

National Water-Quality Assessment Program

Evaluation of Aquatic Biota in Relation to Environmental Characteristics Measured at Multiple Scales in Agricultural Streams of the Midwest: 1993–2004



Scientific Investigations Report 2010–5051

Cover photographs

Left and top: Segment and watershed scale view of an agricultural stream: David L. Lorenz, USGS—Mounds View, Minn.

Bottom: Reach scale view of an agricultural stream: Jeffrey D. Stoner, USGS—Eagan, Minn.

Evaluation of Aquatic Biota in Relation to Environmental Characteristics Measured at Multiple Scales in Agricultural Streams of the Midwest: 1993–2004

By Julie A. Hambrook Berkman, Barbara C. Scudder, Michelle A. Lutz, and Mitchell A. Harris

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Foreword

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

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

National and regional assessments are ongoing in the second decade (2001–2012) of the NAWQA Program as 42 of the 51 Study Units are selectively reassessed. These assessments extend the findings in the Study Units by determining status and trends at sites that have been consistently monitored for more than a decade, and filling critical gaps in characterizing the quality of surface water and groundwater. For example, increased emphasis has been placed on assessing the quality of source water and finished water associated with many of the Nation's largest community water systems. During the second decade, NAWQA is addressing five national priority topics that build an understanding of how natural features and human activities affect water quality, and establish links between sources of contaminants, the transport of those contaminants through the hydrologic system, and the potential effects of contaminants on humans and aquatic ecosystems. Included are studies on the fate of agricultural chemicals, effects of urbanization on stream ecosystems, bioaccumulation of mercury in stream ecosystems, effects of nutrient enrichment on aquatic ecosystems, and transport of contaminants to public-supply wells. In addition, national syntheses of information on pesticides, volatile organic compounds (VOCs), nutrients, trace elements, and aquatic ecology are continuing.

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

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

Matthew C. Larsen
Associate Director for Water

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Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
Length		
millimeter (mm)	0.03937	inch (in.)
centimeter (cm)	.3937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	.6214	mile (mi)
Area		
square meter (m ²)	.0002471	acre
square kilometer (km ²)	.3861	square mile (mi ²)
Volume		
liter (L)	.2642	gallon (gal)
cubic meter (m ³)	264.2	gallon (gal)
cubic centimeter (cm ³)	.06102	cubic inch (in ³)
liter (L)	61.02	cubic inch (in ³)
cubic meter (m ³)	35.31	cubic foot (ft ³)
Flow rate		
meter per second (m/s)	3.281	foot per second (ft/s)
cubic meter per second (m ³ /s)	35.31	cubic foot per second (ft ³ /s)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
 $^{\circ}\text{F}=(1.8\times^{\circ}\text{C})+32$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:
 $^{\circ}\text{C}=(^{\circ}\text{F}-32)/1.8$

Vertical coordinate information is referenced to the North American Vertical Datum of 1988 (NAVD 88).

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (μS/cm at 25°C).

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (μg/L).

Evaluation of Aquatic Biota in Relation to Environmental Characteristics Measured at Multiple Scales in Agricultural Streams of the Midwest: 1993–2004

By Julie A. Hambrook Berkman, Barbara C. Scudder, Michelle A. Lutz, and Mitchell A. Harris

Abstract

This study evaluated the relations between algal, invertebrate, and fish assemblages and physical environmental characteristics of streams at the reach, segment, and watershed scale in agricultural settings in the Midwest. The 86 stream sites selected for study were in predominantly agricultural watersheds sampled as part of the U.S. Geological Survey's National Water-Quality Assessment Program. Species abundance and over 130 biological metrics were used to determine which aspects of the assemblages were most sensitive to change at the three spatial scales. Digital orthophotograph-based riparian land use/land cover was used for analyses of riparian conditions at the reach and segment scales. The percentage area of different land-use/land-cover types was also determined for each watershed. Out of over 230 environmental characteristics examined, those that best explained variation in the biotic assemblages at each spatial scale include the following: 1) reach: bank vegetative cover, fine silty substrate, and open canopy angle; 2) segment: woody vegetation and cropland in the 250-m riparian buffer, and average length of undisturbed buffer; and 3) watershed: land use/land cover (both total forested and row crop), low-permeability soils, slope, drainage area, and latitude. All three biological assemblages, especially fish, correlated more with land use/land cover and other physical characteristics at the watershed scale than at the reach or segment scales. This study identifies biotic measures that can be used to evaluate potential improvements resulting from agricultural best-management practices and other conservation efforts, as well as evaluate potential impairment from urban development or other disturbances.

Introduction

Evaluating changes in land-use practices and the effectiveness of best-management practices (BMPs) in reducing water-quality effects of agriculture is complex because aquatic biota may respond to changes at multiple spatial scales.

Additionally, environmental characteristics at one scale may be highly related to one or more characteristics at other scales (Carter and others, 1996). The importance of spatial scaling has long been recognized in studies of stream geomorphology (Strahler, 1957; Leopold and others, 1964; Hynes, 1975; Friswell and others, 1986; Leopold, 1994). Geology, geomorphology, and climate influence soils and vegetation (Strahler and Strahler, 1978) and, in turn, the slope and soils in a watershed affect the geomorphology and flow of a stream. Determining the relative influences of environmental characteristics at various spatial scales on biological assemblages is difficult; however, this study attempts to define these influences using multivariate techniques (Wiens, 1989; Resh and Rosenberg, 1989; Richards and Host, 1994; Carter and others, 1996; Allan and others, 1997; Dovciak and Perry, 2002; Goldstein and Meador, 2004).

Previous studies that have examined the interaction of multiple aquatic biota (algae, invertebrates, and fish) together with physical and (or) chemical characteristics of streams have found similarities and differences in the way various groups of biota respond to degraded stream conditions (Cuffney and others, 1997; Allen and others, 1999; Lammert and Allan, 1999; Fitzpatrick and others, 2001; Fore, 2003). It is important to identify which aspects (numbers of taxa, relative abundance of taxa, or metrics based on species traits) of the three principal biological assemblages best correspond to stream conditions and changes in management practices at each spatial scale. Efforts have been made to determine environmental preferences of algae (Stevenson and Bahls, 1999; Hill and others, 2000; Fore and Grafe, 2002; Potapova and Charles, 2007; Porter, 2008), invertebrates (DeShon, 1995; Barbour and others, 1996, 1999; Fore and others, 1996; Vieria and others, 2006), and fish (Barbour and others, 1999; Goldstein and Meador, 2004, 2005) to increase the utility of these biological assemblages as monitoring tools for bioassessment of streams. Efforts to improve water quality through changes in land-use and management practices are more likely to be successful if targeted toward environmental characteristics and biological assemblages that are expected to respond most strongly. In other words, to evaluate improvements to the health and

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condition of streams, it may be necessary to select environmental measures that show the greatest relation to desired biotic measures (for example, to benefit fish) and to manage the land use at the scale that would result in this benefit (for example, watershed scale). Conversely, if a management practice is to be applied locally near a stream reach and there is an interest in monitoring benefits, it would make sense to select a biological measure or measures that would likely be the most responsive at a local (reach) scale.

Purpose and Scope

The purpose of this study is to evaluate relations between biota and physical-environmental characteristics of streams at various scales. To do so, the study examined biological and environmental data collected at 86 agricultural stream sites across areas of the Midwestern United States (hereafter “Midwest”), from 1993 through 2004 as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Program. The study objectives were to (1) identify environmental characteristics to which the algal, invertebrate, and fish assemblages show the strongest correlations; (2) examine relations between each biotic assemblage and the most highly correlated environmental characteristics at the reach, segment, and watershed scale; and (3) identify the biological measures (metrics) that show the best potential for monitoring, assessing, and evaluating changes at the various spatial scales to help guide watershed conservation efforts and identify potential land-use changes that could cause impairment to streams in the Midwest. On the basis of previous studies that analyzed multiple biotic assemblages, it was hypothesized that fish assemblages would be more strongly correlated with watershed-scale characteristics than with segment and reach characteristics, whereas algal and invertebrate assemblages would be more strongly influenced by environmental characteristics at the reach and segment scales.

Study Area

The study area included major agricultural watersheds within eight NAWQA study units (fig. 1). The 86 stream sites selected for study (described in Lutz and Kennedy, 2010) were in watersheds characterized as agricultural (median agricultural land cover = 86%), predominantly row crop and pasture, with less than 10% urban land use and remaining area primarily as forest. Drainage-area sizes ranged from 12 to 2,922 km², with a median of 319 km². Sites were predominantly in glaciated ecoregions of the Central, Eastern, and Western Cornbelt Plains; North Central Hardwood Forests; and Southeastern Wisconsin Till Plains (Lutz and Kennedy, 2010). These agricultural watersheds are relatively flat with slopes ranging from about 0.1 to 7 m/km and the median watershed slope is about 1 m/km. Additional environmental characteristics of the study area are given in table 1.

Methods

Physical environmental data and biological samples were collected from 86 sites within the agricultural Midwest from 1993 through 2004 as part of the NAWQA Program. Physical data were collected at three spatial scales—reach, segment, and watershed—whereas biological data were collected only at the reach scale. Data-collection methods were consistent with USGS NAWQA protocols. Protocol changes during this period were generally minor. Reaches established at sampling sites were defined as a length of stream chosen to represent the physical, chemical, and biological conditions within a stream segment. Reaches ranged in length from 150 to 500 m or approximately 20 times the width of the stream channel (Meador and others, 1993a; Fitzpatrick and others, 1998). A stream segment was defined as a length of stream bounded by confluences of tributaries or physical or chemical discontinuities, such as major waterfalls or landform features. Segment lengths began at the downstream end of the reach and extended upstream to a length (in kilometers) equivalent to the base-10 logarithm of the basin area (in square kilometers), or about 7.0 to 9.5 km. A watershed was defined as the land area that drains to the most downstream end of a reach. A subset of the sites was sampled for three or more years and in three replicate reaches for algal, invertebrate, and fish-assemblage data. These replicate samples were used to evaluate the variance of the biological metrics over space and time to assess which were the least variable. For sites where multiple years of data were available, the year selected to represent the site was chosen to be consistent within a study unit and consistent across all study units, where possible. Biological-assemblage data (species relative abundance) and metrics computed from assemblage data (for example, the percentage of carnivore/piscivore fish) were used in analyses.

Data Collection

Biological Assemblages

Biological samples were collected from May through September, generally during periods of stable low flow, according to NAWQA methods as detailed below. All biological data are available from the NAWQA Data Warehouse (USGS, 2005) at <http://water.usgs.gov/nawqa/data>. A list of station identification numbers, needed for data retrieval, can be found in Lutz and Kennedy (2010). Quantitative algal samples were collected from a measured area of substrate to enable estimation of abundance per area. Areas sampled represented either rock or wood habitat in the sampling reach. Separate discrete samples were collected from 25 rocks in riffle areas (5 locations) or from 5 submerged woody snags and composited to form a single algal sample using standard NAWQA methods (Porter and others, 1993; Moulton and others, 2002). Semiquantitative benthic-macroinvertebrate samples were

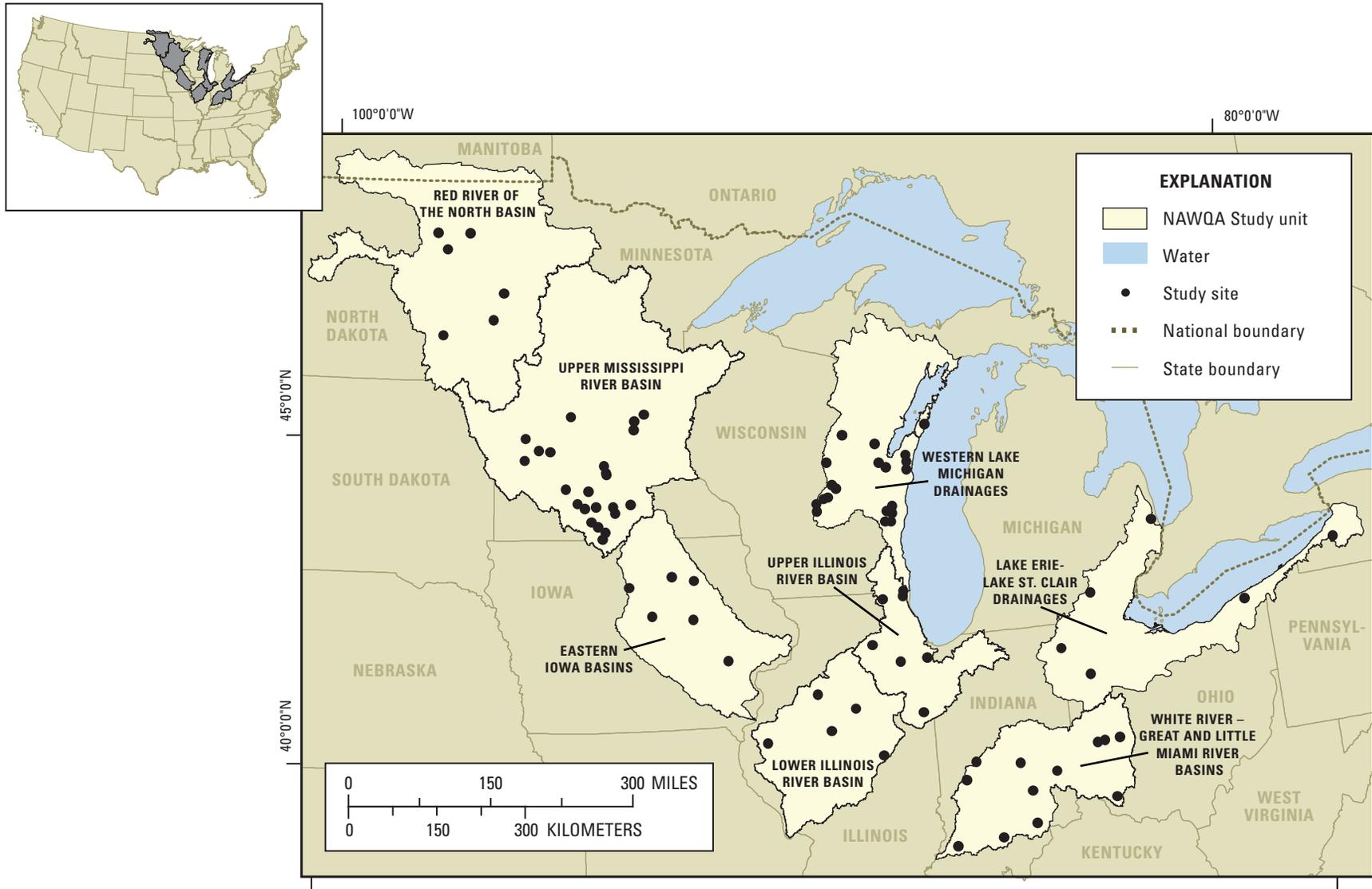


Figure 1. Location of 86 agricultural stream sites in the Midwest within eight study units of the U.S. Geological Survey National Water-Quality Assessment (NAWQA) Program.

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collected from areas where maximum taxa richness was likely to occur (rocks in riffle areas or submerged woody snags), generally the same habitats and locations as the areas sampled for algae, according to NAWQA methods (Cuffney and others, 1993; Moulton and others, 2002). Fish were collected according to NAWQA methods by field personnel using seines and backpack, towed-barge, or boat-mounted electrofishing units (Meador and others, 1993b; Moulton and others, 2002). Fish were identified, weighed, and measured in the field. When species identification could not be confirmed in the field, several

unidentified individuals of that species were archived and their identification confirmed by taxonomists in the laboratory.

Environmental Characteristics

Physical-habitat data used for each site were those that were collected closest to the sampling dates for the algal, invertebrate, and fish assemblages, generally in the same year and season. Physical-habitat data were collected within each sampling reach as described in Meador and others (1993a) for

Table 1. Selected environmental characteristics, with metric abbreviations and range of data (minimum, median or 50th percentile, and maximum) for 86 agricultural stream sites in the Midwest, listed by spatial scale.

Metric abbreviation	Metric description	Unit	Minimum	Median	Maximum
Reach characteristics					
BankEros	Bank erosion	percent	8.3	83.3	100
BankVeg	Bank vegetative cover	percent	13	55.5	90
Fine	Fine silty substrate	percent	0	12.1	100
Froude	Froude number	dimensionless	0	.13	.43
OpCanAng	Open canopy angle	degrees	6.42	64.17	165
OpCanCV	Open canopy angle variation	percent	2.13	44.2	265.6
Riffle	Riffles in reach	percent	.00	18.4	81.3
Velocity	Average stream velocity	m/s	.01	.26	.87
WetWdth	Average wetted channel width	meters	2.50	11.4	59.6
W/D	Average wetted-channel width-depth ratio	ratio	5.86	28.49	163.32
WV50	Woody vegetation in the 50-m buffer	percent	0	41.6	98.0
Segment characteristics					
BuffFrag	Number of buffer fragments per kilometer in stream margin	number/km	0	8.4	16.5
BuffLngth	Average length of undisturbed buffer per kilometer	m/km	0	.2	1.4
Crop250	Percentage of cropland in the 250-m buffer	percent	0	40.7	84.9
SegGrad	Gradient of stream in segment	m/km	0	2.8	15.0
SINUOS	Stream sinuosity	ratio	1	1.32	3.05
WV150	Woody vegetation in the 150-m buffer	percent	.44	33.5	93.7
Watershed characteristics					
DrainArea	Drainage area	km ²	11.9	319	2922
Forest	Percentage of total forest land cover	percent	.36	6.28	57.1
Latitude	Latitude	decimal degrees	38.571	43.742	48.197
MxForest	Percentage mixed forest land cover	percent	.01	.46	29.5
Perml	Percentage of soils that have low-permeability	cm/hr, percent	.21	.895	6.11
RowCrop	Percentage of row-crop land cover	percent	5.71	64.8	94.7
Slope	Average watershed slope	m/km	.07	.94	6.86
WetXArea/DA	Average wetted reach cross-sectional area/drainage area	m ² /km ²	0	13.4	134.5

sites sampled during 1993–97 and as modified by Fitzpatrick and others (1998) for all remaining sites. All physical-habitat data are available from the NAWQA Data Warehouse (USGS, 2005) at <http://water.usgs.gov/nawqa/data>. At the reach scale, measurements were collected from multiple transects for more than 100 reach-level habitat characteristics. Data such as fine sediment, bank erosion, bank vegetative cover, velocity, width, depth, and Froude number were collected and calculated from averaging across the transects. A total of six transects per reach were used for sites sampled during 1993–97; 11 transects per reach were used for sites sampled after 1997. Segment data (more than 80 characteristics) included measures such as gradient, sinuosity, and riparian land use/land cover (LULC). Watershed data (more than 50 characteristics) included drainage area, LULC, geographic location, and watershed slope.

Watershed-scale LULC values in this report are percentages of total watershed area. Watershed boundaries were delineated in a Geographic Information System (GIS) by using 1:24,000- to 1:250,000-scale digital topographic and hydrologic maps (Nakagaki and Wolock, 2005) or 30-m resolution Elevation Derivatives for National Applications (EDNA) reach catchments (U.S. Geological Survey, 2002). LULC information was obtained from 30-m resolution National Land Cover Data (NLCD) that were based on satellite imagery from the early to mid-1990s (Vogelmann and others, 2001) and modified and enhanced (NLCDe 92) with Geographic Information Retrieval and Analysis System (GIRAS) data to give 25 LULC categories, as described in Nakagaki and Wolock (2005).

To develop more detailed descriptions of riparian land cover, the LULC features in buffer areas surrounding each stream were digitized in a GIS environment using orthophotographs as templates (Lutz and Kennedy, 2010). The digitized LULC features within each buffer area were categorized into eight categories: barren, cropland, farmstead, grassland, open water, urban/built-up land, woody vegetation, and wetland (Lutz and Kennedy, 2010). Areas of LULC features were computed within 25-m and 50-m buffers on each side of the stream along the reach length and within 50-m, 100-m, 150-m, and 250-m buffers on each side along the segment length. The length of segment buffers began at the downstream end of the reach and extended upstream to a length (in kilometers) equivalent to the log₁₀ of the watershed area (in square meters) (Lee and others, 2001). Segment lengths were not always exactly the same as the length from which segment slope and sinuosity were determined, but they represent the same scale relative to the biological collection site.

In addition, a narrow segment-length buffer area (stream margin) was created 15 m from the determined bankfull width and intersected with the LULC data layer. The LULC data for the 15-m stream margin were summarized as follows: (1) total number of individual fragments (changes from one land use to another along both sides of the stream margin (minus one)) per kilometer, (2) average length of like (similar) fragments, (3) percentage of total margin length populated by each fragment type, and (4) the length of the fragments considered (a) undisturbed (defined as those with land cover of wetland

and woody vegetation) or (b) disturbed (defined as those with land cover in all other categories, except open water). Because the land cover was calculated from the center or midline of the stream, the open-water category was considered a surrogate for stream size; therefore, the open-water category was removed from the analyses. The digitized LULC features surrounding each stream are available online (Lutz and Kennedy, 2010).

Data Analysis

Biological-assemblage data (species relative abundance) and over 130 biological metrics computed from assemblage data were used in analyses with environmental characteristics.

Biological Assemblages

Algal, invertebrate, and fish assemblages were identified to the lowest possible taxon. Algae were identified, generally to species, and enumerated as described in Charles and others (2002). The relative abundance (cells per square centimeter as a percentage of total algal cell abundance) and relative biovolume (cubic micrometers per square centimeter as a percentage of total algal biovolume) of each taxon were calculated for each sample. Algal-assemblage data analyses were based on 640 algal taxa identified from the 86 sites and a reduced dataset of 244 taxa, which included all taxa with relative abundance greater than 5 percent at 2 or more sites or occurrence at 4 or more sites such that “rare” taxa were excluded. In some cases, inclusion of rare taxa can reduce the number and strength of correlations in bioassessment and may unnecessarily complicate model outputs (Marchant, 2002; Van Sickle and others, 2007). Invertebrate samples were identified and enumerated by the USGS National Water-Quality Laboratory in Denver, Colorado, following methods described by Moulton and others (2000). Invertebrate-assemblage data included 458 taxa (all taxa identified) and a reduced dataset of 246 taxa (all taxa with abundance greater than 5 percent at 3 or more sites such that “rare” taxa were excluded). Fish analyses included all 116 fish taxa identified from the 86 sites.

Biological Metrics

Algal metrics were calculated from relative-abundance data using autecological information on taxon-specific sensitivity to environmental requirements (Porter, 2008). Taxonomic richness (number of taxa) and biovolume metrics were computed using PhycoAide (Sprouffske and others, 2006), which summarizes the algal taxonomic data by categories such as percentages of various taxonomic groups (for example, diatoms, green algae, blue-green algae). Algal metrics were selected to characterize trophic ecology, oxygen tolerance, salinity sensitivity, organic enrichment (saprobien), motility

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(siltation index), potential nitrogen fixation, and the proportion of the assemblage consisting of benthos (attached species) and seston (floating species).

Invertebrate metrics were based on raw and relative abundances of invertebrate taxa, and were computed by use of the Invertebrate Data Analysis System (IDAS) (Cuffney, 2003). Invertebrate metrics that are commonly used in water-quality assessment were computed after use of IDAS to resolve ambiguous taxa (when data are reported at one or more lower or higher taxonomic levels within the taxonomic hierarchy; Cuffney and others, 2007). Types of metrics computed reflected abundance (number of individuals) and richness (number of taxa), similarity and diversity, tolerance, and functional feeding groups (scrapers, shredders, gather-collectors, filter-collectors, etc.). Functional-feeding-group and regional-tolerance metrics included in the calculations were derived from Appendix B of the U.S. Environmental Protection Agency (USEPA) Rapid Bioassessment Protocol (Barbour and others, 1999).

Fish metrics were based on abundances of fish taxa and computed using Excel. Metrics included species traits such as substrate preference, geomorphic preference, trophic ecology or feeding preference, locomotion, reproductive strategy, and stream-size preference (Goldstein and Meador, 2004). In addition, selected metrics that are commonly included in fish Index of Biotic Integrity (IBI) ratings from the Midwest were also included, such as pollution tolerance, the number of individuals, the number of taxa, and the abundance of certain families (Karr, 1981; Lyons, 1992; Meador and others, 1993b; Barbour and others, 1999).

Environmental Characteristics

At the reach scale, transect-level physical characteristics were summarized into metrics representing minimum, maximum, and average values for each reach; summarized values at this scale were for instream and streambank metrics mentioned earlier. Segment- and watershed-scale metrics other than LULC were computed by use of a GIS and digital coverages, according to Fitzpatrick and others (1998).

Relations between Biological Assemblages, Biological Metrics, and Environmental Characteristics

A combination of multivariate techniques and Spearman rank correlations was used to relate the biological assemblages and environmental characteristics. Species-relative-abundance data were square-root transformed; however, environmental characteristics were standardized and transformed if necessary (generally also square-root or log-transformed). Detrended Correspondence Analysis (DCA) was used to examine nonlinear or unimodal patterns in assemblage data for each group of biota. DCA was performed in CANOCO version 4.5 software

(Hill, 1979; ter Braak and Smilauer, 2002). In addition to characterizing gradients in taxonomic composition, site scores from DCA axes represent latent environmental gradients to which each assemblage corresponds (ter Braak, 1987). Site scores from the first two DCA axes were used as additional biological metrics in Spearman rank correlations and as a guide for selecting the subset of environmental characteristics for subsequent multivariate analyses. To further reduce the subset of environmental characteristics for multivariate analyses, Spearman rank correlations were performed between environmental characteristics and biological metrics, where the critical rho (r_s) value ($n = 86$) occurs when $r_s \geq 0.277$ for $p < 0.01$ and when $r_s \geq 0.212$ for $p < 0.05$ (two-tailed significance level; Zar, 1974). Biological metrics selected for subsequent analyses were those that had correlations ($r_s > 0.27$) with environmental characteristics and could serve as representatives for biological metrics with similar (collinear) relations.

Additional relations between biotic assemblages and environmental patterns were assessed by means of non-parametric multivariate analyses based on the approach described by Clarke and Ainsworth (1993). For each biological assemblage, a Bray Curtis similarity (resemblance) matrix of sites was created. For environmental characteristics, a Euclidean distance dissimilarity (resemblance) matrix of the sites was created with the transformed data at all three spatial scales (reach, segment, and watershed). A two-phase regression analysis was used to select environmental characteristics that best explain patterns in the biological assemblages, by maximizing a rank correlation between the biotic and environmental resemblance matrices and their respective elements. The first regression analysis (BVSTEP) performs a stepwise search over the environmental characteristics, selects the single environmental variable with the strongest correlation and adds additional variables, one at a time, to find the combination of variables with the strongest overall correlation. This analysis was used initially for the large number of trial characteristics to select the subset of environmental characteristics that produced the greatest similarity in ranks of the 86 sites. The second regression analysis (BIOENV) calculates all permutations of environmental characteristics, and was run on the reduced set of environmental characteristics that were identified using the first regression analysis. This second procedure identifies the best combination of environmental-characteristic predictor characteristics through an iterative process where every possible combination is tried, in combinations of one, two, three, etc., environmental characteristics. Correlations between individual environmental characteristics of a given site and site similarity (resemblance) values, based on biologic assemblages, were enumerated using this procedure and those characteristics, whether individual or in combination with one or more others, that were most highly correlated with the biotic assemblages were selected for further study. Resemblance matrices, rank correlation between matrices, and the BIOENV and BVSTEP multivariate procedures were performed in Primer-E software, Version 6.0 (Clarke and Gorley, 2006).

To derive a final, reduced subset of 8 to 10 best biological metrics from each assemblage, biotic metrics were analyzed with the subset of environmental characteristics that were identified both as significantly correlated with DCA axes scores and as “best explaining” environmental characteristics from the BIOENV analysis. Metrics from each group of biota (algae, invertebrates, and fish) were used to represent a positive or negative ecosystem health response. Interpretations as to whether the biological response was positive or negative were based on multiple lines of evidence, such as metrics associated with sensitive taxa that have a positive correlation with particular environmental characteristics or metrics associated with tolerant taxa that have a negative correlation with those same characteristics. Through this process, physical characteristics with positive and negative biotic responses were identified.

Spatial and Temporal Variability of Biological Metrics

The spatial and temporal (annual) variance between reaches and between years of the selected biological metrics was examined from approximately 15 percent of the sites by calculating and comparing the coefficient of variation (cv) from three consecutive reaches and years. The coefficient of variation is a unitless measure of relative variability (often multiplied by 100) and is computed by dividing the standard deviation of the sample by the mean of the sample (Zar, 1974).

Relations between Biota and Multiscale Environmental Characteristics

In this regional study of agricultural streams in the Midwest, physical characteristics from the three spatial scales were evaluated, and the relative importance of reach-scale to segment- and watershed-scale characteristics was assessed. The watershed-scale characteristics correlated with all three biotic assemblages but most highly with fish. The stronger relative importance of watershed-scale characteristics was found by means of either Spearman rank correlations with DCA-axis scores or multivariate analyses with the biotic assemblages. The biological assemblages as a whole generally had stronger correlations than individual metrics that represented specific aspects of the assemblages.

Biological Assemblages with Environmental Characteristics

Summary statistics for those environmental characteristics to which algal, invertebrate, and fish assemblages showed

the strongest relations are presented in table 1. The results of the analyses identified environmental characteristics from each of the three scales that together best explained the variation in biological assemblages from the 86 sites (table 2).

Combining characteristics from all spatial scales improved the correlations slightly for algal and invertebrate assemblages but not for the fish assemblages. Instead, fish assemblages were associated ($r_s > 0.6$) with a combination of five watershed features: drainage area, soil permeability, latitude, mixed forest, and row-crop land cover. The biological metrics that were identified as the most responsive to the measured environmental characteristics are summarized in table 3.

At the Reach Scale

The characteristics at the reach scale that best explained variations in biological assemblages, based on multivariate analyses, were bank vegetative cover (*BankVeg*), percentage of fine sediment in the stream substrate (*Fine*), open canopy angle variation (*OpCanCV*), and percentage of woody vegetation in the 50-m buffer (*WV50*) (table 2). For both algal and fish assemblages, average wetted channel width (*WetWdth*) and Froude number (*Froude*) were also included. The Froude number is a dimensionless metric representing the ratio of the inertial forces to the gravitational forces in the stream channel; thus, it relates to the hydrology and the geomorphology of the channel.

When the biological-assemblage data, represented by DCA axes, were examined, additional reach-scale environmental metrics were identified that had significant correlations with one or more biotic assemblages (tables 4, 5, and 6). Additional reach-scale characteristics most strongly correlated with algal assemblages included average stream velocity (*Velocity*), *WetWdth*, and average wetted-channel width-to-depth ratio (*W/D*) (table 4). The other reach-scale characteristics most strongly correlated with invertebrate assemblages, as represented by DCA axes, were bank erosion (*BankEros*), *Froude*, open-canopy angle (*OpCanAng*), percentage of riffles (*Riffle*), *W/D*, *WetWdth*, and *Velocity* (table 5). The first two invertebrate DCA axes (*IDCA_AX1* and *IDCA_AX2*) appeared to represent tolerant taxa, reflected by negative correlations with *BankVeg*, *Riffle*, *Velocity*, and *WV50* and positive correlations with *Fine* and *OpCanAng*. Reach characteristics also highly correlated with fish assemblages were *WetWdth*, *W/D*, *Froude*, *Riffle*, *Velocity*, and *OpCanAng* (table 6). Increasing values of *Riffle*, *BankVeg*, and *WV50* were correlated with increases in positive-indicator metrics for all three biological assemblages.

As mentioned above, selected metrics from each group of biota (algae, invertebrates, and fish) were predefined to represent a positive or negative ecosystem health response. Positive-indicator metrics correlating with *BankVeg* were blue-green algal biovolume (*BGA_bv*), percentage of nitrogen-fixing algal cells (*pNFIKERS*), and percentage of the diatom *Achnanthydium minutissimum* (*pAch_min*) for the algae; caddisfly richness (*R_TRICH*) and percentage abundance of

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mollusks and crustaceans (*pMOLCRU*) for the invertebrates; and percentages of cobble-substrate fish (*pCOBBfish*) and pollution-sensitive fish (*pSENSfish*) for the fish. Negative-indicator biotic metrics correlated with *BankVeg* were percentage of tolerant diatom taxa (*pTOLBPCI*), percentage of

eutrophic algal taxa (*pEUTROPH*), invertebrate taxa richness composed of non-midge Diptera and non-insects (*R_ODIPNI*), average USEPA pollution tolerance value for invertebrates based on taxa richness (*RichTOL*), ratio of taxa richness of Orthocladiinae taxa midges to all midges (*pR_ORTHO_CH*),

Table 2. Summary of correlations between biological assemblages and selected environmental characteristics at each physical scale for agricultural stream sites in the Midwest.

[(#), number of characteristics contributing to the best combinations or highest correlation between biotic assemblages and environmental characteristics; bold numbers indicate characteristics that contributed to the BIOENV combined correlation selections; bold abbreviations indicate characteristics that contributed to correlations with all three biological assemblages. Metric definitions are given in table 1]

Metric abbreviation	Algae	Invertebrate	Fish
Reach characteristics—BIOENV combined correlation	0.375 (¹ 7)	0.365 (6)	0.358 (7)
BankEros	.021	.137	.055
BankVeg	.100	.153	.187
Fine	.125	.114	.055
Froude	.117	.117	.111
OpCanCV	.194	.195	.125
Velocity	.071	.127	.057
WetWdth	.127	.094	.325
W/D	.071	.054	.241
WV50	.081	.045	.070
Segment characteristics—BIOENV combined correlation	.259 (3)	.235 (² 6)	.276 (³ 3)
BuffFrag	.080	.083	.134
BuffLngh	.009	- .056	- .005
Crop250	.191	.138	.143
SegGrad	.113	- .047	- .088
SINUOS	.088	- .089	- .081
WV150	.177	.150	.130
Watershed characteristics—BIOENV combined correlation	.445 (5)	.510 (5)	.604 (5)
DrainArea	.273	.364	.460
Forest	.311	.254	.319
Latitude	.143	.252	.243
MxForest	.244	.264	.409
Perml	.246	.213	.307
RowCrop	.321	.268	.309
Slope	.273	.172	.255
WetXArea/DA	.185	.319	.276
All scales of variables combined	.534 (9)	.523 (7)	.604 (5)

¹ Also row crops in 15-m stream margin (0.031).

² Also woody vegetation in the 250-m buffer (1.92), wetland in the 15-m stream margin (0.050), and urban land in the 250-m buffer (0.043).

³ Also wetland in the 250-m buffer (0.109).

percentage richness of omnivorous invertebrates (*pR_OMNI*), and percentages of creeper locomotion (*pCREEPfish*) fish, percentages of detritivore (*pDETRfish*) fish, and omnivorous (*pOMNIfish*) fish, which all had negative correlations (tables 4–6). The positive-indicator metrics that correlated with *Riffle* were similar to those for *BankVeg*.

At the Segment Scale

Based on multivariate analyses, the only segment-scale characteristic that explained variation in all three biological assemblages combined was the number of buffer fragments in the margin (*BuffFrag*), that is, changes from one type of land cover in the buffer to another along the segment length. Land cover in the buffer area was the other most important feature, whether cropland or woody vegetation. However, Spearman

Table 3. Selected biological metrics, with metric abbreviations and range of data (minimum, median or 50th percentile, and maximum) for 86 agricultural stream sites in the Midwest, listed by biological assemblage.

Metric abbreviation	Metric description	Unit	Minimum	Median	Maximum
Algae					
BGA_bv	Blue-green algal biovolume	µm ³ /cm ²	0.0	1.5 x 10 ⁷	1.3 x 10 ¹⁰
pAch_min	Percentage ¹ of diatom <i>Achnanthydium minutissimum</i>	percent	0	.6	56.0
pBRACKSL	Percentage of brackish-water algal taxa	percent	0	4.3	59.4
pEUTROPH	Percentage of eutrophic algal taxa	percent	.2	24.9	77.3
pNFIKERS	Percentage nitrogen fixing algal cells	percent	0	.0	99.1
pSENBPC3	Percentage of sensitive diatom taxa	percent	0	11.4	58.2
pSESTON	Percentage of seston	percent	0	7.8	86.8
pTOLBPC1	Percentage of tolerant diatom taxa	percent	0	2.8	32.3
ShWTTax	Shannon Wiener, diversity total taxa	dimensionless	.1	3.7	5.3
Silt_Ind	Diatom Siltation Index (percent of total taxa)	percent	.7	47.8	92.8
Invertebrates					
Margalef	Margalef Diversity of total taxa	unitless	3.5	9.1	16.7
pFC_abund ²	Percentage of filtering collectors	percent	1.2	28.0	80.3
pGC_abund ³	Percentage of gathering collectors	percent	.9	27.4	73.5
pMOLCRU	Percentage of molluscs and crustaceans	percent	0	1.4	53.0
pR_OMNI	Percent richness of omnivores	percent	0	4.2	18.5
pR_ORTHO_CH	Percent richness of Orthocladinae midges to all midges	percent	0	33.3	62.5
R_ODIPNI	Richness composed of non-midge Diptera and non-insects	number	1.0	7.5	17.0
R_TRICH	Richness composed of caddisflies (Trichoptera)	number	1.0	5.0	12.0
RichTOL	Average USEPA tolerance values for sample based on richness	dimensionless	3.6	4.9	6.7
Fish					
pCARNfish	Percentage of carnivore/piscivore fish	percent	0	15.7	94.8
pCOBBfish	Percentage of cobble-substrate preference fish	percent	0	32.7	100.0
pCREEPfish	Percentage of creeper locomotion fish	percent	0	13.9	82.7
pDETRfish	Percentage of detritivorous fish	percent	0	24.9	81.4
pMUDfish	Percentage of mud-substrate preference fish	percent	0	17.9	76.7
pOMNIfish	Percentage of omnivorous fish	percent	0	17.1	74.5
pSENSfish	Percentage of pollution-sensitive fish	percent	0	6.7	64.3
R_TAXAfish	Richness of fish taxa	number	2	18	35

¹ Percentage refers to the percent relative abundance of cells or individuals of a specific type compared with the total in a sample.

² Better for rocks.

³ Better for snags.

rho values for all of the segment characteristics together were low ($r_s = 0.235$ for algae to 0.276 for fish) and were the weakest of the three physical scales for explaining patterns in the biotic assemblages (table 2).

Positive-indicator metrics for all three assemblages were correlated with increasing percentages of woody vegetation in the 150-m buffer (*WV150*), whereas negative-indicator metrics correlated with the percentage of cropland in the 250-m buffer (*Crop250*) on both sides of the streams (tables 4–7). Increasing percentage of woody vegetation in the stream margin and increasing average length of undisturbed buffer (*BuffLength*) were correlated with positive-indicator metrics for algae and fish, whereas the *Crop250* was correlated with negative-indicator metrics.

For segment-scale characteristics, algal assemblages were the most highly correlated to *Crop250* and *WV150*. Invertebrate and fish assemblages were also most highly correlated with these two characteristics, as well as *BuffLength*. Sinuosity (*SINUOUS*) correlated significantly for invertebrates only. Woody vegetation, in this case *WV150*, was selected to be representative of the other woody vegetation buffers that were highly correlated with it, for example with the 250-m ($r_s = 0.97$) and the 15-m ($r_s = 0.87$) woody vegetation buffers. If a significant correlation is found between a biological metric and a particular LULC, one might expect similar correlations for that LULC as measured at different buffer widths. A negative correlation for algae with *SINUOS* appeared to be due to reduced current velocity, which correlated with a higher percentage of seston (phytoplankton) in the benthic samples.

At the Watershed Scale

At the watershed scale, variation in each of the three biotic assemblages was best explained by changes in soil permeability (*Perml*), latitude, and percentage of land cover/land use—either total forest (*forest*), mixed forest (*MxForest*), or row crop (*RowCrop*). Drainage area (*DrainArea*) had a greater correlation with the fish and invertebrate assemblages, whereas average watershed slope (*Slope*) best explained variation in algal assemblages (table 2). Average wetted-reach cross-sectional area as normalized by drainage area (*WetXArea/DA*) had mixed results among assemblages; for example, it showed positive correlations with positive-indicator algal metrics such as percentage of *Achnanthydium minutissimum* (*pAch_min*) and negative correlations with percentage of seston (*pSESTON*), a negative-indicator metric (table 4). *WetXArea/DA* was positively correlated with all positive-indicator fish metrics except diversity, and was negatively correlated with all negative-indicator fish metrics (table 6). Both the individual characteristics and the multivariate combinations had the strongest correlations at the watershed scale for algae, invertebrates, and fish.

When the analysis was run with characteristics combined from all three scales, algal and invertebrate correlations improved when fine silty substrate (*Fine*) and open-canopy

angle variation (*OpCanCV*) from the reach scale, and woody vegetation in the 150-m buffer (*WV150*) from the segment scale were included. Although the correlations for the fish assemblages with environmental characteristics were as numerous and strong as the algal and invertebrate assemblages, none of the reach or segment characteristics improved the correlation found with watershed characteristics (table 2).

Spearman rank correlation using DCA axis scores to represent the biological assemblages were compared with the metrics derived from more specific aspects of the biological assemblages such as the number of detritivores (tables 4–6). In many cases, correlations to environmental characteristics were stronger with DCA axes representing the assemblages than with the more specific biotic metrics (see table 5). Some metrics followed a response pattern similar to one of the DCA axes but at varying strengths. One example of this was the similarity in response between the metrics for detritivorous fish (*DETRfish*) and fish DCA axis 1 (*FDCA_AXI*). *DETRfish* correlated with land cover at all three physical scales, showing a negative correlation at the reach scale to woody vegetation in the 50-m buffer (*WV50*) ($r_s = -0.28$) and positive correlations at the segment scale with cropland in the 250-m buffer (*Crop250*) ($r_s = 0.50$) and at the watershed scale with percentage of row-crop land cover (*RowCrop*) ($r_s = 0.56$).

In general, biotic assemblages were positively correlated to watershed characteristics, with the exception of negative correlations with increasing amounts of *RowCrop* and *DrainArea* (table 7). Although diversity and taxa richness increased for algae and fish, respectively, with *DrainArea*, the resulting community was more tolerant (tables 4, 6, and 7). For invertebrates, only the relative abundance of filtering collectors (*pFC_abund*) increased with drainage area.

The combination of the best physical characteristics from each spatial scale for each biotic assemblage indicated that in addition to the watershed metrics that correlated most strongly, other important metrics were *Fine* for algae, *OpCanCV* for algae and invertebrates, *BankVeg* for invertebrates and fish, and *WV150* for algae (table 7). These reach- and segment-scale characteristics correlated strongly enough to be included as characteristics in the combined model (table 2).

Biological Metrics with Environmental Characteristics

Biological metrics that describe specific aspects of a biological assemblage are useful for assessing how a particular taxonomic or functional group might vary with changes in some environmental characteristic. However, metrics like DCA axis scores that more broadly described assemblages had generally stronger correlations with environmental characteristics than did the more specific biological metrics. This finding agrees with other studies that recommend the use of multimetric approaches for bioassessment (for example, see Karr, 1981; Lücke and Johnson, 2009).

Algal Metrics

The selection of algal metrics that correlated most strongly with the environmental characteristics was based on both positive and negative character of the biological metrics (table 4). Indicators of impairment, such as percentage of tolerant diatom taxa (*pTOLBPC1*), the Diatom Siltation Index (*Silt_Ind*), and percentage of seston (*pSESTON*) in the benthic samples were selected to represent negative-indicator metrics (Bahls, 1993; Porter, 2008). In contrast, metrics such as percentage of the diatom *Achnanthydium minutissimum* (*pAch_min*) and biovolume of blue-green algae (*BGA_bv*) consistently correlated opposite to designated negative-indicator metrics and thus were considered to be positive-indicator metrics. Percentage of sensitive diatom taxa (*pSENBPC3*) and *BGA_bv* were negatively correlated with fine silty substrate (*Fine*) at the reach scale; however, *pSENBPC3* and *pAch_min* were positively correlated with average length of undisturbed buffer (*BuffLngth*) at the segment scale (table 4). Positive-indicator metrics such as *pAch_min* and percentage of nitrogen-fixing cells (*pNFXERS*) correlated negatively with drainage-area size (*DrainArea*) and increasing percentages of row crop in the watershed (*RowCrop*), and correlated positively with forest land cover (both *Forest* and *MxForest*). The negative-indicator metric *pSESTON* increased with the percentage of cropland in the buffer (*Crop250*) and row-crop land cover. The Shannon-Wiener diversity index for algae (*ShWTTax*) correlated with many environmental characteristics, but the direction of the response was similar to negative-indicator metrics (table 4). Overall algal taxa richness (metric not shown) did not have significant correlations with environmental characteristics but was correlated with the abundance of diatoms (not shown) and *ShWTTax*. As a result, we selected *ShWTTax* as the representative algal diversity metric that was most responsive to environmental characteristics.

Invertebrate Metrics

The invertebrate metrics selected were those that correlated most highly with measured environmental characteristics (tables 3 and 5); in general, the final invertebrate metrics selected were only weakly to moderately correlated with each other except for *MOLCRUp* and *ODIPNIR* ($r_s = 0.71$), and for the percentage abundances of filtering collectors (*pFC_abund*) and gathering collectors (*pGC_abund*) ($r_s = -0.71$). Correlations between environmental characteristics and invertebrate assemblages, as represented by DCA axes, were generally slightly higher if rare taxa were excluded. Therefore, multivariate analyses used the invertebrate dataset with rare taxa excluded. Correlations at a higher r_s were more numerous for invertebrates collected from rocks and snags combined ($n=86$) and rocks alone ($n=45$) than from snags alone ($n=41$). For this reason, and to allow examination of patterns among 86 sites across the three groups of biota, subsequent discussions focus on results for all invertebrate taxa and for rocks

and snags combined (noting important differences in results by substrate).

Positive-indicator metrics for invertebrates represented qualities of healthy assemblages based on studies in the literature (Lenat, 1988; Kerans and Karr, 1994; Barbour and others, 1996). In contrast, negative-indicator metrics are generally associated with degraded assemblages. In our study, the strongest positive-indicator metrics for invertebrates were taxa richness of caddisflies (*R_TRICH*), the percentage abundance of mollusks and crustaceans (*pMOLCRU*), *pFC_abund* and *pGC_abund* (of these two, the former was better for rocks and the latter was better for snags), and Margalef diversity (Margalef, 1958) (table 5). With degrading water quality, invertebrate assemblages may change from those dominated by aquatic insect larvae, such as members of the pollution-sensitive orders Ephemeroptera-Plecoptera-Trichoptera (EPT; mayflies-stoneflies-caddisflies), to those dominated by non-insects. Percent richness of EPT taxa (*pR_EPT*) was less useful as a positive-indicator metric in this study due to the lack of correlations for snag samples. Significant correlations for *pR_EPT* were found for rock samples, such as with woody vegetation and cropland in the buffer ($r_s = 0.33$ for *WV100* to $r_s = 0.43$ for *WV250*; $r_s = -0.33$ for percentage of cropland in the segment 15-m margin to $r_s = -0.37$ for *Crop250*). Metrics based on EPT taxa can be affected by relatively tolerant mayfly or caddisfly taxa, as seemed to be the case in this study. Tolerant mayfly taxa such as *Baetis flavistriga*, *B. intercalaris*, and *Caenis* spp. and tolerant caddisfly taxa such as *Cheumatopsyche* spp., *Hydropsyche* spp., and *Hydroptila* spp. were dominant at many sites and may have led to the decreased sensitivity of the EPT metrics.

The best negative-indicator metrics for invertebrates were taxa richness composed of non-midge Diptera and non-insects (*R_ODIPNI*), USEPA pollution tolerance value based on taxa richness (*RichTOL*), ratio of taxa richness of Orthocladinae taxa midges to all midges (*pR_ORTHO_CH*), and percentage taxa richness of omnivorous invertebrates (*pR_OMNI*). *RichTOL* values decreased with increasing woody vegetation in the buffer (*WV150*) but increased from narrow to wider cropland buffer (*Crop250*). As mentioned earlier, the first two DCA axes appeared to represent primarily tolerant taxa, as evidenced by correlations with negative-indicator metrics and physical characteristics such as cropland. Several other metrics showed relatively high correlations to selected environmental characteristics; however, they were redundant with the above-mentioned metrics and did not correlate highly with as many physical characteristics.

Fish Metrics

Fish metrics found to be the most strongly correlated with physical environmental characteristics in this study (tables 2 and 6) included the positive-indicator metrics percentage of cobble-substrate fish (*pCOBBfish*), pollution-sensitive fish (*pSENSfish*), and carnivore or piscivore fish (*pCARNfish*);

Table 4. Correlations between algal metrics and selected environmental characteristics for agricultural stream sites in the Midwest.

[bold, $p < 0.01$, based on the Spearman correlation coefficient r_s (Zar, 1974); n, number of sites in comparison. Metric definitions are given in tables 1 and 3]

Ecosystem health indicators			Positive					Negative					n
Metric abbreviation	ADCA_AX1	ADCA_AX2	pAch_min	pSENBPC3	BGA_bv	pNFIKERS	ShWTTax Diversity	pTOLBPC1	Silt_Ind	pSESTON	pBRACKSL	pEUTROPH	
Reach characteristics													
BankEros	-0.15	-0.21	.33	0.01	0.26	0.08	-0.13	-0.07	-0.11	-0.26	-0.04	0.01	85
BankVeg	- .08	- .38	.33	- .14	.41	.39	- .38	- .41	- .28	- .28	- .04	- .35	85
Fine	- .12	- .14	- .11	- .28	- .24	.06	- .06	.00	.10	.15	- .02	- .16	85
Froude	- .11	- .45	.35	- .02	.21	.22	- .41	- .34	- .32	- .38	- .38	- .24	71
OpCanAng	.21	.15	- .19	- .04	- .15	- .18	.19	.13	.21	.25	.21	.00	85
OpCanCV	- .19	- .09	.16	- .08	.18	.23	- .15	- .11	- .07	- .18	- .14	- .02	85
Riffle	.01	- .20	.36	.11	.30	.21	- .28	- .36	- .23	- .31	- .17	- .07	67
Velocity	- .11	- .44	.30	.07	.18	.15	- .35	- .24	- .30	- .39	- .04	- .17	71
WetWdth	.15	- .37	- .12	.27	.01	- .38	.31	.23	.20	- .32	.39	.22	85
W/D	.34	.45	- .02	.16	.11	- .35	.30	.17	.22	.31	.25	.11	79
WV50	- .18	- .25	.33	.10	.26	.02	- .38	- .41	- .32	- .23	- .26	- .12	86
Segment characteristics													
BuffFrag	- .04	- .04	- .12	- .25	- .05	.07	- .08	- .09	.14	.02	- .10	- .16	86
BuffLngth	- .18	- .10	.35	.23	.11	.11	- .10	- .09	- .36	- .23	- .06	.03	86
Crop250	.32	.33	- .42	- .17	- .21	- .32	.34	.37	.55	.47	.22	.07	86
SegGrad	.34	- .09	.15	- .12	- .03	.08	.01	- .10	- .01	.14	- .13	- .14	86
SINUOS	.22	.25	- .33	- .16	- .19	.03	.16	.13	.28	.34	.12	- .22	85
WV150	- .29	- .34	.32	.11	.30	.27	- .41	- .33	- .43	- .37	- .26	- .17	86
Watershed characteristics													
DrainArea	.24	.41	- .34	.10	- .28	- .39	.28	.21	.25	.34	.39	.11	86
Forest	- .32	- .35	.65	.07	.31	.35	- .41	- .39	- .57	- .42	- .37	- .20	86
Latitude	.14	- .30	- .08	- .09	- .19	.23	- .16	- .14	- .26	- .22	- .04	- .09	86
MxForest	.02	- .37	.47	.02	.11	.30	- .25	- .25	- .36	- .36	- .36	- .14	86
Perml	- .05	- .43	.52	.10	.26	.28	- .21	- .40	- .54	- .46	- .47	- .21	86
RowCrop	.34	.33	- .55	- .04	- .26	- .42	.43	.41	.57	.41	.39	.20	86
Slope	- .40	- .40	.49	- .07	.26	.38	- .40	- .39	- .42	- .37	- .38	- .29	86
WetXArea/DA	- .31	- .37	.40	.08	.37	.27	- .27	- .19	- .23	- .32	- .23	- .00	79

Table 5. Correlations between invertebrate metrics and selected environmental characteristics for agricultural stream sites in the Midwest.

[bold, $p < 0.01$, based on the Spearman correlation coefficient r_s (Zar, 1974); n, number of sites in comparison. Metric definitions are given in tables 1 and 3]

Ecosystem health indicators			Positive					Negative				n
Metric abbreviation	IDCA_AX1	IDCA_AX2	R_TRICH	pMOLCRU	pFC_abund	pGC_abund	Margalef Diversity	R_ODIPNI	RichTOL	pR_ORTHO_CH	pR_OMNI	
Reach characteristics												
BankEros	-0.35	-0.20	0.33	0.25	-0.15	0.24	0.11	0.26	-0.10	0.18	-0.19	85
BankVeg	- .42	- .10	.37	.33	- .26	.23	.16	.32	- .38	.34	- .34	85
Fine	.29	.22	.02	- .10	- .02	- .11	- .29	- .10	.08	- .11	.06	85
Froude	- .41	- .23	.36	.13	- .03	.10	- .04	.22	- .42	.42	- .35	71
OpCanAng	.28	.34	- .09	- .17	.29	- .04	- .29	- .24	.06	- .04	- .17	85
OpCanCV	- .28	- .31	.08	.23	- .27	.00	.22	.18	.09	.05	.12	85
Riffle	- .56	- .27	.43	.45	- .22	.20	.14	.49	- .18	.16	- .24	67
Velocity	- .38	- .24	.30	.07	.05	.10	- .06	.18	- .41	.40	- .36	71
WetWdth	.39	.14	- .19	- .42	.30	- .24	.07	- .34	- .08	- .34	.21	85
W/D	.40	.37	- .20	- .36	.18	- .18	.11	- .27	- .09	- .29	.21	79
WV50	- .29	- .38	.15	.17	- .12	.09	.32	.34	- .23	.00	- .15	86
Segment characteristics												
BuffFrag	.03	.02	- .05	- .03	.09	- .05	- .13	.05	.09	- .06	- .01	86
BuffLngh	- .27	- .12	.16	.16	- .17	.11	.23	.11	- .16	.09	.05	86
Crop250	.56	.36	- .27	- .41	.22	- .19	- .29	- .37	.25	- .15	.04	86
SegGrad	- .44	- .17	.17	.21	- .13	.16	- .01	.21	- .37	.30	- .40	86
SINUOS	.31	.35	- .22	- .14	.05	.00	- .15	- .14	.07	- .13	- .07	85
WV150	- .43	- .38	.22	.21	- .10	.07	.36	.27	- .33	.04	- .11	86
Watershed characteristics												
DrainArea	.67	.23	- .32	- .62	.40	- .26	- .09	- .48	.02	- .40	.22	86
Forest	- .60	- .27	.22	.42	- .18	.16	.21	.28	- .21	.17	- .15	86
Latitude	- .15	- .17	.08	.20	- .14	.27	- .12	.13	- .04	.29	- .39	86
MxForest	- .50	- .16	.29	.33	- .28	.46	.09	.23	- .29	.48	- .38	86
Perml	- .50	- .36	.36	.34	- .34	.39	.06	.31	- .37	.35	- .30	86
RowCrop	.67	.37	- .24	- .52	.14	- .12	- .20	- .36	.09	- .22	.12	86
Slope	- .51	- .36	.33	.29	- .18	.12	.19	.27	- .26	.19	.02	86
WetXArea/DA	- .71	- .35	.34	.54	- .28	.19	.21	.45	- .10	.32	- .17	79

Table 6. Correlations between fish metrics and selected environmental characteristics for agricultural stream sites in the Midwest.

[bold, $p < 0.01$, based on the Spearman correlation coefficient r_s (Zar, 1974); n, number of sites in comparison. Metric definitions are given in tables 1 and 3]

Ecosystem health indicators			Positive				Negative				n
Metric abbreviation	FDCA_AX1	FDCA_AX2	pCOBBfish	pSENSfish	pCARNfish	R_TAXAfish (Diversity)	pDETRfish	pOMNIfish	pMUDfish	pCREEPfish	
Reach characteristics											
BankEros	-0.25	-0.00	0.14	0.17	0.42	-0.36	-0.34	-0.27	-0.24	-0.10	85
BankVeg	- .45	.29	.42	.32	.25	- .22	- .36	- .33	- .23	- .43	85
Fine	.06	- .42	- .27	- .32	.02	- .04	.23	.25	.11	.07	85
Froude	- .47	.33	.44	.36	.25	- .28	- .37	- .38	- .33	- .29	71
OpCanAng	.10	- .31	- .30	- .26	- .12	.10	.40	.37	.21	.10	85
OpCanCV	- .19	.20	.26	.11	.16	- .24	- .41	- .33	- .11	- .10	85
Riffle	- .31	.44	.55	.41	.31	- .30	- .44	- .27	- .38	- .04	67
Velocity	- .41	.33	.45	.36	.26	- .25	- .33	- .32	- .34	- .22	71
WetWdth	.75	.06	- .21	.07	- .39	.62	.44	.36	.02	.42	85
W/D	.60	.13	- .21	.04	- .43	.58	.40	.34	.18	.34	79
WV50	- .22	.36	.40	.38	.15	- .10	- .28	- .23	- .28	- .16	86
Segment characteristics											
BuffFrag	- .12	- .25	- .20	- .07	.03	- .04	.23	- .01	.02	- .06	86
BuffLngth	.02	.33	.33	.22	.06	- .01	- .29	- .27	- .29	.06	86
Crop250	.26	- .48	- .50	- .45	- .25	.28	.50	.56	.50	.35	86
SegGrad	- .34	- .09	.13	- .12	.17	.02	.36	.06	.15	- .15	86
SINUOS	.08	- .23	- .25	- .16	- .30	.16	.32	.35	.35	.07	85
WV150	- .11	.59	.59	.56	.14	- .03	- .38	- .37	- .43	- .20	86
Watershed characteristics											
DrainArea	.61	- .21	- .37	- .24	- .38	.52	.50	.44	.20	.43	86
Forest	- .28	.40	.49	.46	.46	- .33	- .51	- .46	- .52	- .35	86
Latitude	- .77	- .19	.13	- .17	.31	- .48	- .27	- .21	.02	- .36	86
MxForest	- .61	.16	.35	.23	.45	- .47	- .46	- .35	- .28	- .43	86
Perml	- .54	.32	.47	.46	.41	- .35	- .45	- .44	- .51	- .47	86
RowCrop	.38	- .34	- .48	- .44	- .47	.15	.56	.48	.48	.37	86
Slope	- .22	.27	.33	.40	.32	- .29	- .44	- .34	- .42	- .36	86
WetXArea/DA	- .25	.49	.49	.56	.32	- .31	- .49	- .45	- .46	- .23	79

Table 7. Summary of ecosystem health indicators for environmental characteristics with the strongest relations to biotic assemblages for agricultural stream sites in the Midwest.

[Bold, variables selected when all scales combined; “-” negative indicator for the biological assemblage; “+”, positive indicator for the biological assemblage; “+/-”, mixed indicators for the biological assemblage; blank spaces, no definitive indicator for the biological assemblage; metric definitions are given in table 1]

Metric abbreviation	Algae	Invertebrates	Fish
Reach characteristics			
BankEros	+	+/-	+
BankVeg	+	+	+
Fine	-	-	-
Froude	+	+	+
OpCanCV	-	+	-
Riffle	+	+	+
Velocity	+	+	+
WetWdth	+/-	+	+/-
W/D		-	+/-
WV50	+	+	+
Segment characteristics			
BuffFrag	-		-
BuffLngth	+		+
Crop250	-	-	-
SINUOS	-		
WV150	+	+	+
Watershed characteristics			
DrainArea	-	+/-	-
Forest	+	+	+
Latitude			+/-
MxForest	+	+	+
Perml	+	+/-	+
RowCrop	-	-	-
Slope	+	+	+
WetXArea/DA	+	+/-	+

negative-indicator metrics were the percentages of omnivore fish (*pOMNIfish*), detritivore fish (*pDETRfish*), and mud-substrate preference fish (*pMUDfish*). Although *pOMNIfish* and *pDETRfish* were intercorrelated ($r_s = 0.83$), these metrics represent distinct aspects of the fish assemblage and *pOMNIfish* is a common metric used by watershed managers to assess impact. Omnivore fish have broad diets that include plant and animal material as well as detritus. The *pDETRfish* also had a positive correlation ($r_s = 0.55$) with the algal metric *pSESTON*, which is considered a negative algal water-quality indicator for streams. Although the fish assemblages were more strongly correlated with watershed characteristics, as shown by the multivariate results, there were many correlations between fish metrics and reach-level characteristics. As with invertebrate assemblages, fish assemblages and metrics were highly correlated with land cover: negatively with the percentage of cropland and positively with woody vegetation in the riparian buffer at reach and segment scales (15- to 250-m buffer widths) but especially at the segment-scale 250-m buffer. The *pSENSfish* and *pCOBBfish* metrics, as well as the positive-indicator metrics for algae (*BGA_bv*, *pSENBPC1*), were positively correlated with increased riffle area (*Riffle*) and negatively correlated with percentage of fine silt substrate (*Fine*).

Spatial and Temporal Variability of Metrics

Biological metrics were less variable between reaches and between years for metrics based on taxonomic richness than those based on percentage abundance, as shown by coefficients of variation for these metrics (table 8). Richness metrics (*R_TAXAalg*, *R_Diatom*, *RichTOL*, *R_ODIPNI*, *R_TAXAinv*, and *R_TAXAfish*) and diversity indices (*ShWTTax*, *Margalef*) had relatively low variance (generally <20%),

whereas Bahl’s siltation index (*Silt_Index*) and pollution tolerance index (*TOLBPC1*) for algae varied from relatively low to high variance. The percentage abundances for the fish metrics were less variable than those for the algae metrics (*pAch_min*) and invertebrate metrics (*pFC_abund*), which were more consistent between reaches but varied between years (figs. 2 and 3). Wang and others (2006) found that habitat and fish assemblage measures can vary greatly among years, especially at sites with low habitat diversity.

Discussion of Findings and Comparison with Other Studies

Few regional studies have examined patterns of multiple biological-assemblage associations with environmental characteristics representing multiple scales. Allen and others (1999) assessed environmental gradients and patterns of taxonomic composition among multiple biotic assemblages in lakes in New England, and Fore (2003) assessed biological indications of stream condition in the mid-Atlantic area. General findings from these assessments were concordant with our study and supported the observation by Carter and others (1996) regarding the confounding influence of watershed-scale features on benthic assemblages, which is due to dependence of smaller-scale characteristics and processes on larger-scale characteristics and processes. Although physical factors at one scale may influence those at another—and categorizations into reach-segment-watershed are somewhat arbitrary in some cases—it is useful to attempt to understand the relative importance of these scales of environmental characteristics to different groups of biota. In Allen and others (1999) and in Fore (2003), the fish communities also correlated most with landscape and watershed-scale characteristics compared to reach-scale characteristics. Fore (2003) suggested that

Table 8. Values for median coefficient of variation between selected biological metrics from samples collected among multiple reaches and multiple years at each site for agricultural stream sites in the Midwest.

[Coefficient values are in percent; n, number of sites. Metric definitions are given in table 3]

Algae	n	Low variability					High variability			
		ShWTTax	R_TAXAalg	R_Diatom	Silt_Ind	pAch_min	pSESTON	BGA_bv	pTOLBPC1	
Multiple reach	11	7.7	9.7	10.4	17.0	19.6	62.3	68.4	69.0	
Multiple year	11	10.8	9.9	10.9	32.2	72.0	75.6	72.0	75.1	
Invertebrates		RichTOL	R_TAXAinv	Margalef	R_ODIPNI	pFC_abund	pR_OMNI	pR_ORTHO_CH	pGC_abund	
Multiple reach	14	3.2	11.4	17.1	24.4	20.2	29.3	30.3	32.2	
Multiple year	14	12.3	16.4	11.1	15.6	66.6	26.1	22.6	25.8	
Fish		R_TAXAfish	pCOBBfish	pCARNfish	pOMNIfish	pCREEPfish	pDETRfish	pMUDfish	pSENSfish	
Multiple reach	16	11.0	16.0	26.0	27.5	34.0	34.0	40.9	44.8	
Multiple year	16	12.2	28.4	27.4	40.0	23.1	29.8	36.0	49.4	

the different sensitivity to watershed characteristics across assemblage groups might reflect the relative size of the organisms, as discussed by Allen and others (1999) from their study of New England lakes. In that study, large-bodied organisms responded more strongly to watershed-scale characteristics than to local-scale characteristics. These results correspond to those in this study where it was observed that fish assemblages correlated strongly to the watershed-scale attributes including latitude, drainage area, and mixed-forest land cover. Assemblages of smaller-bodied organisms (diatoms and zooplankton) generally have been found to have stronger correlations with more local-scale characteristics than to broader watershed-scale characteristics (Allen and others, 1999; Fore, 2003; Allan and others, 1997).

Allan and others (1997) suggested that the broader issue of landscape influence across multiple scales needs further study to distinguish the relative importance of local and regional influences. They concluded that the extent of agricultural land at the subcatchment (watershed) scale was the best single predictor of local stream conditions as evaluated by fish IBI values for the River Raisin, Michigan. In contrast, Stauffer and others (2000) found that local-scale characteristics such as riparian cover were more important to fish assemblages. In the current study, although the percentage of cropland and woody vegetation at various widths at the segment-scale were the best predictors for all three biotic assemblages at the segment scale, the segment-scale characteristics generally were not the best predictors of stream conditions across all study sites when compared to watershed-scale characteristics. Fitzpatrick and others (2001) found that fish metrics responded most to watershed-scale characteristics, and fish IBI scores were most affected by land use in the watershed and buffer widths less than 200 m in the entire stream network. As Allan and others (1997) and Stewart and others (2001) suggest, these contrasting results could be due to the geographic extent of individual studies and the complex influences of landscape characteristics at various scales.

Fore (2003) found no relations between diatoms and watershed size; although correlations were found between diatoms and other watershed characteristics, these relations were the weakest among all three assemblages analyzed. The current study included analysis of non-diatom metrics such as the biovolume of blue-green algae and the percentage of seston (planktonic species *pSESTON*) found in benthic samples. In typical watersheds, the amount of seston increases from the headwater streams to wider, downstream sites, where more light and nutrients are typically available and residence times are longer (Vannote and others, 1980). Percentage of seston in the current study increased with drainage area, correlating with the percentage of cropland in the 250-m buffer (*Crop250*) and with the mean length of margin areas that are in crop land cover. Frothingham and others (2002) recognized the occurrences of open canopies in headwater prairie streams and considered the functions of these streams as a reversal of the river continuum in east-central Illinois, where natural land cover has been eradicated by agriculture and croplands now extend

to the margins of streams throughout the headwaters. In these settings, riparian forest remains only along medium and large rivers. The removal of streamside vegetation that accompanies channelization reduces shading, thereby increasing diurnal temperature variations, eliminating cover for fish, and decreasing organic inputs. Some fish species have been extirpated by channelization (Frothingham and others 2002) and although diversity has increased in channelized streams over time, habitat still limits fish assemblages in these streams.

Implications for the Use of Aquatic Biota in Evaluations of Agricultural Best-Management Practices

The findings of this study have implications for the design of monitoring programs to evaluate ecological benefits to streams that may result from the implementation of best-management practices (BMPs) to agricultural lands. In general terms, if management practices are implemented at only a local scale, then algal or invertebrate metrics might be the best choice to assess site-specific changes. Because the current study indicates that fish may respond to watershed-scale factors, fish metrics might be the best choice to evaluate BMPs across an entire watershed. Typically, BMPs are implemented progressively in isolated areas of a watershed rather than simultaneously over the entire watershed. Because fish are mobile and may travel throughout a watershed, they might be appropriate targets for long-term watershed monitoring; special attention could be placed on changes to physical stream characteristics that result in fewer detritivores and mud-substrate fish and more pollution-sensitive fish, carnivorous fish, and cobble-substrate fish in suitable reaches. Additional, more specific implications are discussed below.

Implementation of BMPs can produce changes to geomorphological and ecological dynamics of streams. The selection of biological metrics to use as measures of progress could take into consideration the intermediate disturbance hypothesis described by Connell (1978), which relates species richness to dynamic variability in disturbance. This hypothesis suggests that intermediate disturbance may result in high taxa richness when it interrupts the process of competitive elimination among species. In the current study, in terms of multireach and multiyear variation, biological metrics that represent taxa richness were the least variable at sites for all three groups of biota. In contrast, the biological metrics that represent a percentage of the assemblage were much more variable because they included not only an individual characteristic but also its relation to the rest of the assemblage. The annual stage of development of the assemblage (life-history characteristics), interannual differences in population dynamics, and sampling error are also important. It has been suggested that taxa richness and the Shannon-Wiener Diversity Index generally characterize biotic integrity (Stevenson and Bahls, 1999). In

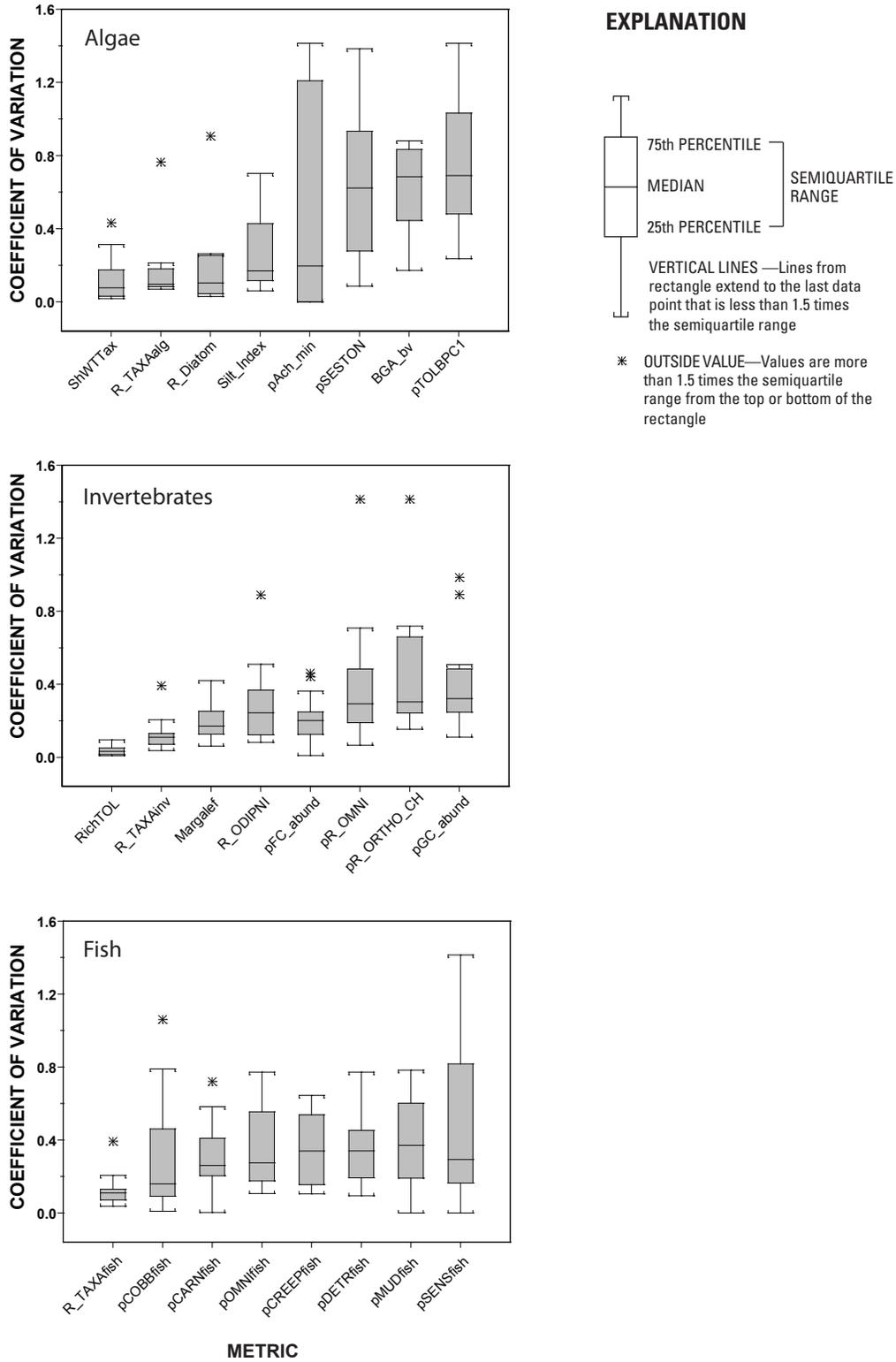


Figure 2. Coefficient of variation between biological metrics from samples collected among multiple reaches at agricultural stream sites in the Midwest. [Metric definitions are given in table 3.]

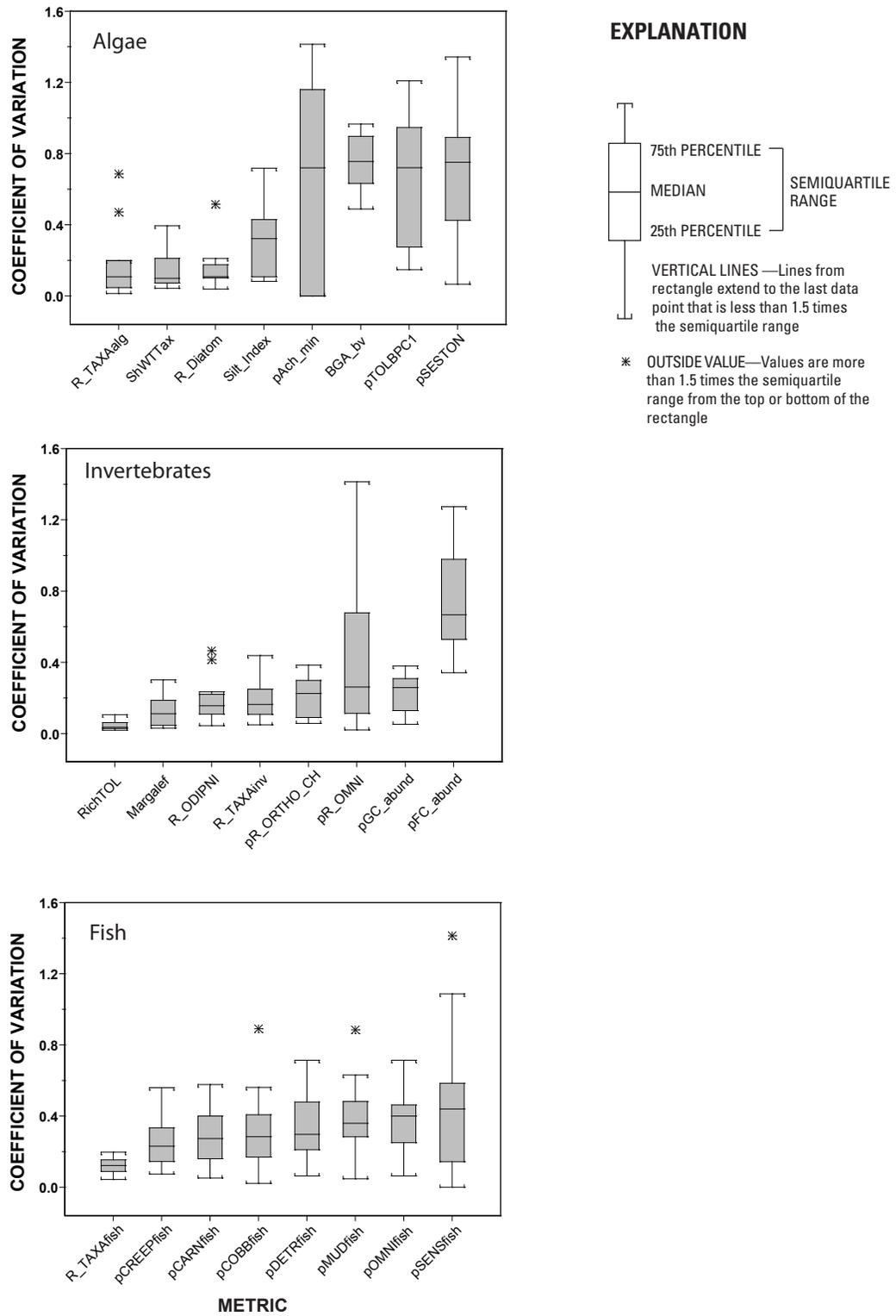


Figure 3. Coefficient of variation between biological metrics from samples collected among multiple years at agricultural stream sites in the Midwest. [Metric definitions are given in table 3.]

contrast, metrics in the current study—such as average USEPA tolerance value for a sample based on richness (*RichTOL*) and the percentage of sensitive diatom taxa (*pSENBPC3*)—are more specifically related to ecological conditions and causes of impairment. These results indicate that using richness as a measure of integrity may be problematic because richness can be relatively insensitive; for example, some species (possibly sensitive) could be displaced by other species (possibly tolerant), so richness would not necessarily show a net gain or loss and therefore would appear stable, and CVs would be low. It may be especially important to consider the measures of ecological condition as well as taxa richness in the assessment of the prairie streams of the Midwest, where higher fish-taxa richness has been associated with channelized streams (Frothingham and others, 2002).

Biological metrics selected for this study have potential for monitoring, assessing, and evaluating changes at multiple spatial scales and therefore may help guide watershed-conservation efforts in the Midwest. Physical characteristics that are frequently modified to improve ecological condition in streams through BMPs were analyzed to identify correlations with one or more biotic metrics. The results suggest that fish and algal metrics (including percentage of cobble-substrate fish, percentage of pollution-sensitive fish, biovolume of blue-green algae, and percentage of pollution-sensitive diatom taxa) could serve as metrics for assessing rehabilitation of riparian buffer and reduction of sediment runoff. Invertebrate data collected from riffle samples were correlated more strongly with physical characteristics of streams than invertebrate data collected from snag samples. This indicates the importance of considering both the metric and the substrate in selecting monitoring tools. Positive-indicator metrics for all three groups of biota correlated positively with percent bank vegetative cover. This indicates that multiple biotic assemblages could be used in future studies targeted at assessing improvements in the ecological condition of agricultural streams after implementation of best management practices that included restoration of streambank vegetation.

Goldstein and Meador (2005) suggest using fish-trait category frequencies to examine relations between potential sources of degradation and specific “functional” responses. This approach was followed in the current study by identifying, where possible, the functional traits of various algal, invertebrate, and fish taxa. For example, percentage of seston (*pSESTON*) algae is a function of available light and nutrients, as well as hydrologic characteristics; percentage richness of invertebrate omnivores (*pR_OMNI*) is a response measure for the amount and diversity of available food, usually increasing with algal biomass; and percentage detritivorous fish (*pDE-TRfish*) may serve as a measure of available benthic organic matter that can accumulate in poorly managed systems. These results suggest that metrics that provide information about the structure and function of aquatic assemblages may be of greater importance than purely chemical measures to monitoring recovery of stream integrity and therefore in assessing BMP effectiveness. In contrast, some biological metrics have

been developed as indicators of water chemistry (Potopova and Charles, 2007) and may have limited application for BMP assessment. In addition, the use of metrics based on species traits may also be less geographically constrained than analyses that rely on taxonomic designations (Goldstein and Meador, 2005). Trait data have recently been published for fish (Goldstein and Meador, 2004), invertebrates (Vieira and others, 2006), and algae (Porter, 2008) in North America.

Based on results from this study in the agricultural Midwest, where low-gradient streams are common, it may be possible to use functional traits to identify a spectrum of degradation due to siltation. In other words, a continuum may be identified that includes streams that are still functioning as lotic systems at one end of the spectrum to those that are functioning more like silted ponds (lentic systems) at the opposite end of the spectrum. There were several aspects of algal and fish functional groups that showed similar response across the study area. Conditions that favored sestonic algae and silt-tolerant diatoms were correlated with omnivorous and mud-substrate fish, whereas increases in a silt-intolerant diatom (*Achnantheidium minutissimum*) and benthic blue-green algae occurred along with carnivorous, cobble-substrate, and pollution-sensitive fish.

The ability of researchers to accurately relate biological response to BMPs will be enhanced as the areal extent of BMPs becomes documented and available in GIS database coverages. When these coverages are available, it should be possible to collect and analyze data in terms of the scale at which BMPs are applied throughout watersheds. Shields and others (2006) state that further work is needed to develop a conceptual model or index based on monitoring data associated with specific agricultural BMPs at multiple scales; such a model or index would allow us to build a scientific foundation to be established for assessing improvements to rivers in the Midwest.

Summary and Conclusions

This report evaluated relations between biota and physical environmental characteristics of streams measured at three spatial scales—reach, segment, and watershed. Data on algae, invertebrates, fish, and environmental characteristics were collected by the USGS with nationally-consistent protocols at 86 stream sites from 1993 through 2004. Sites were located within agricultural river basins of the Midwest in eight USGS NAWQA study units. Data analyses (1) identified environmental characteristics to which algal, invertebrate, and fish assemblages showed the strongest correlations; (2) examined relations between each biotic assemblage and the most highly correlated physical environmental characteristics at the reach, segment, and watershed scale; and (3) identified biological measures (metrics) that showed the most potential for monitoring, assessing, and evaluating changes at the various spatial

scales to assess the effectiveness of BMPs in agricultural areas in the Midwest.

Characteristics that best explained variation in the biotic assemblages were bank vegetative cover, fine silty substrate, and open-canopy angle at the reach scale; woody vegetation and cropland in the 250-m riparian buffer and average length of undisturbed buffer at the segment scale; and land cover (both total forested and row crop), low-permeability soils, slope, drainage area, and latitude at the watershed scale. As hypothesized, variations among fish assemblages correlated more with watershed-scale characteristics than with segment and reach characteristics, whereas algal and invertebrate assemblages correlated more with physical environmental characteristics at reach and segment scales.

The strong relations between stream biota and watershed characteristics found in this study reinforce the importance of managing whole watersheds to maintain healthy streams and underscore difficulties with measuring responses implemented at smaller scales. These response patterns in agricultural watersheds across the Midwest have relevance to state and federal farm-management-related agencies. The relative importance of environmental characteristics should be taken into consideration to measure the effects of BMPs designed to improve water quality in agricultural watersheds. Because fish appear to be more strongly influenced by watershed-scale features, algal and invertebrate metrics may prove to be better indicators for BMPs applied locally (at the reach scale).

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