# EFFECTS OF MULTI-SCALE ENVIRONMENTAL CHARACTERISTICS ON AGRICULTURAL STREAM BIOTA IN EASTERN WISCONSIN

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# EFFECTS OF MULTI-SCALE ENVIRONMENTAL CHARACTERISTICS ON AGRICULTURAL STREAM BIOTA IN EASTERN WISCONSIN[1]

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ABSTRACT: The U.S. Geological Survey examined 25 agricultural streams in eastern Wisconsin to determine relations between fish, invertebrate, and algal metrics and multiple spatial scales of land cover, geologic setting, hydrologic, aquatic habitat, and water chemistry data. Spearman correlation and redundancy analyses were used to examine relations among biotic metrics and environmental characteristics. Riparian vegetation, geologic, and hydrologic conditions affected the response of biotic metrics to watershed agricultural land cover but the relations were aquatic assemblage dependent. It was difficult to separate the interrelated effects of geologic setting, watershed and buffer land cover, and base flow. Watershed and buffer land cover, geologic setting, reach riparian vegetation width, and stream size affected the fish IBI, invertebrate diversity, diatom IBI, and number of algal taxa; however, the invertebrate FBI, percentage of EPT, and the diatom pollution index were more influenced by nutrient concentrations and flow variability. Fish IBI scores seemed most sensitive to land cover in the entire stream network buffer, more so than watershed-scale land cover and segment or reach riparian vegetation width. All but one stream with more than approximately 10 percent buffer agriculture had fish IBI scores of fair or poor. In general, the invertebrate and algal metrics used in this study were not as sensitive to land cover effects as fish metrics. Some of the reach-scale characteristics, such as width/depth ratios, velocity, and bank stability, could be related to watershed influences of both land cover and geologic setting. The Wisconsin habitat index was related to watershed geologic setting, watershed and buffer land cover, riparian vegetation width, and base flow, and appeared to be a good indicator of stream quality. Results from this study emphasize the value of using more than one or two biotic metrics to assess water quality and the importance of environmental characteristics at multiple scales.
(KEY TERMS: aquatic ecosystems; water quality; fish; benthos; algae.)

## INTRODUCTION

Many investigators have examined the correlations among environmental characteristics and aquatic biota; however, the relative influence of geologic, geomorphic, and land-cover characteristics on species distributions of stream organisms remains elusive (Poff, 1997). The importance of watershed versus riparian land-cover characteristics is a subject of debate. Results from recent studies are conflicting regarding the interactions between physical and chemical characteristics at various spatial and temporal scales. Differences in results can be attributed to: (1) regional variations in responses, such as those influenced by climatic or geologic variability or lack thereof; (2) different responses among aquatic assemblages; (3) use of different types of metrics or indexes used to represent a particular aquatic assemblage; and (4) differences in the methods or spatial scales used to measure environmental characteristics (Hunsaker and Levine, 1995; Allan *et al.,* 1997; Allen *et al.,* 1999).

Researchers recognize the importance of placing streams and stream habitats in a geographic, spatially nested hierarchy (Godfrey, 1977; Lotspeich and Platts, 1982; Bailey, 1983; Frissell *et al.,* 1986). The watershed refers to the area less than 1 square kilometer to many thousands of square kilometers that contributes water, sediment, and dissolved materials to a common outlet along a stream channel. Geology, climate, topography, soils, and land cover at the watershed scale influence the transfer of water, sediment, nutrients, and organic material (Langbein and

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Schumm, 1958; Schumm and Lichty, 1965; Frissell *et al.,* 1986). However, geology, geomorphology, and land cover along the riparian buffer at the segment scale, a length of stream approximately 1 to 15 km bounded by tributary junctions or major waterfalls, may also influence aquatic habitat and biota. Conditions at the reach scale, a length of stream generally less than 1000 m, may locally affect aquatic habitat and biota. The reach scale is the scale at which aquatic habitat and biotic data are usually measured (Fitzpatrick *et al.,* 1998). Typical reach measurements include descriptions of channel, bank, substrate, and habitat cover features, as well as riparian buffer conditions.

The scale of investigation may influence the relative importance of predictive characteristics in study outcomes (Carter *et al.,* 1996; Allan *et al.,* 1997; Lammert and Allan, 1999; Isaak and Hubert, 2000). Carter *et al.* (1996) found that species composition of invertebrates was more correlated to segment- and reach-scale environmental characteristics than physical characteristics at the invertebrate sampling point; however, interpretations were complicated by correlations among environmental characteristics at differing scales. Some studies of Midwestern streams have shown that land cover at the watershed and segment scales is an important determinant of fish and habitat quality, although watershed land cover may be more important than segment land cover such as that within a riparian buffer (Roth *et al.,* 1996; Richards *et al.,* 1996; Wang *et al.,* 1997; Lyons *et al.,* 2000). Other studies have also shown the direct benefits of segment or reach scale riparian buffers for fish productivity, trophic structure, trophic interactions, and recruitment dynamics (Beschta and Platts, 1986). Fish assemblages in Minnesota and North Dakota streams appeared to be more related to reach-scale habitat and riparian and hydrologic conditions than to watershed agricultural land cover (Goldstein et al., 1996; Allan *et al.,* 1997). Another study of Minnesota streams found that fish assemblages were primarily related to riparian land cover and secondarily to runoff potential (Stauffer *et al.,* 2000). In Wisconsin, fish assemblage composition was related to environmental characteristics at a variety of scales: ecoregion, watershed area, stream slope, and water temperature (Lyons, 1996).

Various types of organisms, whether fish or invertebrates or algae, respond to different scales of environmental characteristics in sometimes conflicting ways and these responses may vary regionally. Lammert and Allen (1999) observed that fish assemblages were most related to flow variability and riparian land cover, whereas invertebrate assemblages were most strongly correlated with substrate type. Statzner and Higler (1986) found that physical characteristics related to streamflow were the most important characteristics affecting benthic invertebrate populations worldwide. Invertebrate assemblages in New Zealand streams were most affected by watershed land cover, nutrient enrichment, and slope. Flow variability was less important; however, land cover affected benthic algae less than invertebrates (Quinn and Hickey, 1990). Richards and Host (1994) examined streams along Lake Superior and determined that although invertebrate assemblage richness and composition were most strongly correlated to reach characteristics – substrate characteristics and presence of coarse woody debris – algal abundance was most related to watershed land cover, which in turn was correlated to substrate characteristics. In Washington, Leland (1995) found that the effects of watershed geology and land cover on benthic algal assemblage structure varied regionally.

A major goal of this study was to examine the relations among fish, invertebrate, and algal assemblages represented by several metrics and environmental characteristics at multiple scales. We examined 25 agricultural streams in eastern Wisconsin to investigate the relative importance of watershed-, segment-, and reach-scale environmental characteristics on fish, benthic invertebrate and algal assemblages, and aquatic habitat (Figure 1).

## STUDY AREA

Streams studied were located in the North-Central Hardwood Forests and Southeastern Wisconsin Till Plains ecoregions (Omernik and Gallant, 1988). These streams were tributaries on the western side of Lake Michigan (Figure 1). Row-crop agriculture in the watersheds ranged from 5 to 82 percent (median = 30.9 percent) and is mainly composed of corn and alfalfa for dairy farming (Rheaume *et al.,* 1996a). The remaining land cover was composed primarily of forest, grassland, and wetland. Less than 1 percent of the watersheds contained urban land. Surficial deposits in the study varied from clayey tills and glacial lacustrine deposits to sandy till and outwash. The watersheds had relatively little topographic relief, ranging from 30 to 137 m, and watershed slopes of less than 23 m/km.

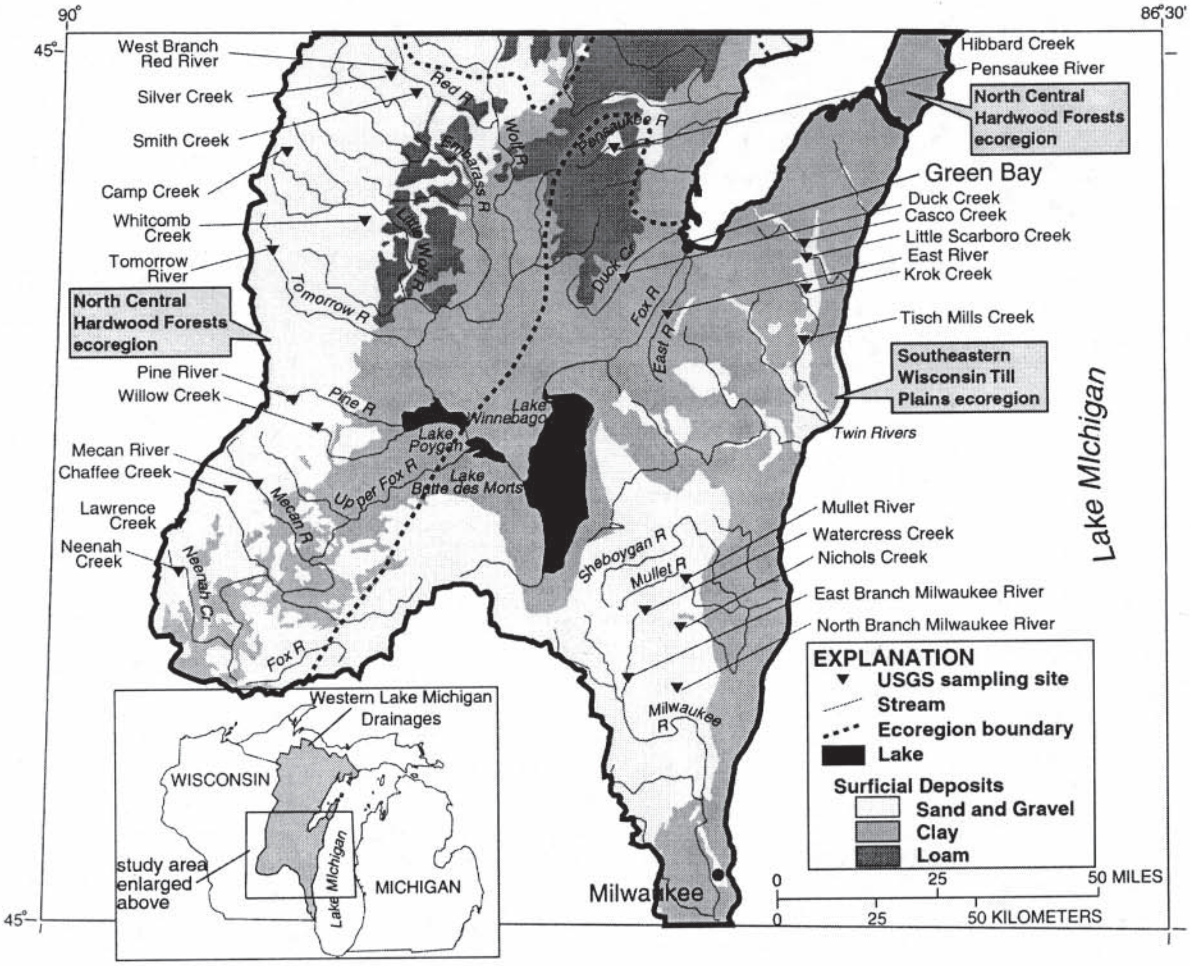


Figure 1. Location of 25 Stream Sites in the Study Area Showing Geologic Setting and Ecoregions.

## METHODS

### *Study Design*

The U.S. Geological Survey's National Water-Quality Assessment Program (NAWQA) collected biological, physical, and chemical data at 25 sites from 1993 to 1995 in the Western Lake Michigan Drainages study unit. Twenty of the sites were part of a separate study and were selected for the purpose of defining healthy biotic communities in eastern agricultural areas of Wisconsin; these sites are referred to as benchmark sites in this report. Site selection for these streams was based on historical invertebrate or fisheries data that indicated good to excellent water quality. These sites were located on streams ranging from first to fourth order, most were second order streams. Streams were located in areas dominated by agriculture but had fewer agriculturally-related impacts than most streams in the area, possibly due to land management protection (Rheaume *et al.,* 1996a). The assemblages and habitat were sampled once. Algae and habitat were sampled in May to June 1993, invertebrates in April 1995, and fish in July and August 1993 or 1995. Five additional third- to fifth-order agricultural streams were sampled from 1993 to 1995 as part of the water-quality monitoring network for the NAWQA study. Assemblage and habitat data from 1993 were used for this analysis. For all sampled streams, stream lengths (the distance from the headwaters to the sample site, using a 7.5 ft. map) varied from about 1 to 32 km. Detailed descriptions of all sites can be found in Rheaume *et al.* (1996a) and Sullivan *et al.* (1995), respectively. Results from separate analyses of assemblage data for fish, invertebrates, algae, and habitat have been previously published (Fitzpatrick *et al.,* 1996; Rheaume *et al.,* 1996b; Fitzpatrick and Giddings, 1997; Sullivan, 1997; Sullivan and Peterson, 1997; Lenz and Rheaume, 2000; Scudder and Stewart, 2001).

*Data Collection*

Several biotic metrics were used to represent each aquatic assemblage to determine differences, if any, related to environmental characteristics (Tables 1 and 2). Physical and chemical environmental characteristics were collected at multiple spatial scales.

**Fish.** Fish were collected with direct-current electrofishing gear (Meador *et al.,* 1993a; Sullivan and Peterson, 1997; Sullivan, 1997). Backpack-mounted electrofishing units were used on small streams and a towed barge unit was used on larger streams. One sampling pass was made at most sites to minimize injury or disturbance to salmonids. Two passes were made at five benchmark sites; however, no new species were collected on the second pass except at one site. The length of the sampling reach was determined to be 20 times the average wetted channel width, or at least 150 m to a maximum of 300 m, in order to encompass at least one meander sequence and represent all habitat types in a reach. Four metrics were calculated using fish assemblage data (Table 1). An Index of Biotic Integrity (IBI) was calculated using either the index for coldwater streams (Lyons *et al.,* 1996) or warmwater streams (Lyons, 1992) as appropriate. Higher IBI scores reflect better stream quality. The other three fish metrics used were number of tolerant species, percentage of intolerant individuals, and total number of species.

**Invertebrates.** Benthic invertebrates were collected with a Slack kick sampler (fitted with a 425 micrometer mesh net) from riffles (Cuffney *et al.,* 1993a; 1993b). Three 0.5 $m^2$ samples were collected from a single riffle in each reach at the 20 benchmark sites. Each sample was processed separately as described by Hilsenhoff (1987) (Rheaume *et al.,* 1996b). The mean of the three separate samples were used for data analysis. At the remaining five sites each sample consisted of kick samples collected and composited from two riffles and processed (Cuffney *et al.,* 1993a; 1993b) as per the NAWQA protocol, except that six kick samples were composited instead of five. Data for all sites were summarized into three metrics, including the Family-level Biotic Index (FBI) (Hilsenhoff, 1988); the percentage of the total number of Ephemeroptera, Plecoptera, and Trichoptera individuals to the total number of individuals in the sample (percent EPT) (Lenat, 1988); and a diversity index (Margalef, 1969; Rosenberg and Resh, 1993). The FBI is an assessment of the effects of nutrients and values increase with increasing levels of nutrients. Higher percent EPT values indicate better water quality. Margalef's diversity index is widely used in the Great Lakes area. Higher diversity generally indicates better stream quality although small headwater streams with good water quality may appear less diverse as a result of low productivity, limited habitat or insufficient flow. Diversity also may increase in slightly enriched streams (Rosenberg and Resh, 1993). The

TABLE 1. Biotic Metrics and Indexes Used to Represent Assemblage Data for 25 Stream Sites in Eastern Wisconsin. In the fish index of biotic integrity (Lyons *et al.,* 1996; Lyons, 1992), scores are ranked as 90 to 100 (excellent), 60 to 80 (good), 30 to 50 (fair), 10 to 20 (poor), and 0 or no score (very poor). In the Hilsenhoff family-level biotic index (Hilsenhoff, 1988), scores are ranked as 0 to 3.75 (excellent), 3.76 to 4.25 (very good), 4.26 to 5.0 (good), 5.01 to 5.75 (fair), 5.76 to 6.5 (fairly poor), 6.51 to 7.25 (poor), 7.26 to 10.0 (very poor), diversity index from (Margalef, 1969). In the diatom siltation index from Bahls (1993), scores are ranked as 4 (excellent), 3 (good), 2 (fair), 1 (poor); percentage of pollution tolerant diatoms based on tolerance values from Lange-Bertalot (1979) and Bahls (1993).

| Biotic Metric or Index | Abbreviation | Median | Minimum | Maximum |
|---|---|---|---|---|
| **FISH** | | | | |
| Index of Biotic Integrity | FIIBI | 70 | 10 | 100 |
| Number of Tolerant Species | TOLSP | 3 | 0 | 6 |
| Percentage of Intolerant Individuals | INTIN | 30.7 | 0 | 100 |
| Total Number of Species | FISSP | 7 | 2 | 20 |
| **INVERTEBRATES** | | | | |
| Hilsenhoff Family-Level Biotic Index | INFBI | 4.2 | 1.9 | 10.0 |
| Percent Ephemeroptera-Plecoptera-Trichoptera Individuals | INEPT | 32 | 1.0 | 82 |
| Diversity Index | INMDI | 3.4 | 0.2 | 4.2 |
| **ALGAE** | | | | |
| Number of Algal Taxa (Taxa Richness) | ALGTA | 49 | 27 | 71 |
| Diatom Siltation Index | DIASI | 22 | .67 | 67 |
| Percentage of Pollution Tolerant Diatoms | DIAPT | 5.3 | 0.66 | 25 |
| Diatom Index of Biological Integrity | DIIBI | 3 | 2 | 4 |

TABLE 2. Summary Statistics for Physical and Chemical Characteristics for 25 Stream Sites in Eastern Wisconsin. [**Bolded** characteristics are those used in multivariate analysis; watershed scale characteristics are for the watershed outside the 50-m buffer of the entire stream network (buffer) except for land cover specified for the buffer.] Methods for watershed-scale characteristics are described in Meador *et al.,* 1993b; reach-scale water chemistry methods are described in Shelton (1994); Great Lakes Environmental Assessment Section (GLEAS) Index (Michigan Department of Natural Resources, 1991) scores are ranked as 111 to 135 (excellent), 75 to 102 (good) 39 to 66 (fair), 0 to 30 (poor); the Wisconsin Habitat Index (Simonson *et al.,* 1994) scores are ranked as 74 to 100 (excellent), 50 to 74 (good) 25 to 49 (fair), and 0 to 24 (poor).

| Environmental Characteristic | Abbreviation | Median | Minimum | Maximum |
|---|---|---|---|---|
| **WATERSHED SCALE** | | | | |
| Drainage Density (km/km$^2$) | DRDEN | 0.3 | 0.04 | 0.67 |
| Relief (m) | RELIE | 67 | 30 | 137 |
| Slope (m/km) (log-10 transformed) | WSLOP | 3.19 | 0.74 | 22.7 |
| **Sandy Surficial Deposits** (percent) (log-10 transformed) | **WSANDL** | 40 | 1 | 96 |
| Permeability (cm/hr) | PERME | 13 | 4.2 | 28 |
| **Drainage Area** (km2) (log-10 transformed) | **WAREAL** | 42 | 2.3 | 250 |
| Stream Length (km) | STLEN | 9.5 | 0.80 | 32 |
| Cumulative Stream Length (km) (log-10 transformed) | CSTLE | 12 | 0.80 | 53 |
| Stream Order (categorical) | ORDER | 2 | 1 | 5 |
| **Watershed Agriculture** (percent) | **WBUAG** | 32 | 1 | 83 |
| Watershed Grassland (percent) | WBUGR | 17 | 3 | 40 |
| Watershed Forest (percent) | WBUFO | 31 | 5 | 56 |
| Watershed Wetland (percent) | WBUWE | 11 | 1 | 37 |
| Buffer Agriculture (percent | BUFAG | 6 | 0 | 67 |
| **Buffer Grassland** (percent) | **BUFGR** | 5 | 0 | 20 |
| **Buffer Forest** (percent) | **BUFFO** | 14 | 0 | 36 |
| **Buffer Wetland** (percent) | **BUFWE** | 61 | 8 | 76 |
| **SEGMENT SCALE** | | | | |
| Sinuosity (ratio) | SINUO | 1.17 | 1.00 | 1.66 |
| **Slope** (m/km) | **SSLOP** | 24.6 | 0.5 | 84.9 |
| **Riparian Vegetation Width** (m) | **SVEGE** | 152 | 27.3 | 876 |
| **REACH SCALE** | | | | |
| Riffle (percent) | RIFFLE | 25 | 0 | 74 |
| Run (percent) | RRUNS | 59 | 25 | 86 |
| Pool (percent) (log-10 transformed) | POOLS | 14 | 0 | 51 |
| Woody Debris (percent) | WOODY | 9 | 0 | 19 |
| Canopy Angle (degrees) | CANOP | 40 | 0 | 120 |
| **Width/Depth Ratio** (log-10 transformed) | **WDRATL** | 18.5 | 5.80 | 118 |
| Velocity (m/s) (log-10 transformed) | VELOC | 0.30 | 0.11 | 0.87 |
| Substrate (categorical) (log-10 transformed) | **SUBSTL** | 4.6 | 2.2 | 5.7 |
| Microhabitat Substrate (log-10 transformed) | INSUB | 3.2 | 2.0 | 7.0 |
| Bank Stability Index (categorical) (log-10 transformed) | BASTI | 7.8 | 6.1 | 13 |
| Embeddedness (categorical) | **EMBAV** | 3.07 | 0 | 4.47 |
| Embeddedness in Riffles (categorical) | EMBRI | 2.8 | 0 | 4.8 |
| Michigan Habitat Index (categorical) | GLEAS | 86 | 45 | 110 |
| **Wisconsin Habitat Index** (categorical) | WIHAB | 69 | 40 | 80 |
| **Riparian Vegetation Width** (m) (log-10 transformed) | **RVEGEL** | 76.8 | 7.6 | 1110 |
| **Base Flow/Drainage Area** (m$^3$/s/km$^2$) (log-10 transformed) | **QBASEL** | 0.41 | 0.30 | 1.96 |
| Estimated Two-Year Flood/Drainage Area (m$^3$/s/km$^2$) (log-10 transformed) | Q2-YR | 13 | 6.1 | 93 |
| **Flow Variability (Estimated 2-Year Flood/Base Flow)** (log-10 trans.) | **QVARIL** | 37.1 | 3.6 | 1000 |
| Conductance (microsiemens/cm) (log-10 transformed) | CONDU | 407 | 137 | 770 |
| **Ammonium, Dissolved** (mg/L as N) (log-10 transformed) | **NAMMOL** | 0.02 | 0.007 | 0.38 |
| **Nitrate Plus Nitrite, Dissolved** (mg/L as N) (log-10 transformed) | **NO2NOL** | 1.4 | 0.048 | 6.15 |
| Total Organic Plus Ammonia Nitrogen (mg/L as N) | NORAM | 0.55 | 0.1 | 1.55 |
| **Phosphorus, Total** (mg/L as P) (log-10 transformed) | **PHOSPL** | 0.02 | 0.005 | 0.68 |

FBI and percent EPT indicate tolerance to water-quality degradation, and fewer EPT taxa and individuals will result in higher FBI values; however, unlike the FBI and percent EPT metrics, the diversity index is unrelated to the abundance of tolerant or intolerant taxa. Although sampling methods differed for the two groups of sites the FBI should be relatively insensitive to these differences (Lenz and Miller, 1996). Percent EPT also should be relatively unaffected by sampling differences since it is based on percent composition and is dependent upon the most abundant species, and qualitative studies have used percent EPT (Lenat, 1988). The degree to which the diversity index was affected by sampling differences in our study is unknown.

**Algae.** Quantitative and qualitative samples of benthic algae were collected from natural substrates and processed with the NAWQA protocol (Porter *et al.,* 1993). Five quantitative samples were collected, primarily from riffles, in each of five locations from the reach and composited into a single sample for the reach. Algae were removed from a defined surface area on rocks using a stiff brush and a modified syringe barrel with attached o-ring. If rocks were unavailable, sections of woody debris were collected, attached algae removed, and the surface area estimated. Four algal metrics were calculated for this study, including number of algal taxa (taxa richness), a diatom siltation index (Bahls, 1993), the percentage of pollution-tolerant diatoms (tolerance ratings from Lange-Bertalot, 1979; Bahls, 1993), and an IBI for diatoms (Bahls, 1993). The diatom siltation index is computed as the percentage of diatoms in the genera *Navicula, Nitzschia, Surirella,* and *Cylindrotheca* relative to all diatoms.

**Physical Characteristics.** Physical data were collected at three spatial scales: watershed, segment, and reach per the NAWQA protocol as described in Meador *et al.* (1993b) (also see Fitzpatrick *et al.,* 1996; and Fitzpatrick and Giddings, 1997). Watershed characteristics included ecoregion, drainage density, relief, slope, percentage of sandy surficial deposits, permeability, drainage area, stream length, cumulative stream length, stream order, and land cover (Table 2). Segment characteristics included sinuosity, slope, and riparian vegetation width. Reach characteristics included percentage of riffles-runs-pools, amount of woody debris, canopy angle, channel, width to depth ratio, average velocity, average substrate, bank stability index, embeddedness, and riparian vegetation width. The bank stability index (Fitzpatrick *et al.,* 1998) was used to characterize streambank conditions. Unstable banks have high bank stability indexes.

The reach-scale habitat data also were summarized into two habitat indexes: the qualitative Great Lakes Environmental Assessment Section (GLEAS) index of the Michigan Department of Natural Resources (1991) and the WI habitat index (Simonson *et al.,* 1994). Both indexes have been used to evaluate stream condition in the region. Scores for the Wisconsin and Michigan habitat indexes increase as stream quality increases. The Michigan habitat index was based on the following characteristics: bottom substrate type, embeddedness, velocity, flow stability, amount of bottom deposition, variety and quality of habitats (pools-riffles-runs-bends), bank stability, bank vegetative stability, and stream-side cover. The Wisconsin habitat index was based on seven measures: riparian vegetation width, bank erosion, pool area, width: depth ratio, riffle: riffle or bend: bend ratio, amount of fine sediments, and cover for fish. Characteristics that were included in the Michigan habitat index were weighted into three groups (listed in order of importance): (1) substrate condition, (2) channel morphology, and (3) riparian bank structure. Similar characteristics in the Wisconsin habitat index were equally weighted and seem to comprise a more useful index of overall environmental impacts. Velocity and flow stability are included in the Michigan but not in the Wisconsin habitat index. The reach-scale, riparian vegetation width is included in the Wisconsin habitat index; however, the Michigan habitat index has a more general characteristic that reflects the type of vegetation along the stream bank. Thus, the Michigan habitat index is more reflective of substrate and channel conditions than riparian conditions.

Land-cover data was estimated at watershed, segment, and reach scales. Watershed land-cover percentages (agriculture, grassland, forest, wetland, water, and urban) were calculated from the Wisconsin Initiative for Statewide Cooperation on Landscape Analysis and Data (WISCLAND) land-cover data, derived from 1992-93 satellite imagery (Lillesand *et al.,* 1998). These data were collected by Landsat Thematic Mapper and had a 30-m resolution. Land-cover percentages were calculated within a 50-m buffer on each side of the stream along the entire stream network above the site. The 50-m buffer will be referred to as "the buffer" in subsequent text. In order to remove any effects of the buffer land cover on overall watershed land cover, buffer percentages were subtracted from the entire watershed to calculate the percentage of land cover within the watershed but outside the buffer. Land cover outside the buffer is referred to as watershed land cover in this document. Riparian vegetation width was measured at the segment and reach scales with a stereoscope and 1:40,000-scale National Aerial Photography Program

(NAPP) aerial photographs. Three width measurements were taken at evenly distributed intervals through the reach and 10 width measurements were taken in the segment, as allowable by the size and resolution of the photographs. The riparian vegetation width was considered the width of forest, grassland (excluding pasture), and wetland (forested and non forested) vegetation adjacent to the stream.

Streamflow measurements were collected during aquatic biota sampling under base-flow conditions. Base-flow measurements were normalized by drainage area. Two-year flood estimates were based on regional flood-frequency regression curves (Rantz *et al.,* 1982; Krug *et al.,* 1992). Five sites had USGS gaging stations, allowing additional accuracy in flood estimates. Flow variability was calculated by dividing the two-year flood estimate by base flow.

**Water Chemistry.** Most sites had limited water chemistry data available because they were not part of the NAWQA water-quality monitoring network. Temperature, pH, dissolved oxygen, and conductance were measured during biological sampling at all sites. Conductance measurements from 1993 only were used for data analyses. Two samples for dissolved ammonium, nitrate plus nitrite, total organic plus ammonia nitrogen, and total phosphorus were collected in April and June/July 1995 during relatively low flows according to the NAWQA protocols (Shelton, 1994; Rheaume, 1996a). Means of water chemistry characteristics were used for data analyses.

### *Statistical Analyses*

Descriptive statistics were calculated for all data using DataDesk 6.1 statistical software. Nonparametric Spearman rank correlation was used to determine statistical significance and relative strength of correlations between biotic metrics and environmental characteristics (Iman and Conover, 1983; Johnson and Wichern, 1992). Unless stated otherwise, significant correlations presented were those with Spearman rho $(r) \geq \pm 0.40$ and p-values $< 0.05$. Correlations referred to as highly significant were those with $r \geq \pm 0.62$ and p-values $< 0.001$. Distribution patterns of correlated data were examined with scatter plots. Redundancy Analysis (RDA) was used to examine the data (Hill, 1979; Ter Braak, 1986; Ter Braak and Smilauer, 1998). RDA is a direct gradient analysis that describes the variation between a linear response data set (in this case biotic metrics) and a predictor data set (environmental characteristics) (Ter Braak, 1986). Some environmental characteristics were log- transformed to achieve normal distributions prior to RDA (Table 2). Prior to the RDA, characteristics were excluded that either did not significantly correlate to any of the biotic metrics or were highly correlated with other environmental characteristics.

The RDA was used to examine the responses of the biotic metrics to gradients of environmental characteristics. Metrics were plotted in ordination diagrams with vectors representing gradients in selected environmental characteristics using a symmetric focus for scaling. The length of the arrow reflects the importance of the environmental characteristic and the amount of change in the biotic metric along that environmental characteristic. Arrows in opposite directions from each other represent characteristics that were negatively correlated with each other. Monte Carlo permutation tests were used to determine whether the RDA axes were significant ($p < 0.05$). The proximity of a biotic metric to certain environmental characteristics in an RDA biplot represents the relative influence of the environmental characteristic on that metric. The proximity of metrics to other metrics identifies those that behave similarly under a given set of environmental conditions.

## RESULTS

### *Correlations Among Environmental Characteristics*

Simple scatter plots and Spearman correlation analysis of environmental characteristics revealed significant correlations among environmental characteristics at multiple scales (Table 3). The watershed-scale characteristics that reflected the size of the watershed (drainage area, stream length, cumulative stream length, watershed slope, and stream order) were highly intercorrelated ($r > \pm 0.65$). Watershed agriculture was negatively correlated with sandy surficial deposits (Figure 2A). In contrast, watershed forest was negatively correlated to watershed agriculture and positively correlated to sandy surficial deposits. Watershed permeability, sandy surficial deposits, and grassland were significantly intercorrelated. The grassland category in the WISCLAND data was somewhat problematic in that it included both natural grassland areas and pasture. This made it difficult to distinguish the relative importance of geologic setting from watershed land cover. Land cover in the buffer was similar to watershed land cover ($r > 0.65$). Watershed agriculture also negatively correlated with the summed percentage of wetland, grassland, and forest in the buffer. In addition, streams with a higher percentage of watershed forest contained more buffer forest and larger segment and reach riparian vegetation widths.

TABLE 3. Spearman Rank Correlations Among Selected Environmental Characteristics for 25 Stream Sites in Eastern Wisconsin. [**Bolded** correlation coefficients have p-values < 0.001; —, Not significant, p-value >0.05; watershed scale characteristics are for the watershed outside the 50-m buffer of the entire stream network (buffer) except for land cover specified for the buffer.]

| Environmental Characteristic | Characteristic Abbreviation | Sandy Surficial Deposits | Permeability | Watershed Agriculture | Watershed Grassland | Watershed Forest | Buffer Agriculture | Segment Riparian Vegetation Width | Reach Riparian Vegetation Width | Wisconsin Habitat Index | Baseflow/ Drainage Area |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **WATERSHED SCALE** | | | | | | |
| Slope (m/km) | WSLOP | 0.56 | 0.58 | -0.45 | 0.50 | 0.52 | **-0.65** | – | – | 0.52 | 0.54 |
| Sandy Surficial Deposits (percent) | WSAND | 1.00 | **0.70** | **-0.76** | **0.62** | **0.74** | **-0.68** | 0.54 | **0.71** | **0.66** | 0.58 |
| Permeability (cm/hr) | PERME | **0.70** | 1.00 | **-0.62** | **0.68** | **0.66** | -0.61 | – | 0.44 | – | 0.53 |
| Drainage Area ($km^2$) | WAREA | -0.44 | – | 0.43 | – | -0.41 | 0.56 | -0.46 | – | -0.43 | -0.42 |
| Stream Order (categorical) | ORDER | -0.58 | -0.43 | 0.54 | -0.52 | -0.57 | **0.70** | -0.54 | -0.44 | -0.6 | **-0.67** |
| Watershed Forest (percent) | WBUFO | **0.74** | **0.66** | **-0.91** | 0.52 | 1.00 | **-0.77** | 0.58 | 0.61 | – | 0.46 |
| Buffer Agriculture (percent) | BUFAG | **-0.68** | -0.61 | **0.82** | -0.57 | **-0.77** | 1.00 | -0.60 | -0.48 | -0.59 | **-0.66** |
| Buffer Grassland (percent) | BUFGR | – | 0.44 | – | 1.00 | – | – | – | – | 0.45 | 0.58 |
| Buffer Forest (percent) | BUFFO | **0.72** | – | **-0.66** | – | **0.67** | -0.57 | 0.53 | 0.52 | 0.54 | 0.50 |
| Buffer Grassland, Forest, Wetland (percent) | BUFGR | **0.68** | 0.46 | **-0.76** | 0.49 | **0.70** | **-0.93** | **0.72** | 0.54 | – | **0.68** |
| | | | | | **SEGMENT SCALE** | | | | | | |
| Slope (m/km) | SSLOP | 0.50 | – | – | – | – | -0.52 | 0.59 | 0.46 | **0.66** | 0.54 |
| Riparian Vegetation Width (m) | SVEGE | 0.54 | – | -0.59 | – | 0.58 | -0.60 | 1.00 | **0.77** | – | 0.46 |
| | | | | | **REACH SCALE** | | | | | | |
| Run (percent) | RRUNS | – | – | -0.58 | – | 0.55 | -0.51 | – | – | – | – |
| Width/Depth Ratio | WDRAT | **-0.65** | -0.46 | **0.71** | -0.42 | **-0.72** | **0.70** | -0.44 | – | -0.54 | -0.52 |
| Velocity (m/s) | VELOC | 0.61 | 0.40 | -0.44 | **0.65** | – | -0.48 | – | 0.41 | **0.69** | 0.60 |
| Bank Stability Index (categorical) | BASTI | -0.40 | -0.52 | 0.56 | -0.42 | -0.51 | 0.46 | – | – | – | -0.49 |
| Embeddedness (categorical) | EMBAV | – | – | 0.44 | – | -0.49 | – | – | – | – | – |
| Substrate (categorical) | SUBST | – | – | – | – | – | – | – | – | – | – |
| MI Habitat Index (categorical) | GLEAS | – | – | – | – | – | – | 0.43 | – | 0.54 | 0.43 |
| Wisconsin Habitat Index (categorical) | WIHAB | **0.66** | – | – | **0.65** | – | -0.59 | 0.53 | 0.48 | 1.00 | **0.84** |
| Riparian Vegetation Width (m) | RVEGE | 0.71 | 0.44 | -0.56 | 0.42 | 0.61 | -0.48 | **0.77** | 1.00 | 0.48 | 0.40 |
| Base Flow/Drainage Area ($m^3/s/km^2$) | QBASE | 0.58 | 0.53 | -0.41 | **0.71** | 0.47 | **-0.66** | 0.46 | – | **0.84** | 1.00 |
| Flow Variability (Estimated 2-year Flood/Base Flow) | QVARI | -0.58 | -0.51 | 0.50 | **-0.71** | -0.47 | **0.56** | -0.40 | – | **-0.70** | **-0.87** |
| Conductance (microsiemens/cm) | CONDU | **-0.67** | -0.50 | **0.84** | – | **-0.76** | **0.63** | **-0.68** | **-0.71** | – | – |
| Ammonium, Dissolved (mg/L as N)-0.51 | NAMMO | -0.51 | -0.56 | – | **-0.65** | – | – | – | -0.43 | -0.55 | **-0.64** |
| Nitrate plus Nitrite, Dissolved (mg/L as N) | NO2NO | – | – | – | 0.48 | – | – | – | – | 0.49 | 0.46 |
| Total Organic Plus Ammonia Nitrogen (mg/L as N) | NORAM | -0.58 | **-0.62** | – | **-0.71** | -0.46 | – | – | -0.47 | -0.60 | **-0.71** |
| Phosphorus, Total (mg/L as P | PHOSP | -0.54 | -0.56 | – | **-0.70** | – | – | – | -0.56 | -0.60 | **-0.69** |

In general, correlations between reach scale characteristics and watershed agriculture or percent sandy surficial deposits were more numerous and stronger than correlations between reach characteristics and buffer agriculture or segment and reach riparian vegetation width (Table 3). The two habitat indexes correlated with different environmental characteristics. The Wisconsin habitat index increased with sandy surficial deposits, watershed grassland, and riparian vegetation width and base flow, and decreased with buffer agriculture. Although the Michigan index was correlated to the Wisconsin index, the Michigan index was only weakly correlated to segment riparian vegetation width. Neither index correlated with watershed agriculture; however, the Wisconsin habitat index decreased with increasing buffer agriculture. One of the highest correlation coefficients was observed between base flow and the Wisconsin habitat index (Figure 2D). Base-flow measurements were not part of the characteristics used to calculate the index. Flow characteristics such as base flow and flow variability correlated to sandy surficial deposits and multiple spatial scales of land cover. For example, base flow increased with higher percentages of sandy surficial deposits and watershed grassland but decreased with increasing watershed and buffer agriculture (Table 3, Figure 2B and 2C). In contrast, flow variability increased with higher percentages of watershed and buffer agriculture but decreased with higher percentages of sandy surficial deposits and watershed grassland.

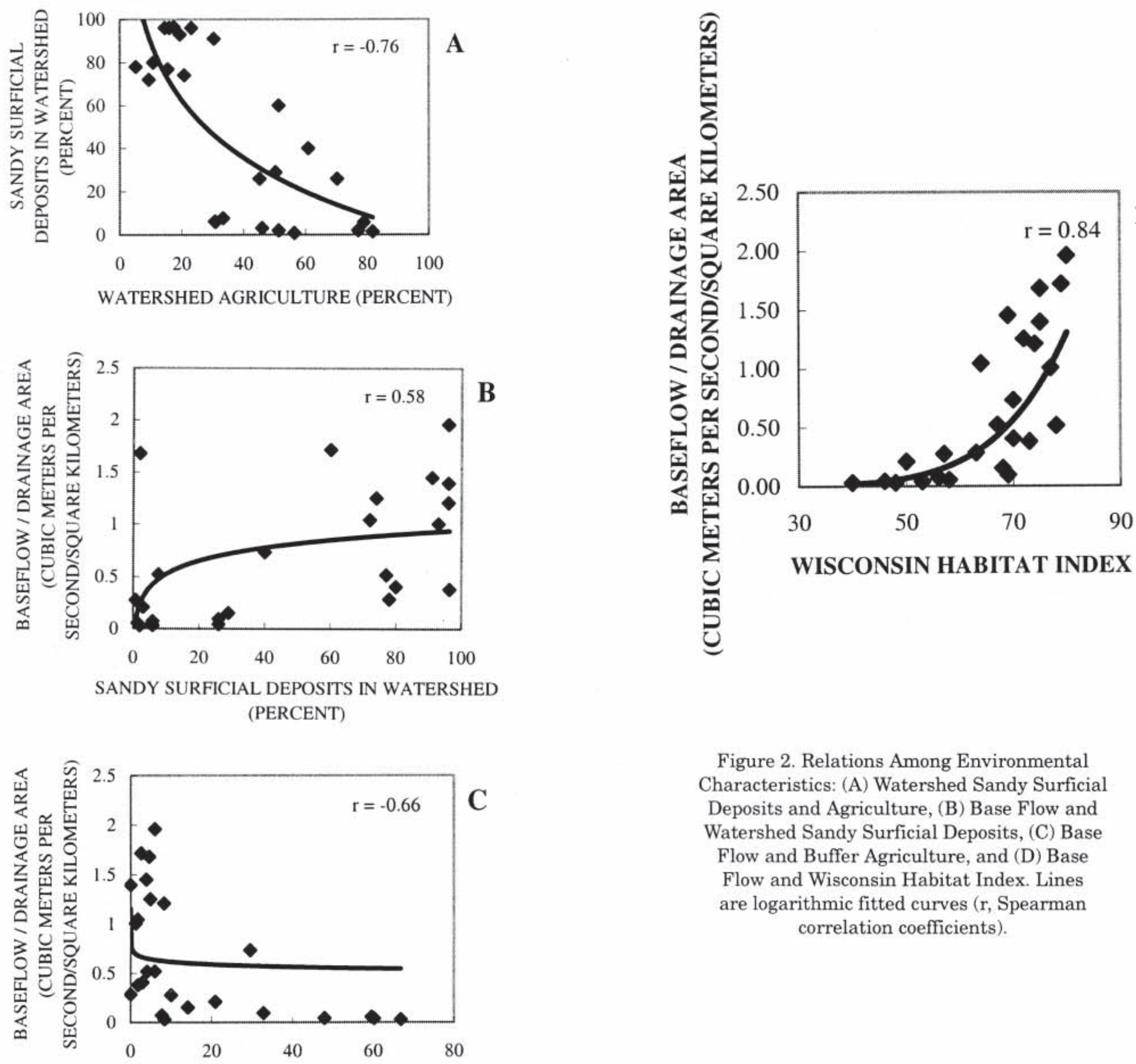


Figure 2. Relations Among Environmental Characteristics: (A) Watershed Sandy Surficial Deposits and Agriculture, (B) Base Flow and Watershed Sandy Surficial Deposits, (C) Base Flow and Buffer Agriculture, and (D) Base Flow and Wisconsin Habitat Index. Lines are logarithmic fitted curves (r, Spearman correlation coefficients).

Conductance and nutrients responded differently to multiple scales of environmental characteristics, mainly watershed and buffer land cover, geologic setting, segment and reach riparian vegetation, and base flow. Conductance increased with increasing percentages of watershed and buffer agriculture; however, none of the four nutrient concentrations correlated with watershed or buffer agriculture. Instead, dissolved ammonium, total organic plus ammonia nitrogen, and total phosphorus increased with decreasing sandy surficial deposits, permeability, and reach riparian vegetation width.

### *Correlations Among Biotic Metrics and Environmental Characteristics*

Environmental characteristics at multiple spatial scales were important influences on aquatic assemblages (Table 4). Not surprisingly, biotic metrics within the same aquatic assemblage sometimes correlated to different characteristics. Environmental characteristics that did not correlate with any biotic metrics were watershed relief and several reach scale characteristics: percentage of pools, canopy angle, average reach substrate, average microhabitat substrate, bank stability index, average embeddedness, and average riffle embeddedness.

In general, fish metrics had more and higher correlations with environmental characteristics at a variety of spatial scales compared to invertebrate and algal metrics. Fish metrics correlated with nearly all the environmental characteristics measured, indicating very complex relations among fish assemblages and their environment (Table 4). Fish IBI and percentage of intolerant fish individuals responded similarly. Fish IBI decreased with increasing number of tolerant species. This is not surprising because the fish IBI incorporates the other three fish metrics into the index scores. Fish IBI scores increased with decreasing stream order, buffer agriculture, width/depth ratio, and increasing Wisconsin habitat index, segment riparian vegetation width, and base flow, suggesting that these characteristics may have equal importance in affecting fish assemblages. None of the fish metrics correlated to the Michigan habitat index even though the Wisconsin habitat index correlated with all fish metrics, indicating that the two habitat indexes emphasized different aspects of physical condition. The total number of fish species increased with increasing watershed size and associated characteristics.

The influence of watershed agriculture and the moderating effects of buffers and riparian vegetation on fish IBI scores are illustrated in Figure 3. Watershed agriculture had some impact on fish IBI scores, with scores dropping to fair or poor (< 60) when watershed agriculture increased above 30 percent (Figure 3A). However, scores also dropped below good when buffer agriculture increased above approximately 10 percent (Figure 3B) or segment riparian vegetation width dropped below approximately 200 m (Figure 3C). A few streams were able to maintain a good or higher fish IBI scores with greater than 30 percent watershed agriculture or less than 200 m of segment riparian vegetation; however, all but one stream with more than approximately 10 percent buffer agriculture had fair or poor IBI scores.

Correlation coefficients between invertebrate metrics and environmental characteristics were generally lower than those for fish metrics (Table 4). In addition, the three invertebrate metrics tended to respond somewhat differently to environmental characteristics. For example, two of the strongest correlations were between the FBI and percent EPT and the Wisconsin habitat index, although the invertebrate diversity index did not correlate at all with the Wisconsin habitat index. Percent EPT decreased with increasing total organic plus ammonia nitrogen and total phosphorus. Unlike fish metrics, the invertebrate metrics did not correlate with watershed area or buffer agriculture. Environmental characteristics that correlated with two or more invertebrate metrics include watershed and buffer grassland, segment slope, segment and reach riparian vegetation width, both habitat indexes, reach riparian vegetation width, base flow, flow variability, dissolved nitrate plus nitrite, and total organic plus ammonia nitrogen, and total phosphorus. Only a limited number of low correlations were found between invertebrate diversity and environmental characteristics measured.

Similar to the invertebrate metrics, algal metrics had fewer correlations with environmental characteristics than did the fish metrics (Table 4). The strongest correlations with environmental characteristics generally occurred for percentage of pollution tolerant diatoms. The percentage of pollution tolerant diatoms increased with decreasing permeability and watershed grassland, and it increased with increasing dissolved ammonium, total organic plus ammonia nitrogen, and total phosphorus. Environmental characteristics that correlated with three or more algal metrics included: watershed land cover, segment and reach riparian vegetation width, and conductance. The strengths of the correlation coefficients were similar for watershed agriculture and reach riparian width, suggesting equal importance between the two spatial scales, although all four algal metrics significantly correlated with reach riparian width. The strongest correlation for the diatom IBI occurred with reach riparian vegetation width.

TABLE 4. Statistically Significant Spearman Correlation Coefficients for Fish, Invertebrate, and Algal Measures Versus Land Cover, Hydrologic Characteristics, Watershed Geomorphic and Geologic Characteristics, Habitat Scores, and Conductance. [**Bolded** correlation coefficients have p-values < 0.001(–, not significant, p-value > 0.05; IBI, Index of Biotic Integrity; FBI, Hilsenhoff (family-level) Biotic Index; Percent EPT, Percentage of Ephemeroptera, Plecoptera, and Trichoptera individuals; MDI, Margalef's Diversity Index; watershed characteristics are for the watershed outside the buffer (50-m buffer of entire stream network); percent land cover in the buffer is listed separately).]

| Environmental Characteristic | Abbreviation | Fish IBI | Number of Tolerant Species | Percentage of Intolerant Individuals | Total Number of Species | FBI | Percent EPT Individuals | Diversity Index | Number of Algal Taxa | Diatom Siltation Index | Percentage of Pollution Tolerant Diatoms | Diatom IBI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **WATERSHED SCALE** | | | | | | | | | | | | |
| Slope (m/km) | WSLOP | 0.60 | **-0.74** | – | **-0.78** | – | – | – | – | – | – | – |
| Sandy Surficial Deposits (percent) | WSAND | 0.60 | -0.60 | 0.60 | **-0.72** | – | 0.47 | – | – | -0.57 | -0.49 | – |
| Permeability (cm/hr) | PERME | – | -0.56 | – | -0.54 | – | – | – | – | – | -0.65 | – |
| Drainage Area (km$^2$) | WAREA | -0.52 | 0.61 | -0.46 | **-0.69** | – | – | -0.45 | – | – | – | – |
| Stream Length (km) | STLEN | -0.50 | **0.69** | -0.44 | **0.71** | – | – | – | – | – | – | – |
| Cumulative Stream Length (km) | CSTLE | -0.49 | **0.73** | – | **0.70** | – | – | -0.41 | – | – | – | – |
| Stream Order (categorical) | ORDER | **-0.81** | **0.74** | **-0.65** | **0.80** | – | 0.44 | – | – | – | – | – |
| Watershed Agriculture (percent) | WBUAG | -0.56 | 0.56 | **-0.62** | **0.64** | – | – | – | -0.57 | 0.54 | – | -0.40 |
| Watershed Grassland (percent) | WBUGR | 0.58 | -0.53 | – | -0.56 | -0.58 | 0.59 | – | – | – | **-0.70** | 0.40 |
| Watershed Forest (percent) | WBUFO | 0.60 | **-0.63** | 0.60 | **-0.65** | – | – | – | 0.56 | -0.43 | – | 0.41 |
| Watershed Grassland, Forest, Wetland (percent) | WBUG_ | 0.60 | -0.52 | **0.65** | -0.59 | – | – | – | 0.55 | -0.49 | – | 0.44 |
| Buffer Agriculture (percent) | BUFAG | **-0.76** | **0.71** | **-0.62** | **0.76** | – | – | – | -0.42 | – | – | – |
| Buffer Grassland (percent) | BUFGR | – | – | – | – | -0.49 | 0.48 | – | – | – | -0.46 | – |
| Buffer Forest (percent) | BUFFO | 0.54 | – | 0.57 | -0.54 | – | – | – | 0.44 | – | – | – |
| Buffer Wetland (percent) | BUFWE | **0.71** | -0.50 | **0.63** | -0.59 | -0.42 | – | – | – | -0.40 | – | – |
| Buffer Grassland, Forest, Wetland (percent) | BUFG_ | **0.76** | -0.59 | **0.67** | **-0.75** | – | – | – | – | -0.40 | – | – |
| **SEGMENT SCALE** | | | | | | | | | | | | |
| Slope (m/km) | SSLOP | 0.54 | -0.50 | 0.49 | -0.58 | – | 0.54 | 0.53 | – | – | – | – |
| Rparian Vegetation Width (m) | SVEGE | **0.72** | -0.49 | **0.68** | -0.58 | – | 0.41 | 0.50 | 0.48 | 0.44 | – | 0.54 |
| **REACH SCALE** | | | | | | | | | | | | |
| Width/Depth Ratio | WDRAT | **-0.67** | **0.70** | -0.57 | **0.80** | – | – | – | – | – | – | – |
| Velocity (m/s) | VELOC | 0.52 | -0.42 | – | -0.54 | – | 0.54 | – | – | – | -0.59 | – |
| Bank Stability Index | BASTI | – | – | – | – | – | – | – | – | – | – | – |
| Embeddedness (categorical) | EMBAV | – | – | – | – | – | – | – | – | – | – | – |
| Substrate (categorical) | SUBST | – | – | – | – | – | – | – | – | – | – | – |
| Michigan Habitat Index | GLEAS | – | – | – | – | – | 0.51 | 0.53 | – | – | – | – |
| Wisconsin Habitat Index | WIHAB | **0.74** | -0.58 | 0.49 | **-0.71** | **-0.63** | **0.74** | – | – | – | -0.45 | – |
| Riparian Vegetation Width (m) | RVEGE | 0.55 | -0.46 | 0.56 | -0.53 | – | 0.55 | 0.52 | 0.50 | -0.52 | -0.40 | **0.65** |
| Base Flow/Drainage Area (m$^3$/s/km$^2$) | QBASE | **0.64** | -0.48 | 0.40 | **-0.63** | -0.56 | 0.61 | – | – | – | -0.55 | – |
| Estimated 2-yr Flood/ Drainage Area (m$^3$/s/km$^2$) | Q2-YR | – | – | – | – | – | – | – | – | 0.43 | 0.50 | – |

TABLE 4. Statistically Significant Spearman Correlation Coefficients for Fish, Invertebrate, and Algal Measures Versus Land Cover, Hydrologic Characteristics, Watershed Geomorphic and Geologic Characteristics, Habitat Scores, and Conductance (cont'd.). [**Bolded** correlation coefficients have p-values < 0.001(–, not significant, p-value > 0.05; IBI, Index of Biotic Integrity; FBI, Hilsenhoff (family-level) Biotic Index; Percent EPT, Percentage of Ephemeroptera, Plecoptera, and Trichoptera individuals; MDI, Margalef's Diversity Index; watershed characteristics are for the watershed outside the buffer (50-m buffer of entire stream network); percent land cover in the buffer is listed separately).]

| Environmental Characteristic | Abbreviation | Fish IBI | Number of Tolerant Species | Percentage of Intolerant Individuals | Total Number of Species | FBI | Percent EPT Individuals | Diversity Index | Number of Algal Taxa | Diatom Siltation Index | Percentage of Pollution Tolerant Diatoms | Diatom IBI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **REACH SCALE (cont'd.)** | | | | | | | |
| Flow Variability (estimated 2-yr flood/base flow) | QVARI | -0.49 | – | – | 0.43 | 0.48 | -0.45 | – | -0.41 | – | 0.57 | – |
| Conductance (microsiemens/cm) | CONDU | -0.49 | – | -0.55 | 0.44 | – | – | – | **-0.68** | 0.59 | – | -0.56 |
| Ammonium, Dissolved (mg/L as N) | NAMMO | – | – | – | – | – | -0.56 | – | – | – | **0.68** | – |
| Nitrate plus Nitrite, Dissolved (mg/L as N) | NO2NO | – | – | – | – | -0.5 | 0.52 | 0.40 | – | – | – | – |
| Nitrogen, Total Organic+ Ammonia (mg/L as N) | NORAM | -0.45 | 0.45 | – | 0.53 | 0.54 | **-0.66** | – | – | – | **0.63** | – |
| Phosphorus, Total (mg/L as P) | PHOSP | -0.46 | 0.41 | – | 0.50 | 0.43 | **-0.63** | -0.41 | – | 0.47 | **0.67** | – |

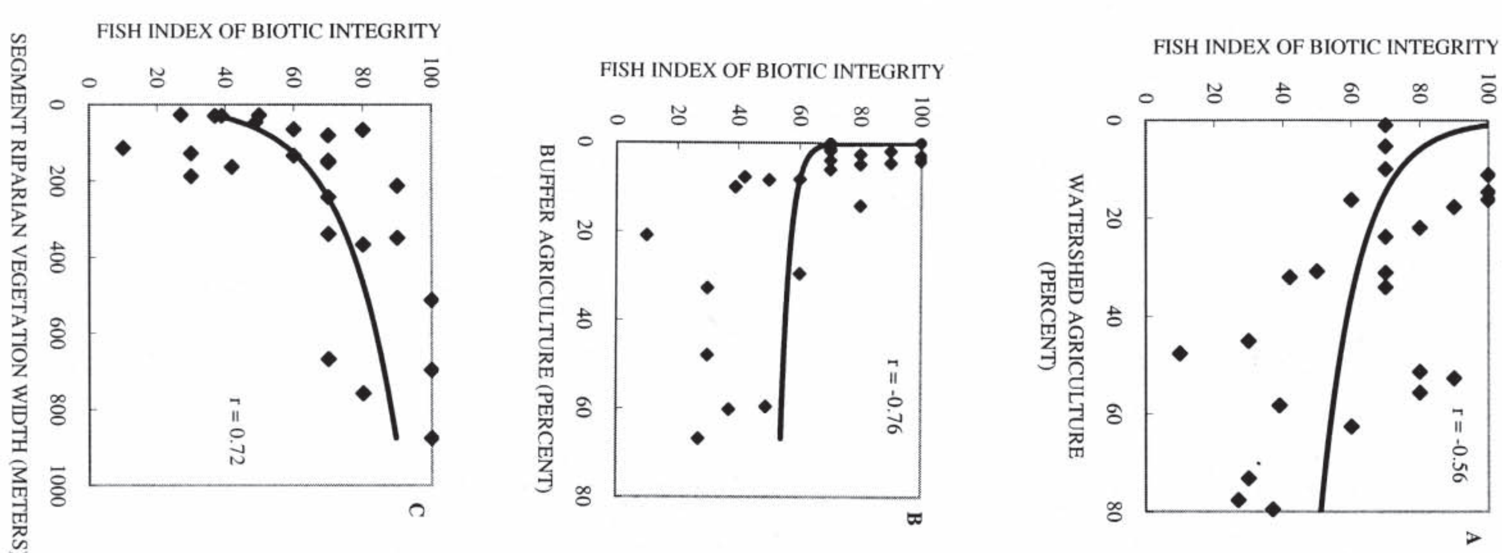


Figure 3. Relations Among Fish Index of Biotic Integrity and (A) Watershed Agriculture, (B) Buffer Agriculture, and (C) Segment Riparian Vegetation Width. Lines are logarithmic fitted curves (r, Spearman correlation coefficients).

Sixteen environmental characteristics were selected for the RDA and these are shown in bold in Table 2. Results from the Spearman correlation analysis were used to reduce the number of environmental characteristics used in the RDA. Some characteristics were eliminated due to high correlations with other characteristics or no correlations with biotic metrics. For example, drainage area was used as the representative characteristic for watershed size and sandy surficial deposits was used to represent permeability. Some reach characteristics were not included, such as substrate size or bank stability index, because they did not correlate with any biotic metrics. The first four axes of RDA explained 97 percent of the variance in the metric-environment relation (Table 5). The first axis explained 64 percent of the variance. Monte Carlo permutation tests indicated that all axes were significant ($p = 0.005$).

The RDA biplot of selected environmental characteristics and biotic metrics illustrates the overlapping gradients of watershed area, sandy surficial deposits, watershed and buffer land cover, segment riparian vegetation width, and width/depth ratio (Figure 4). All these relations also were found using Spearman rank correlations. These environmental characteristics plotted very closely along RDA Axis 1. Another group of environmental characteristics plotted between RDA Axes 1 and 2. These included segment slope, Wisconsin habitat index, base flow, flow variability, and nutrient concentrations. Buffer grassland plotted most closely along RDA Axis 2.

The RDA biplot also shows the differing responses of the biotic metrics to multiple scales of environmental characteristics (Figure 4). The fish and diatom IBIs, number of algal taxa, and invertebrate diversity plotted near each other along the positive side of Axis 1. The length and direction of the arrows on the biplot indicate the relative strength of relations among the biotic metrics and environmental characteristics. Fish and diatom integrity, number of algal taxa, and invertebrate diversity plotted closely to sandy surficial deposits, buffer wetland, reach riparian vegetation width, base flow, and the Wisconsin habitat index. Environmental characteristics that plotted directly opposite these same biotic metrics, indicating a negative correlation, included width/depth ratio, watershed area, total phosphorus, and flow variability. Unlike invertebrate diversity, the invertebrate FBI, percent EPT, and the percentage of pollution-tolerant diatoms plotted between Axis 1 and Axis 2. The invertebrate FBI and percentage of pollution-tolerant diatoms plotted near arrows for flow variability and dissolved ammonium. Directly opposite, percent EPT plotted near dissolved nitrate plus nitrite concentrations.

## DISCUSSION

The results from this study indicate that environmental characteristics at all spatial scales were important influences on aquatic biota. Watershed and buffer land cover, as well as segment and reach riparian vegetation width, influenced some metrics from all three types of biota. The following interpretations can be made based on the strength of Spearman correlation coefficients and results from the RDA. For fish, the following environmental characteristics are important (listed in order of decreasing importance): watershed area, buffer land cover, watershed land cover, segment riparian vegetation width, sandy surficial deposits, and base flow. For invertebrates, percent EPT and FBI responded differently than invertebrate diversity. Reach habitat followed by nutrient concentrations were most important for percent EPT and FBI, whereas invertebrate diversity responded similarly to the fish IBI, although correlations were much weaker. For algae, diatom IBI and number of algal taxa also responded similarly to the fish IBI, whereas other algal metrics correlated with nutrients and flow but not reach habitat.

Distinguishing the importance of one spatial scale of land-cover characteristics over the other was confounded because of the spatial overlap of geologic setting with land cover. In eastern Wisconsin and

TABLE 5. Summary of Redundancy Analysis (RDA) of 16 Multi-Scale Environmental Characteristics and Biotic Metrics and Indexes for 25 Stream Sites in Eastern Wisconsin.

| Summary Statistic | Axis 1 | Axis 2 | Axis 3 | Axis 4 |
|---|---|---|---|---|
| Eigenvalues | 0.588 | 0.179 | 0.082 | 0.030 |
| Metrics/Indexes and Environment Correlations | 0.987 | 0.924 | 0.910 | 0.807 |
| Cumulative Percent of Variance Explained in Biotic Metrics/Indexes | 58.8 | 76.7 | 84.9 | 87.9 |
| Cumulative Percent of Variance Explained in Biotic Metrics/Indexes-Environment Relation | 65.0 | 84.8 | 93.8 | 97.1 |

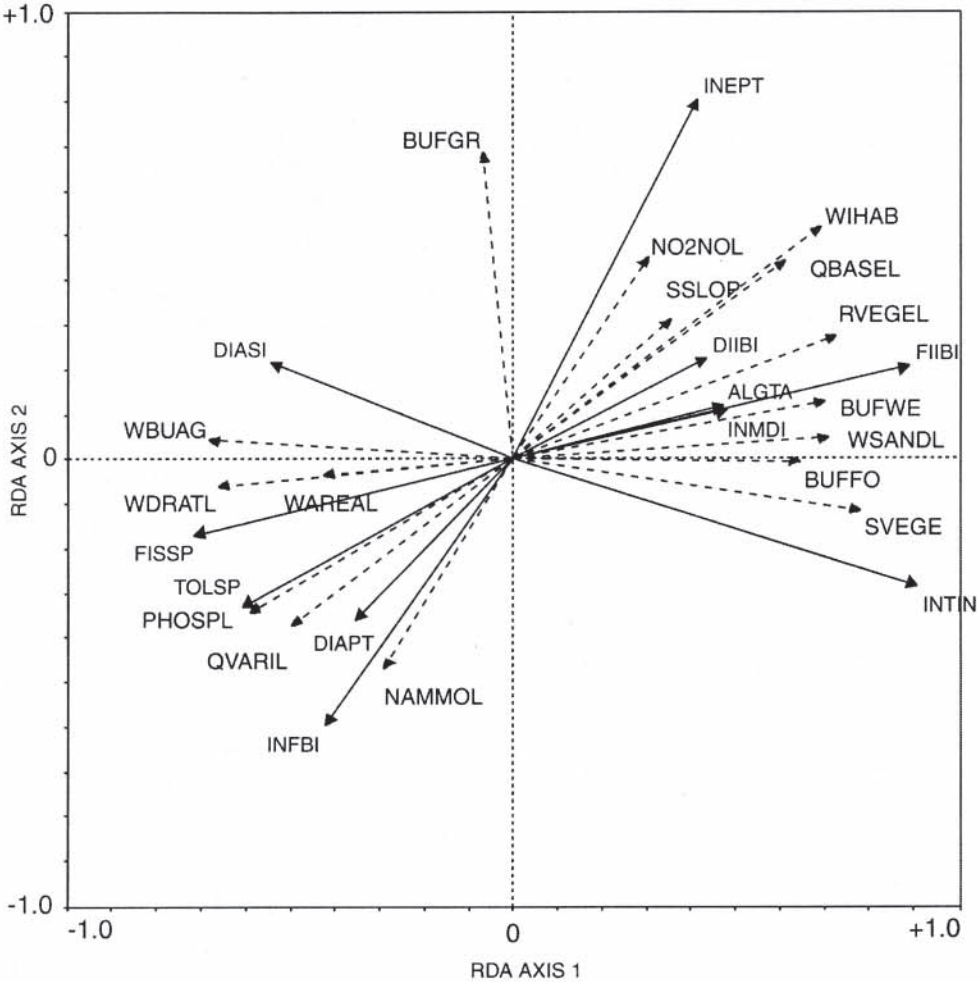


Figure 4. Redundancy Analysis (RDA) Biplot of Representative Environmental Characteristics in Relation to Aquatic Biotic Metrics. Refer to Tables 1 and 2 for definition of biota and environmental characteristic abbreviations, respectively. Arrows are dashed for environmental characteristics.

elsewhere in the Midwest, geologic setting affects the amount of land used for agriculture. Base flow and flow variability are linked in our study with both sandy surficial deposits and land cover. Areas of sandy surficial deposits in eastern Wisconsin often tend to be less suitable for row-crop agriculture. These deposits allow for higher recharge rates due to their greater permeability, and streams in sandy areas of Wisconsin tend to have higher base flow. In addition, a high percentage of agricultural land in less sandy areas leads to less infiltration, which further limits the potential for base flow. Streams in areas with high percentages of watershed agricultural land and high percentages of clayey surficial deposits will have high runoff, low base flow, and thus high flow variability. Watershed size also related to watershed land cover and thus geologic setting.

Although excessive increases in dissolved nitrate plus nitrite might be expected as negative effects of agriculture, increases in dissolved nitrate plus nitrite correlated with increased permeability, watershed grassland, and base flow (Table 3). Shallow ground water in sandy areas within the study area also had higher dissolved nitrate concentrations than shallow ground water from loamy and clayey deposits in the study area (Saad, 1997). This is likely due to higher permeability and lower organic matter content of sandy surficial deposits compared to loamy/clayey surficial deposits, allowing greater infiltration of nitrogen fertilizers used in agriculture (Saad, 1997).

It is also difficult to completely separate the effects of multiple scales of land cover. In eastern Wisconsin, streams with nonagricultural land cover in their watersheds tend to have buffers with high percentages of forest or wetland along the entire stream network as well as at the segment and reach scale. The presence of buffers in agricultural watersheds varies depending on topography near the stream and the percentage of private or public wildlife preserves or natural areas along the riparian corridor. In this study, large watersheds had more agriculture throughout the watershed and within the buffer. However, comparison of fish IBI scores with multi-scale land cover data indicated that land cover in the buffer along the entire stream network was very important for maintaining high IBI scores, and that as little as 10 percent agriculture in the buffer could quickly decrease IBI scores. All but one stream with more than 10 percent buffer agriculture had fair or poor fish IBI scores. The one stream with a higher fish IBI score above 10 percent buffer agriculture was Hibbard Creek, located near the tip of the peninsula that separates Green Bay from Lake Michigan. Perhaps agricultural practices in this area differ in some manner compared to the rest of the study area. Although watershed agriculture also influenced fish IBI scores, some streams could maintain higher IBI scores even with 50 to 60 percent watershed agriculture as long as the stream network buffer contained less than 10 percent agriculture. Some streams also maintained good or better fish IBI scores with less than 200 m of segment riparian vegetation width, perhaps indicating that for fish, localized pockets of agriculture within a 200-m buffer were less influential than general conditions of buffer land cover throughout the stream network upstream of the sampling site.

In examining the environmental characteristics in a hierarchical sense, we would expect that watershed characteristics would impact physical and chemical characteristics at the segment and reach scale, which then would in turn affect biotic assemblages. Watershed characteristics determine hydrologic and sediment supply characteristics, which subsequently affect geomorphic processes and chemical concentrations. Geomorphic processes in turn affect segment and reach scale habitat characteristics that are related to channel conditions (substrate, width/depth ratios, velocity, bank conditions, and Embeddedness). Studies such as Richards *et al.* (1996) have shown that surficial geology and land use patterns influence channel morphology that then influenced local habitat characteristics. In this study, watershed and buffer land cover and geologic setting influenced width/depth ratio, velocity, bank stability, and the Wisconsin habitat index, but not embeddedness, substrate, or the Michigan habitat index (Table 3). The categorical nature of the methods used for collection of embeddedness and substrate may not have been sensitive enough to quantify the response of channel substrate conditions to changes in land cover. An evaluation of spatial variations in reach-scale habitat data at the five monitored sites indicated that substrate and embeddedness measurements were not adequate to represent the range of substrate conditions in the reach (Fitzpatrick and Giddings, 1997). In 1998, the NAWQA habitat protocol was revised to include more transects; categorical substrate measurements are now collected at 33 points in the channel instead of 11 points (Fitzpatrick *et al.,* 1998). Embeddedness measurements were updated from categorical units to percent and are also now collected at 33 instead of 11 points.

The two habitat indexes showed different responses to environmental characteristics and to the biotic metrics even though both were based on semi-quantitative reach measurements. The differences in correlation coefficients between the Michigan and Wisconsin habitat indexes suggest that the indexes were measuring different aspects of aquatic habitat. The Wisconsin index correlated more with sandy surficial deposits and the three scales of riparian land cover characteristics than did the Michigan index. This may be because one of the seven characteristics that make up the Wisconsin habitat index is the reach scale riparian vegetation width. The Michigan habitat index had a more general metric of streamside cover that reflected the type of vegetation along the stream bank. The Wisconsin habitat index was very sensitive to multi-scale environmental characteristics (Table 3), and, similar to the fish IBI, it appeared to be useful for assessing anthropogenic and natural impacts on stream quality. The Michigan habitat index related less to fish metrics and more to invertebrate metrics. Past studies have shown that habitat at the point of collection can be as important to benthic invertebrates as water quality (Rheaume *et al.,* 1996b) which may explain why the Michigan habitat index related better than the Wisconsin habitat index to invertebrate assemblages.

Some biotic metrics appeared to be more sensitive to environmental degradation than others. Fish IBI scores were correlated to a variety of environmental characteristics at multiple spatial scales, indicating that in this geographic region the IBI is highly useful for assessing anthropogenic and natural impacts on stream quality. For invertebrates, percent EPT correlated to more environmental characteristics than did the FBI and diversity index. The lack of correlations with the FBI may be an artifact of our collection techniques and warrants further investigation. The invertebrate diversity index most closely related to the fish IBI, although correlation coefficients with the same environmental characteristics were much weaker. For algal metrics, the strongest correlation coefficients with environmental characteristics were for the percentage of pollution tolerant diatoms, although as stated earlier, the diatom IBI and number of algal taxa responded to environmental characteristics in a similar way as the fish IBI.

Invertebrate metrics in our study reflected decreases in favorable conditions of water chemistry and reach habitat that were generally associated with increased agricultural land cover. Other studies have found invertebrates to be good indicators of water quality changes due to agriculture (Hilsenhoff, 1987; Lenat, 1988; Cuffney *et al.,* 1997). Richards and Host (1993; 1994) found that invertebrates responded mostly to variation in local substrate characteristics and suggested an indirect effect of agriculture on invertebrate assemblages, such as through the deleterious effects of increasing fine sediment on substrate. The difference in collection and processing methods among sites may have influenced the usefulness of the diversity metric for invertebrates. Rosenberg and Resh (1993) found that site-specific characteristics could confound relations between metrics such as diversity and environmental characteristics. In particular, small first-order streams may have good water quality but may lack diversity because of lack of habitat or food for invertebrates. Whiles *et al.* (2000) found that a modified Hilsenhoff index was less effective at discriminating between agricultural sites than was EPT, and that invertebrate metrics were related to riparian land cover. The lower taxonomic resolution of the invertebrate data used in our study, compared to the fish and algal data, might explain the lower sensitivity of the invertebrate metrics to gradients in watershed and buffer land cover. For example, the family-scale FBI is considered to be less sensitive than the generic- and species-scale biotic index by Hilsenhoff (1988) but may be a useful screening tool.

In addition to the effects of differing scales of environmental characteristics, the scales of biotic metrics may have affected the number and strength of correlations with the environmental characteristics tested. The fish metrics used were reach scale, whereas the benthic invertebrate and algal metrics were based on a smaller scale, that is, habitat found in only part of the reach. For this reason, the fish metrics in our study might be expected to generally correlate with more and larger scale environmental characteristics than the invertebrate and algal metrics.

In a study by Cuffney *et al.* (1997), fish showed an almost linear decline in assemblage condition as watershed agriculture increased; however, benthic invertebrates and algal assemblages did not show a linear response and instead appeared to deteriorate rapidly once a relatively low threshold of watershed agriculture was reached. The post-threshold assemblages of invertebrates and algae showed little response to further increases in agricultural intensity. They felt, therefore, that it was critical to assess the responses of invertebrate and algal assemblages at low levels of agriculture. Our results also emphasize the need to consider assemblage responses and metrics for more than one trophic group of aquatic biota in order to accurately examine biotic community responses due to land management practices. Results from this study also indicate that physical and chemical characteristics measured at the segment and reach scale, in addition to watershed scale, are needed to characterize environmental differences in streams and account for unknown variability. Study designs focusing on the watershed scale may be missing some important characteristics that may be influencing stream quality, and study designs focusing on local scales may be unable to detect effects of watershed or regional environmental characteristics.

## CONCLUSIONS

The relative influence of environmental characteristics on species distribution, abundance, and assemblage composition of aquatic organisms was highly complex and interrelated. It was difficult to separate the effects of geologic setting from watershed or buffer land cover and base flow. All were interrelated and all had some effect on aquatic biota.

Fish metrics, specifically the fish IBI, appeared to be the best indicator of land-cover effects that might occur at a variety of spatial scales. Watershed and buffer land cover, geologic setting, segment and reach riparian vegetation width, and stream size affected the fish IBI. In particular, fish IBI scores seemed most sensitive to land cover in the entire stream network buffer, more so than watershed land cover and segment or reach riparian vegetation width. As little as 10 percent agriculture in the stream network buffer related to fish IBI scores of fair or poor. In addition, segment riparian vegetation widths of less than 200 m also corresponded to fish IBI scores of fair or poor, although a few streams maintained good IBI scores at widths less than 200 m.

Invertebrate diversity, the diatom IBI, and number of algal taxa showed similar relations as with fish IBI in the RDA although correlation coefficients were much weaker. In contrast, the invertebrate FBI, percentage of EPT, and the diatom pollution index were influenced more by nutrient concentrations and flow variability. In general, the invertebrate and algal metrics used in this study were not as sensitive to watershed and buffer land cover; this may be due to the scale at which invertebrate data are processed, or the manner in which the algal species data are summarized into metrics. Perhaps fish are more integrative of watershed and buffer land cover because of their mobility, whereas less mobile invertebrates and algae reflect reach conditions.

The Wisconsin habitat index seemed to be a better indicator of stream quality than the Michigan habitat index in this study. The two habitat indexes responded differently to watershed characteristics. Both indexes were designed to assess the impacts of land use. However, the Wisconsin habitat index related more to watershed geologic setting and watershed and buffer land cover, riparian vegetation width, and base flow, whereas the Michigan habitat index only weakly correlated with segment riparian vegetation width and base flow.

Studies that use different biotic metrics to define an aquatic assemblage and assess water quality may come to different conclusions as to the importance of one environmental factor over another. Examination of a variety of metrics among different aquatic assemblages, coupled with a thorough understanding of potential geologic and hydrologic variability, are needed to more thoroughly understand the relations among land cover and biotic integrity.

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