically are not toxic to organisms, (2) of the uncertainty associated with an absence of an organism, and (3) the presence of an organism indicates a possible response to nutrients. In almost all cases, the removal of sites preserved a stressor gradient. Other variables that are covariant may still remain, and removal of sites with water-quality exceedances, nonnutrient-related data outliers, and absent organisms should produce a greater number of significant nutrient-biological relations. The most statistically and ecologically significant relations were subsequently examined for breakpoint response. The number of sites removed because a species did not occur varied for each significant response variable and ranged from 0 to 52 for the invertebrate dataset, 42 to 74 for the headwater (fish) dataset, and 2 to 88 for the wadable (fish) dataset.

Identification of Significant Relations among Variables and Determination of Breakpoints

The identification of the most statistically and ecologically significant relations among the stressor and response variables (biological organisms, community attributes, and metrics) was aided by the reduction of the large number of response variables. First, the stressor variables were related to the response variables using Spearman rank order correlation. Environmental data typically are not normally distributed and when the sample size is greater than 20 for most variables, a nonparametric analysis, such as Spearman's correlations (r_s), is the preferred method for determining relations between variables (Helsel and Hirsch, 2002). In this study, all potential and strong correlations were identified using $|r_s|$.

In this report, for a correlation to be considered statistically significant, the Spearman's correlation was required to have, at most, a 5-percent significance level ($\alpha = 0.05$) based on the sample size. Although an r_s with a significance level of 0.05 is considered significant, there is a possibility of introducing a Type I error in which the relation is declared present when the relation is not present (Helsel and Hirsch, 2002). 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 at a specific alpha level. Although useful in reducing Type I error, this technique increases the chance of producing a Type II error, in which no relation is declared when a relation is present. Because of the low number of significant relations between nutrients (TN and TP), periphyton and seston CHL*a*, and turbidity to the biological organisms and community attributes and metrics, no Bonferroni corrections were applied in this analysis because of concerns of increasing a Type II error. Response variables were removed that were not statistically correlated ($p < 0.05$) to the stressor variables. The remaining response variables were assessed for collinearity; for the response variables that were collinear, only the variables with the highest $| r_s |$, or known ecological significance, were kept for further analysis. This analysis produced a list of candidate response variables for the three datasets, one invertebrate and two fish, used in the breakpoint analysis.

After the list of candidate response variables was completed for the three datasets used in the breakpoint analysis—one invertebrate and two fish—the mean, standard deviation, variance, skewness, and kurtosis were calculated for each variable. In addition, a mix of box plots, histograms, Normal-Probability (N-P) plots, and Probability-Probability (P-P) plots were used to determine the distribution and normality of the data. Nonnormal data must be transformed before it can be used in a statistical procedure that requires normality, unless nonparametric techniques are used.

The second step in reducing the number of response variables was the use of cluster analysis as a visual censoring tool to select the final response variables by partitioning data into significant groups (clusters). This technique visually identified covariance among response variables by placing similar variables together. The most ecologically significant response variable with the highest $| r_s |$ from each cluster grouping was retained for the breakpoint analysis.

A regression-tree analysis was used in S-Plus (Insightful Corporation, 2001) to determine the concentration where the greatest change was seen for each metric or attribute from the finalized list of statistically and ecologically significant response variables. For each of the stressor variables (TN, TP, seston and periphyton CHL*a*, and turbidity), the median breakpoints for the most significant metrics and attributes from the invertebrate and the headwater and wadable fish datasets were determined.

Bootstrapping was used to determine the confidence intervals at the 90th percentile of the median breakpoints identified in the regression-tree analysis. Bootstrapping simulates the results of repeated experiments based on the observed data by randomly selecting subsets of the observed data. The approach used in the regression tree bootstrap preserved sampling along a gradient and allowed for the variables of the distribution to change along that gradient. This was done by defining groups of data along the environmental gradient and forcing the bootstrap to resample within those groups, thus preserving the gradient and also the variability of the distribution.

Assessment of the Stressor Variables and the Biological Communities

The data was evaluated in several ways to aid understanding of the relations among the stressor variables and between stressor variables and the biological-community. First, the biological community data were examined to determine if natural variability, such as basin size, in invertebrate or fish community data made it necessary to subset the data. Second, the biological communities were assessed for composition to determine if the species reflected nutrient-enriched conditions. Third, the stressor variables were (1) examined for distribution, (2) examined for relations among each other, and (3) compared to Dodds' trophic classification levels. Finally, the biological communities were assessed to determine where the breakpoints in a species or biological community attribute or metric occurred in response to a stressor variable of interest.

Influence of Basin Size on the Biological-Community Composition

The MDS of the invertebrate community data of the 74 sites visually indicated they were slightly affected by basin size; however, the MDS stress level of 0.25 indicates that not much reliance should be placed on the relation between invertebrate composition and basin size. Consequently, the invertebrate community data was not subset based on basin size. The MDS of the fish community dataset of the 232 sites indicated that they were affected by basin size; the MDS stress level of 0.18 statistically supports the influence of basin size on the fish community. Therefore, the fish community data was subset into three categories based on basin size: headwater (n=98), wadable (n=117), and boatable (n=17). Because the number of boatable sites was less than 20, that dataset was not analyzed further.

Biological-Community Composition within the Sampled Basins

The invertebrate community consisted of 16,499 individuals, representing 59 families, and ranged from 7 to 18 families per site (appendix 3). Only one family, Chironomidae (non-biting midges), was found at all 74 sites. Chironimidae accounted for 41.7 percent of the total relative abundance, followed by Hydropsychidae – net-spinning caddisflies (collected at 73 sites with a total relative abundance of 17.3 percent), and Baetidae – mayflies (collected at 71 sites with a total relative abundance of 10.2 percent). The three dominant invertebrate families accounted for 69.2 percent of the total relative abundance. Recent studies have shown that invertebrate family-level identification can be as effective as genus-level identification in relating stressors, such as nutrients, to the biological community (Carlisle and others, 2007). Many

genera of the Chironomidae and Hydropsychidae families are considered highly nutrient tolerant (Carlisle and others, 2007), are an important food source for fish, and are often found in enriched freshwater habitats. In addition, as members of the order Dipteran, Chironomidae, are often the dominant taxa in freshwater habitats with extreme environmental conditions (Ward, 1992). Hydropsychidae are often used as indicator species in water-quality assessments, because some genera are sensitive to pollution (Ward, 1992).

The headwater fish community (n=98) consisted of 16,896 individuals, representing 71 taxa, and ranged from 1 to 23 taxa per site (appendix 5). The creek chub (*Semotilus atromaculatus*), central stoneroller (*Campostoma anomalum*), western blacknose dace (*Rhinichthys obtusus*), bluntnose minnow (*Pimephales notatus*), white sucker (*Catostomus commersonii*), orangethroat darter (*Etheostoma spectabile),* johnny darter (*Etheostoma nigrum),* and striped shiner (*Luxilus chrysocephalus*) accounted for 52.8 percent of the number of individuals collected. The most abundant species collected were creek chub (collected at 89 sites with a total relative abundance of 15.2 percent), central stoneroller (collected at 52 sites with a total relative abundance of 10.4 percent), and western blacknose dace (collected at 40 sites with a total relative abundance of 9.3 percent). In addition, these species are moderately tolerant to increases in turbidity (Robison and Buchanan, 1984). The central stoneroller populations, tolerant to increased turbidity and stream temperatures, can explode in warm nutrient-rich systems characterized by abundant algal growth (Ohio Department of Natural Resources, 2009). The headwater-fish communities were dominated by nutrient-tolerant species (Meador and Carlisle, 2007).

The wadable fish community (n=117) consisted of 40,795 individuals, representing 117 taxa, and ranged from 4 to 40 taxa per site (appendix 5). The central stoneroller, bluntnose minnow, longear sunfish (*Lepomis megalotis*), striped shiner, bluegill (*Lepomis macrochirus*), rainbow darter (*Etheostoma caeruleum*), spotfin shiner (*Cyprinella spiloptera*), greenside darter (*Etheostoma blenniodes*), and sand shiner (*Notropis stramineus*) accounted for 50.8 percent of the number of individuals collected. The most abundant species collected were central stoneroller (collected at 116 sites with a total relative abundance of 13.3 percent), bluntnose minnow (collected at 101 sites with a total relative abundance of 7.9 percent) and longear sunfish (collected at 84 sites with a total relative abundance of 7.8 percent). As with the headwater fish community dataset, the wadable fish communities were dominated by nutrient-tolerant species. Two species, spotfin shiner and sand shiner, were highly tolerant of nitrate (Meador and Carlisle, 2007) and were dominant in the wadable, but not the headwater, sites. Two species, western blacknose dace and white sucker, which are highly tolerant of nutrients (Meador and Carlisle, 2007), were dominant in the headwater but not in the wadable sites. The dominance of the invertebrate- and fish-community composition by nutrient-tolerant taxa provides evidence that the streams sampled in the study are eutrophic at times throughout the year.

Distribution of the Stressor Variables

Mueller and Spahr (2006) found that streams within the Midwest, including Indiana, have some of the highest nutrient loadings in the United States. Although streams for this study were sampled during periods of stable low flow, which typically has lower nutrient levels (Lowe and others, 2008), many streams within the state were nutrient enriched, based on the trophic classification by Dodds and others (1998). The summary statistics show only slight differences between the one invertebrate and two fish datasets used in these analyses (table 2). Within all three datasets, concentrations of stressor variables ranged from 0.30 to 11 mg/L for TN and from 0.025 to 1.33 mg/L for TP (table 2). For the stressor variables periphyton and seston CHL*a*, concentrations ranged from 2.9 to 768 mg/m^2 and from 0.37 to 42 µg/L, respectively. Turbidity ranged from 0.8 to 65.4 NTU (table 2).

Relations between the Stressor Variables

The strongest and most frequently significant correlations (r_s) between the stressor variables were between concentrations of TP and turbidity for all three datasets (table 3). Turbidity is a measure of the suspended solids within the water column and includes clay particles and seston algae. The strong correlations between TP and turbidity reflect the propensity for phosphorus to bind with clay particles (Hem, 1989). The concentrations of TP and mean seston CHL*a* were strongly correlated in the invertebrate and fish wadable sites datasets, and this was partially a function of the presence of both chlorophyll and phosphorus in algae (Van Nieuwenhuyse and Jones, 1996; Morgan and others, 2006). Both of these factors may explain the significant correlations between seston CHL*a* and turbidity in the fish headwater and wadable sites. Turbidity was inversely related to periphyton CHL*a* in the fish headwater dataset, a finding that concurs with previous studies (Lowe and others, 2008). Periphyton CHL*a* was positively correlated with TN in the fish headwater and wadable datasets and negatively correlated with TP in the invertebrate dataset. Concentrations of TN and TP were weakly positively correlated in the fish headwater and wadable site datasets.

Table 2. Summary statistics for stressor variables used in the breakpoint analysis study, 2001–05.

[TN, total nitrogen; mg/L, milligrams per liter; TP, total phosphorus; CHL*a*, chlorophyll *a*; mg/m^2, milligrams per square meter; µg/L, micrograms per liter; NTU, Nephelometric turbidity units; n, number of samples]

Statistic	TN[1] (mg/L)	TP[1] (mg/L)	Periphyton CHL*a*[1] (mg/m^2)	Seston CHL*a*[1] (µg/L)	Turbidity[1] (NTU)
Invertebrate sites (n=74)					
Minimum	0.79	0.025	7.3	0.50	3.7
25th percentile	3.0	.052	37	2.1	9.9
Median	3.6	.078	62	3.2	14.1
Mean	3.8	.117	76	4.5	17.1
Maximum	8.0	1.13	310	26	65.4
Fish headwater sites (n=98)					
Minimum	0.39	0.025	2.9	0.37	0.8
25th percentile	.94	.037	20	1.3	7.0
Median	2.7	.065	48	3.3	10.3
Mean	3.4	.105	71	3.7	14.1
Maximum	11	1.33	540	26	52.4
Fish wadable sites (n=117)					
Minimum	0.30	0.025	4.7	0.66	2.7
25th percentile	1.7	.050	32	2.2	9.2
Median	3.4	.071	60	3.5	12.5
Mean	3.2	.091	96	5.5	15.1
Maximum	8.2	.417	768	42	65.4

[1] The mean of all the values was used in this analysis.

Table 3. Spearman rho correlations among the stressor variables used in the breakpoint analysis study, 2001–05.

[TN, total nitrogen; mg/L, milligrams per liter; TP, total phosphorus; CHL*a*, chlorophyll *a*; mg/m^2, milligrams per square meter; µg/L, micrograms per liter; NTU, Nephelometric turbidity units; n, number of samples; bold text, $p < 0.05$]

Stressor	TN[1] (mg/L)	TP[1] (mg/L)	Periphyton CHL*a*[1] (mg/m^2)	Seston CHL*a*[1] (µg/L)	Turbidity[1] (NTU)
Invertebrate sites (n=74)					
TN (mg/L)		0.219	0.184	-0.207	-0.071
TP (mg/L)	0.219		**-.315**	**.311**	**.454**
Periphyton CHL*a* (mg/m^2)	.184	**-.315**		.000	-.122
Seston CHL*a* (mg/L)	-.207	**.311**	.000		.204
Turbidity (NTU)	-.071	**.454**	-.122	.204	
Fish headwater sites (n=98)					
TN (mg/L)		**0.265**	**0.214**	-0.014	**0.258**
TP (mg/L)	**0.265**		-.166	.044	**.474**
Periphyton CHL*a* (mg/m^2)	**.214**	-.166		-.035	**-.253**
Seston CHL*a* (mg/L)	-.014	.044	-.035		**.240**
Turbidity (NTU)	**.258**	**.474**	**-.253**	**.240**	
Fish wadable sites (n=117)					
TN (mg/L)		**0.236**	**0.221**	-0.034	0.172
TP (mg/L)	**0.236**		-.109	**.457**	**.473**
Periphyton CHL*a* (mg/m^2)	**.221**	-.109		.004	-.181
Seston CHL*a* (mg/L)	-.034	**.457**	.004		**.310**
Turbidity (NTU)	.172	**.473**	-.181	**.310**	

[1] The mean of all the values was used in this analysis.

Comparison of Trophic Classification for the Stressor Variables

Dodds and others (1998) classified trophic conditions as oligotrophic, mesotrophic, and eutrophic on the basis of criteria for TN, TP, and CHL*a* (periphyton and seston) using existing data. Based on the Dodds' criteria, streams in this study were most often classified as eutrophic for TN. In particular, the streams in the invertebrate dataset were almost all considered eutrophic based on Dodds' classification for TN. Based on the Dodds' criteria for TN, 94.6 percent of the invertebrate sites, 67.7 percent of the fish headwater sites, and 78.6 percent of the fish wadable sites would be classified as eutrophic (fig. 2). The eutrophic classifications for TP were similar among the three datasets: 42.9 percent of the invertebrate sites, 39.8 percent of the fish headwater sites, and 46.2 percent of the fish wadable sites (fig. 3). For periphyton CHL*a*, 43.2 percent of the invertebrate sites, 35.7 percent of the fish headwater sites, and 41.0 percent of the fish wadable sites would be classified as eutrophic (fig. 4).

Of all the stressor variables examined based on the trophic classification of Dodds and others (1998), the least number of streams would be considered eutrophic using seston CHL*a* data. Only about 1 percent of the fish wadable site data and none of the invertebrate and headwater fish site data would be classified as eutrophic based on seston CHL*a* (fig. 5). Part of the reason for the low number of streams classified as eutrophic based on seston CHL*a* in this study is that the large river streams were not included in the analysis because of the sample size. Typically, seston CHL*a* concentrations are greater in large rivers than in small rivers (Vannote and others, 1980; Lowe and others, 2008). Turbidity and seston CHL*a* concentrations are closely related (Lowe and others, 2008), and as with seston CHL*a*, few streams in this study would be considered impacted based on turbidity. Based on a biological impact classification for turbidity as used by Nelson (1993), less than 3 percent of the sites were impacted adversely by turbidity (fig. 6). Part of the reason for the low turbidity values in this study was that samples were only collected during stable low flow conditions to minimize the scour effect on algae from elevated streamflow. Of all the stressor variables, seston CHL*a* and turbidity could be the most misleading when assessing the trophic level of streams, because headwater and wadable streams generally have lower seston CHL*a* and turbidity. This is especially true for study designs that sample only during stable low flow conditions, but the alternative design of collecting samples during elevated flow would underestimate periphyton CHL*a* because of scour. These findings support the use of multiple lines of evidence when assessing the trophic levels in streams.

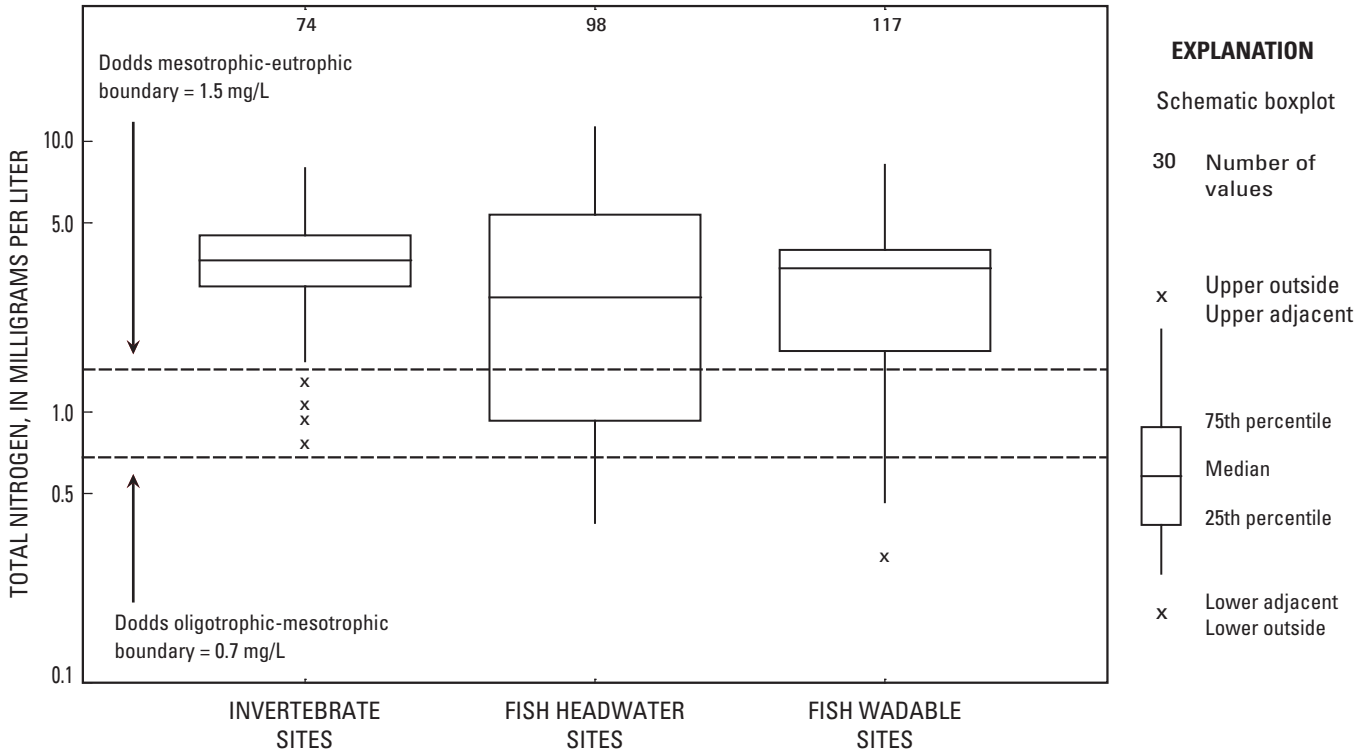

Figure 2. Distribution of the mean total nitrogen log transformation of concentrations compared to Dodd and others (1998) trophic classifications in the three selected datasets used in the breakpoint analysis study, 2001–05.

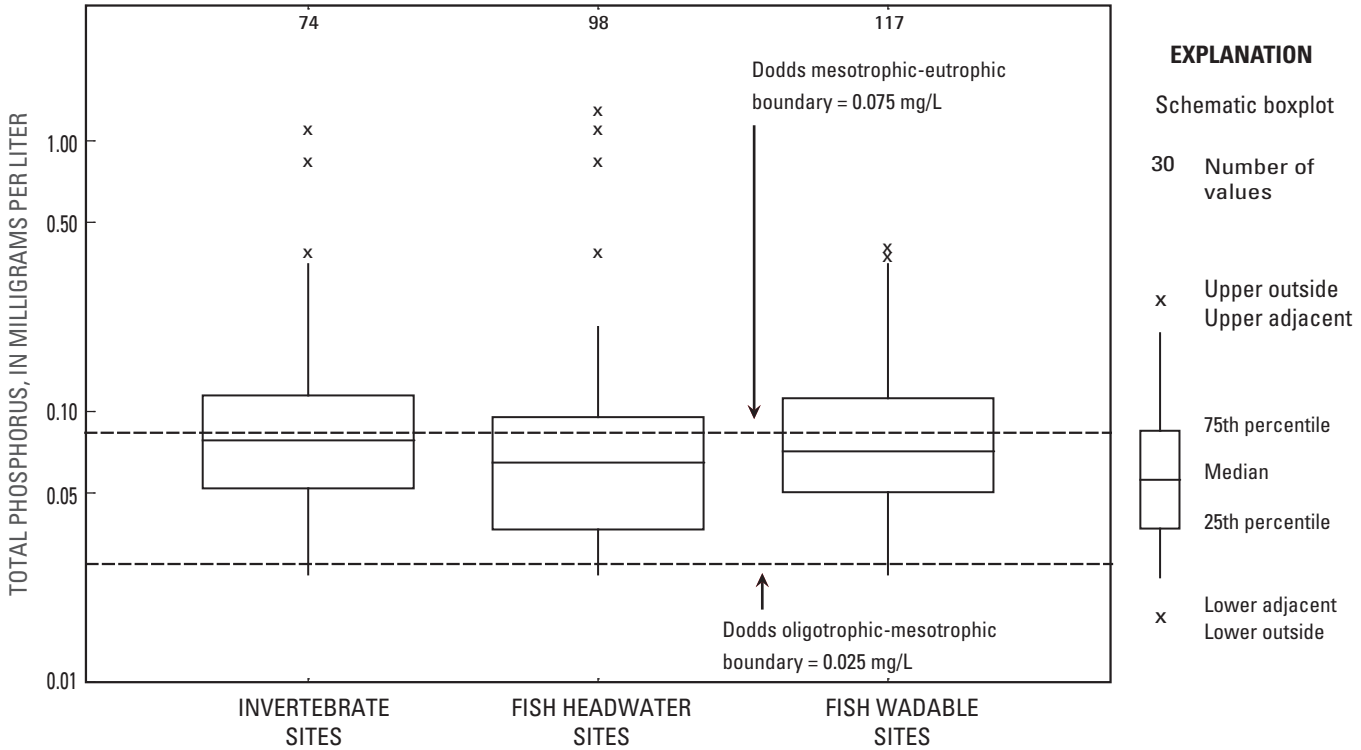

Figure 3. Distribution of the mean total phosphorus log transformation of concentrations compared to Dodds and others (1998) trophic classifications in the three selected datasets used in the breakpoint analysis study, 2001–05.

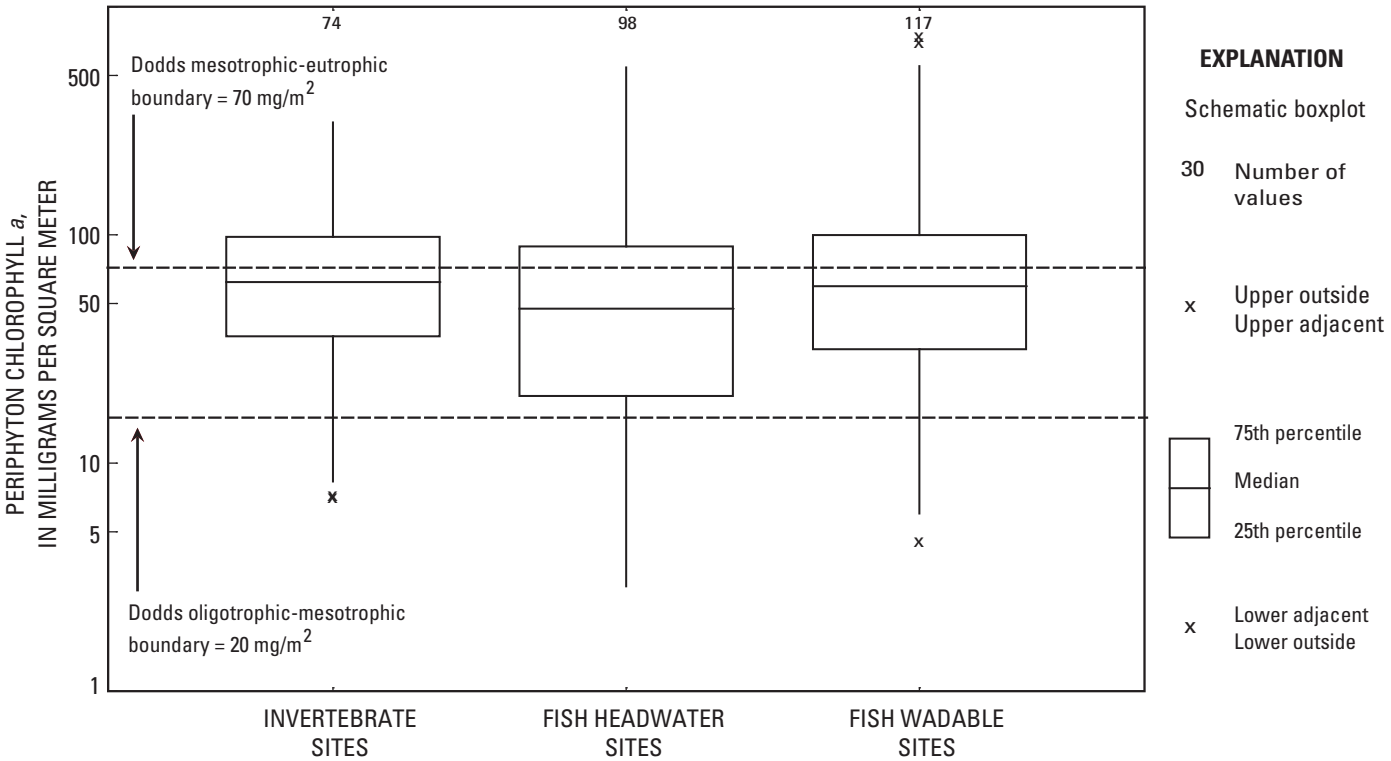

Figure 4. Distribution of the mean periphyton chlorophyll *a* log transformation of concentrations compared to Dodds and others (1998) trophic classifications in the three selected datasets used in breakpoint analysis study, 2001–05.

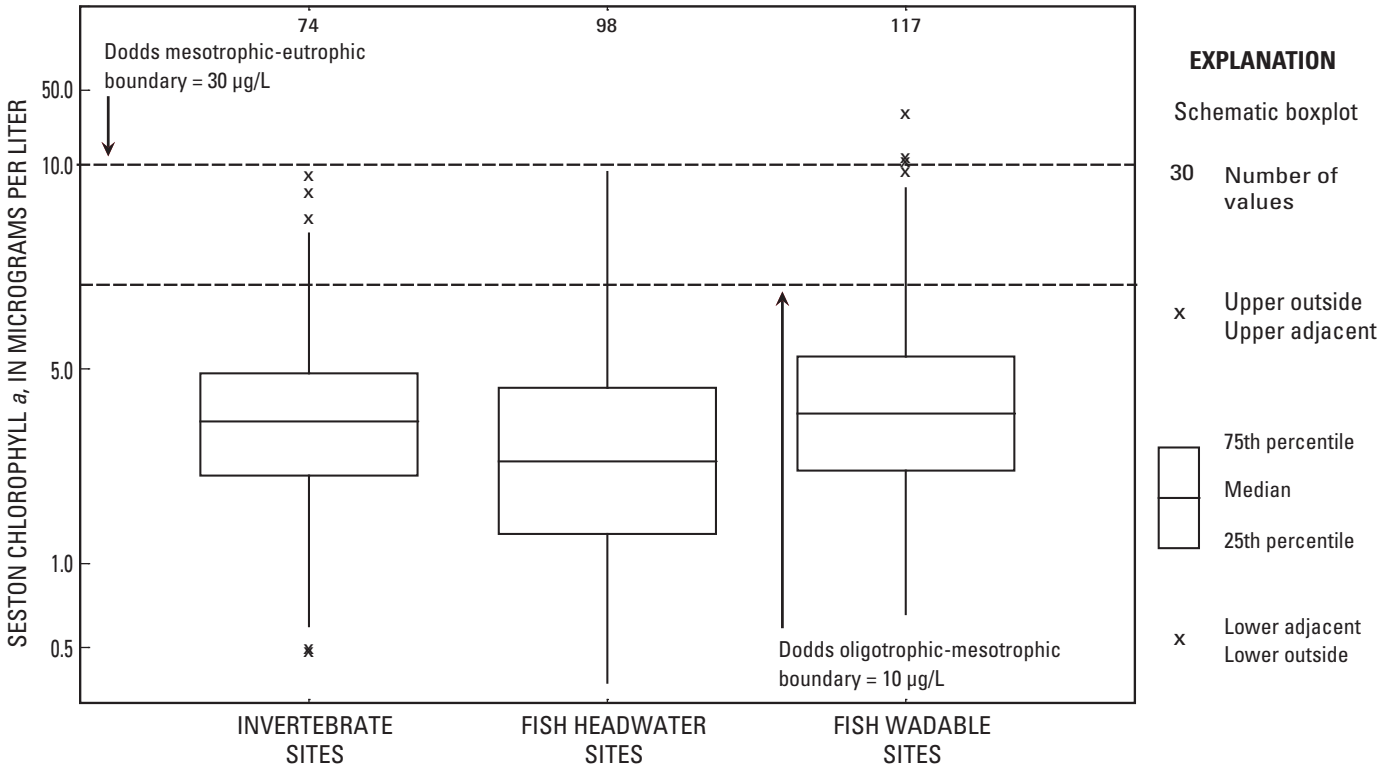

Figure 5. Distribution of the mean seston chlorophyll *a* log transformation of concentrations compared to Dodds and others (1998) trophic classifications in the three selected datasets used in the breakpoint analysis study, 2001–05.

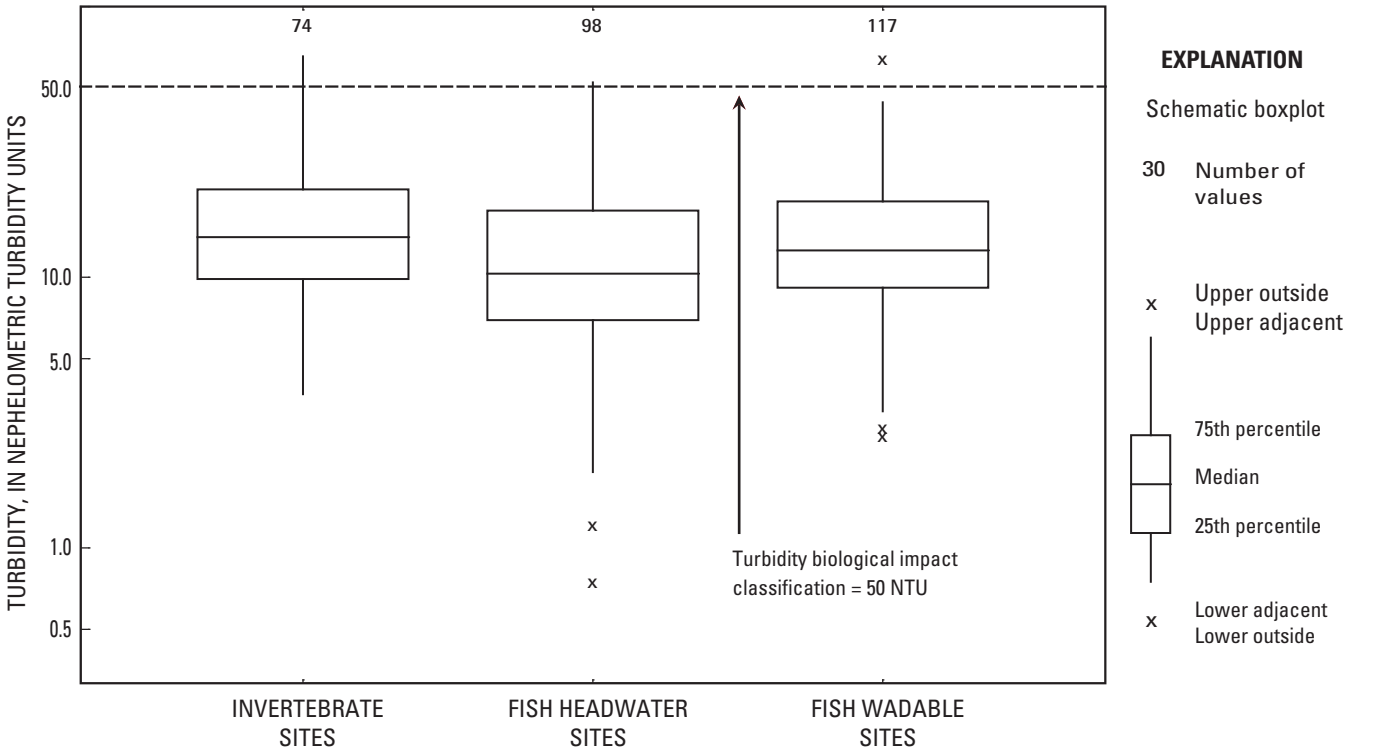

Figure 6. Distribution of the mean turbidity log transformation of concentrations compared with turbidity biological impact classifications (Nelson, 1993) in the three selected datasets used in the breakpoint analysis study, 2001–05.

Biological Breakpoints of the Stressor Variables and Implications for Developing Nutrient Criteria

Regression tree analysis was conducted on the most statistically and ecologically significant stress-response relations. The strongest and most ecologically significant of these relations and the breakpoints are listed in table 4. The strongest correlations $|r_s|$ included both invertebrate and fish measures.

For mean TN, the median breakpoints of the three most significant biological response variables ranged from 2.4 to 3.3 mg/L. The most significant relation was between mean TN to percentage of biomass composed of white suckers in the fish headwater dataset (table 4 and fig. 7). In this positive relation, mean TN increased as the percentage of biomass composed of white suckers increased with a median breakpoint of 2.9 mg/L (fig. 7). White suckers are highly tolerant of nutrients, specifically, nitrate and TP (Meador and Carlisle, 2007), and the relative percentage of white suckers would be expected to increase with high nutrients. The TN breakpoints presented in this study fall between one-half to one standard deviation greater than the statewide mean of 1.9 mg/L for TN, which was based on data collected from 1996 to 2005 (Steven A. Newhouse, Indiana Department of Environmental Management, oral commun., 2008). The mean TN concentration associated with the biological breakpoints in this study (table 4) is high compared with most TN breakpoint values within the Midwest. Specifically, the breakpoints for this study are more than two times higher than the TN breakpoint of 1.2 mg/L to seston CHL*a* found in a Wisconsin study (Robertson and others, 2006). In addition, the median breakpoint for TN of 2.9 mg/L found in this study is almost two times higher than the levels Dodds and others (1998) described as eutrophic.

Table 4. Breakpoint summary for the stressor variables used in the breakpoint analysis study, 2001–05.

[n, number of sites in analysis; TN, total nitrogen; mg/L, milligrams per liter; TP, total phosphorus; CHL*a*, chlorophyll *a*; mg/m^2, milligrams per square meter; µg/L, micrograms per liter; NTU, Nephelometric turbidity units]

Data site type	Biological response variable	Stressor variable to biological attribute Spearman rho	n	Median bootstrap breakpoint	Bootstrap confidence interval
		TN (mg/L)			
Invertebrate	Hydroptilidae (percent relative abundance)	0.337	40	3.3	2.3–4.0
Fish headwater	White sucker (percentage of biomass)	.316	50	2.9	2.8–5.9
Fish wadable	Number of pioneer taxa	.314	114	2.4	2.0–4.0
		TP (mg/L)			
Invertebrate	Percentage of abundance composed of molluscs and crustaceans	0.422	38	0.090	0.057–0.110
Fish headwater	Striped shiner (percentage of biomass)	-.337	34	.042	.042–.077
Fish wadable	Green sunfish (percent relative abundance)	.296	88	.129	.099–.176
		Periphyton CHL*a* (mg/m^2)			
Invertebrate	Percentage of total richness composed of shredders	-0.519	22	60	50.4–87.8
Fish headwater	Number of sensitive taxa	.390	56	68	18.1–99.4
Fish wadable	Largemouth bass (percent relative abundance)	-.266	31	54	19.1–58.6
		Seston CHL*a* (µg/L)			
Invertebrate	Richness composed of filtering-collectors	0.388	74	4.7	3.0–7.4
Fish headwater	Bluegill (g)	.367	35	4.5	4.3–4.9
Fish wadable	Number of sunfish species	.330	115	7.5	5.0–10.3
		Turbidity (NTU)			
Invertebrate	Hydropsychidae (percent relative abundance)	0.335	70	14.1	12.4–22.9
Fish headwater	Number of sensitive species	-.422	56	16.1	6.7–31.0
Fish wadable	Spotted sucker (percentage of biomass)	.499	29	14.6	12.2–19.0

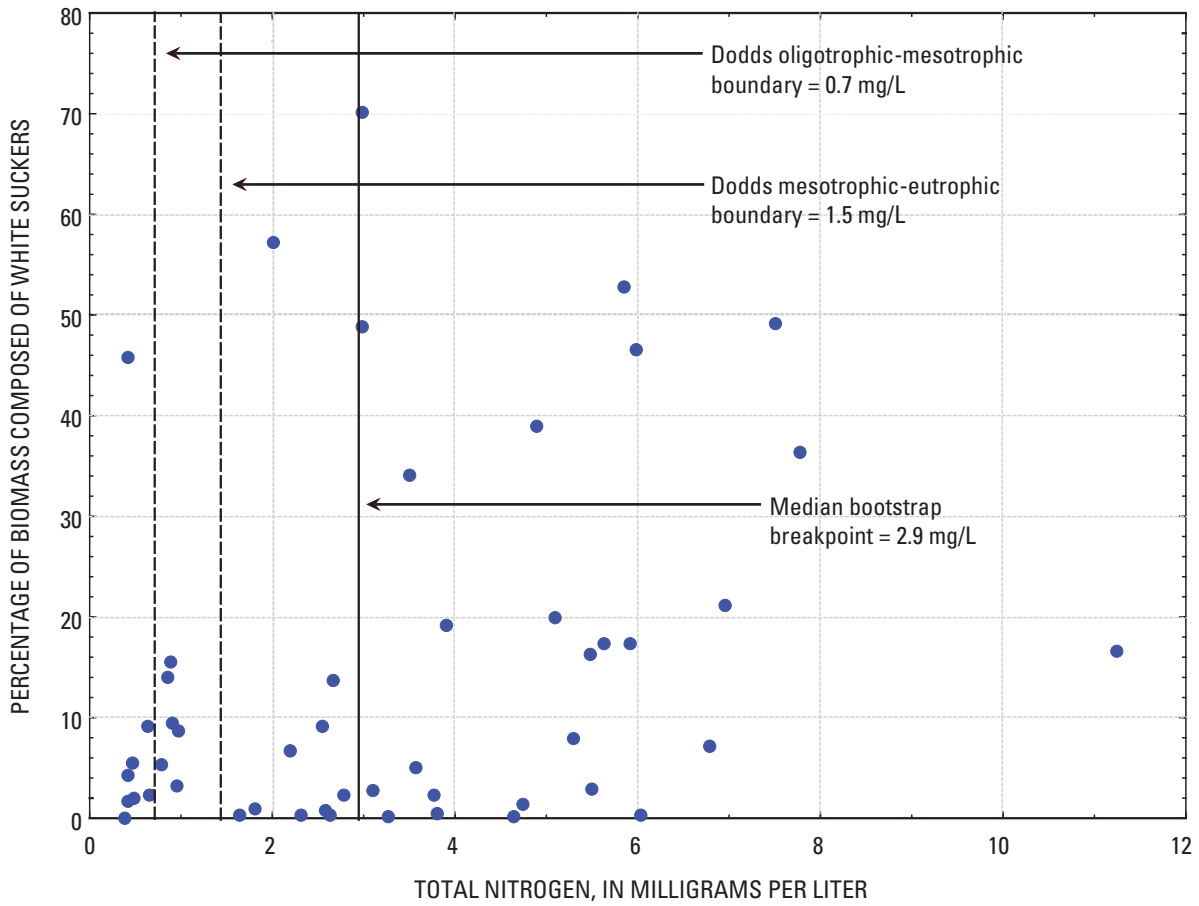

Figure 7. Scatter plot of mean total nitrogen concentrations to the percentage of biomass composed of white suckers at headwater fish sites for the breakpoint analysis study, 2001–05. (Trophic classifications from Dodds and others, 1998.)

Of the five stressor variables examined, all the TN median breakpoints would be classified as eutrophic using the method of Dodds and others (1998). These findings indicate that a mean TN concentration target of 2.9 mg/L might be an appropriate attribute for showing changes in the fish community from hypereutrophic to eutrophic stream classification, but might not be appropriate for showing change to meso- or oligotrophic conditions (fig. 7). In the nutrient-saturated Midwest, this may be a possible first target for showing improvements in the hypertrophic headwater streams. This relation is another line of evidence that indicates many streams within the dataset are eutrophic.

For mean TP, the median breakpoints of the three most significant biological response variables ranged from 0.042 to 0.129 mg/L. The most significant relation was between mean TP to the percentage of biomass composed of striped shiners in the fish headwater dataset (table 4 and fig. 8). In this negative relation, as the mean TP increased the percentage of biomass composed of striped shiners decreased with a median breakpoint of 0.042 mg/L (fig. 8). Striped shiners are intolerant of high phosphorus levels (Meador and Carlisle, 2007). Two of the median breakpoints for TP found in this study are higher than the TP breakpoint of 0.07 mg/L found in a Wisconsin study (Robertson and others, 2006), but the striped shiner metric is lower than the value found in Wisconsin. The breakpoint in this study falls between one-half and one standard deviation below the statewide mean TP of 0.09 mg/L, which was based on TP data collected from 1996 to 2005 (Steven A. Newhouse, Indiana Department of Environmental Management, oral commun., 2008). These findings indicate that a TP concentration target of 0.042 mg/L based upon striped shiner biomass might be an appropriate concentration for showing changes from eutrophic or mesotrophic stream classification to oligotrophic conditions for headwater streams.

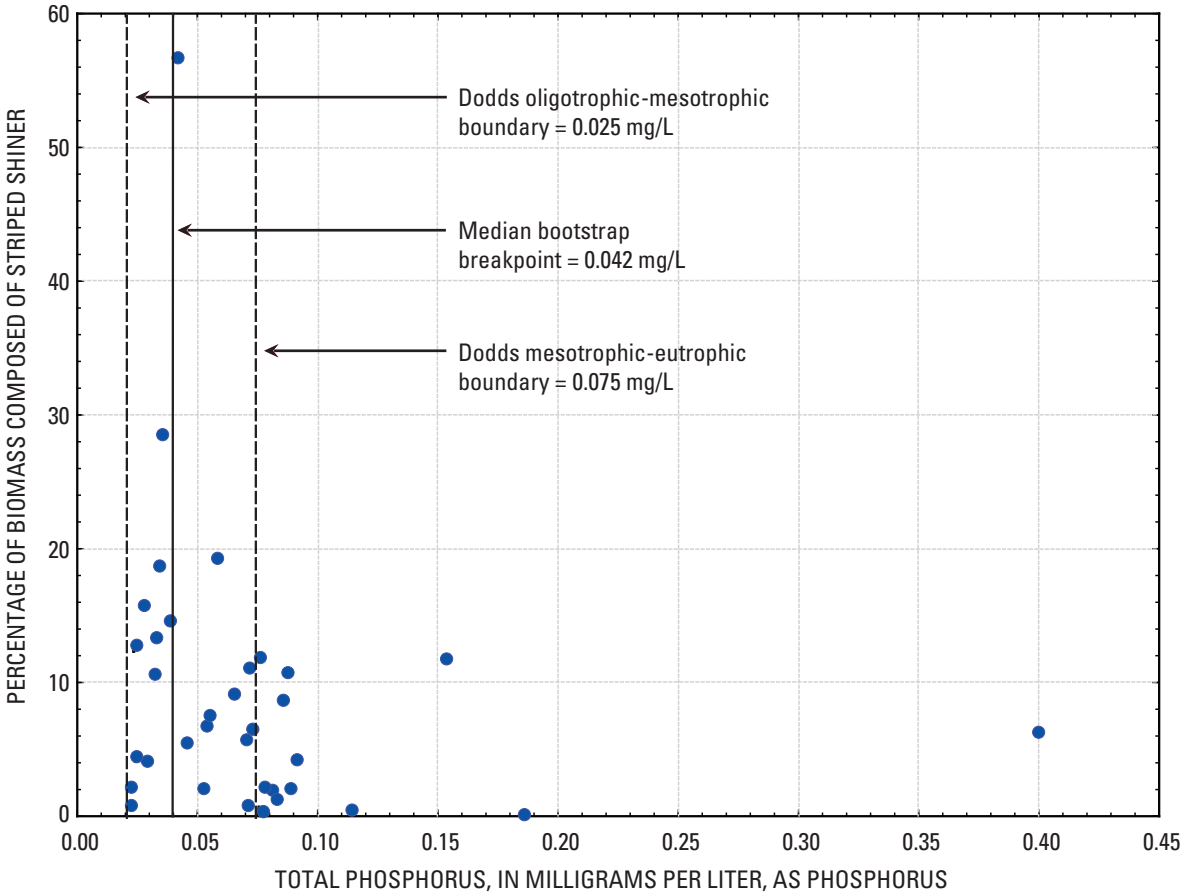

Figure 8. Scatter plot of mean total phosphorus concentrations to the percentage of biomass composed of striped shiners at headwater fish sites for the breakpoint analysis study, 2001–05. (Trophic classifications from Dodds and others, 1998.)

For mean periphyton CHL*a*, the median breakpoints of the three most significant biological response variables ranged from 54 to 68 mg/m^2 (table 4). The most significant relation was between mean periphyton CHL*a* to percentage of total richness composed of shredders in the invertebrate dataset (table 4 and fig. 9). In this negative relation, as the mean periphyton CHL*a* increased the percentage of total richness composed of shredders decreased with a median breakpoint of 60 mg/m^2 (fig. 9). Although this relation is statistically significant, the ecological significance of this relation is most likely a function of habitat and stream size, and not periphyton CHL*a* concentrations. As stream size increases more light reaches the stream and periphyton CHL*a* increases (Vannote and others, 1980). Shredders are widely considered to be of central importance in invertebrate communities because they process coarse detritus (Ward, 1992). Headwater streams typically have more closed canopy, a source of coarse detritus, than wadable streams (Vannote and others, 1980), and as stream size increases the source of detritus decreases; therefore, the role of shredders decreases. Although the strongest breakpoint for mean periphyton CHL*a* (table 4) was the invertebrate dataset, the fish headwater (number of sensitive taxa) and fish wadable (percentage of relative abundance of largemouth bass) datasets had similar breakpoints, 60 and 54, respectively. All three of these values are close to the mesotrophic/eutrophic boundary of 70 mg/m^2 found by Dodds and others (1998). Although a possible habitat signature influence was observed, these findings indicate that a target of 60 mg/m^2 for periphyton CHL*a* or 70 mg/m^2 for the Dodds and others (1998) classification for eutrophic systems might be appropriate for showing changes in the invertebrate community from eutrophic to mesotrophic stream classification for headwater and wadable streams.

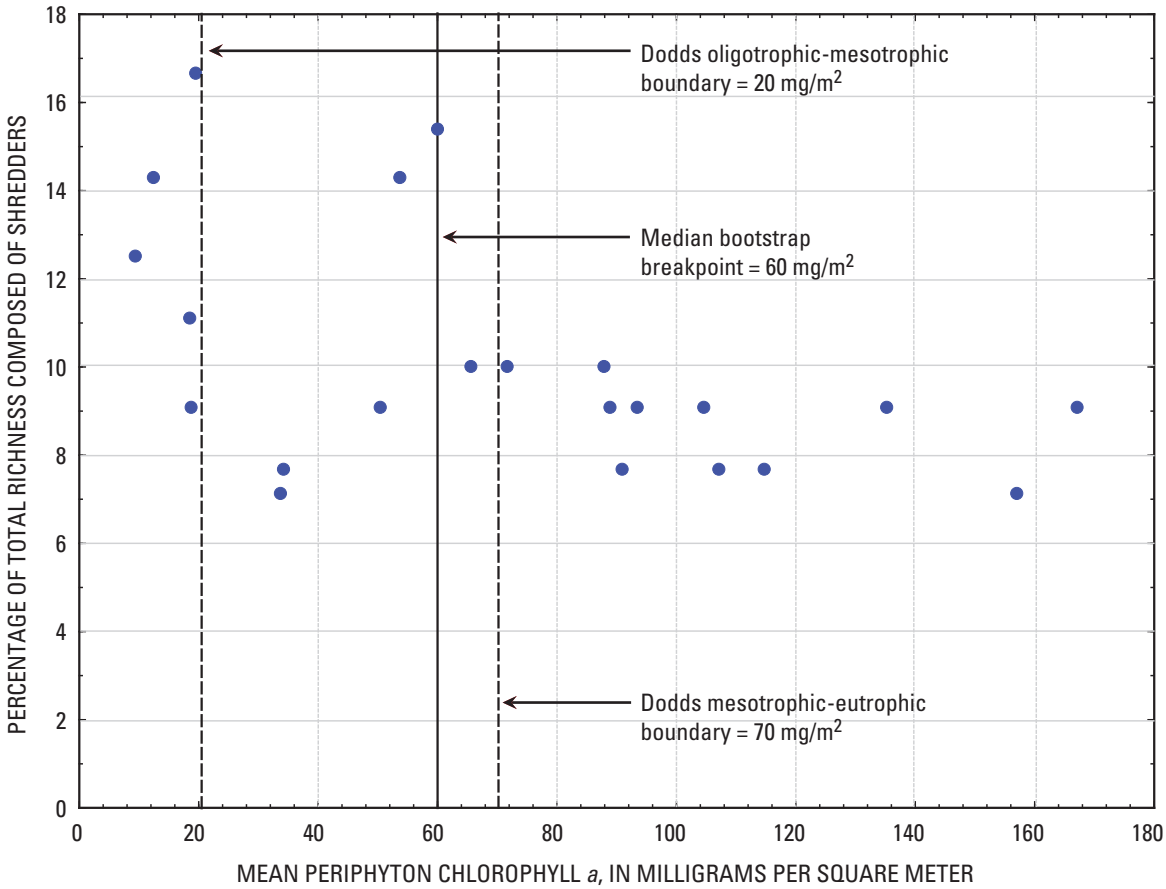

Figure 9. Scatter plot of mean periphyton chlorophyll *a* concentrations to the percentage of total richness composed of shredders at invertebrate sites for the breakpoint analysis study, 2001–05. (Trophic classifications from Dodds and others, 1998.)

For mean seston CHL*a*, the median breakpoints of the three most significant biological response variables ranged from 4.5 to 7.5 µg/L. The most significant relation was between mean seston CHL*a* to bluegill biomass in the fish headwater dataset (table 4 and fig. 10). In this positive relation, as mean seston CHL*a* increased the biomass of bluegill increased with a median breakpoint of 4.5 µg/L (fig. 10). The ecological significance of this relation is not readily discernible. In headwater streams bluegills are primarily juveniles, they typically feed on small crustaceans and insects, and they are intolerant of continuous high turbidity and siltation (Robison and Buchanan, 1984). Lowe and others (2008) noted that as basin size increased, seston CHL*a* and turbidity increased in Indiana streams. In this study, seston CHL*a* and turbidity were significantly related (table 3), and more than 75 percent of the seston CHL*a* samples collected between 2001–05 had values less than 6 µg/L (Lowe and others, 2008). It indicates that the positive relation might be because the data heavily favored low seston CHL*a* concentrations, and there may have been an eventual negative relation if stream size increased or a larger seston CHL*a* gradient was sampled. Lowe and others (2008) found that 75 percent of the seston CHL*a* samples had values less than 6 µg/L which is five times lower than Dodds and others (1998) eutrophic level. However, Dodds and others (1998) based their results on a dataset collected during the growing season from Van Nieuwenhuyse and Jones (1996) that included many larger streams with drainage areas greater than 1,000 km^2, and larger streams were not included in this study. Seston CHL*a* values are greater in larger streams (Vannote and others, 1980; Lowe and others, 2008), and in streams larger than 1,000 km^2 the median seston CHL*a* value of 22.1 µg/L was about 10 times greater than the headwater or wadable median seston CHL*a* values (Lowe and others, 2008). Consequently, the observed increase in the bluegill biomass in this study could be the result of stream size, specifically the use of headwater and wadable sites for this analysis, and not a direct link to increased seston CHL*a*. These findings indicate that a seston CHL*a* value between the 4.5 µg/L breakpoint for biomass of bluegill metric and the Dodds and others (1998) oligotrophic classification of 10 µg/L might be an appropriate concentration for showing changes to oligotrophic conditions in headwater streams. Based on these findings, multiple lines of evidence should be considered in analysis of whether TN, TP, periphyton CHL*a*, or turbidity indicate more eutrophic or impacted conditions.

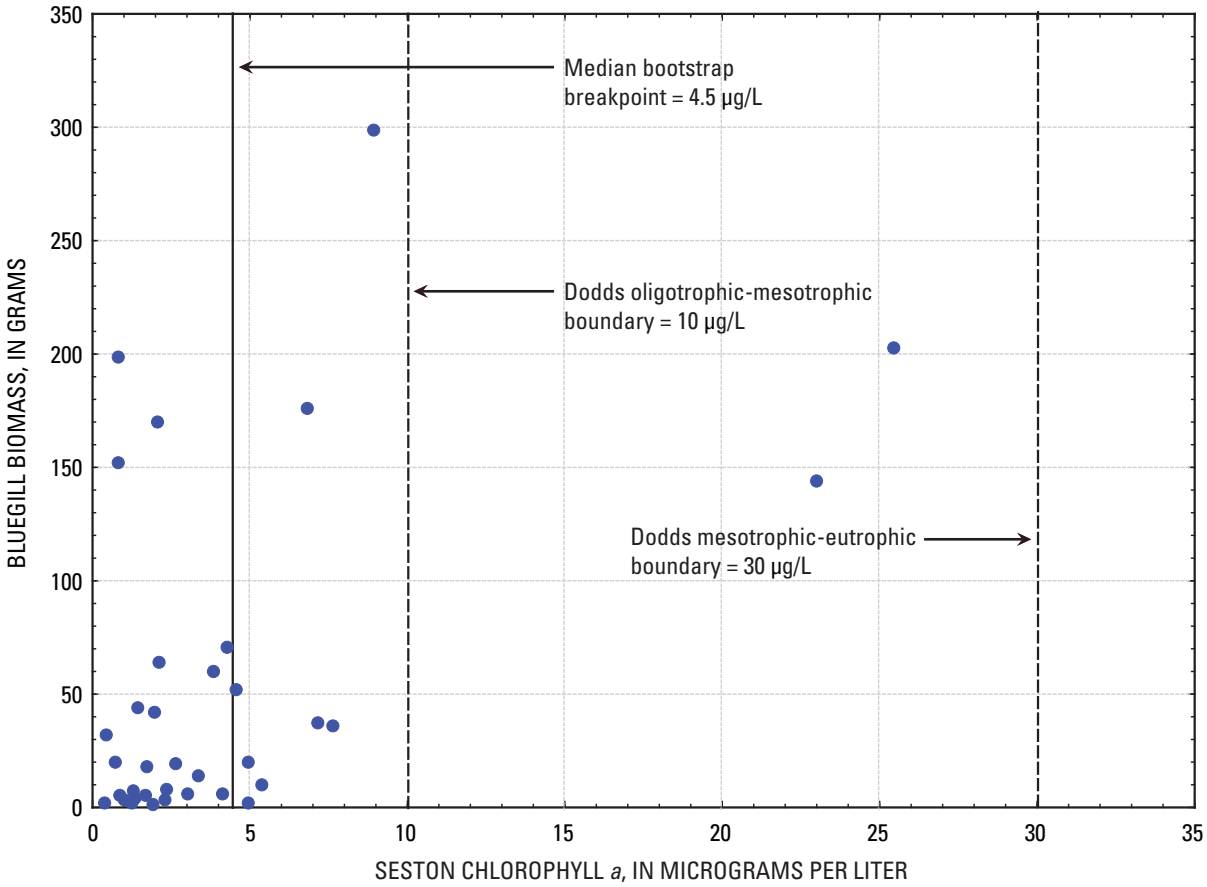

Figure 10. Scatter plot of mean seston chlorophyll *a* concentrations to bluegill biomass at headwater fish sites for the breakpoint analysis study, 2001–05. (Trophic classifications from Dodds and others, 1998.)

For mean turbidity, the median breakpoints of the three most significant biological response variables ranged from 14.1 to 16.1 NTU. The most significant relation was between mean turbidity to percentage of biomass composed of spotted suckers in the fish wadable dataset (table 4 and fig. 11). In this positive relation, as the mean turbidity increased the percentage of biomass composed of spotted suckers increased with a median breakpoint of 14.6 NTU (fig. 11). Increases in turbidity can result in detrimental impacts to fish reproduction and larval survival when concentrations are greater than 50 NTU (Nelson, 1993). In contrast, the positive relation in this study indicates that as turbidity increases there is an increase in spotted suckers. This is unexpected because spotted suckers are thought to be particularly intolerant of turbid waters (Trautman, 1981) or slightly turbid waters (Robison and Buchanan, 1984); but because the turbidity values from this study were low, less the 50 NTU, the relation indicates that the percentage of biomass composed of spotted suckers would increase until the turbidity reaches 50 NTU, followed by a subsequent decrease. This finding is most likely because of the significant positive correlation between turbidity and TP; phosphorus can bind with clay and provide nutrients essential for algal growth, one food source for spotted suckers. In addition, spotted suckers can be found in all sizes of rivers, including the Mississippi River (Iowa Department of Natural Resources, 2009). However, since large rivers, which typically have higher turbidity (Lowe and others, 2008) were not included in this analysis, there may have been a decrease in the percentage biomass of spotted suckers if the data from the large river sites had been included in this analysis, because large river systems typically have turbidity concentrations greater than 50 NTU's which has been shown to impact fish reproduction. Since about 97 percent of the turbidity samples in this analysis reported turbidity concentrations less than the 50 NTU and this analysis indicates that a target breakpoint of 16.1 NTU for turbidity, it was concluded that the calculated breakpoint of 16.1 NTU would lack the sensitivity to show changes in the fish community in wadable streams; but use of Nelson's (1993) turbidity impact classification of 50 NTU would protect aquatic biota from turbidity impacts.

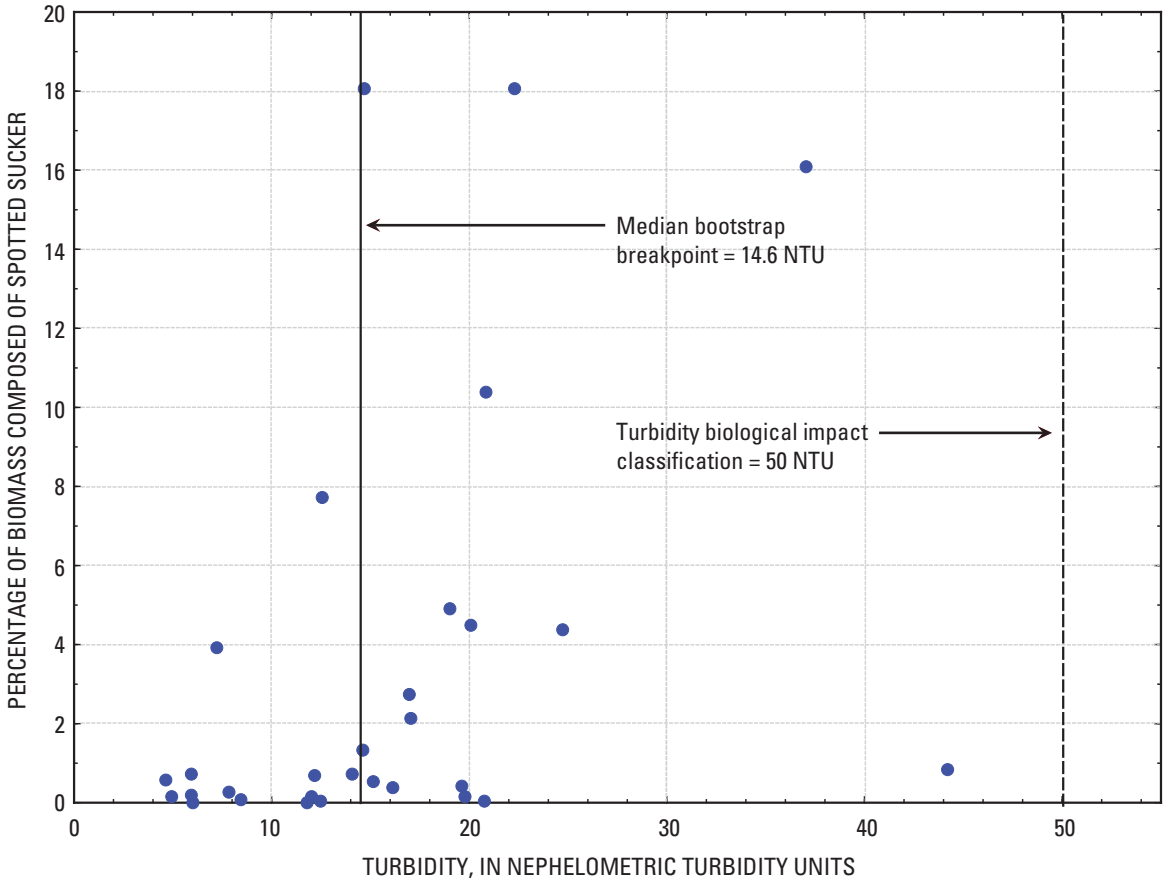

Figure 11. Scatter plot of mean turbidity concentrations to the percentage of biomass composed of spotted suckers at wadable fish sites for the breakpoint analysis study, 2001–05. (Turbidity biological impact classification from Nelson, 1993.)

Overall, the results from this study indicate some breakpoints between the stressor and the response variables could be used to support the development of nutrient criteria. Trophic classifications from Dodds and others (1998) may also be appropriate, because even though they were developed based on only chemistry data, breakpoints in the biological communities appear to generally follow these trophic classifications. It is apparent from the relatively weak correlations between the stressor and response variables and lack of a gradient for some of the stressor variables in this study (seston CHL*a* and turbidity), that the assessment of the true nutrient condition within a stream or the development of nutrient criteria needs to rely on multiple lines of evidence that includes measures of nutrients (TN and TP), algal biomass (periphyton and seston CHL*a*), turbidity, and biological attributes. Development of a nutrient Index of Biotic Integrity that would incorporate fish and invertebrate attributes and metrics indicative of oligotrophic, mesotrophic, and eutrophic levels to show changes in the biological communities also could aid the assessment of stream nutrient conditions.

Summary

Nutrients are essential to the development, health, and diversity of plants and animals in surface waters, yet excessive inputs of nutrients into streams have potential human-health, economic, and ecological consequences. Indiana is part of the nutrient-rich Midwest that leads the nation in corn and soybean production, which require the use of significant amounts of nutrients—primarily from fertilizer and manure for the production of these crops. Streams sampled within the Midwest have some of the highest nutrient concentrations and loading in the United States. Earlier studies in Indiana found weak relations between nutrients and algal biomass and that other indicators, such as biological community attributes and metrics, could be helpful in developing nutrient criteria for streams in such nutrient-rich regions.

Water chemistry and biological community data were collected from 321 sites from 2001 through 2005 to determine if the changes among biological species and attributes were statistically related to changes in stressor (TN, TP, periphyton and seston CHL*a*, and turbidity) variables. Because of the typically weak relations among the stressor and response variables, statistical methods were used to eliminate biological stressors that could override relations of interest. This included removing sites that were biologically impaired by other known factors, removing outliers greater that two standard deviations of the mean, and removing sites from the biological datasets when a species of interest was not collected because it could not be confirmed that the species was from the site in response to the stressor variable of interest. The invertebrate and fish species and attributes were related to stressor variables to determine the most statistically and ecologically significant stressor-response relations for each community. Breakpoint analysis was used for the most statistically significant and ecologically relevant relations to find the concentration of the stressor variables where the greatest change occurred with the biological species and community attributes and metrics.

Within all three datasets, concentrations of stressor variables ranged from 0.30 to 11 mg/L for TN and from 0.025 to 1.33 mg/L for TP. For the stressor variables periphyton and seston CHL*a*, concentrations ranged from 2.9 to 768 mg/m^2 and from 0.37 to 42 µg/L, respectively. Turbidity concentrations ranged from 0.8 to 65.4 NTU. Most streams would be classified as eutrophic due to TN, and the least number of streams would be classified as eutrophic due to seston CHL*a*. Of all the stressor variables, seston CHL*a* could be the most misleading when assessing the trophic level of streams, especially at headwater and wadable sites.

In this study, the relative total abundance of invertebrates was dominated by Chironimidae, 41.7 percent, Hydropsychidae, 17.3 percent, and Baetidae, 10.2 percent. Many genera of the Chironomidae and Hydropsychidae families are considered highly nutrient tolerant. The percentage of richness composed of shredders and filtering-collectors were the only invertebrate attributes to be statistically related to periphyton and seston CHL*a*, respectively. Of the three dominant invertebrate families, only Hydropsychidae was found to be related to a response variable (turbidity). Turbidity and TP were found to be correlated, and thus it was expected that a positive relation between these two variables would be found because turbidity concentrations were well below a level that would impact biological organisms. The two other invertebrate families, Hydroptilidae (microcaddisflies or purse-case caddisflies) and Tipulidae (crane flies), were found to be statistically related to nutrients, TN and TP, respectively.

This study found that the relative total abundance in the fish communities were dominated by algivores and nutrient-tolerant species, specifically central stonerollers, 13.3 percent, creek chubs, 9.9 percent, and bluntnose minnow, 9.3 percent. Although they are not the dominant taxa, bluegill, spotted sucker, white sucker, and striped shiner species were statistically significant with the stressor variables.

The breakpoint for TN indicates that a target concentration of 2.9 mg/L could be an appropriate attribute to show changes in the biomass of white suckers from hypereutrophic to eutrophic stream conditions. The breakpoint for TP indicates a target concentration of 0.042 mg/L based upon striped shiner biomass might be an appropriate concentration to show changes from eutrophic or mesotrophic stream classification to oligotrophic conditions for headwater streams. The breakpoint for mean periphyton CHL*a* indicates that a target concentration of around 60 mg/m^{2,} found in this study, to 70 mg/m^{2,} based on Dodds and others (1998) eutrophic classifications, might be an appropriate attribute to show changes in the invertebrate community from eutrophic to mesotrophic stream classification for headwater and wadable streams. The breakpoint for mean seston CHL*a* indicates a target concentration between the 4.5 µg/L breakpoint for biomass of bluegill metric and 10 µg/L, from Dodds and others (1998) oligotrophic classification, might be an appropriate concentration to show changes to oligotrophic conditions in headwater streams. Based on these findings, multiple lines of evidence should be considered during assessments of whether or not TN, TP, periphyton CHL*a*, or turbidity indicate more eutrophic or impacted conditions. The breakpoints for mean turbidity lack the sensitivity to show changes in headwater or wadable streams, and the Nelson (1993) turbidity biological impact classification would provide a starting point to develop turbidity criteria.

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Table 1. Location of Indiana Department of Environmental Management Watershed Monitoring Program sampling sites used in the breakpoint analysis study, 2001–05.

[USGS, U.S. Geological Survey; STAID, station identification; IDEM, Indiana Department of Environmental Management; dd.mm.ss, degrees minutes seconds; CK, creek; FT, feet; US, upstream; ST, street; IN, Indiana; CR, county road; SR, state route; DS, downstream; INDPLS, Indianapolis; NR, Near; E, east; RD, road; FK, fork; N, North; MI, mile; USHWY, US highway; M, middle; RMI, river mile, R, river; bus, business]

Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
1	400620086050901	INRB01-302	40 06 20	86 05 09	HINKLE CK 1500 FT US EAST 216TH ST AT DEMING, IN	Headwater
2	393410086085901	INRB01-304	39 34 10	86 08 59	HONEY CR 2300 FT US SR 135 AT STONES CROSSING, IN	Headwater
[1]3	392659086181801	INRB01-306	39 26 59	86 18 18	SOUTH PRONG STOTTS CK 1200 FT US SR 44 AT COPE, IN	Wadable
4	401606085532301	INRB01-311	40 16 06	85 53 23	POLYWOG CK 3000 FT US CR 300S AT NEW LANCASTER, IN	Headwater
5	384949087024201	INRB01-313	38 49 49	87 02 42	KANE DITCH 1200 FT DS CR 700E NR EPSOM, IN	Headwater
6	395256086242601	INRB01-316	39 52 56	86 24 26	BEAMAN DITCH 3800 FT US CR 800N NR BROWNSBURG, IN	Headwater
[1]7	394734086133501	INRB01-318	39 47 34	86 13 35	LITTLE EAGLE CREEK 2100 FT US WEST 16TH ST INDPLS	Headwater
8	401451086110701	INRB01-319	40 14 51	86 11 07	KIGIN DITCH 300 FT US CR 900W NR EKIN, IN	Headwater
[1]9	393046086573501	INRB01-320	39 30 46	86 57 35	BIG WALNUT CK 3700 FT US 1-70 NR PLEASANT GARDENS	Wadable
[1]10	385116086565701	INRB01-321	38 51 16	86 56 57	UNNAMED CREEK 3500 FT DS CR 1100E NR FARLEN, IN	Headwater
[1]11	393855086245601	INRB01-322	39 38 55	86 24 56	W FK WHITE LICK CK 1500 FT US I-70 NR PLAINFIELD	Wadable
[1]12	401449085311801	INRB01-323	40 14 49	85 31 18	JAKES CREEK 600 FT DS CR 700W AT BETHEL, IN	Headwater
[1]13	395003086410401	INRB01-324	39 50 03	86 41 04	BIG WALNUT CREEK 1600 FT US CR 900E NR BARNARD, IN	Wadable
[1]14	400046085370901	INRB01-327	40 00 46	85 37 09	FALL CREEK 1300 FT DS CR 300E NR EMPORIA, IN	Wadable
[1]15	393022086313401	INRB01-330	39 30 22	86 31 34	LAMBS CREEK 1600 FT DS BEREAN RD NR WILBUR, IN	Headwater
[1]16	394648086432701	INRB01-332	39 46 48	86 43 27	PLUM CREEK 1800 FT US CR 800N AT NEW MAYSVILLE, IN	Headwater
[1]17	400831084552901	INRB01-335	40 08 31	84 55 29	WHITE RIVER 2800 FT DS CR 200S NR WINCHESTER, IN	Headwater
[1]18	393713086213101	INRB01-338	39 37 13	86 21 31	E FK WHITE LICK CK 1400FT US OLD 67 MOORESVILLE, IN	Wadable
19	401237085510001	INRB01-339	40 12 37	85 51 00	LAMBERSON DITCH 1300 FT US CR 700N NR AROMA, IN	Headwater
20	392755086444401	INRB01-340	39 27 55	86 44 44	MILL CREEK 3800 FT US CR 1150N AT WALLACE JUNCTION, IN	Wadable
21	384333087082001	INRB01-341	38 43 33	87 08 20	N FK PRARIE CK 3000 FT US CR 100E NR WASHINGTON, IN	Wadable
[1]22	395935086163801	INRB01-342	39 59 35	86 16 38	EAGLE CREEK 2600 FT US WEST 146TH ST AT ZIONSVILLE, IN	Wadable
23	392116087052501	INRB01-344	39 21 16	87 05 25	TURKEY CREEK 500 FT US CR 665 NR SALINE CITY, IN	Headwater
24	393402086384301	INRB01-346	39 34 02	86 38 43	MUD CREEK 5300 FT DS CR 1100W AT LITTLE POINT, IN	Wadable

Table 1. Location of Indiana Department of Environmental Management Watershed Monitoring Program sampling sites used in the breakpoint analysis study, 2001–05. —Continued

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Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
25	401219085344901	INRB01-347	40 12 19	85 34 49	KILLBUCK CREEK 500 FT DS CR 800N NR GILMAN, IN	Wadable
26	394307086474301	INRB01-348	39 43 07	86 47 43	BLEDSOE BRANCH 500 FT US CR 400N NR FILLMORE, IN	Headwater
[1]27	395620085090801	INRB02-401	39 56 20	85 09 08	WHITEWATER R 400 FT DS HOOVER RD NR HAGERSTOWN, IN	Wadable
[1]28	394041084562001	INRB02-402	39 40 41	84 56 20	RICHLAND CK 3800 FT US CR 100W NR CLIFTON, IN	Headwater
[1]29	393827085001401	INRB02-403	39 38 27	85 00 14	E FK WHITEWATER R 2700 FT US SR 44 NR LIBERTY, IN	Wadable
[1]30	392010085163801	INRB02-404	39 20 10	85 16 38	SALT CK 1500 FT DS ENOCHSBURG RD AT ENOCHSBURG, IN	Wadable
31	393249085113501	INRB02-407	39 32 49	85 11 35	N BR GARRISON CK 200 FT DS CR 650S AT ALPINE, IN	Headwater
[1]32	395649084545201	INRB02-408	39 56 49	84 54 52	FOUNTAIN CK AT WHITEWATER RD AT FOUNTAIN CITY, IN	Headwater
[1]33	395350085054001	INRB02-409	39 53 50	85 05 40	MORGAN CK 2000 FT DS SR 38 NR GREENS FK, IN	Headwater
34	392254084554401	INRB02-410	39 22 54	84 55 44	BIG CEDAR CK 1.8 MI US USHWY52 NR CEDAR GROVE, IN	Wadable
[1]35	393609085125801	INRB02-411	39 36 09	85 12 58	FALL CK 1000 FT DS CR 425W NR COLUMBIA, IN	Headwater
36	392251085074801	INRB02-412	39 22 51	85 07 48	PIPE CK 900 FT US PUMPHOUSE RD NR OAK FOREST, IN	Wadable
[1]37	395148085074801	INRB02-413	39 51 48	85 07 48	MARTINDALE CK 3900 FT US I-70 NR JACKSONBURG, IN	Wadable
38	393514084580001	INRB02-415	39 35 14	84 58 00	UNNAMED TRIB 1800 FT DS CR 175W NR ROSEBURG, IN	Headwater
[1]39	395158084535701	INRB02-416	39 51 58	84 53 57	W FK E FK WHITEWATER R 2000 FT DS I-70 NR RICHMOND, IN	Headwater
[1]40	394735084561101	INRB02-417	39 47 35	84 56 10	LICK CK 2200 FT US ABINGTON PIKE NR RICHMOND, IN	Headwater
[1]41	393439085092401	INRB02-419	39 34 39	85 09 24	WHITEWATER R 1200 FT US CR 480S AT NULLTOWN, IN	Wadable
[1]42	392645085071101	INRB02-420	39 26 45	85 07 11	WHITEWATER R 1.6 MI DS USHWY 52 AT METAMORA, IN	Wadable
43	395344085064201	INRB02-421	39 53 44	85 06 42	OSER CK 2700 FT DS SR 38 NR HAGERSTOWN, IN	Headwater
[1]44	392616085042301	INRB02-422	39 26 16	85 04 23	WHITEWATER R 4 MI DS USHWY 52 AT YELLOW BANK, IN	Wadable
45	393913084491301	INRB02-423	39 39 13	84 49 13	LITTLE FOUR MI CK 2 MI US SR 44 NR FAIRHAVEN, OH	Headwater
46	394904085002401	INRB02-425	39 49 04	85 00 24	CROWN CK 400 FT DS MCMINN RD AT CENTERVILLE, IN	Headwater
[1]47	394318085073301	INRB02-427	39 43 18	85 07 33	WHITEWATER R 1.4 MI US CR 440N AT BEESONS, IN	Wadable
[1]48	394649085023001	INRB02-429	39 46 49	85 02 30	NOLANDS FK 2600 FT DS MCCONAHA RD NR PINHOOK, IN	Wadable

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Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
[1]49	392142085152501	INRB02-430	39 21 42	85 15 25	SALT CK 2300 FT US CR 250W NR HAMBURG, IN	Wadable
50	393259084502801	INRB02-431	39 32 59	84 50 28	INDIAN CK 900 FT DS SAND RUN RD NR CONTRERAS, OH	Headwater
[1]51	395301084511501	INRB02-432	39 53 01	84 51 15	M FK E FK WHITEWATER R 0.5 MI US SR 227 MIDDLEBORO, IN	Wadable
[1]52	394527084541701	INRB02-433	39 45 27	84 54 17	ELKHORN CK 5000 FT US USHWY 27 NR LOCUST GROVE, IN	Headwater
53	394545084540201	INRB02-434	39 45 45	84 54 02	ELKHORN CK 1.3 MI US USHWY 27 NR LOCUST GROVE, IN	Headwater
[1]54	393450085061301	INRB02-435	39 34 50	85 06 14	WILSON CK 700 FT DS SR 1 NR EVERTON, IN	Headwater
55	392755085181001	INRB02-436	39 27 55	85 18 10	BULL FK 2 MI US STIPPS HILL RD NR BUENA VISTA, IN	Headwater
[1]56	394550085073301	INRB02-437	39 45 50	85 07 33	WHITEWATER R 2 MI DS E MILTON RD NR MILTON, IN	Wadable
[1]57	392638085063101	INRB02-438	39 26 38	85 06 31	WHITEWATER R 2 MI DS USHWY 52 NR METAMORA, IN	Wadable
[1]58	392809085103601	INRB02-439	39 28 09	85 10 38	WHITEWATER R 2 MI DS LAUREL RD NR LAUREL, IN	Wadable
[1]59	395822084572701	INRB02-440	39 58 21	84 57 28	MORGAN CK 2600 FT US MEYERS RD NR WILLIAMSBURG, IN	Headwater
[1]60	395346085023501	INRB02-441	39 53 48	85 02 37	GREENS FK 1200 FT US SR 38 AT GREENS FK, IN	Wadable
[1]61	394147085064301	INRB02-443	39 41 47	85 06 43	WHITEWATER R 2900 FT DS CR 440N AT WATERLOO, IN	Wadable
62	394540085031701	INRB02-445	39 45 40	85 03 17	CENTERAL RUN 1.3 MI US CHAPEL RD NR PINHOOK, IN	Headwater
[1]63	395651085222501	INRB02-501	39 56 51	85 22 26	BIG BLUE R 400 FT DS NRFK-WSTRN RR AT NEW CASTLE,	Wadable
[1]64	390637085350201	INRB02-502	39 06 36	85 35 02	SAND CK 1.5 RMI US CR 800N NR BREWERSVILLE, IN	Wadable
[1]65	392348085315501	INRB02-503	39 23 48	85 31 55	CLIFTY CK 840 FT US CR 400N NR ADAMS, IN	Wadable
66	384632086094201	INRB02-504	38 46 32	86 09 42	E FK WHITE R 1 RMI US MUSCATATUCK R NR MEDORA, IN	Boat
[1]67	392221085483701	INRB02-505	39 22 21	85 48 37	FLATROCK R 300 FT DS CR 150W NR FLATROCK, IN	Wadable
68	385657086275601	INRB02-506	38 56 57	86 27 56	LITTLE SALT CK 400 FT DS CR 650N NR COVEYVILLE, IN	Wadable
[1]69	394628085321301	INRB02-509	39 46 28	85 32 13	BIG BLUE R 1 RMI DS CR 450W NR KNIGHTSTOWN, IN	Wadable
70	391917085520901	INRB02-510	39 19 17	85 52 09	FLATROCK R 1.5 RMI DS CR 900N AT ST LOUIS CROSSING	Wadable
[1]71	385539085431501	INRB02-511	38 55 39	85 43 15	VERNON FK MUSC R 1.2 RMI US CR 500S NR HAYDEN, IN	Wadable
72	384810086284601	INRB02-512	38 48 10	86 28 46	E FK WHITE R 3 RMI DS GUTHRIE CK NR YOCKEY, IN	Boat

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Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
73	385836086062401	INRB02-515	38 58 36	86 06 24	LITTLE SALT CK 1 RMI DS SR 258 AT FREETOWN, IN	Headwater
74	383934086463201	INRB02-516	38 39 34	86 46 32	BEAVER CK 3000 FT DS SR 150 AT IRONTON, IN	Wadable
[1]75	390659085383801	INRB02-518	39 06 59	85 38 38	WYALOOSING CK 1500 FT DS SR 3 NR SARDINIA, IN	Wadable
[1]76	390745085273801	INRB02-519	39 07 45	85 27 38	SUGAR CK 4000 FT US N FK MUSC R NR ZENAS, IN	Headwater
[1]77	392751085291801	INRB02-521	39 27 51	85 29 18	LITTLE FLATROCK R 700 FT US CR 1000S NR MILROY, IN	Wadable
[1]78	385730086102801	INRB02-522	38 57 30	86 10 28	S FK SALT CK 1 RMI DS SR 58 NR KURTZ, IN	Wadable
[1]79	384717085320901	INRB02-523	38 47 17	85 32 09	BIG CK 3000 FT US HARVERTS CK AT VOLGA, IN	Wadable
80	383107086502501	INRB02-524	38 31 07	86 50 25	E FK WHITE R 800 FT US WOLFE CK NR THALES, IN	Boat
[1]81	392508085545301	INRB02-525	39 25 08	85 54 53	BIG BLUE R 3 RMI US USHWY 65 NR MT AUBURN, IN	Wadable
82	384832086340901	INRB02-526	38 48 32	86 34 09	E FK WHITE R 2 RMI US CR 400S NR COXTON, IN	Boat
83	384838085383401	INRB02-527	38 48 32	85 38 34	BIG CK 1800 FT US SR 3 NR DEPUTY, IN	Wadable
[1]84	382939087060901	INRB02-528	38 48 39	87 06 09	BEAR CK 200 FT DS CR 800N NR OTWELL, IN	Headwater
85	384635086041401	INRB02-530	38 46 35	86 04 14	MUSCATATUCK R 3 RMI US SR 135 NR MILLPORT, IN	Boat
86	390939086202001	INRB02-531	39 09 39	86 20 20	N FK SALT CK 2250 FT US SR 46 AT BELMONT, IN	Wadable
87	384433086434501	INRB02-532	38 44 33	86 43 45	E FK WHITE R 2 RMI US DEVILS ELBOW NR HURON, IN	Boat
88	393950085544601	INRB02-533	39 39 50	85 54 46	SUGAR CK 1500 FT DS CR 1000N NR PLEASANT VIEW, IN	Wadable
[1]89	390028085365701	INRB02-534	39 00 28	85 36 57	VERNON FK MUSC R 1150 FT US WWTF AT N VERNON, IN	Wadable
[1]90	391848085333801	INRB02-535	39 18 48	85 33 38	UNNAMED TRIB 3000 FT US SR 3 AT EWINGTON, IN	Headwater
91	384525086014401	INRB02-536	38 45 25	86 01 44	MUSCATATUCK R 1 RMI US DELANEY CK NR MILLPORT, IN	Boat
[1]92	393249085294401	INRB02-537	39 32 49	85 29 44	FLATROCK R 500 FT DS CR 450S NR MILROY, IN	Wadable
93	385607085325901	INRB02-539	38 56 07	85 32 59	GRAHAM CK 1.7 RMI US SR 7 AT WALNUT RIDGE, IN	Wadable
94	392105085592401	INRB02-542	39 21 06	85 59 24	BIG BLUE R 2500 FT DS SR 31 AT EDINBURGH, IN	Wadable
95	384858085525501	INRB02-543	38 53 04	85 35 05	VERNON FK MUSC R 2300 FT DS CR 530S NR RETREAT, IN	Wadable
96	383726086202701	INRB02-544	38 37 26	86 20 26	LOST R 1.5 MI US POTATO RD NR ORLEANS, IN	Wadable

Table 1. Location of Indiana Department of Environmental Management Watershed Monitoring Program sampling sites used in the breakpoint analysis study, 2001–05. —Continued

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Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
97	392544085585801	INRB02-545	39 25 44	85 58 58	SUGAR CK 2600 FT DS USHWY 65 AT AMITY, IN	Wadable
[1]98	403857085593501	INRB03-601	40 38 58	85 59 31	PIPE CK 100 FT US PIPE CK AT SANTA FE, IN	Wadable
99	401643084521501	INRB03-602	40 16 43	84 52 13	HARSHMAN CK 0.9 MI US MISSISSINEWA R NR SALEM, IN	Headwater
[1]100	404805086333701	INRB03-603	40 48 04	86 33 37	GALBREATH DITCH 0.5 MI DS CR 200N NR LK CICOTT, IN	Headwater
[1]101	402554085305901	INRB03-604	40 25 54	85 30 53	MISSISSINEWA R 0.8 MI US SR 26 NR FOWLERTON, IN	Wadable
102	405236086495201	INRB03-605	40 52 36	86 49 53	BIG MONON CK 0.5 MI US SR 16 NR MONON, IN	Wadable
[1]103	405243085564701	INRB03-606	40 52 43	85 56 47	PAW PAW CK 0.3 MI US CR 800N NR ROANN, IN	Wadable
[1]104	401943086385101	INRB03-607	40 19 43	86 38 50	S FK WILDCAT CK 0.6 MI US CR 800W NR MULBERRY, IN	Wadable
105	410301086184501	INRB03-608	41 02 59	86 18 42	MUD CK 0.3 MI US GRUBE DITCH NR PERSHING, IN	Wadable
106	403712086593901	INRB03-609	40 37 11	86 59 39	MEYERS DITCH 820 FT US I-65 NR BADGER GROVE, IN	Headwater
[1]107	410543085373101	INRB03-610	41 05 42	85 37 27	CLEAR CK 980 FT US SPRING CK AT SOUTH WHITLEY, IN	Headwater
[1]108	404156086024501	INRB03-611	40 41 57	86 02 48	LITTLE PIPE CK 900 FT DS CR 150E NR SOUTH PERU, IN	Headwater
109	403633085115401	INRB03-612	40 36 34	85 11 53	ROCK CK 770 FT US CR 900S NR PETROLEUM, IN	Headwater
[1]110	403542086305901	INRB03-613	40 35 42	86 30 59	DEER CK 0.7 MI US MERIDIAN LINE RD NR CAMDEN, IN	Wadable
[1]111	405208085093701	INRB03-614	40 52 09	85 09 37	EIGHTMILE CK 0.4 MI US SR 1 AT OSSIAN, IN	Wadable
112	403603086325101	INRB03-615	40 36 03	86 32 51	DEER CK 0.6 MI DS SR 75 AT CAMDEN, IN	Wadable
113	411707085593601	INRB03-616	41 17 12	85 59 45	EASTERDAY DITCH 0.3 MI DS CR 400N NR ATWOOD, IN	Headwater
[1]114	402612086094301	INRB03-617	40 26 12	86 09 40	E FK LITTLE WILDCAT CK 880 FT US CR200W AT ALTO, IN	Headwater
[1]115	404912086080401	INRB03-619	40 49 14	86 08 03	UNNAMED TRIB 1.2 MI US EEL R AT MEXICO, IN	Headwater
[1]116	410526085291501	INRB03-622	41 05 27	85 29 19	STONY CK 720 FT US MERIDIAN RD AT PEABODY, IN	Headwater
[1]117	402423086423501	INRB03-623	40 24 23	86 42 38	MID FK WILDCAT CK 0.4 MI DS HOG RUN AT PETTIT, IN	Wadable
118	411753085493801	INRB03-624	41 17 52	85 49 39	TIPPECANOE R 880 FT DS CR 100E NR ISLAND PARK, IN	Wadable
119	403639086402301	INRB03-625	40 36 39	86 40 23	WABASH R 1.9 MI US SR 39 NR DELPHI, IN	Boat
120	404739085210101	INRB03-626	40 47 45	85 21 05	ROCK CK 0.4 MI US SR 3 NR MARKLE, IN	Wadable

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Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
121	402135086211701	INRB03-627	40 21 36	86 21 17	KILMORE CK 500 FT US CR 800E NR FOREST, IN	Wadable
[1]122	410048086350101	INRB03-628	41 00 48	86 35 00	MILL CK 0.6 MI US TIPPECANOE R NR WINAMAC, IN	Wadable
[1]123	404248086341901	INRB03-629	40 42 48	86 34 18	UNNAMED TRIB 0.8 MI US BURNETTS CK AT LOCKPORT, IN	Headwater
[1]124	404203085274901	INRB03-630	40 42 04	85 27 50	DETAMORE DITCH 700 FT DS I-69 NR WARREN, IN	Headwater
[1]125	401517084495201	INRB03-631	40 15 18	84 49 53	LITTLE MISSISSINEWA R 2.8 MI DS SR28 NR COSMOS, OH	Headwater
[1]126	402739085413701	INRB03-633	40 27 38	85 41 39	DEER CK 0.25 MI US CR 100W NR WEAVER, IN	Wadable
[1]127	401401085413701	INRB03-634	40 13 58	85 09 58	ELKHORN CK 0.25 MI US CR 500N NR FARMLAND, IN	Headwater
128	405501086490201	INRB03-635	40 55 04	86 49 01	BIG MONON CK 0.2 MI US BIG MONON DITCH NR MONON, IN	Headwater
129	402425084574101	INRB03-636	40 24 25	84 57 42	LITTLE SALAMONIE R 0.4 MI US CR 120S AT LIBER, IN	Headwater
[1]130	410106085191601	INRB03-638	41 01 07	85 19 15	ABOITE CK 0.3 MI US USHWY 24 AT TIMBERCREST, IN	Wadable
131	402710086510501	INRB03-639	40 27 10	86 51 04	WILDCAT CK 150 FT US SR 25 AT LAFAYETTE, IN	Wadable
132	412029086435701	INRB04-701	41 20 29	86 43 57	KANAKEE R 1320 FT US SR39 NR HANNA, IN	Wadable
133	410737087273101	INRB04-703	41 07 37	87 27 31	LAWLER D 2900 FT DS OF N400W AT LAKE VILLAGE, IN	Headwater
134	411021087020901	INRB04-705	41 10 21	87 02 09	WOLF CK 60 FT DS E1100N NR WHEATFIELD, IN	Headwater
135	412400086420601	INRB04-706	41 24 00	86 42 06	KANAKEE R 3.89 MI DS OF W1200S NR HANNA, IN	Wadable
136	405002087233501	INRB04-708	40 50 02	87 23 35	IROQUOIS R 3430 FT US OF S100W NR BROOK, IN	Wadable
137	411752087120701	INRB04-709	41 17 52	87 12 07	UNNAMED TRIB 900 FT US OF USHWY231 NR HEBRON, IN	Headwater
138	412251086110301	INRB04-710	41 22 51	86 11 03	YELLOW R 1700 FT US OF E 8TH ST NR BREMEN, IN	Wadable
139	403952087271401	INRB04-712	40 39 52	87 27 14	SUGAR CK 8120 FT DS OF N600W NR EARL PARK, IN	Wadable
140	405728087033201	INRB04-713	40 57 28	87 03 32	RYAN D 4840 FT US OF S250W NR RENSSELAER, IN	Wadable
141	413841086391401	INRB04-714	41 38 41	86 39 14	COLLINS D 3100 FT US OF SR2 NR LA PORTE, IN	Headwater
142	412118087182401	INRB04-715	41 21 18	87 18 24	STONY RUN 60 FT US OF IOWA ST NR CROWN POINT, IN	Headwater
143	404940087182801	INRB04-716	40 49 40	87 18 28	WEISS D 510 FT DS OF SR 55 NR BROOK, IN	Headwater
144	411619086501501	INRB04-717	41 16 19	86 50 15	KANAKEE R 3890 FT DS OF S650W AT ENGLISH LAKE, IN	Boat

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Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
145	411814086334801	INRB04-718	41 18 14	86 33 48	EAGLE CK 150 FT DS OF N700E NR KNOX, IN	Wadable
146	411056087204401	INRB04-719	41 10 56	87 20 44	KANAKEE R 1370 FT DS OF SR55 AT SHELBY, IN	Boat
147	403551087282201	INRB04-720	40 35 51	87 28 22	MINIER LATERAL 960 FT US S800W NR FREELAND PARK, IN	Headwater
148	412247087081101	INRB04-723	41 22 47	87 08 11	WOLF CK 6320 FT DS OF W300S NR KOUTS, IN	Headwater
149	405127087211401	INRB04-724	40 51 27	87 21 14	IROQUOIS R 2150 FT US E LK KENOYER RD AT BROOK, IN	Wadable
150	411541087024601	INRB04-725	41 15 41	87 02 46	KANAKEE R 4320 FT DS OF SR49 NR KOUTS, IN	Boat
151	405248087262301	INRB04-727	40 52 48	87 26 23	CLARK D 1770 FT US OF S325W AT ADE, IN	Headwater
152	404335087232101	INRB04-728	40 43 35	87 23 21	UNNAMED TRIB 4400 FT US OF W1800S NR KENTLAND, IN	Headwater
153	405552087102801	INRB04-729	40 55 52	87 10 28	IROQUOIS R 5700 FT DS OF USHWY231 AT RENSSELAER, IN	Wadable
154	405210087043401	INRB04-732	40 52 10	87 04 34	HOWE D 850 FT DS OF SR 16 NR RENSSELAER, IN	Headwater
155	411556086513401	INRB04-733	41 15 56	86 51 34	KANAKEE R 2 MI DS S650W NR ENGLISH LAKE, IN	Boat
156	411635086265901	INRB04-734	41 16 35	86 26 59	YELLOW R 2650 FT US OF S UPAS RD NR CULVER, IN	Wadable
157	411324087300401	INRB04-735	41 13 24	87 30 04	WEST CK 3750 US OF W223RD AV NR SCHNEIDER, IN	Wadable
158	413216086262301	INRB04-736	41 32 16	86 26 23	POTATO CK 1830 FT US SMILAX RD AT NORTH LIBERTY, IN	Wadable
159	412759086453401	INRB04-738	41 27 59	86 45 34	MILL CK 770 FT US OF LONG LANE NR UNION MILLS, IN	Wadable
160	411514087054501	INRB04-741	41 15 14	87 05 45	HODGE D 2230 FT US N300W NR WHEATFIELD, IN	Headwater
161	412211086145001	INRB04-742	41 22 11	86 14 50	YELLOW R 3360 FT DS OF N JARRAH RD NR PLYMOUTH, IN	Wadable
162	394017087302701	INRB04-805	39 40 17	87 30 27	BROUILLETS CK 3760 FT US OF SR163 AT BLANFORD, IN	Wadable
163	385709087190101	INRB04-808	38 57 09	87 19 01	MARLA CK 500 FT US OF CR 500E NR CARLISLE, IN	Headwater
164	400204087175301	INRB04-810	40 02 04	87 17 53	PRAIRIE CK 1000 FT US OF S170W NR YEDDO, IN	Headwater
165	380500087555301	INRB04-812	38 05 00	87 55 53	HAWTHORNE CK 2170 FT DS OLD SAND RD AT NEW HARMONY, IN	Headwater
166	400753086324501	INRB04-815	40 07 53	86 32 45	SPRING CK 6000 FT DS NORTH RD AT GARDEN PARK, IN	Headwater
167	395117086531201	INRB04-817	39 51 17	86 53 12	BIG RACCOON CK 2900 FT US USHWY 231 AT RACCOON, IN	Wadable
168	395506087143101	INRB04-819	39 55 06	87 14 31	W PRONG GREEN CK 1030 FT DS USHWY 41 NR KINGMAN, IN	Headwater

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Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
169	375936087434901	INRB04-820	37 59 36	87 43 49	LITTLE CK 1500FT US WILDEMAN RD AT ST PHILLIP, IN	Headwater
170	394256087260001	INRB04-821	39 42 56	87 26 00	UNNAMED TRIB 600 FT US OF E1150S NR CLINTON, IN	Headwater
171	403034086504401	INRB04-823	40 30 34	86 50 44	BURNETT CK 160 FT US NORTHEAST RD AT BATTLE GROUND, IN	Wadable
172	383357087361701	INRB04-824	38 33 57	87 36 17	SWAN POND D 790 FT DS S6TH ST RD NR ST FRANCISVILLE, IL	Wadable
173	402248087290801	INRB04-826	40 22 48	87 29 08	JORDAN CK 4125 FT DS OF N800W NR TAB, IN	Wadable
174	401533086201701	INRB04-827	40 15 33	86 20 17	SCOTT WINCOOP D 230 FT DS 900E NR HILLISBURG, IN	Headwater
175	384046087322101	INRB04-828	38 40 46	87 32 21	WABASH R 1400 FT DS OF USHWY 50 BUS AT VINCENNES, IN	Boat
176	393931087174801	INRB04-829	39 39 31	87 17 48	ROCK RUN 1900 FT DS OF S325W NR ROSEDALE, IN	Headwater
177	391444087185101	INRB04-830	39 14 44	87 18 51	BUSSERON CK 600 FT US CR 1100N NR FARMERSBURG, IN	Headwater
178	381003087510301	INRB04-832	38 10 03	87 51 03	UNNAMED TRIB 2900 FT US NEW HARMONY RD STEWARTSVILLE, IN	Headwater
179	402122087190901	INRB04-834	40 21 22	87 19 09	BIG PINE CK 2300 FT DS OF W300N NR CARBONDALE, IN	Wadable
180	375434088010601	INRB04-836	37 54 34	88 01 06	WABASH R 2.72 MI DS OF SR62 NR MT VERNON, IN	Boat
181	394855087025501	INRB04-841	39 48 55	87 02 55	S FK LITTLE RACCOON CK 200 FT DS N1000E NR MILLIGAN, IN	Headwater
182	400252087255101	INRB04-842	40 02 52	87 25 51	WABASH R 830 FT DS OF SR32 AT PERRYSVILLE, IN	Boat
183	403351087084501	INRB04-843	40 33 51	87 08 45	LITTLE PINE CK 3000 FT US OF CR 300S NR OXFORD, IN	Headwater
184	385114087320601	INRB04-844	38 51 14	87 32 06	WABASH R 13.6 MI US OF USHWY50 NR RUSSELLVILLE, IN	Boat
185	393948087045101	INRB04-845	39 39 48	87 04 51	ROCKY FORK CK 125 FT DS GREENCASTLE RD NR MANSFIELD, IN	Wadable
186	391647087363201	INRB04-846	39 16 47	87 36 32	WABASH R 21.2 MI DS OF I-70 AT DARWIN, IL	Boat
187	411545084562601	INRB05-009	41 15 45	84 56 26	ST JOSEPH RIVER 800 FT US CUBA RD NR GRABILL, IN	Wadable
188	413836085385001	INRB05-018	41 38 36	85 38 50	LITTLE ELKHART R 200 FT US CR 1150 W NR MIDDLEBURY, IN	Wadable
189	412805085355901	INRB05-023	41 28 05	85 35 59	ELKHART RIVER 3900 FT DS SR 5 AT LIGONIER, IN	Wadable
190	405814084512701	INRB05-024	40 58 14	84 51 27	FLATROCK CREEK 2100 FT DS CR 101 AT MONROEVILLE, IN	Headwater
191	413856086032001	INRB05-026	41 38 56	86 03 20	BAUGO CREEK 4100 FT US ASH ROAD AT OSCEOLA, IN	Wadable
192	414137085182601	INRB05-029	41 41 37	85 18 26	PIGEON RIVER 2400 FT US CR 600 EAST NEAR MONGO, IN	Wadable

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Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
193	412750087140001	INRB05-030	41 27 50	87 14 00	DEEP RIVER 3600 FT DS RANDOLPH ST AT DEEP RIVER, IN	Wadable
194	414143085584801	INRB05-042	41 41 43	85 58 48	CHRISTIANA CREEK 1300FT US N MAIN ST AT ELKHART, IN	Wadable
195	414224086461401	INRB05-047	41 42 24	86 46 14	E BR TRAIL CK 1830 FT US CR400 W AT SPRINGFIELD, IN	Headwater
196	414046086493201	INRB05-052	41 40 46	86 49 32	UNNAMED TRIB 20FT US I-94 AT ORCHARD HIGHLANDS, IN	Headwater
197	412718085502501	INRB05-061	41 27 18	85 50 25	KIEFFER D 20 FT US OLD STATE RD NR MILFORD JUNCTION, IN	Headwater
198	382108086164601	INRB05-103	38 21 08	86 16 46	BLUE R 230 FT DS OF SR 64 AT MILLTOWN, IN	Wadable
199	383339085454001	INRB05-104	38 33 39	85 45 40	MILLER FK 1680 FT US HEBRON CHURCH RD AT HENRYVILLE, IN	Headwater
200	382806085470801	INRB05-106	38 28 06	85 47 08	UNNAMED TRIB 200FT US EBENEZERCHURCH RD NR MEMPHIS, IN	Headwater
201	375731086420401	INRB05-109	37 57 31	86 42 04	UNNAMED TRIB 950 FT DS OF ASTER RD NR TELL CITY, IN	Headwater
202	382151085561201	INRB05-110	38 21 51	85 56 12	INDIAN CK 875 FT DS OF NAVILLETON RD AT GALENA, IN	Headwater
203	380537086290701	INRB05-111	38 05 37	86 29 07	LITTLE OIL CK 975 FT DS OF PARKS RD NR MAGNET, IN	Headwater
204	390432085070401	INRB05-112	39 04 32	85 07 04	SOUTH HOGAN CK 6100 FT DS OF SR 101 NR MILAN, IN	Wadable
205	380309085594301	INRB05-113	38 03 09	85 59 43	MOSQUITO CK 1.2MI DS BUENA VISTA RD NR BUENA VISTA, IN	Headwater
206	391155084542401	INRB05-116	39 11 55	84 54 24	BRUSHY FK 1230FT US SAWDON RIDGE RD NR GUILFORD, IN	Headwater
207	381121086184001	INRB05-119	38 11 21	86 18 40	BLUE R 0.6MI DS OLD FOREST RD SW NR LEAVENWORTH, IN	Wadable
208	381345086072001	INRB05-121	38 13 45	86 07 20	INDIAN CK 8730 FT US OF SR337 AT CORYDON, IN	Wadable
209	384615085243501	INRB05-122	38 46 15	85 24 35	DEANS BR 1050 FT US OF SR 1 AT NORTH MADISON, IN	Headwater
210	390511085032701	INRB05-124	39 05 11	85 03 27	ALLEN BR 4450FT US OF IRELAND RD NR MOORES HILL, IN	Headwater
211	380620087041401	INRB05-125	38 06 20	87 04 14	LITTLE PIGEON CK 0.2MI US PIGEON SWITCH GENTRYVLLE, IN	Wadable
212	383221085352801	INRB05-126	38 32 21	85 35 28	FOURTEENMILE CK 3140FT US ROGERS RUN NR NEW MARKET, IN	Wadable
213	380926086344501	INRB05-127	38 09 26	86 34 45	OIL CK 5580 DS OF BRANCHVILLE RD AT BRANCHVILLE, IN	Headwater
214	385822085024401	INRB05-128	38 58 22	85 02 44	HAYES BR 1620 FT DS W LAUGHERY CK RD NR MILTON, IN	Headwater
215	391015084523101	INRB05-132	39 10 15	84 52 31	SALT FK 7380 FT US OF SR 1 NR LAWRENCEBURG, IN	Headwater
216	390707084521701	INRB05-136	39 07 07	84 52 17	TANNERS CK 6300 FT US SCHENLEY PL AT GREENDALE, IN	Wadable

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Site identifier	USGS STAID	IDEM site number	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	USGS station name	Site type
217	380313087075701	INRB05-141	38 03 13	87 07 57	LITTLE PIGEON CK 4550FT US E525 RD NR TENNYSON, IN	Wadable
218	382349085433601	INRB05-142	38 23 49	85 43 36	SILVER CK 5850FT DS GREENLEAF DR AT SELLERSBURG, IN	Wadable
219	382309086145001	INRB05-145	38 23 09	86 14 50	BLUE R 2 MI US OF E TOTTEN FORD RD NR MILLTOWN, IN	Wadable
220	390550085144001	INRB05-148	39 05 50	85 14 40	LAUGHERY CK 4.3 MI US E PERRY ST NR VERSAILLES, IN	Wadable
221	382620086032101	INRB05-150	38 26 20	86 03 21	BEAR CK 730FT US E MARTINSBURG FIRE RD MARTINSBURG, IN	Headwater
222	381333086164201	INRB05-153	38 13 33	86 16 42	BLUE R 3.64 MI DS OF SR 462 NR LEAVENWORTH, IN	Wadable
223	382852085421201	INRB05-156	38 28 52	85 42 12	SUGAR RUN 760 FT DS OF SR 160 NR CHARLESTOWN, IN	Headwater
224	381947086283401	INRB05-157	38 19 47	86 28 34	LITTLE BLUE R 4200FT DS OF SPEARS ST AT ENGLISH, IN	Wadable
225	385130085152601	INRB05-158	38 51 30	85 15 26	INDIAN KENTUCK CK 8070 FT DS OF SR 62 NR CANAAN, IN	Wadable
226	380443086464901	INRB05-159	38 04 43	86 46 49	ANDERSON R 1500FT US N AVERY RIDGE RD NR HUFFMAN, IN	Wadable
227	385955084565501	INRB05-162	38 59 55	84 56 55	LAUGHERY CK 4900 FT DS OF COLE LANE AT HARTFORD, IN	Wadable
228	383640085324401	INRB05-164	38 36 40	85 32 44	UNNAMED TRIB 2100FT US SR 362 NR NEW WASHINGTON, IN	Headwater
229	381044086374601	INRB05-167	38 10 44	86 37 46	WINDING BR 3630 FT US OF CR46 NR APALONA, IN	Headwater
230	384635085044401	INRB05-172	38 46 35	85 04 44	INDIAN CK 9200 FT US OF KNOX FORD RD NR VEVAY, IN	Wadable
231	382341086150801	INRB05-179	38 23 41	86 15 08	BLUE R 4.1MI US OF E TOTTEN FORD RD NR MILLTOWN, IN	Wadable
232	381705086163001	INRB05-185	38 17 05	86 16 30	BLUE R 1.35 MI US OF CR 30 NR MILLTOWN, IN	Wadable

[1] Invertebrate community sample collected.

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