In cooperation with the Wisconsin Department of Natural Resources

Nutrient Concentrations and Their Relations to the Biotic Integrity of Nonwadeable Rivers in Wisconsin

Professional Paper 1754

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

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By Dale M. Robertson, Brian M. Weigel, and David J. Graczyk

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Conversion Factors

Multiply	By	To obtain
	Length	
micrometer (µm)	0.00003927	inch (in.)
millimeter (mm)	0.03927	inch (in.)
centimeter (cm)	0.3937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Area	
square kilometer (km^2)	247.1	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
liter (L)	0.2642	gallon (gal)
cubic meter (m^3)	35.31	cubic foot (ft^3)
	Flow	
cubic meter per second (m^3/s)	70.07	acre-foot per day (acre-ft/d)
cubic meter per second per square kilometer]($(m^3/s)/km^2$]	91.49	cubic foot per second per square mile [($(ft^3/s)/mi^2$]
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)
microgram (µg)	0.00000003527	ounce, avoirdupois (oz)
milligram (mg)	0.00003527	ounce, avoirdupois (oz)

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

°F=(1.8×°C)+32

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius.

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

Abbreviations and Symbols

Ag %	percentage of agricultural land in basin
AIC	Akaike’s Information Criterion
BIN	Biotic Index of total Nitrogen
BIP	Biotic Index of total Phosphorus
DEM	digital elevation model
DFA	Driftless Area, level III ecoregion
DP	dissolved phosphorus
EPT	Ephemeroptera, Plecoptera, or Trichoptera (insect orders)
EtOH	ethanol
EV	explained variance
EWI	equal-width-increment
GIS	geographic information system
N	nitrogen
NCHF	North Central Hardwood Forest, level III ecoregion
NLF	Northern Lakes and Forests, level III ecoregion
NH_4-N	dissolved ammonia
NO_3-N	dissolved nitrite plus nitrate
NWIS	National Water Information System
p	probability
P	phosphorus
PP	particulate phosphorus
r	Pearson correlation coefficient
r_s	Spearman correlation coefficient
R^2	coefficient of determination
Res	residualized (X*)
RDA	redundancy analysis
SCHL	suspended chlorophyll *a*
SD	Secchi-tube depth
SSC	suspended sediment concentration
SWTP	Southeastern Wisconsin Till Plains, level III ecoregion
STATSGO	State Soil Geographic database
STORET	Storage and Retrieval database
TKN	total Kjeldahl nitrogen
TN	total nitrogen
TP	total phosphorus
Urb %	percentage of urban land in basin

USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WDNR	Wisconsin Department of Natural Resources
<	less than

Abbreviations for Macroinvertebrate Indices

%CHIRON	percentage of individuals from the family Chironomidae, within the order Diptera, from the class Insecta
%DEPOS	percentage of individuals tolerant to depositional habitat
%DIPT	percentage of individuals from the order Diptera, from the class Insecta
%EPHEM	percentage of individuals from order Ephemeroptera, from the class Insecta
%EPTN	percentage of individuals that are EPT
%EPTTX	percentage of EPT taxa
%GATHER	percentage of individuals that are gatherers (feeding function)
HBI	Hilsenhoff Biotic Index
MPTV	mean pollution tolerance value
%PLEC	percentage of individuals from the order Plecoptera, from the class Insecta
%SCRAP	percentage of individuals that are scrapers (feeding function)
%SHRED	percentage of individuals that are shredders (feeding function)
SPECIES	species richness
%TRICHOP	percentage of individuals from the order Trichoptera, from the class Insecta

Abbreviations for Fish Indices

#INTOL	number of species considered intolerant of degradation
#NATIVESP	number of native species (not exotic)
#RIVERSP	number of riverine species
#SUCKER	number of sucker species in the family Catastomidae
%DISEASE	percentage of fish with disease or deformities
%INSECT	percentage of total biomass accounted for by insectivores
%LITSPAWN	percentage of individuals that are lithophilic spawners
%RIVERSP	percentage of total biomass accounted for by individuals that are riverine species
%SUCKER	percentage of total biomass accounted for by round-bodied suckers (genera Cycleptus, Hypentelium, Minytrema, and Moxostoma)
IBI	Wisconsin large-river index of biotic integrity
WPUE	weight of fish caught per unit effort (excluding tolerant fish)

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Nutrient Concentrations and Their Relations to the Biotic Integrity of Nonwadeable Rivers in Wisconsin

By Dale M. Robertson[1], Brian M. Weigel[2], and David J. Graczyk[1]

Abstract

Excessive nutrient [phosphorus (P) and nitrogen (N)] input from point and nonpoint sources is frequently associated with degraded water quality in streams and rivers. Point-source discharges of nutrients are fairly constant and are controlled by the U.S. Environmental Protection Agency's (USEPA) National Pollutant Discharge Elimination System. To reduce inputs from nonpoint sources, agricultural performance standards and regulations for croplands and livestock operations are being proposed by various States. In addition, the USEPA is establishing regionally based nutrient criteria that can be refined by each State to determine whether actions are needed to improve water quality. More confidence in the environmental benefits of the proposed performance standards and nutrient criteria would be possible with improved understanding of the biotic responses to a range of nutrient concentrations in different environmental settings.

To achieve this general goal, the U.S. Geological Survey and the Wisconsin Department of Natural Resources collected data from 282 streams and rivers throughout Wisconsin during 2001 through 2003 to: (1) describe how nutrient concentrations and biotic-community structure differ throughout the State, (2) determine which environmental characteristics are most strongly related to the distribution of nutrient concentrations and biotic-community structure, (3) determine reference conditions for water quality and biotic indices for streams and rivers in the State, (4) determine how the biotic communities in streams and rivers in different areas of the State respond to differences in nutrient concentrations, (5) determine the best regionalization scheme to describe the patterns in reference conditions and the corresponding responses in water quality and the biotic communities (primarily for smaller streams), and (6) develop algorithms to estimate nutrient concentrations in streams and rivers from a combination of biotic indices. The ultimate goal of this study is to provide the information needed to guide the development of regionally based nutrient criteria for Wisconsin streams and rivers. In this report, data collected, primarily in 2003, from 42 nonwadeable rivers are used to describe nutrient concentrations and their relations to the biotic integrity of rivers in Wisconsin. In a separate report by Robertson and others (2006a), the data collected from 240 wadeable streams are used to describe these relations in streams in Wisconsin.

Reference water-quality conditions for nonwadeable rivers were found to be similar throughout Wisconsin (approximately 0.035 milligrams per liter (mg/L) for total P (TP), 0.500 mg/L for total N (TN), 4 micrograms per liter for suspended chlorophyll *a* (SCHL), and greater than 110 centimeters for Secchi-tube depth (SD)). For each category of the biotic community (SCHL, macroinvertebrates, and fish), a few indices were more strongly related to differences in nutrient concentrations than were others. For the indices most strongly related to nutrient concentrations, reference conditions were obtained with a regression approach, from values corresponding to the worst 75th-percentile value from a subset of minimally impacted streams (streams having reference nutrient concentrations), and from the best 25th-percentile value of all the data.

Concentrations of TP and TN in nonwadeable rivers increased as the percentage of agricultural land in the basin increased; these increases resulted in increased SCHL concentrations and decreased SDs. The responses in SDs and SCHL concentrations to changes in nutrient concentrations were similar throughout most of the State except in rivers in the southeastern part, where SCHL concentrations were lower than would be expected given their nutrient concentrations. Rivers in the southeastern part of the State had high concentrations of total suspended sediment compared to the SCHL concentrations.

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Many biotic indices responded to increases in nutrient concentrations, which indicates that nutrients have direct or indirect effects on the composition of the biotic community. Higher nutrient concentrations and poorer biotic index scores, indicative of poorer water quality, were found in agricultural areas in the southern half of the State. Most of the biotic indices were more strongly related to changes in TP concentrations than to changes in TN concentrations. Many of the responses to changes in nutrient concentrations were nonlinear and, therefore, thresholds or breakpoints were identified where a small change in nutrient concentrations corresponded to a relatively large change in the biotic communities. The thresholds in the responses to changes in TP concentrations ranged from 0.03 to 0.15 mg/L, whereas thresholds to changes in TN concentrations ranged from about 0.5 to 2.0 mg/L. The thresholds for many of the biotic responses were only slightly higher than the reference TP concentrations estimated for rivers throughout the State.

The biotic communities in a river reflect its overall ecological integrity; they integrate the effects of many different stressors and thus provide a broad measure of the stressors' aggregate effect. Nutrient concentrations by themselves, however, explained only 1–11 percent of the total variance in the components of the biotic communities or about 2–25 percent of the explained variance. Nutrient concentrations were most important in affecting SCHL concentrations.

Three biotic indices were combined to create two new multiparameter indices [Biotic Index of total Phosphorus (BIP) and Biotic Index of total Nitrogen (BIN)] to estimate TP and TN concentrations from biotic data collected in the rivers. The BIP predicted TP concentrations better than the BIN predicted TN concentrations (63 and 51 percent of the variances, respectively). The difference in the accuracy of these indices was consistent with biotic indices that were more correlated with TP concentrations than with TN concentrations. This result indicates that P is more important than N in affecting most biotic communities in rivers.

Distributions of water quality and biotic indices for nonwadeable rivers, in general, were similar to those found for wadeable streams, with best conditions in the northern (forested) part of the State. The main differences between wadeable streams and nonwadeable rivers include: nonwadeable rivers had a smaller range in nutrient concentrations (less extreme concentrations, especially lower maximum concentrations), although median concentrations were similar; nonwadeable rivers had higher percentages of P and N in particulate forms; nonwadeable rivers had SCHL concentrations that were higher and had a stronger relation with nutrient concentrations; most biotic indices in nonwadeable rivers were more strongly related to nutrient concentrations; most biotic indices in nonwadeable rivers had a less consistent wedge-shaped response to changes in nutrient concentrations (the wedge-shaped response in wadeable streams resulted from biotic indices that ranged widely at low nutrient concentrations, but were consistently poor at high nutrient concentrations); and the biota in nonwadeable rivers had a slightly larger range in the thresholds in the responses to changes in TP concentrations.

Although specific mechanisms of how nutrients affect the biota in wadeable streams and nonwadeable rivers were not examined in this study, the results indicate that nutrients are important in controlling their biotic health. Although the biotic-community structure represents the overall ecological integrity of the stream or river, nutrients alone explained only a small part of the variance in the biotic community. Therefore, it is difficult to predict the exact result of reducing nutrient concentrations without also modifying the factors typically associated with high nutrient concentrations. Nutrient concentrations in many streams and rivers, especially in agricultural areas, are well above the threshold concentrations; therefore, small reductions in nutrient concentrations in these streams and rivers are not expected to have large effects on the biotic community. Even with these limitations, however, it is expected that reducing nutrient concentrations will improve the biotic communities of most streams and rivers, improve their beneficial ecological functioning, and improve the quality of downstream nutrient-limited receiving waters.

Introduction

Elevated concentrations of nutrients are some of the most common stressors (contaminants) affecting rivers and streams throughout the United States. Problems associated with elevated nutrient concentrations in surface water are not new, but they are among the most persistent. According to the National Water Quality Inventory: 1996 Report to Congress by the U.S. Environmental Protection Agency (USEPA), 50 States, Tribes, and other jurisdictions surveyed water-quality conditions in 19 percent of the Nation's 3.6 million miles of rivers and streams and found overenrichment of nutrients to be the second-most common reason for impairment after the combined effects of suspended sediment and siltation (U.S. Environmental Protection Agency, 1996). Excessive nutrients in rivers and streams can result in the overgrowth of benthic algae in shallow areas and in areas with fast currents, and an overabundance of phytoplankton and macrophytes in deep areas with slow currents. High algal and macrophyte biomass can cause severe diurnal fluctuations in dissolved oxygen and pH associated with biotic production and respiration, and can cause low dissolved oxygen concentrations when part of the population dies (Welch and others, 1992). Low dissolved oxygen concentrations, in turn, can cause an increase in the availability of toxic substances, reduction in available aquatic habitat, modifications to the composition of the biotic communities especially if fish die off, and a decrease in the overall usefulness of the stream (Miltner and Rankin, 1998; Dodds and Welch, 2000). In addition to local effects, excessive transport of nutrients has also been linked to eutrophication of downstream lakes and impoundments, outbreaks of *Pfiesteria* in bays and estuaries in various Gulf and Mid-Atlantic States, and hypoxia in the Gulf of Mexico (U.S. Environmental Protection Agency, 2000a).

Under recommendations of the Clean Water Action Plan released in 1998, the USEPA has developed a National strategy to develop waterbody-specific nutrient criteria for lakes and reservoirs, rivers and streams, wetlands, and estuaries (U.S. Environmental Protection Agency, 1998); this study is concerned with criteria for rivers and streams. The intention of this strategy is to get all States and Tribes to establish nutrient standards, that, if enforced, will reduce nutrient concentrations and improve the beneficial ecological uses of surface waters. The best way to control nutrient concentrations is to reduce the part contributed by humans, not the part contributed naturally (U.S. Environmental Protection Agency, 1998). Various environmental characteristics, such as land use, geology, soils, climate, and hydrology (including human modifications and hydrologic structures) are important in determining water quality (Monteith and Sonzogni, 1981; Clesceri and others, 1986; and Robertson, 1997). Because these characteristics differ greatly across the United States, the determination of regionally specific background or reference nutrient concentrations and the establishment of regionally specific nutrient criteria make scientific sense.

The USEPA has taken the initial step in developing a regional framework for nutrient criteria based on combining Omernik's 84 level III ecoregions into 14 national nutrient ecoregions for the conterminous United States (U.S. Environmental Protection Agency, 1998; Rohm and others, 2002). On a subregional basis, such as a specific State, each of these 14 nutrient ecoregions can be further subdivided into the original level III ecoregions. Wisconsin is subdivided into two national nutrient ecoregions (ecoregions 7 and 8) that are further subdivided into four level III ecoregions: Northern Lakes and Forests (NLF; national nutrient ecoregion 8), and North Central Hardwood Forests (NCHF), Southeastern Wisconsin Till Plains (SWTP), and the Driftless Area (DFA) that are in national ecoregion 7 (Omernik and others, 2000; fig. 1A). In addition, Wisconsin includes small parts of the Western Cornbelt Plains and the Central Cornbelt Plains ecoregions (not labeled in fig. 1A). The nutrient ecoregions provide an initial regionalization scheme for developing nutrient criteria; however, the USEPA expects individual States and Tribes to evaluate and possibly develop alternative regionalization schemes (U.S. Environmental Protection Agency, 2000b). Robertson and others (2006b) demonstrated that the ecoregions in the Midwest primarily reflect differences in land use and developed a regionalization technique that removed the effects of land use. They subdivided the Midwest into five environmental phosphorus zones that were delineated primarily on the basis of differences in soils and surficial deposits (primarily based on differences in the amount of till and clay in the soils; fig. 1B).

After relatively homogenous geographic areas have been chosen, several approaches can be used to define quantitative nutrient criteria. The approach suggested by the USEPA to define possible criteria is based on the reference or potential water quality of each area—in other words, on the conditions that are attainable in the geographic location of each river or stream (U.S. Environmental Protection Agency, 2000a). Reference concentrations for total phosphorus (TP), total nitrogen (TN), suspended chlorophyll *a* (SCHL, also referred to as sestonic chlorophyll), and a measure of turbidity have been defined from the frequency distribution of all available data (from USEPA's Storage and Retrieval, STORET, database) for each national nutrient ecoregion and most level III ecoregions. It has been suggested that the lower (best water quality) 25th percentile of all data for an area may represent this reference condition (25th-percentile approach; U.S. Environmental Protection Agency, 2000b); that is, 25 percent of all the sites have water quality at least as good as this reference condition. Defining reference conditions based on this approach can result in reference nutrient concentrations that are biased high in predominantly agricultural areas where more than 75 percent of the sites are impacted by nutrient influx, and biased low in predominantly forested areas where fewer than 75 percent of the sites are impacted. Therefore, it has also been suggested that the upper (worst water quality) 75th percentile of the data for a subset of rivers or streams thought to be minimally impacted for a defined area may represent

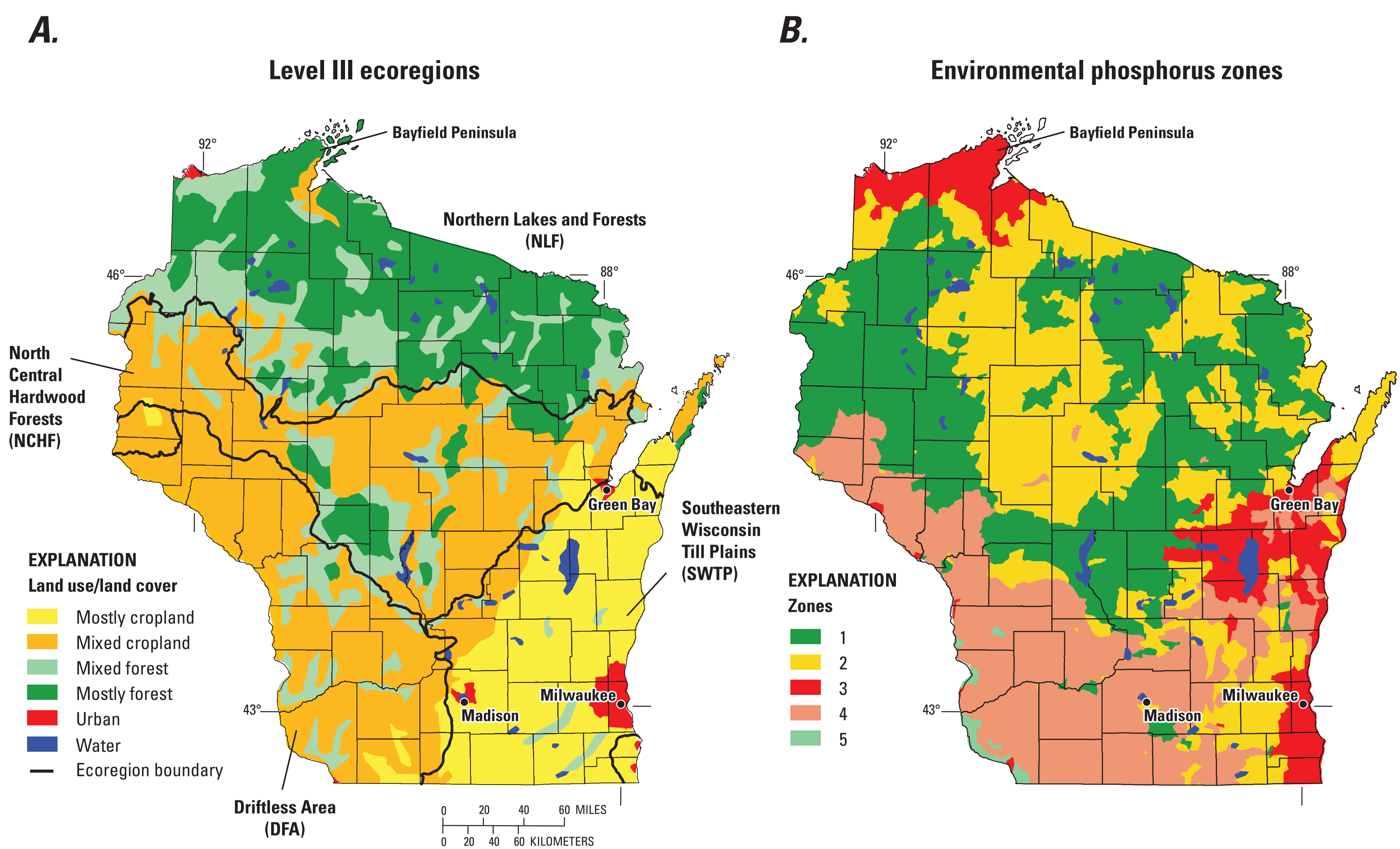

Figure 1. Two regionalization schemes considered for streams and rivers in Wisconsin: ***A***, level III ecoregions (Omernik and others, 2000) with major land-use/land-cover categories (Wisconsin Department of Natural Resources, 1998) and ***B***, environmental phosphorus zones (Robertson and others, 2006b).

this reference condition (75th-percentile approach); that is, 75 percent of the minimally impacted sites have water quality at least as good as this reference condition.

Defining reference conditions based upon a percentile approach is arbitrary. An alternative approach to estimate reference concentrations is to develop a multiple linear-regression model from data for a specific area that relates water quality to anthropogenic factors or characteristics such as the percentages of agricultural and urban area in the watershed (Dodds and Oakes, 2004). With this approach, the estimated concentration of a constituent or value of an index in the absence of human activities (with 0-percent agricultural and 0-percent urban areas) represents the reference or background condition. These relations or equations can also be used to place confidence intervals on the reference conditions.

A few studies have shown observational linkages between nutrient concentrations and the health of the biotic communities in streams. Nutrients have been shown to directly affect the productivity and species composition of primary producers, such as macrophytes and benthic and suspended algae, and indirectly affect the primary and secondary consumers in controlled nutrient-enrichment experiments (for example, Mundie and Simpson, 1991; Peterson and others, 1993; Perrin and Richardson, 1997). Miltner and Rankin (1998) reported that macroinvertebrate- and fish-assemblage indices were negatively correlated with TN and TP concentrations in wadeable streams in Ohio. Zorn (2003) reported that TP was one of the important variables for predicting the presence or absence of specific fish species in Michigan streams. Heiskary and Markus (2003) also reported significant negative correlations between macroinvertebrate- and fish-assemblage characteristics and TP and TN concentrations in nonwadeable rivers in Minnesota.

Most empirical relations between nutrient concentrations and biotic indices, trophic status, primary production, and food-web dynamics, however, have generated mixed results (for example, Vannote and others, 1980; Junk and others, 1989; Sedell and others, 1989; Dodds and others, 1998; and Thorp and Delong, 2002). These mixed results demonstrate that models developed in specific areas may not be applicable elsewhere, or that the biotic community reflects more than just the nutrient concentrations that are or were present in the stream. The biotic community represents the overall ecological integrity of the stream (physicochemical habitat and biotic integrity) and thus provides a broad measure of the aggregate effect of all stressors (Barbour and others, 1999). The physicochemical habitats of the stream where the biota live are in turn controlled by watershed characteristics such as geomorphology, geochemistry, and land use/land cover; therefore, watershed characteristics are also important factors affecting the biotic community (Frissell and others, 1986; Poff, 1997).

Because biota respond to stresses from multiple spatial and (or) time scales and several pathways including habitat and water chemistry, monitoring macroinvertebrate and fish communities, in addition to water quality, is valuable for determining natural and anthropogenic influences on stream and river resources (Karr, 1981; Ohio Environmental Protection Agency, 1987; Rosenberg and Resh, 1993). Macroinvertebrate-based assessments have become important tools in rapid bioassessment protocols for indicating ecological condition (Plafkin and others; 1989, Kerans and Karr, 1994; DeShon, 1995; Weigel, 2003). Likewise, protocols for assessing ecosystem health often use combinations of fish-assemblage measures as indices of biotic integrity (Karr and others, 1986; Simon and Lyons, 1995; Lyons and others, 2001). Macroinvertebrate and fish assessments complement each other because one may be more responsive to particular stressors or spatial scales than the other (Davis and Simon, 1995; Barbour and others, 2000; Davies and Jackson, 2006). One of the goals of establishing nutrient criteria is to protect the biotic community. An alternative approach to define nutrient criteria is to use biotic models or measured data to define thresholds in the response between nutrient concentrations and biotic indices such as the amount of suspended algae in a stream or river (as quantified by SCHL), water clarity (as quantified by SD), or macroinvertebrate or fish biotic indices (U.S. Environmental Protection Agency, 2000a).

One of the greatest impediments to understanding nutrient–biota relations is that biota may respond to nutrient enrichment in the same way that they respond to other stressors (Yoder and Rankin, 1995; Karr and Chu, 1999). Furthermore, generally the high correlations between environmental variables make it difficult to differentiate spurious correlations from cause-and-effect relations (Miltner and Rankin, 1998; Wang and others, 2003; Dodds and Oakes, 2004).

If relations between nutrient concentrations and biotic integrity are used to define nutrient criteria, the final criteria should be chosen to minimize degradation in the biotic integrity of rivers and streams. In other words, the criteria should be the nutrient concentrations that would not result in high algal concentrations or values of biotic indices that reflect a degraded environment. One of the difficulties in defining nutrient criteria is determining the values of the biotic index (such as a SCHL concentration) for which a river or stream is considered degraded or impaired. Whichever approach is used, the final criteria must be stringent enough to protect the specific site and cause no adverse effects in downstream waters.

The USEPA developed preliminary criteria based on the 25^{th}-percentile approach and the distribution of median concentrations of all the data measured at each site within a specified region rather than the mean concentrations. Median values were used because they represent the concentration most frequently present in the stream, and because a statistical summary based on median values reduces the effects of outliers and values reported as less than their respective detection limits. The USEPA has provided preliminary criteria for TP, TN, SCHL, and turbidity for the national nutrient ecoregions and most level III ecoregions (table 1; U.S. Environmental Protection Agency, 2000b and 2001). Robertson and others (2006b) developed an alternative regionalization scheme for the Midwest (environmental phosphorus zones) and estimated reference concentrations for each zone by use of multiple linear-regression models that related TP, TN, SCHL, and SD to two anthropogenic characteristics (percentages of agricultural and urban areas in the watershed; Dodds and Oakes, 2004). Robertson and others (2006b) found similar reference TP concentrations for the entire State, but reference concentrations of TN and SCHL and SD were different in areas with high clay-content soils (Zone 3; from Green Bay to Milwaukee in southeastern Wisconsin and Bayfield Peninsula in northwestern Wisconsin; table 1 and fig. 1B).

There would be more confidence in the potential environmental benefits of enforcing nutrient criteria and standards for streams and rivers in Wisconsin if the criteria and standards were based on the most appropriate regionalization scheme and if the criteria and standards reflect appropriate regionally defined thresholds to biotic response. Defined nutrient criteria and thresholds for biotic indices would enable the use of monitoring data to identify rivers and streams affected by excessive nutrients and would be useful to water-resource managers in directing rehabilitation efforts.

Purpose and Scope

In 2001, the U.S. Geological Survey (USGS) and the Wisconsin Department of Natural Resources (WDNR) began a collaborative study to (1) describe how nutrient concentrations and biotic-community structure in rivers and streams differ throughout Wisconsin, (2) determine which environmental characteristics of watersheds are most strongly related to the distribution of nutrient concentrations and biotic-community structure, (3) determine reference water-quality and biotic conditions for different areas of the State, (4) determine how the biotic communities of rivers and streams in different areas of the State respond to differences in nutrient concentrations, (5) determine the best regionalization scheme to describe the patterns in reference conditions and the corresponding responses in water quality and the biotic communities (primarily for smaller streams), and (6) develop new algorithms to predict nutrient concentrations in rivers and streams from a combination of biotic indices. The ultimate goal of this study is to provide the information needed by water-resource managers to develop regionally based nutrient criteria for rivers and streams in Wisconsin.

This study was divided into two parts (nonwadeable rivers and wadeable streams) because (1) the natural physicochemical and biotic attributes are not comparable between nonwadeable rivers and wadeable streams, (2) the biotic response was expected to vary as a function of stream size, and (3) biota in wadeable streams are sampled by means of different techniques than nonwadeable rivers. The first part of the study involved sampling 240 wadeable streams in 2001–03, and the second part involved sampling 42 nonwadeable rivers in 2003. The results of the first part of this study were described in the USGS publication entitled “Nutrient concentrations and their relations to the biotic integrity of wadeable streams in Wisconsin” by Robertson and others (2006a) and the biotic relations are summarized in an article entitled “Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: implication to nutrient criteria development” by Wang and others (2007). The second part of the study, “Nutrient concentrations and their relations to the biotic integrity of nonwadeable rivers in Wisconsin,” is presented in this report, and the biotic relations are summarized in an article entitled “Identifying biotic integrity and water chemistry relations in nonwadeable rivers of Wisconsin: towards the development of nutrient criteria,” by Weigel and Robertson (2007).

Table 1. Reference concentrations for total phosphorus, total nitrogen, and suspended chlorophyll *a* concentrations, and turbidity in various ecoregions (U.S. Environmental Protection Agency, 2000b and 2001), Zones from the Upper Midwest study (Robertson and others, 2006b), and wadeable streams study (Robertson and others, 2006a; Wang and others, 2007).

[USEPA, U.S. Environmental Protection Agency; NCHF, North Central Hardwood Forests; DFA, Driftless Area; SWTP, Southeastern Wisconsin Till Plains; mg/L, milligram per liter; µg/L, microgram per liter; >, greater than; --, no data]

Region	USEPA criteria	Upper Midwest study			Wadeable streams study		
		Median	Upper 95-percent confidence limit	25th percentile of all sites	Median	Upper 95percent confidence limit	25th percentile of all sites
	Total phosphorus (mg/L)						
Ecoregion 7	0.033	0.016	0.024	0.040	0.038	0.049	0.058
NCHF-51[a]	.029	--	--	--	.041	.060	.054
DFA-52[a]	.070	--	--	--	.040	.057	.053
SWTP-53[a]	.080	--	--	--	.025	.044	.072
Ecoregion 8	.010	.015	.019	.010	.032	.036	.024
Areas with high clay-content soils (Zone 3)	--	.012	.017	.020	.029	.043	.053
Rest of State (Zones 1, 2, 4, and 5)	--	.021–.023	.026–.030	.030–.060	.032–.042	.043–.054	.027–.061
	Total nitrogen (mg/L, calculated/reported)				Total nitrogen (mg/L, calculated)		
Ecoregion 7	0.54/0.54	--	--	--	0.775	0.974	1.555
NCHF-51[a]	.46/.71	--	--	--	1.132	1.637	1.188
DFA-52[a]	1.88/1.51	--	--	--	.643	.901	1.489
SWTP-53[a]	1.59/1.30	--	--	--	.811	1.409	2.020
Ecoregion 8	.20/.38	--	--	--	.509	.587	.403
Areas with high clay-content soils (Zone 3)	--	--	--	--	.367	.601	1.318
Rest of State (Zones 1, 2, 4, and 5)	--	--	--	--	.557–.690	.676–1.050	.486–1.780
	Turbidity (nephelometric/formazin turbidity units)				Secchi-tube depth (cm)[b]		
Ecoregion 7	1.7/2.32	--	--	--	>120	>120	66.0
NCHF-51[a]	.84/2.14	--	--	--	104.4	116.9	102.3
DFA-52[a]	3.38/2.4	--	--	--	118.6	>120	67.5
SWTP-53[a]	--/2.74	--	--	--	>120	>120	47.8
Ecoregion 8	.81/1.3	--	--	--	116.5	119.8	>120
Areas with high clay-content soils (Zone 3)	--	--	--	--	109.5	>120	51.5
Rest of State (Zones 1, 2, 4, and 5)	--	--	--	--	>115	>120	65.3–>120

Table 1. Reference concentrations for total phosphorus, total nitrogen, and suspended chlorophyll *a* concentrations, and turbidity in various ecoregions (U.S. Environmental Protection Agency, 2000b and 2001), Zones from the Upper Midwest study (Robertson and others, 2006b), and wadeable streams study (Robertson and others, 2006; Wang and others, 2007)—Continued.

[USEPA, U.S. Environmental Protection Agency; NCHF, North Central Hardwood Forests; DFA, Driftless Area; SWTP, Southeastern Wisconsin Till Plains; mg/L, milligram per liter; µg/L, microgram per liter; >, greater than; --, no data]

Region	USEPA criteria	Upper Midwest study: Median	Upper Midwest study: Upper 95-percent confidence limit	Upper Midwest study: 25th percentile of all sites	Wadeable streams study: Median	Wadeable streams study: Upper 95-percent confidence limit	Wadeable streams study: 25th percentile of all sites
	Chlorophyll *a* (µg/L, fluorometric/spectrophotometric/trichromatic methods)				Chlorophyll *a* (µg/L, trichromatic method)		
Ecoregion 7	1.54/3.50/5.8	--	--	--	1.50	1.94	1.78
NCHF-51[a]	1.03/8.76/--	--	--	--	1.73	2.65	1.49
DFA-52[a]	1.00/2.32/--	--	--	--	1.48	2.02	1.75
SWTP-53[a]	0.55/3.52/--	--	--	--	1.44	2.71	2.02
Ecoregion 8	0.60/2.60/4.3	--	--	--	1.49	1.74	1.08
Areas with high clay-content soils (Zone 3)	--	--	--	--	1.03	1.55	1.62
Rest of State (Zones 1, 2, 4, and 5)	--	--	--	--	1.17–1.71	1.93–2.23	1.37–1.86

[a] U.S. Environmental Protection Agency level III ecoregion identification numbers.

[b] A lower 95-percent confidence limit and 75th percentile of all data are given here because higher values represent better conditions for Secchi-tube depth.

Approach

Wisconsin has approximately 40 nonwadeable rivers with a combined length of more than 2,500 km as river and 1,500 km as impounded habitat (Weigel and others, 2006). A river reach is considered nonwadeable if it has more than 3 km of continuous channel too deep to sample effectively by wading during summer base flow. Because simultaneously collected hydrological, water-quality, and biological data were not available to determine how the biotic integrity of Wisconsin's rivers is related to differences in nutrient concentrations, a network of sites was selected to represent the nonwadeable rivers throughout the State. The locations of the 42 sites on 35 nonwadeable rivers are shown in figure 2 and listed in appendix 1. Sites were chosen from throughout the State and represented the types of rivers and the kinds and intensities of stress upon each river type. The drainage basins of these rivers represent approximately 88 percent of the area of the State. Watersheds of several basins extend into adjacent States. Multiple sampling sites were chosen on several of the major rivers in the State; however, the sites were widely separated. The discharge and water quality at each site were sampled monthly during a 6-month period (May through October, 2003). Macroinvertebrate data were collected during summer 2003. Fish-population data, however, were not necessarily collected as part of this study; most data were available from past surveys. All fish-population data were collected between 1996 and 2004.

For each site, the drainage basin upstream of the sampling site was digitized and a geographic information system (GIS) was used to describe the environmental characteristics of the watershed. Several multivariate statistical approaches were then used to determine how the environmental characteristics of the watershed were related to water quality and biotic-community structure. For the wadeable streams, the water-quality and biotic data were used to evaluate different regionalization schemes. It was previously found that there were slight differences in reference conditions for TN, SCHL, and water clarity in wadeable streams; however, reference TP concentrations were similar throughout the State (table 1; Robertson and others, 2006a). Streams in areas with high clay-content soils (Zone 3 in fig. 1) had slightly lower reference concentrations of TN and SCHL and poorer clarity, and the TP concentrations were more responsive to differences in land use than in streams in the rest of the State. Despite variation in nutrient concentrations among wadeable streams in different regions (especially lower nutrient concentrations in the northern part of the State), the responses of all of the biotic indices to changes in nutrient concentrations were similar throughout the State. The watersheds of the nonwadeable rivers, however, usually crossed several ecoregions and environmental phosphorus zones; therefore, different regionalization schemes were not evaluated in detail for the nonwadeable rivers.

Reference concentrations for TP, TN, SCHL, water clarity (SD), and biotic indices for the nonwadeable rivers were estimated by use of the multiple linear-regression and percentile approaches. Water-quality data in nonwadeable rivers were statistically compared with biotic indices describing SCHL, macroinvertebrates, and fish to determine (1) how the biotic integrity of rivers is related to differences in nutrient concentrations, (2) whether thresholds in TP and (or) TN concentrations can be defined above which the biota are adversely affected, and (3) the relations between environmental variables and the structure of macroinvertebrate and fish communities. Two indices were then developed to estimate TP and TN concentrations in rivers on the basis of a combination of three indices describing the structure of the biotic community. Results from the nonwadeable rivers were then compared with those from the wadeable streams (Robertson and others, 2006a; Wang and others, 2007).

Methods of Data Collection and Analysis

Field Methods

Discharge, Water Chemistry, and Suspended Chlorophyll *a* Concentrations

Discharge and water quality in each river were sampled monthly over a 6-month period (May through October 2003). This 6-month period represents the typical growing season for Wisconsin's rivers and streams. Each site was sampled near the middle of the month regardless of flow conditions. If the site had an operating continuous-record gage, discharge was determined on the basis of the current stage/discharge relation (Rantz and others, 1982). If the site had a discontinued continuous-record gage, discharge was determined on the basis of the last stage/discharge relation that was in effect when the gage was operational. If the site never had a continuous-record gage, discharge was estimated on the basis of relations developed between previous discharge measurements at the site and measurements, adjusted for the drainage-area ratio, from a nearby continuous-record gage.

Figure 2. Sites on nonwadeable rivers in Wisconsin included in this study. Water-quality and biological data for each site are given by site-identification number in the appendixes.

During each visit, field parameters (specific conductance (SC), water temperature, dissolved oxygen, and pH) were measured at the time of sampling by use of a multiparameter meter. The meters were calibrated daily. Water clarity was also measured by use of a 120-cm Secchi tube [also referred to as a transparency tube (U.S. Environmental Protection Agency, 2006)]. The Secchi tube was filled with water collected from near the surface of the river. The tube was then held perpendicular to the ground and drained until the Secchi disk at the bottom of the tube became visible. The water level in the tube was read to the nearest centimeter and defined as the Secchi-tube depth (SD). If the disk was visible when the tube was full, the value was reported as greater than 120 cm.

During each visit, water samples were collected with depth-integrating samplers according to the equal-width-increment (EWI) method (Edwards and Glysson, 1999). If the river was wadeable at the time of sampling, a handheld sampler was used. When the river was too deep or velocities too high, a cable-suspended sampler was used. Samples from each location in a transect were composited into a churn splitter and split into appropriate bottles for laboratory analysis. Samples to be analyzed for dissolved constituents were filtered in the field through 0.45-µm membrane filters. Samples to be analyzed for SCHL were obtained by filtering a known volume of water through a 5-µm membrane filter. The filter was then placed in a labeled petri dish and wrapped in aluminum foil. All samples were chilled until they were delivered to the Wisconsin State Laboratory of Hygiene for analysis, except samples to be analyzed for SCHL, which were frozen, kept in the dark, and delivered to the WDNR Research Laboratory. All samples were analyzed for TP, dissolved phosphorus (DP), total Kjeldahl nitrogen (TKN), dissolved nitrite plus nitrate nitrogen (NO_3-N), dissolved ammonia nitrogen (NH_4-N), SCHL, and suspended sediment (SSC). Total nitrogen was computed as the sum of TKN and NO_3-N. Particulate phosphorus (PP) was computed as the difference between TP and DP. All chemical analyses of water samples (except SCHL) were done by the Wisconsin State Laboratory of Hygiene in accordance with standard analytical procedures described in the “Manual of Analytical Methods, Inorganic Chemistry Unit” (Wisconsin State Laboratory of Hygiene, 1993). At the WDNR Research Laboratory, the filters for SCHL analysis were placed in tubes containing 90 percent acetone, stored at least 24 hours, sonicated for 15 minutes, and stored an additional 24 hours in a freezer. The trichromatic chlorophyll *a* (SCHL) concentration in the samples was determined by means of a USEPA-approved method (Greenberg and others, 1992). Throughout this report, the water-chemistry, water-clarity, and SCHL data are collectively referred to as “water-quality data.”

Macroinvertebrates

Macroinvertebrates samples were collected at each site by the WDNR by use of modified Hester-Dendy artificial-substrate samplers during summer 2003. Sampling methods were based upon those of the Ohio Environmental Protection Agency (1987) regarding sampler construction and deployment. Three samplers were deployed at each site and macroinvertebrates were allowed to colonize them for 6 weeks starting in mid-June. Samplers were then retrieved, organisms were scraped off, sample contents were combined, and the organisms were preserved with ethanol (EtOH) for laboratory processing. Samples were sorted and identified by J. Dimick at the laboratory of Dr. Stanley Szczytko at the University of Wisconsin, Stevens Point. A randomized grid-pan subsorting procedure (Hilsenhoff, 1988) in combination with a large-and-rare organism search (Vinson and Hawkins, 1996) was used to select at least 500 individual organisms and reduce the sample to a manageable size. All macroinvertebrates were identified to the lowest possible taxon.

Fish

Standard WDNR methods were used for collecting fish-population data (Lyons and others, 2001). Sampling occurred once in each river during daylight between June and September from 1996 to 2004. An electrofishing boat was steered downstream along one randomly chosen shore for 1.6 km, a distance at which estimates of species richness were shown to be asymptotic and insensitive to variation in sampling effort (Lyons and others, 2001). One person used a 19-mm (3/8-in. stretch-mesh) dip net and tried to capture all of the fish seen. All captured fish were identified, counted, weighed in aggregate by species, and then released, except for a few specimens to confirm species identification. All sites had considerable flow during sampling.

Data Summaries

All of the water-quality data collected in this study were input into the USGS National Water-Quality Information System (NWIS) database (U.S. Geological Survey, 1998) and are summarized by median values for each site in appendix 1. Median values were computed from the six individual monthly measurements. All data reported as less than the detection limit were set to one-half of the detection limit, and all SD data greater than 120 cm were set to 120 cm prior to any statistical and graphical analyses.

Fourteen assemblage measures were computed to summarize the macroinvertebrate data, including species richness (1 index), habitat (1) and pollution tolerance (2), feeding ecology (3), and insect order (7). Tolerance to depositional zones or substrates and feeding ecologies were largely estimated based upon Merritt and Cummins (1996). The Hilsenhoff Biotic Index (HBI; Hilsenhoff, 1988) and mean pollution tolerance value index (MPTV; Lillie and Schlesser, 1994) represent the stress response of the macroinvertebrate assemblage to organic pollution. HBI and MPTV are on a 0–10 scale, with 0 representing no apparent organic pollution. The macroinvertebrate index values for each site are summarized in appendix 2.

Eleven assemblage measures were computed to summarize the fish data, including number of species and percentage of individuals of native or riverine fish (three indices), number of species in the sucker family Catastomidae, percentage of the biomass accounted for by round-bodied suckers of the genera *Cylceptus*, *Hypentelium*, *Minytrema*, and *Moxostoma* (Lyons and others, 2001), number of species intolerant of degradation, weight per unit effort (WPUE; one unit of effort equates to 1.6 km; this value excludes tolerant fish as defined by Lyons and others, 2001), percentage of fish that spawn in stony substrate (lithophilic), percentage of fish biomass that are insectivores, percentage of fish with diseases, and an overall index of biotic integrity (IBI; Lyons and others, 2001). All of the sites were in warm-water reaches (except one site, described below), where summer water temperatures excluded resident salmonids. Therefore a warm-water IBI was computed (Lyons and others, 2001) that ranges from 0 to 100 with qualitative categories at 20-point increments (for example, 80 to 100 represents an excellent fishery). The fish metrics for each site are summarized in appendix 3. Biotic data from one of the sites (White River near Ashland) was not used in the analyses because it had cold water and a cold-water fish assemblage that was not comparable to the warm-water assemblages at the other sites. Therefore, only 41 sites were used to examine the response of the biotic communities to differences in water quality.

Watershed Boundaries and Environmental Characteristics

Watershed boundaries for the sampled rivers were manually digitized from 1:24,000-scale USGS topographic quadrangle maps (Henrich and Daniel, 1983). Coverages of the environmental characteristics thought to affect the water quality and biota in the rivers were compiled for each watershed used in this study: land use/land cover (based on data collected in 1992; Wisconsin Department of Natural Resources, 1998), soil characteristics (from the USSOILS digital coverage of the State Soil Geographic data base, STATSGO; Schwarz and Alexander, 1995), types of surficial deposits (Fullerton and others, 2003), annual average air temperature and annual total precipitation (National Climatic Data Center, 2002), annual evaporation (Farnsworth and others, 1982), annual runoff (Gebert and others, 1987), river length (length of the river between the sampling location and the most upstream location identified on the 1:24,000 hydrology GIS coverage; Wisconsin Department of Natural Resources, 2004), and mean basin slope [computed based on 30-m digital-elevation-model (DEM) data resampled to 100 m at the sampling site and at the most upstream location identified on the 1:24,000 hydrology GIS coverage and the river length; U.S. Geological Survey, 1999; Wisconsin Department of Natural Resources, 2004].

Coverages of all basin characteristics were compiled in digital form by use of a GIS and were then used to compute the average or percentage value for each environmental characteristic for each of the 42 watersheds. A summary of the environmental characteristics (with the specific metric describing each environmental characteristic) for all of the watersheds used in this study is given in table 2.

Statistical Methods

The SAS statistical software package (SAS Institute, Inc., 1989) was used for all statistical analyses except for the redundancy analyses, which were done with the CANOCO statistical software package (ter Braak and Smilauer, 2002), and the regression-tree analyses, which were done with the SPLUS statistical software package (Lam, 2001).

Table 2. Summary statistics for median monthly water quality collected in 2003 and environmental characteristics of the watersheds of the study sites in nonwadeable rivers in Wisconsin.

[mg/L, milligram per liter; cm, centimeter; C, degrees Celsius; µS/cm, microsiemen per centimeter, %, percent; km^2, square kilometer; mm, millimeter; mm/yr, millimeter per year; $(m^3/s)/km^2$, (cubic meter per second) per square kilometer; km, kilometer; mm/hr, millimeter per hour; >, greater than; --, unitless]

Characteristic	Abbreviation	Units	Transformation	Count	Median	Mean	Standard deviation	Minimum	Maximum
Water-quality characteristics									
Total phosphorus	TP	mg/L	log	42	0.109	0.132	0.111	0.023	0.497
Dissolved phosphorus	DP	mg/L	log	42	.041	.053	.042	.012	.156
Particulate phosphorus	PP	mg/L	log	42	.053	.079	.077	.011	.391
Total nitrogen	TN	mg/L	log	42	1.268	1.707	1.280	.266	5.485
Dissolved nitrite plus nitrate	NO_3-N	mg/L	log	42	.395	.837	1.036	.011	3.770
Dissolved ammonia	NH_4-N	mg/L	log	42	.024	.034	.027	.007	.134
Total Kjeldahl nitrogen	TKN	mg/L	log	42	.620	.848	.547	.255	2.850
Suspended chlorophyll *a*	SCHL	µg/L	log	42	7.31	18.47	25.3	1.74	130
Secchi-tube depth[a]	SD	cm	none	42	60.5	71.8	40.0	12	>120
Suspended sediment	SSC	mg/L	log	42	14.0	24.1	24.4	1.0	87.5
Water temperature	WTemp	C	none	42	19.6	19.7	1.33	16.8	22.1
Specific conductance	SC	µS/cm	none	42	295	364	234	78	904
pH	pH	standard	none	42	8.18	8.15	.30	7.55	8.75
Color	Color	--	none	42	34.4	36.3	21.2	5	80
Land-use characteristics									
Urban	Urb %	%	none	42	0.005	0.013	0.023	0.000	0.109
Agriculture (row crops)	AgRow %	%	none	42	.122	.152	.134	.001	.479
Agriculture (other)	AgOther %	%	none	42	.138	.146	.122	.004	.639
Total agriculture	Ag %	%	none	42	.294	.298	.230	.007	.867
Grassland	Grass %	%	none	42	.098	.095	.049	.002	.188
Wetland (open)	WetO %	%	none	42	.074	.080	.046	.001	.211
Wetland (forested)	WetF %	%	none	42	.079	.079	.057	.000	.222
Barren	Barren %	%	none	42	.009	.011	.009	.000	.036
Forest (all)	For %	%	none	42	.481	.471	.243	.067	.865
Basin characteristics									
Watershed area	Area	km^2	log	42	2,000	4,890	6,420	655	27,000
Air temperature	ATemp	C	none	42	5.9	6.1	1.3	4.1	8.3
Precipitation	PPT	mm	none	42	835	834	25.3	781	902
Evaporation	Evap	mm	none	42	828	826	86.9	711	991
Runoff	Runoff	mm/yr	none	42	255	260	49.1	356	181
River length	Length	km	log	42	99.6	141	104	41.3	534
Basin slope	BSlope	degrees	none	42	1.18	1.24	.737	.370	4.17
Flow per unit area	Flow	$(m^3/s)/km^2$	log	42	.008	.011	.010	.003	.051

Table 2. Summary statistics for median monthly water quality collected in 2003 and environmental characteristics of the watersheds of the study sites in nonwadeable rivers in Wisconsin—Continued.

[mg/L, milligram per liter; cm, centimeter; C, degrees Celsius; µS/cm, microsiemen per centimeter, %, percent; km^2, square kilometer; mm, millimeter; mm/yr, millimeter per year; $m^3/s/km^2$, cubic meter per second per square kilometer; km, kilometer; mm/hr, millimeter per hour; >, greater than; --, unitless]

Characteristic	Abbreviation	Units	Transformation	Count	Median	Mean	Standard deviation	Minimum	Maximum
				Soil characteristics					
Clay content	SClay	%	none	42	12.4	13.8	6.71	5.69	30.6
Erodibility	Erod	--	none	42	.217	.218	.050	.146	.369
Organic-matter content	OM	%	none	42	9.42	7.78	4.52	.393	15.2
Permeability	Perm	mm/hr	none	42	4.67	4.60	1.62	1.24	8.28
Soil slope	SSlope	%	none	42	6.59	7.44	3.17	3.76	15.1
				Surficial deposits					
Nonglacial deposits	NonGlac	%	none	42	0.3	18.5	32.5	0.0	1.0
Clay	SDClay	%	none	42	.1	6.7	9.9	.0	27.0
Loam	Loam	%	none	42	.0	3.1	6.0	.0	30.8
Peat	Peat	%	none	42	.0	.7	1.0	.0	3.8
Sand	Sand	%	none	42	30.2	39.3	31.9	.0	97.1
Sand and gravel	SG	%	none	42	24.0	31.7	28.4	.0	89.4

[a] All values greater than 120 cm were set to 120 centimeters for computation of summary statistics; as a result, the mean values were biased low.

Normalization

Before statistical analyses were done, all water-quality data except the SD data were logarithmically transformed (base 10) to improve the normality of the data. The normality of the data improved, although not always to the 5-percent-significance level (Shapiro-Wilk normality test; SAS Institute, Inc., 1989). In addition, watershed areas were logarithmically transformed prior to statistical analyses.

Correlations and Regressions

Spearman correlation analyses were used to determine the correlation (r_s) between water-quality characteristics, biotic indices, and environmental characteristics. This nonparametric procedure was chosen to reduce the influence of the assumption of normal-data distributions. The statistical significance of each correlation was obtained by applying the Student's t-test to the t statistic (Spiegel and others, 2000); correlations were also examined by accounting for the effects of the number of tests on the significance level by use of a Bonferroni correction (Tukey, 1977). Pearson correlation (r) analyses were also used to determine the relation between each water-quality characteristic and each environmental characteristic prior to the use of multiple regressions and forward stepwise-regression analyses [with a probability (p) less than (<) 0.05 as the critical level for entry]. This procedure was used to determine the magnitude of the interaction between environmental characteristics and water-quality characteristics, as well as to determine the best multivariate relation to estimate concentrations at a specific site as a function of the environmental characteristics in its watershed.

Simultaneous Partial Residualization

Other studies (such as Robertson and others, 2006b) have shown that land use in the watershed not only directly affects the water quality in a river, but it is often strongly correlated with the environmental characteristics used to define regions of similar water quality (through the indirect effects of land use). Therefore, in order to determine the relation between the water-quality characteristics and the nonanthropogenic or natural characteristics, a simultaneous partial-residualization approach (Robertson and others, 2006b), related to partial correlation, was used to remove agricultural and urban effects from the TP and TN concentrations and from the measures of each of the environmental characteristics.

In simple regression, the relation between the dependent variable Y (for example, logarithmically transformed TP) and a predictor variable X_1 (for example, the clay content of the soil in the basin) can be measured by the sample correlation r_{YX1}. If the variable X_1 is regressed on the variable X_2 (for example, the percentage of agricultural area), the estimated regression equation $X_{1,2} = \beta_0 + \beta_1 X_2$ would be obtained. To adjust X_1 for the effects of X_2, a residualized X_1, X_1^*, can be obtained by computing $X_1^* = X_1 - X_{1,2}$. In a manner similar to simple correlation, the strength of the relation between Y and X_1 adjusted for X_2 (in this case, land use) can be obtained by the correlation between the residuals for Y on X_2 (Y^*) and the residuals for X_1 on X_2 (X_1^*). The resulting correlation is the partial correlation of Y and X_1 adjusted for X_2; the strength of the relation between Y and X_1 has been adjusted for the effects of X_2. This approach, described by Weisberg (1980), is easily extended to control for more than one variable; X_2 can be replaced by an arbitrary set of variables. In this study, the residualization approach was used to remove the effects of the percentages of agricultural and urban areas to allow examinations of the relations between the dependent variables TP, TN, SCHL, and SD and all of the nonanthropogenic environmental characteristics. Residualizations of TP, TN, and SCHL were done on logarithmically transformed data to account for the nonlinear relations between water-quality concentrations and the percentages of agricultural and urban land uses. Spearman correlations and forward-stepwise regressions were done with raw data and with residualized data to determine which environmental characteristics best described the distribution of each water-quality characteristic. The residualization approach was not used to examine relations between environmental characteristics and the biotic indices.

Regression-Tree Analysis to Define Thresholds or Breakpoints

One approach to defining nutrient criteria is to identify thresholds or breakpoints in the response between nutrient concentrations and biotic indices (U.S. Environmental Protection Agency, 2000a). Defining a specific threshold or breakpoint in a response curve of a specific biotic index is straightforward if the curve is well defined and has an abrupt breakpoint (fig. 3A); however, the response curves in many biotic indices are poorly defined and have broad breakpoints (fig. 3B). In the case of indices with broad thresholds, it is very difficult to define the concentration at which the index first begins to change. For the biotic indices, the thresholds or breakpoints were defined as the concentrations at which the rate of response is greatest and, therefore, represents a critical concentration with ecological significance. Regression-tree analyses (Breiman and others, 1984) were used to determine thresholds or breakpoints (most abrupt responses) in the relations between nutrient concentrations and a biotic index. Regression-tree analysis sequentially partitions the data for each independent variable into two groups and examines the differences in the mean values of the dependent variable on the basis of the least-square-error criterion. The least-square-error criterion allows identification of breakpoints that maximize intergroup means relative to the intragroup variance. Only one independent variable (for example, TP or TN) and one dependent variable (for example, IBI) were used at a time; thus, the regression-tree analysis was forced to divide the data for the dependent variable into two groups with highly contrasting means relative to intragroup variances. To determine whether the intergroup means identified by the breakpoints in TP and TN concentrations were statistically different, two-sample student t-tests were done on the basis of assumed equal and unequal variances.

Redundancy Analysis

Redundancy analysis (RDA) is a form of direct-gradient analysis that describes the variation between two multivariate data sets (for example, water-quality characteristics and biotic-assemblage data) as a function of multiple axes that are combinations of the explanatory characteristics (ter Braak and Prentice, 1988). The correlation of the explanatory characteristics with each axis indicates the strength of its relationship with the water-quality, fish, or macroinvertebrate characteristics. RDA is appropriate for data sets having short gradients and linear responses by the dependent variables. Detrended correspondence analysis demonstrated that gradient lengths were less than 2.4, which indicated that RDA was the appropriate form of analysis to be used. RDA was used to determine the relative importance of specific explanatory characteristics to specific nutrient, fish, or macroinvertebrate characteristics and the most important characteristics within a specific category of characteristics (such as watershed characteristics).

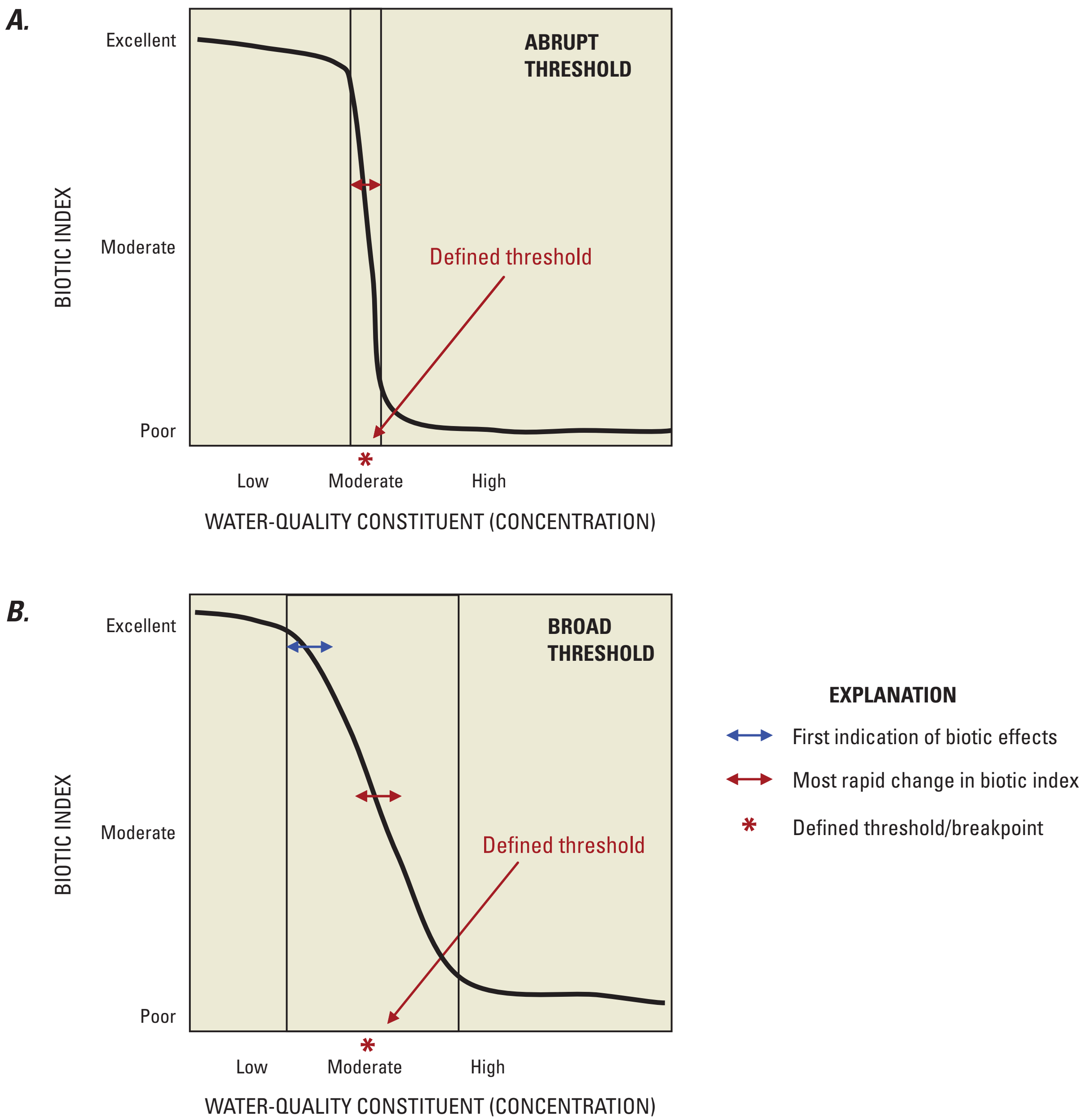

Figure 3. Definition of water-quality thresholds in responses of biotic indices to changes in water quality: ***A***, biotic indicator with an abrupt threshold and ***B***, biotic indicator with a broad threshold.

In addition, partial RDA (Richards and others, 1996) was used to determine the fraction of the variance in the water-quality characteristics explained by specific categories of environmental characteristics (such as land-use, basin, and soil/surficial-deposit characteristics) and to determine the fraction of the variance in the macroinvertebrate- and fish-assemblage data explained by specific categories of environmental characteristics (such as nutrient characteristics and environmental characteristics). Monte Carlo permutation tests with 100 iterations, the default number of iterations in CANOCO, were used to determine the validity of the total and partial RDA results. Monte Carlo tests were done by randomly permutating the assignment of the independent (environmental) data to the dependent (water-quality or biological) data and repeating the ordinations (Richards and others, 1996; Johnson and others, 1997).

Statistical Differences among Groups

To determine whether any apparent differences among groupings of data (such as sites with nutrient concentration near reference concentrations compared to sites with high nutrient concentrations) were statistically significant, a nonparametric Kruskal-Wallis rank-analysis-of-variance test was used (SAS Institute, Inc., 1989). The probability of all statistically significant differences occurring by chance was less than 5 percent ($p < 0.05$), unless otherwise specified.

Graphical Techniques

Bivariate scatterplots were used to demonstrate relations between specific variables graphically. On the bivariate scatterplots between nutrient concentrations and specific biotic indices, LOESS-smoothing lines with 95-percent confidence intervals (computed with SAS) were used to highlight trends. LOESS is a nonparametric method of estimating regression surfaces while making no assumptions about the relation (Cleveland and others, 1988). The default method within SAS was used to determine the value of the smoothing parameter in the LOESS fit, unless otherwise specified. The default method minimizes a bias-corrected Akaike's Information Criterion (AIC) (Hurvich and others, 1998), which balances the residual sum of squares against the smoothness of the fit. A smoothing parameter larger than the default value was used in some cases to smooth the relation between two variables further.

Water Quality and Its Relations with Environmental Characteristics in the Watershed

U.S. Geological Survey personnel processing water samples and collecting streamflow measurements.

Median TP concentrations at the 42 sites ranged from 0.023 to 0.497 mg/L (table 2, pages 13 and 14). The overall median and mean of the 42 individual medians were 0.109 and 0.132 mg/L, respectively. Highest concentrations were measured in the southern and western parts of the State, and lowest concentrations were measured in the most northern rivers (fig. 4). There was a distinct seasonality in TP concentrations, with highest concentrations measured in July and lowest concentrations measured in October (table 3).

Median DP concentrations ranged from 0.012 to 0.156 mg/L (table 2). The overall median and mean were 0.041 and 0.053 mg/L, respectively. Similar to TP, the highest concentrations were measured in the southern and western parts of the State, and lowest concentrations were measured in the most northern rivers (fig. 4). Median DP concentrations were strongly correlated to TP concentrations (r_s = 0.89; table 4). However, unlike TP, there was little seasonality in DP concentrations (table 3). DP represented about 23 to 36 percent of the TP.

Median TN concentrations ranged from 0.266 to 5.485 mg/L (table 2). The overall median and mean were 1.268 and 1.707 mg/L, respectively. Highest median TN concentrations were measured in the southern and western parts of the State and lowest concentrations were measured in the northern part of the State (fig. 5). Highest TN concentrations were measured in May and then concentrations slowly decreased as summer progressed (table 3).

Median NO_3-N concentrations ranged from 0.011 to 3.770 mg/L (table 2). The overall median and mean were 0.395 and 0.837 mg/L, respectively. Median NH_4-N concentrations ranged from 0.007 mg/L (less than the 0.013 mg/L detection limit) to 0.134 mg/L. The overall median and mean were 0.024 and 0.034 mg/L, respectively. Median TKN concentrations ranged from 0.255 to 2.850 mg/L. The overall median and mean were 0.620 and 0.848 mg/L, respectively. Nitrogen (N) was about equally divided between dissolved and particulate forms in all months. Highest NO_3-N, NH_4-N, and TKN concentrations were generally found in southern parts of the State, and lowest concentrations were found in the northern part of the State except for NH_4-N, for which lowest concentrations were found in the western and central parts of the State (fig. 5).

Median SCHL concentrations ranged from 1.74 to 130 µg/L (table 2). The overall median and mean were 7.31 and 18.47 µg/L, respectively. Highest SCHL concentrations were in the southern quarter of the State, and lowest concentrations were in northern part of the State (fig. 6). Highest SCHL concentrations were measured in August, and lowest concentrations were measured in May and October (table 3).

Median SDs ranged from 12 cm to greater than 120 cm (table 2). A few sites consistently had clarities greater than the length of the Secchi tube. The overall median and mean were 60.5 and 71.8 cm, respectively. Highest SDs (the best clarities) were in rivers in the northern quarter of the State, and lowest SDs (the worst clarities) were in rivers in the southern quarter of the State (fig. 6). Lowest SDs were measured during May through July, and the highest SDs were measured during September and October (table 3).

Relations between Water Quality and Environmental Characteristics in the Watershed

Correlations between Individual Characteristics

Spearman correlation coefficients (r_s values) between the water-quality characteristics are given in table 4. Concentrations of TP and DP were significantly correlated with TN, NO_3-N, and TKN (r_s values ranged from 0.51 to 0.89). Correlations between TP and the N species were slightly stronger than between DP and the N species. Concentrations of TN were strongly correlated to NO_3-N and TKN (r_s = 0.88 and 0.75, respectively) because each made up about 50 percent of the N. Concentrations of NH_4-N were more strongly correlated to TKN (r_s = 0.40) than they were to TN and NO_3-N (r_s = 0.30 and 0.22, respectively). NH_4-N is a larger part of TKN than of TN; therefore, a stronger correlation between NH_4-N and TKN was expected.

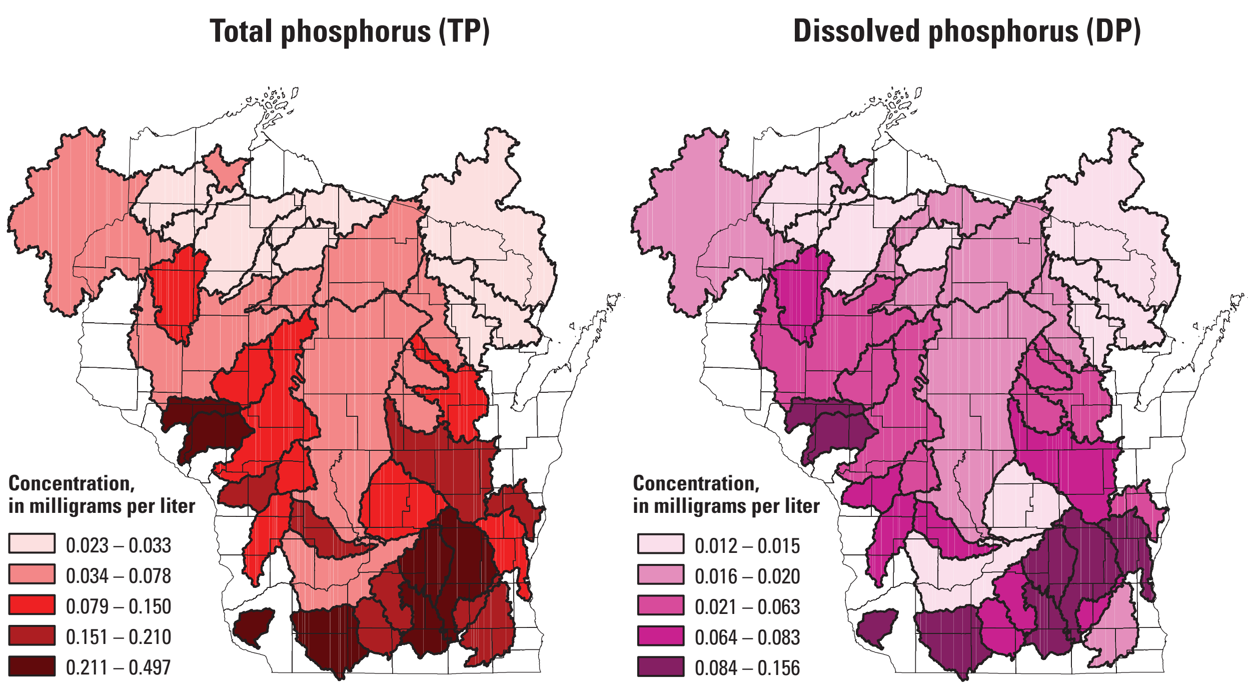

Figure 4. Distributions (quintiles) for median monthly total phosphorus (TP) and dissolved phosphorus (DP) concentrations for the studied nonwadeable rivers in Wisconsin, 2003.

Table 3. Median and average monthly concentrations for total, dissolved, and particulate phosphorus, suspended chlorophyll *a*, total nitrogen, nitrite plus nitrate, ammonia, Kjeldahl nitrogen, and Secchi-tube depth in the studied nonwadeable rivers in Wisconsin, 2003.

[All concentrations are in milligrams per liter, except chlorophyll *a*, which is in micrograms per liter, and Secchi-tube depth, which is in centimeters]

Month	Total phosphorus		Dissolved phosphorus		Particulate phosphorus	
	Median	Average	Median	Average	Median	Average
May	0.094	0.122	0.034	0.044	0.054	0.078
June	.118	.142	.031	.052	.066	.090
July	.136	.164	.045	.062	.064	.102
August	.105	.145	.024	.060	.059	.086
September	.110	.133	.039	.064	.048	.069
October	.070	.099	.023	.043	.036	.057
May–October	**.109**	**.132**	**.041**	**.053**	**.053**	**.079**

Month	Suspended chlorophyll *a*		Secchi-tube depth		Total nitrogen	
	Median	Average	Median	Average	Median	Average
May	6.95	17.82	57.0	67.6	1.443	1.956
June	8.15	24.97	56.0	64.5	1.369	1.653
July	7.71	27.83	55.5	66.1	1.376	1.983
August	11.3	28.71	59.0	73.1	1.133	1.538
September	7.71	14.53	76.0	74.8	1.260	1.790
October	5.74	16.10	94.5	85.2	1.193	1.525
May–October	**7.31**	**18.47**	**60.5**	**71.8**	**1.268**	**1.707**

Month	Dissolved nitrite plus nitrate		Dissolved ammonia		Total Kjeldahl nitrogen	
	Median	Average	Median	Average	Median	Average
May	0.435	0.997	0.036	0.054	0.840	0.959
June	.337	.786	.043	.053	.710	.867
July	.358	.984	.026	.052	.710	.999
August	.199	.744	.019	.023	.520	.794
September	.545	.975	.022	.057	.680	.814
October	.358	.800	.007	.017	.555	.725
May–October	**.395**	**.837**	**.024**	**.034**	**.620**	**.848**

Table 4. Spearman correlation coefficients (r_s) between median total, dissolved, and particulate phosphorus, total nitrogen, nitrite plus nitrate, ammonia, and total Kjeldahl nitrogen concentrations, median Secchi-tube depths, chlorophyll *a* concentrations, percentages of urban and agricultural areas, and specific environmental (land-use, basin, soil, and surficial-deposit) characteristics for the studied nonwadeable rivers in Wisconsin.

[All r_s values with an absolute value greater than 0.41 are statistically significant ($p < 0.05$) with Bonferroni adjustment and r_s values with an absolute value greater than 0.30 are statistically significant with no Bonferroni adjustment; all r_s values with an absolute value greater than 0.6 are in **bold**]

Characteristic	Total phosphorus	Dissolved phosphorus	Particulate phosphorus	Total nitrogen	Dissolved nitrite plus nitrate	Dissolved ammonia	Total Kjeldahl nitrogen	Secchi-tube depth	Suspended chlorophyll *a* concentration	Percent urban	Percent agriculture
Water-quality characteristics											
Total phosphorus	**1.00**	**0.89**	**0.95**	**0.89**	**0.74**	0.20	**0.69**	**-0.85**	**0.66**	**0.69**	**0.87**
Dissolved phosphorus	**.89**	**1.00**	**.73**	**.79**	**.72**	.28	.51	**-.64**	.42	.53	**.77**
Particulate phosphorus	**.95**	**.73**	**1.00**	**.84**	**.68**	.12	**.71**	**-.91**	**.75**	**.72**	**.83**
Total nitrogen	**.89**	**.79**	**.84**	**1.00**	**.88**	.30	**.75**	**-.73**	.59	**.70**	**.92**
Dissolved nitrite plus nitrate	**.74**	**.72**	**.68**	**.88**	**1.00**	.22	.50	-.52	.37	.51	**.79**
Dissolved ammonia	.20	.28	.12	.30	.22	**1.00**	.40	-.20	.16	.34	.27
Total Kjeldahl nitrogen	**.69**	.51	**.71**	**.75**	.50	.40	**1.00**	**-.75**	**.86**	**.87**	**.75**
Suspended chlorophyll *a*	**.66**	.42	**.75**	.59	.37	.16	**.86**	**-.76**	**1.00**	**.83**	**.64**
Secchi-tube depth	**-.85**	**-.64**	**-.91**	**-.73**	-.52	-.20	**-.75**	**1.00**	**-.76**	**-.75**	**-.75**
Suspended sediment	**.90**	**.70**	**.94**	**.80**	**.65**	.08	**.63**	**-.89**	**.66**	**.62**	**.81**
Water temperature	.51	.34	.53	.43	.20	.09	**.68**	-.54	**.70**	**.67**	.56
Specific conductance	**.70**	**.60**	**.69**	**.81**	**.68**	.40	**.68**	**-.67**	.51	**.69**	**.85**
pH	.50	.32	.52	.46	.31	.03	.58	-.49	**.63**	.52	.58
Color	**-.61**	-.56	-.57	-.52	**-.61**	.13	-.14	.48	-.23	-.28	-.54
Land-use characteristics											
Urban	**.69**	.53	**.72**	**.70**	.51	.34	**.87**	**-.75**	**.83**	**1.00**	**.73**
Agriculture (row crops)	**.85**	**.71**	**.82**	**.91**	**.74**	.27	**.80**	**-.76**	**.70**	**.77**	**.94**
Agriculture (other)	**.85**	**.81**	**.77**	**.86**	**.81**	.22	**.62**	**-.66**	.55	.59	**.94**
Total agriculture	**.87**	**.77**	**.83**	**.92**	**.79**	.27	**.75**	**-.75**	**.64**	**.73**	**1.00**
Grassland	.56	.44	.58	.54	.57	.05	.45	-.53	.45	.41	.50
Wetland (open)	-.21	-.20	-.20	-.23	-.43	.11	.20	.07	.16	.05	-.27
Wetland (forested)	**-.82**	**-.68**	**-.82**	**-.68**	**-.62**	.08	-.41	**.76**	-.47	-.48	**-.73**
Barren	.31	.27	.27	.24	.13	.12	.47	-.29	.49	.47	.24
Forest (all)	**-.88**	**-.78**	**-.83**	**-.92**	**-.74**	-.32	**-.80**	**.78**	**-.68**	**-.76**	**-.95**

Table 4. Spearman correlation coefficients (r_s) between median total, dissolved, and particulate phosphorus, total nitrogen, nitrite plus nitrate, ammonia, and total Kjeldahl nitrogen concentrations, median Secchi-tube depths, chlorophyll *a* concentrations, percentages of urban and agricultural areas, and specific environmental (land-use, basin, soil, and surficial-deposit) characteristics for the studied nonwadeable rivers in Wisconsin—Continued.

[All r_s values with an absolute value greater than 0.41 are statistically significant ($p < 0.05$) with Bonferroni adjustment and r_s values with an absolute value greater than 0.30 are statistically significant with no Bonferroni adjustment; all r_s values with an absolute value greater than 0.6 are in **bold**]

Characteristic	Total phosphorus	Dissolved phosphorus	Particulate phosphorus	Total nitrogen	Dissolved nitrite plus nitrate	Dissolved ammonia	Total Kjeldahl nitrogen	Secchi-tube depth	Suspended chlorophyll *a* concentration	Percent urban	Percent agriculture
Basin characteristics											
Watershed area	-0.22	-0.26	-0.14	-0.23	-0.27	-0.23	0.06	0.11	0.20	0.04	-0.21
Air temperature	**.86**	**.71**	**.85**	**.87**	**.72**	.24	**.76**	**-.79**	**.67**	**.77**	**.93**
Precipitation	**.76**	**.65**	**.76**	**.75**	**.69**	.04	.47	**-.66**	.45	.51	**.73**
Evaporation	**.87**	**.76**	**.83**	**.81**	**.75**	.08	**.60**	**-.73**	.57	**.62**	**.85**
Runoff	**-.90**	**-.80**	**-.87**	**-.87**	**-.77**	-.20	**-.70**	**.74**	**-.65**	**-.69**	**-.87**
River length	-.31	-.34	-.23	-.33	-.31	-.22	-.04	.23	.11	-.03	-.28
Basin slope	-.28	-.18	-.32	-.23	-.03	-.01	-.41	.36	-.47	-.45	-.19
Flow per unit area	-.49	-.44	-.46	-.44	-.33	-.10	-.34	.43	-.25	-.30	-.56
Soil characteristics											
Clay content	**.85**	**.73**	**.82**	**.80**	**.69**	.16	**.61**	**-.81**	.56	**.64**	**.92**
Erodibility	**.73**	**.66**	**.68**	**.69**	.59	.06	.50	**-.65**	.50	.51	**.81**
Organic-matter content	**-.68**	-.59	**-.66**	**-.60**	**-.67**	.18	-.31	.48	-.42	-.34	**-.64**
Permeability	**-.64**	**-.63**	-.56	-.57	-.52	-.01	-.29	.50	-.32	-.31	**-.69**
Soil slope	-.08	-.09	-.05	-.18	.01	-.41	-.53	.13	-.37	-.33	-.23
Surficial-deposit characteristics											
Nonglacial deposits	.58	.47	.59	.41	.45	-.34	.23	-.46	.34	.35	.45
Clay	.09	-.04	.15	.27	.26	.48	.49	-.15	.24	.29	.24
Loam	-.23	-.22	-.18	-.16	.00	-.07	-.13	.17	-.10	-.25	-.15
Peat	-.41	-.27	-.44	-.51	-.46	-.15	-.27	.36	-.17	-.27	-.54
Sand	-.07	-.03	-.08	-.15	-.30	-.01	.06	-.05	.17	.09	-.15
Sand and gravel	-.44	-.45	-.39	-.17	-.18	.27	.04	.33	-.10	-.06	-.24

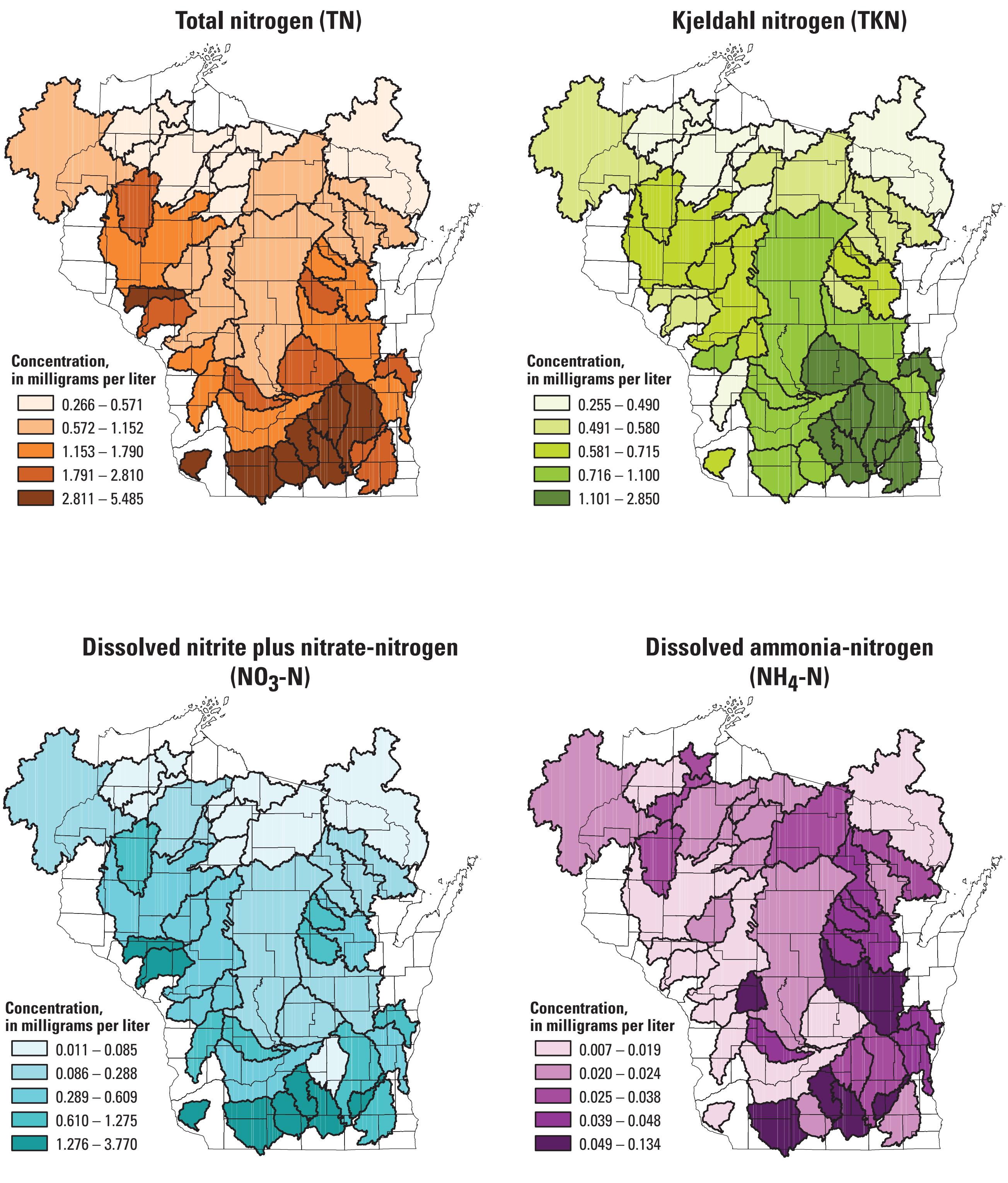

Figure 5. Distributions (quintiles) for median monthly total nitrogen (TN), total Keldahl nitrogen (TKN), dissolved nitrite plus nitrate nitrogen (NO_3-N), and dissolved ammonia nitrogen (NH_4-N) for the studied nonwadeable rivers in Wisconsin, 2003.

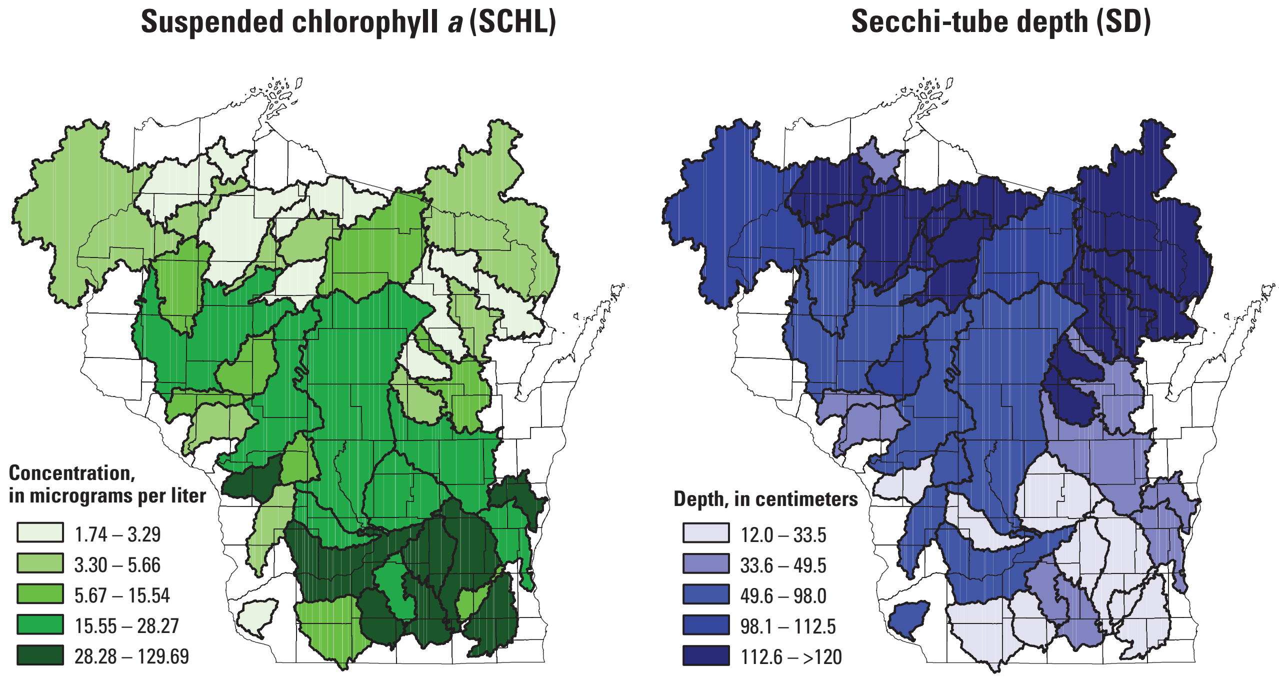

Figure 6. Distributions (quintiles) for median monthly suspended chlorophyll *a* (SCHL) and Secchi-tube depth (SD) for the studied nonwadeable rivers in Wisconsin, 2003.

All water-quality characteristics were significantly correlated with some environmental characteristics (land-use, basin, soil, and surficial-deposit characteristics; table 4); however, they were most strongly correlated with characteristics describing the land use (presence of agriculture or absence of forest), the basin (air temperature, precipitation, evaporation, and runoff), and the soil/surficial deposits (clay content, erodibility, organic-matter content, and permeability). In general, TN, NO_3-N, TKN, TP, and DP were correlated with the same environmental characteristics. Concentrations of NH_4-N were less strongly correlated with the nonanthropogenic or natural environmental characteristics (basin and soil/surficial-deposit characteristics) than the other water-quality characteristics. Concentrations of NH_4-N were most strongly correlated with soil slope and content of clay in the surficial deposits (r_s = -0.41 and 0.48, respectively).

Concentrations of SCHL were significantly correlated with most nutrient constituents: most strongly correlated with TKN and TP (r_s = 0.86 and 0.66, respectively), less strongly correlated with TN, DP, and NO_3-N (r_s = 0.59 to r_s = 0.37), and insignificantly correlated with NH_4-N (r_s = 0.16). Concentrations of SCHL were significantly correlated with SDs (r_s = -0.76), SSC concentrations (r_s = 0.66), and pH (r_s = 0.63). Concentrations of SCHL were significantly correlated with most environmental characteristics; however, they were most strongly correlated with land-use characteristics [positively correlated with the percentages of urban area (Urb %) and total agricultural area (Ag %), and negatively correlated with the percentage of forest (For %)], basin characteristics describing the air temperature, evaporation, and runoff from the watershed, and soil properties in the watershed (clay content and erodibility).

SDs were most strongly correlated with many of the same characteristics as SCHL; however, SDs were more strongly correlated with most characteristics, and were especially more correlated with TN, DP, NO_3-N, SSC, and clay content. Similar results were obtained if only sites with SDs less than 120 cm were included in the analysis; however, the correlation coefficients were slightly smaller.

Many of the natural characteristics were strongly correlated with the land-use characteristics, primarily Ag %, and less strongly with Urb % (table 4). For example, air temperature, precipitation, evaporation, and runoff were strongly correlated with Ag %. Therefore, even if the land-use characteristics were not included in further statistical analyses, their effects could be incorporated into the final results by use of natural characteristics such as air temperature. To examine the relations between the natural characteristics and the water-quality characteristics further, the relations between the land-use characteristics (Ag % and Urb %) and TP, TN, SCHL, and SD were removed by use of simultaneous partial-residualization. Residualized TP, TN, and SCHL concentrations and SDs were computed with equations 1–8 (the coefficient of determination (R^2) is given for each characteristic):

$$\textbf{Log TP}_{\textbf{Res}} = \textbf{Log TP}_{\textbf{Measured}} - \textbf{Log TP}_{\textbf{Predicted}}, \quad (1)$$

where

$$\text{Log TP}_{\text{Predicted}} = -1.455 + 1.384 \text{ Ag \%} + 0.659 \text{ Urb \%, and} \quad (2)$$
$$R^2 = 0.72;$$

$$\textbf{Log TN}_{\textbf{Res}} = \textbf{Log TN}_{\textbf{Measured}} - \textbf{Log TN}_{\textbf{Predicted}}, \quad (3)$$

where

$$\text{Log TN}_{\text{Predicted}} = -0.289 + 1.252 \text{ Ag \%} + 2.044 \text{ Urb \%, and} \quad (4)$$
$$R^2 = 0.84;$$

$$\textbf{Log SCHL}_{\textbf{Res}} = \textbf{Log SCHL}_{\textbf{Measured}} - \textbf{Log SCHL}_{\textbf{Predicted}}, \quad (5)$$

where

$$\text{Log SCHL}_{\text{Predicted}} = 0.596 + 1.052 \text{ Ag \%} - 5.608 \text{ Urb \%, and} \quad (6)$$
$$R^2 = 0.41;$$

$$\textbf{SD}_{\textbf{Res}} = \textbf{SD}_{\textbf{Measured}} - \textbf{SD}_{\textbf{Predicted}}, \quad (7)$$

where

$$\text{SD}_{\text{Predicted}} = 110.4 - 115.9 \text{ Ag \%} - 307.9 \text{ Urb \%, and} \quad (8)$$
$$R^2 = 0.54.$$

Residual transformations were also applied to all of the other water-quality and environmental characteristics.

Residualized concentrations and SDs were still significantly correlated with many residualized environmental characteristics; however, they were not as strongly correlated (table 5). Residualized nutrient concentrations were most strongly correlated with residualized runoff and evaporation. In addition to these characteristics, residualized TN was significantly correlated with residualized clay content.

The correlation coefficients between the residualized variables revealed that highest TP and DP concentrations were measured in areas with low runoff and high evaporation. Highest TN, NO_3-N, and TKN concentrations were measured in areas with low runoff, and soils or surficial deposits with low clay content, low erodibility, high permeability, and high amounts of sand and gravel. Highest NH_4-N concentrations were measured in areas with low evaporation and high organic-matter content. Highest SCHL concentrations were measured in long rivers in large watersheds with low basin slopes. Highest SDs were measured in areas with low evaporation and cool air temperatures.

Many of the natural characteristics, such as clay content, erodibility, and permeability of the soil were so strongly correlated with Ag % that their relations to water quality may have been removed by the residualization approach. Therefore, the use of the residualization approach may not provide a complete description of the characteristics affecting water quality.

Effects of Multiple Environmental Characteristics on Water Quality

Stepwise Regression

Forward stepwise regressions were done with all of the environmental characteristics to determine which three environmental characteristics best described the variance in TP, TN, and SCHL concentrations and SDs, then with only the natural (non-land-use) characteristics, and then with residualized characteristics (whose correlations with land-use characteristics had been removed). Models with more than three variables did not significantly increase the amount of variance explained (accumulative R^2 values).

Runoff was the first variable incorporated into the TP model; the second and third variables were the percentages of forested wetland (WetF %) and For %, respectively (table 6). Collectively, this model explained 89 percent of the variance in TP concentrations. If the land-use characteristics were omitted from the analysis, runoff remained the first variable incorporated into the model, evaporation was second, and the percentage of clay deposits was third; these three variables explained 86 percent of the variance. After the characteristics were adjusted to remove the land-use effects, residualized runoff was the first variable incorporated into the model, residualized clay deposits was second, and residualized air temperature was third; these three variables explained 56 percent of the variance in residualized total phosphorus concentrations (TP_{Res}).

Table 5. Spearman correlation coefficients (r_s) between residualized logarithmically transformed median total, dissolved, and particulate phosphorus, nitrogen, nitrite plus nitrate, total Kjeldahl nitrogen, ammonia, and chlorophyll *a* concentrations, median Secchi-tube depths, and specific residualized environmental (basin, soil, and surficial-deposit) characteristics for the studied nonwadeable rivers in Wisconsin.

[All r_s values with an absolute value greater than 0.41 are statistically significant ($p < 0.05$) with Bonferroni adjustment and r_s values with an absolute value greater than 0.29 are statistically significant with no Bonferroni adjustment; all r_s values with an absolute value greater than 0.41 are in **bold**]

Characteristic	Total phosphorus	Dissolved phosphorus	Particulate phosphorus	Total nitrogen	Dissolved nitrite plus nitrate	Dissolved ammonia	Total Kjeldahl nitrogen	Secchi-tube depth	Suspended chlorophyll *a*
Water-quality characteristics									
Total phosphorus	**1.00**	**0.67**	**0.91**	0.34	0.17	-0.10	0.34	**-0.78**	**0.47**
Dissolved phosphorus	**.67**	**1.00**	.35	.27	.33	.11	-.06	-.34	-.03
Particulate phosphorus	**.67**	.35	**1.00**	.27	.16	-.23	**.41**	**-.80**	**.58**
Total nitrogen	.34	.27	.35	**1.00**	**.77**	.05	.29	-.17	.07
Dissolved nitrite plus nitrate	.17	.33	.16	**.77**	**1.00**	.06	-.09	.03	-.17
Dissolved ammonia	-.10	.11	-.23	.05	.06	**1.00**	.22	.12	-.11
Total Kjeldahl nitrogen	.34	-.06	**.41**	.29	-.09	.22	1.00	-.37	**.76**
Suspended chlorophyll *a*	**.47**	-.03	**.58**	.07	-.17	-.11	**.76**	**-.48**	**1.00**
Secchi-tube depth	**-.78**	-.34	**-.80**	-.17	.03	.12	-.37	**1.00**	**-.48**
Suspended sediment	**.70**	.26	**.81**	.28	.22	-.22	.21	**-.75**	.34
Water temperature	.18	-.19	.32	-.11	-.28	-.16	**.56**	-.19	**.63**
Specific conductance	-.22	-.13	-.24	-.08	-.06	.27	.07	.06	-.20
pH	.07	-.11	.14	-.16	-.13	-.19	.25	-.14	.32
Color	-.28	-.30	-.25	-.03	-.22	.33	**.44**	.24	.15
Basin characteristics									
Watershed area	.06	-.17	.09	-.04	-.14	-.15	.33	-.04	.40
Air temperature	**.43**	.14	**.49**	.03	.03	-.23	.14	**-.58**	.20
Precipitation	.22	.08	.32	.23	.27	-.30	-.27	-.27	-.08
Evaporation	**.46**	.31	**.51**	.25	.25	**-.47**	-.08	**-.41**	.13
Runoff	**-.47**	-.34	**-.48**	**-.41**	**-.47**	.16	-.33	.29	-.30
River length	.03	-.17	.07	-.10	-.09	-.10	.31	-.10	**.41**
Basin slope	-.36	-.05	-.38	-.14	.18	.11	**-.43**	.29	**-.45**
Flow per unit area	-.02	-.03	.01	.27	.27	.06	.02	.01	.03

Table 5. Spearman correlation coefficients (r_s) between residualized logarithmically transformed median total, dissolved, and particulate phosphorus, nitrogen, nitrite plus nitrate, total Kjeldahl nitrogen, ammonia, and chlorophyll *a* concentrations, median Secchi-tube depths, and specific residualized environmental (basin, soil, and surficial-deposit) characteristics for the studied nonwadeable rivers in Wisconsin—Continued.

[All r_s values with an absolute value greater than 0.41 are statistically significant ($p < 0.05$) with Bonferroni adjustment and r_s values with an absolute value greater than 0.29 are statistically significant with no Bonferroni adjustment; all r_s values with an absolute value greater than 0.41 are in **bold**]

Characteristic	Total phosphorus	Dissolved phosphorus	Particulate phosphorus	Total nitrogen	Dissolved nitrite plus nitrate	Dissolved ammonia	Total Kjeldahl nitrogen	Secchi-tube depth	Suspended chlorophyll *a*
Soil characteristics									
Clay content	-0.12	-0.14	-0.07	**-0.49**	-0.31	-0.20	-0.37	-0.14	-0.18
Erodibility	-.03	.01	-.05	-.40	-.21	-.37	-.35	-.02	-.06
Organic-matter content	-.24	-.27	-.25	-.14	-.40	**.51**	**.43**	.09	.08
Permeability	.01	-.08	.05	.39	.16	.26	**.41**	-.04	.16
Soil slope	.06	.09	.10	-.09	.10	-.32	**-.58**	-.09	-.36
Surficial-deposit characteristics									
Nonglacial deposits	.22	.21	.23	-.07	.07	**-.42**	**-.42**	-.21	-.23
Clay	-.07	-.16	-.03	.21	.30	.15	.25	-.05	.06
Loam	-.02	-.12	.03	-.02	.14	-.10	-.08	.07	.13
Peat	.03	.12	-.03	-.09	-.17	-.05	.20	-.01	.24
Sand	.10	.06	.05	-.24	-.37	-.09	.09	-.03	.25
Sand and gravel	-.28	-.30	-.24	.21	.10	.38	.29	.17	.07

Table 6. Results from forward stepwise-regression analyses to explain variability in raw and residualized water-quality concentrations in the studied nonwadeable rivers in Wisconsin.

[All regressions were on log-transformed concentrations; *r*, Pearson correlation coefficient; R^2, coefficient of determination for the one-, two-, and three-variable models; $_{Res}$, residualized characteristics]

Dependent variable	First variable	Second variable	Third variable
	Total phosphorus (TP)		
All environmental characteristics			
TP	Runoff	Wetland (forested)	Forest (all)
r	-0.90	-0.81	-0.87
Accumulative R^2	.81	.87	.89
No land-use characteristics			
TP	Runoff	Evaporation	Clay deposits
r	-.90	.88	-.08
Accumulative R^2	.81	.85	.86
Residualized characteristics			
TP_{Res}	$Runoff_{Res}$	Clay $deposits_{Res}$	Air $temperature_{Res}$
r	-.67	-.15	.53
Accumulative R^2	.45	.53	.56
	Total nitrogen (TN)		
All environmental characteristics			
TN	Forest (all)	Runoff	Wetland (open)
r	-.92	-.89	-.27
Accumulative R^2	.86	.89	.90
No land-use characteristics			
TN	Runoff	Precipitation	Sand-and-gravel deposits
r	-.89	.76	-.22
Accumulative R^2	.80	.83	.87
Residualized characteristics			
TN_{Res}	$Runoff_{Res}$	Clay $content_{Res}$	$Precipitation_{Res}$
r	-.57	-.53	.25
Accumulative R^2	.32	.55	.65
	Suspended chlorophyll *a* (SCHL)		
All environmental characteristics			
SCHL	Total Kjeldahl nitrogen	Dissolved ammonia	River length
r	.89	.21	.18
Accumulative R^2	.79	.83	.84
No land-use characteristics			
SCHL	Total Kjeldahl nitrogen	Dissolved ammonia	River length
r	.89	.21	.18
Accumulative R^2	.79	.83	.84
Residualized characteristics			
$SCHL_{Res}$	Total Kjeldahl nitrogen	Total nitrogen	Organic-matter $content_{Res}$
r	.53	.03	.08
Accumulative R^2	.28	.56	.71

Table 6. Results from forward stepwise-regression analyses to explain variability in raw and residualized water-quality concentrations in the studied nonwadeable rivers in Wisconsin—Continued.

[All regressions were on log-transformed concentrations; *r*, Pearson correlation coefficient; R^2, coefficient of determination for the one-, two-, and three-variable models; $_{Res}$, residualized characteristics]

Dependent variable	First variable	Second variable	Third variable
	Secchi-tube depth (SD)		
All environmental characteristics			
SD	Particulate phosphorus	Suspended sediment	Organic-matter content
r	-0.92	-0.90	0.48
Accumulative R^2	.84	.86	.88
No land-use characteristics			
SD	Particulate phosphorus	Suspended sediment	Organic-matter content
r	-.92	-.90	.48
Accumulative R^2	.84	.86	.88
Residualized characteristics			
SD_{Res}	Air Temperature$_{Res}$	Suspended sediment	Total nitrogen
r	-.52	-.49	-.03
Accumulative R^2	.27	.41	.65

The difference in the amount of variance explained by the first two regression models was caused by the removal of the effects of the land-use characteristics that were not correlated with other environmental characteristics in the second model. The large difference between the amounts of variance explained by the last two regressions was caused by the removal of all of the effects of the land-use characteristics, including the independent (direct) effects and correlated (indirect) effects on the other variables.

For % was the first variable incorporated into the TN model, runoff was the second, and WetO % was the third; these three variables explained 90 percent of the variance in TN concentrations. If the land-use characteristics were omitted from the analysis, runoff was the first variable, precipitation was the second, and the percentage of sand-and-gravel deposits was the third. After the characteristics were adjusted to remove the land-use effects, residualized runoff was the first variable included in the model, residualized clay content was the second, and residualized precipitation was the third. This model collectively explained 65 percent of the variance in residualized total nitrogen concentrations (TN_{Res}).

For both of these constituents, removing the effects of land use moderately reduced the amount of variability explained by the models. When the direct and indirect effects of the land-use characteristics were included in the models, 89 to 90 percent of the variance could be explained with three variables; however, when all of the land-use effects were removed, the models explained about 55 to 65 percent of the variance.

To develop regression models to predict SCHL and SD, all of the water-quality characteristics and environmental characteristics were included. The TKN concentration was the first variable incorporated into the SCHL model, NH_4-N was the second, and stream length was the third; these three variables explained 84 percent of the variance in SCHL concentrations. If the land-use characteristics were omitted from the analysis, similar results were obtained. After the environmental characteristics were adjusted to remove the land-use effects, TKN remained the first variable incorporated in the model; however, TN was the second variable, and residualized organic-matter content was the third; these three variables explained 71 percent of the total variance.

Particulate phosphorus (PP) was the first variable incorporated into the SD model, SSC was the second, and organic-matter content was the third; these three variables explained 88 percent of the variance in SDs. If the land-use characteristics were omitted from the analysis, similar results were obtained. After the environmental characteristics were adjusted to remove the land-use effects, residualized air temperature was the first variable included in the model, SSC was the second, and TN was the third; these three variables explained 65 percent of the total variance.

Removing the land-use effects from the environmental characteristics included in the analysis had little effect on the amount of variability explained by the SCHL and SD models (second model). The land-use characteristics were strongly correlated with nutrient concentrations, and therefore, a similar amount of variability was explained whether or not the land-use characteristics or the nutrient concentrations were included. The models used to predict residualized SCHL and SD provided less predictability because much of the variability in SCHL and SD was removed in the residualization process.

Redundancy Analysis

Each of the four primary water-quality characteristics (TP, TN, SCHL, and SD) has been shown to be related to the land-use characteristics and other characteristics of the watershed upstream from the assessment site. Partial RDA was used to determine the relative importance of each of the general categories of environmental characteristics to the distribution of overall water quality, as defined by these four water-quality characteristics.

The three main categories of environmental characteristics—land-use characteristics, basin characteristics, and soil/surficial-deposit characteristics (table 2)—were used in the partial RDA. A two-step process was used to select three characteristics to describe each category. The characteristics that were most significantly correlated with the individual water-quality characteristics in each category were initially chosen. The final characteristics in each category were then chosen to have minimal correlations among themselves. For example, Ag % and For % were both strongly correlated with water quality; however, both were not chosen for the land-use category because they were strongly correlated to one another. The land-use category was described by Ag %, Urb %, and WetO %. The basin characteristics were described by watershed area (logarithmically transformed), runoff, and basin slope. Soils/surficial deposits were described by the clay content of the soils, organic-matter content of the soils, and soil slope. The land-use category reflects the extent of human intervention—characteristics that may be altered. The basin and soil/surficial-deposit categories reflect the topographical and geological effects—characteristics that cannot be altered.

The total variance in the four water-quality characteristics was separated into five categories: (1) variance explained by the land-use characteristics alone, (2) variance explained by soil/surficial-deposit characteristics alone, (3) variance explained by the basin characteristics alone, (4) variance explained by the interactions of land-use, soil/surficial-deposit, and basin characteristics (variance that could not be assigned to a single category), and (5) variance not explained by these characteristics. Results from the partial RDA indicated that the nine characteristics collectively explained 74 percent of the variance ($p < 0.01$) in water quality (TP, TN, SCHL, and SD). Independently, the land-use characteristics explained 9 percent of the total variance (12 percent of the explained variance; $p < 0.05$; fig. 7), the soil/surficial-deposit characteristics explained 12 percent of the total variance (16 percent of the explained variance; $p < 0.01$), and the basin characteristics explained 10 percent of the total variance (14 percent of the explained variance; $p < 0.01$). The shared contribution or interactions of all three general categories of environmental characteristics explained 43 of the total variance (58 percent of the explained variance). Therefore, much of the variance in water quality could not be explained by a single category of environmental characteristics.

RDA was also used to determine which of the environmental characteristics explained the most variance in overall water quality (TP, TN, SCHL, and SD). In RDA, as in principal-component analysis, the explained variance is separated into a series of ordination (canonical) axes. Almost all of the variance in this analysis was explained on the first canonical axis. The scores on the first axis (table 7) indicate the water-quality characteristics with the most explained variance and the importance of the individual environmental characteristics in explaining this variance. SD had the highest scores (absolute values) on the first canonical axis; these scores indicate that more of its variance was explained by the environmental characteristics than were the variances in TP, TN, and SCHL. The most important characteristics explaining the variance in these four water-quality characteristics in descending order of axis score were Ag %, runoff, clay content, organic-matter content, and Urb %. The relations between the environmental characteristics and water-quality characteristics can be determined by comparing their respective axis scores. Areas with low runoff, high Ag %, high Urb %, and soils with high clay content and low organic-matter content had the highest nutrient and SCHL concentrations and the worst water clarity. These results agree with the findings of the correlation and regression analyses.

Table 7. Results from redundancy analysis between water-quality and environmental (land-use, basin, and soil/surficial deposit) characteristics for the studied nonwadeable rivers in Wisconsin.

[log, logarithm to base 10 transformation]

	First canonical axis score
Water-quality constituents	
Total phosphorus (log)	-0.79
Total nitrogen (log)	-.79
Suspended chlorophyll *a* (log)	-.67
Secchi-tube depth	.86
Land-use characteristics	
Total agriculture	-.85
Urban	-.52
Wetland (open)	.17
Basin characteristics	
Watershed area (log)	.11
Runoff	.85
Basin slope	.27
Soil/surficial-deposit characteristics	
Clay content	-.84
Organic-matter content	.56
Soil slope	-.09

A multiple-regression approach (similar to partial RDA) was done to determine how the total variances in SDs and SCHL, independently, could be separated into four categories: (1) variance explained by nutrients alone (TP, DP, NO_3-N, NH_4-N, and TKN; PP and TN were not included because they were computed from the other constituents), (2) variance explained by environmental characteristics alone (the same nine environmental characteristics that were used in the partial RDA for water quality), (3) variance explained by the interactions between nutrients and environmental characteristics, and (4) variance not explained by these characteristics. In this approach, three regressions were done for SD and for SCHL: multiple regressions with all 15 variables, with only the 6 nutrient constituents, and with only the 9 environmental characteristics. The first regression with all 15 variables was used to determine the total variance explained by all of the variables. The other two regressions were used to partition the variance among the three categories. The amount of variance explained by the interaction of the two categories was determined by equation 9:

$$EV_{Interactions} = EV_{Nutrients} + EV_{Environmental} - EV_{All\ Variables}, \quad (9)$$

where EV is the variance explained by the specified group of variables. The variance explained by each subset of variables alone was then determined by subtracting the variance explained by the interactions between the variables from the total variance explained by a subset of variables.

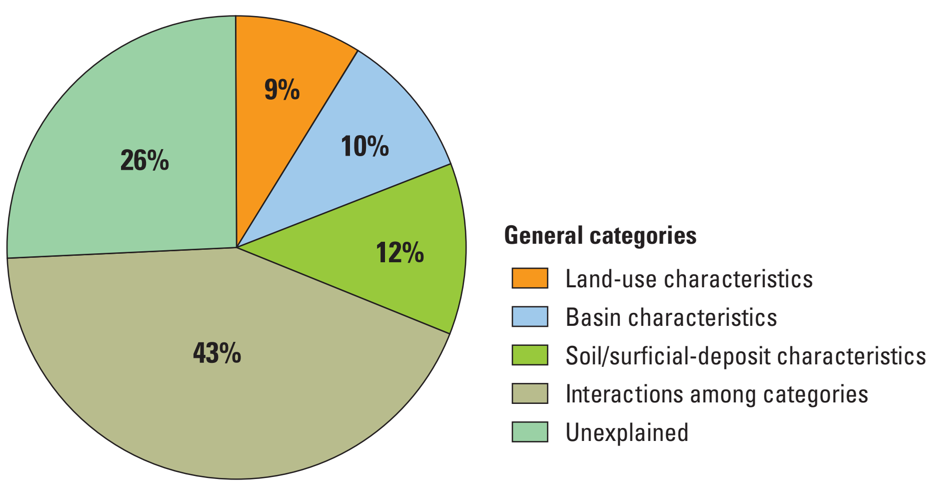

Figure 7. Percentages of variance in water quality [total phosphorus (TP), total nitrogen (TN), and suspended chlorophyll *a* concentrations (SCHL) and Secchi-tube depth (SD)] described by land-use, basin, soil/surficial-deposit characteristics, interactions among categories (variance that cannot be explained by a single category), and unexplained variance for the studied nonwadeable rivers in Wisconsin. [%, percentage of total variance]

For example, the variance explained by the nutrients alone is equal to the explained variance from the nutrient regression minus the variance explained by the interactions:

$$EV_{\text{Nutrients Alone}} = EV_{\text{Nutrients}} - EV_{\text{Interaction}}. \tag{10}$$

Results from this analysis indicated that these 15 characteristics collectively explained 90 and 94 percent of the variance in SCHL and SDs, respectively (fig. 8). Nutrients alone explained 22 and 19 percent of the total variance in SCHL and SDs, respectively. The environmental characteristics alone explained 6 to 7 percent of the variance in SDs and SCHL concentrations, and the interactions between the nutrients and environmental characteristics explained 62 to 68 percent of the total variance in both characteristics. Again, most of the total variability in these two parameters could not be explained by the nutrients alone.

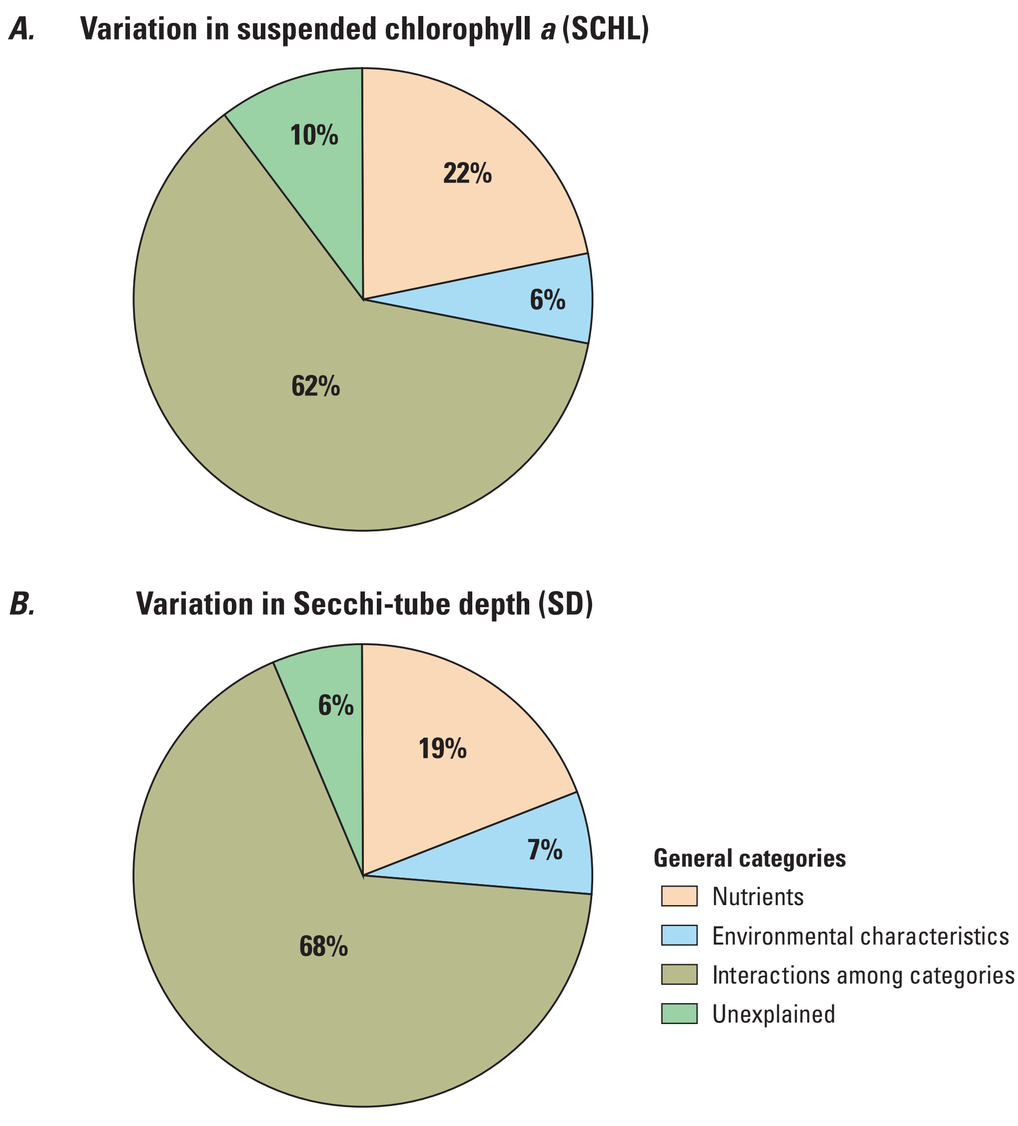

Figure 8. Percentages of variance in ***A***, suspended chlorophyll *a* concentrations (SCHL) and ***B***, Secchi-tube depths (SD) described by nutrients, environmental characteristics (land-use, basin, and soil/surficial-deposit characteristics), interactions among categories (variance that cannot be explained by a single category), and unexplained variance for the studied nonwadeable rivers in Wisconsin. [%, percentage of total variance]

Environmental Characteristics Most Strongly Related to Water Quality

Correlations, stepwise regressions, and redundancy analyses all indicated that the land-use characteristics (primarily For % and Ag %) were the characteristics most strongly related to water quality. Simply omitting the land-use characteristics and reanalyzing the data, however, may not provide a true indication of what other factors affect water quality because some of the remaining factors were strongly correlated with the land-use characteristics of the basins. For example, air temperature and clay content of the soil were both strongly correlated with many water-quality characteristics and with Ag % (table 4). Therefore, it is difficult to determine whether it was these factors or the indirect effects of agriculture that affected water quality. The clay content of the soil has been demonstrated to have a strong effect on the water quality of Midwestern streams (Robertson, 1997; Robertson and others, 2006b); however, the effects of air temperature seem questionable and may be indirectly related to the land-use characteristics.

Various approaches (RDA and residualization analyses) were used to determine which environmental characteristics other than land-use characteristics were most strongly related to water quality. The results of partial RDA indicated that soil characteristics were important; however, much of the variance explained by soil characteristics was also explained by the land-use characteristics. Results of RDA indicated that the most important soil characteristics were the clay content and organic-matter content of the soils; however, many of the soil characteristics were correlated with one another. The results also indicated that the amount of runoff was strongly related to water quality.

Results of the residualization analyses indicated that the natural (non-land-use) environmental characteristics most strongly related to the distribution of TP concentrations were runoff and basin slope. Air temperature and evaporation were also found to be important, but these factors may simply reflect their north/south gradients that also occurs in TP concentrations. The natural environmental characteristics most strongly related to the distribution of TN concentrations were runoff and the clay content and erodibility of the soils. In all cases, high nutrient concentrations were related to low annual runoff values (including residualized values). Lower amounts of total annual runoff and higher nutrient concentrations occurred in the southern part of the State than in the northern part. High nutrient concentrations typically occur during runoff events; however, the concentrations used in this study represent the typical (base-flow) condition.

The natural environmental characteristics most strongly related to the distribution of SDs were air temperature and evaporation, which was similar to that for TP. In addition to the land-use characteristics, results from the stepwise regressions indicated that the distribution of SDs was strongly related to SSC concentrations. The natural environmental characteristics most strongly related to the distribution of SCHL were the size and slope of the basin and the length of the river. All of these factors would affect the travel time of the water in the basin and allow different amounts of time for the algal community to consume nutrients. A few of the natural characteristics, however, such as clay content and erodibility of the soil, were so strongly correlated with Ag % that their relations to water quality may have been reduced by the residualization approach.

Thresholds in Water-Quality Responses and Responses to Changes in Land Use

Concentrations of TP and TN were significantly correlated with Ag %. To define these relations better, Log TP and Log TN concentrations were plotted against Ag % (fig. 9), and regression-tree analyses were done to determine the percentages of agriculture that were the best breakpoints or thresholds in the responses. Regression-tree results indicate that the best statistically significant ($p < 0.001$) breakpoints in the responses of Log TP and Log TN to changes in Ag % were at 24.7 and 18.1 percent, respectively (table 8). In both cases, however, the relations between Log TP and Log TN concentrations and Ag % appear linear; the line determined with linear regression better defined the response than a step change in values (on the basis of a mean-square-error criterion).

Thresholds or breakpoints in the responses to changes in Ag % were also determined for the other logarithmically transformed water-quality constituents, including SSC (table 8). Thresholds ranged from as low as 8.8 percent agriculture for NO_3-N to 43.7 percent for NH_4-N. In almost all cases, the relations between the concentrations and Ag % appear linear; however, for all of these additional constituents a step change better defined the response (on the basis of a mean-square-error criterion).

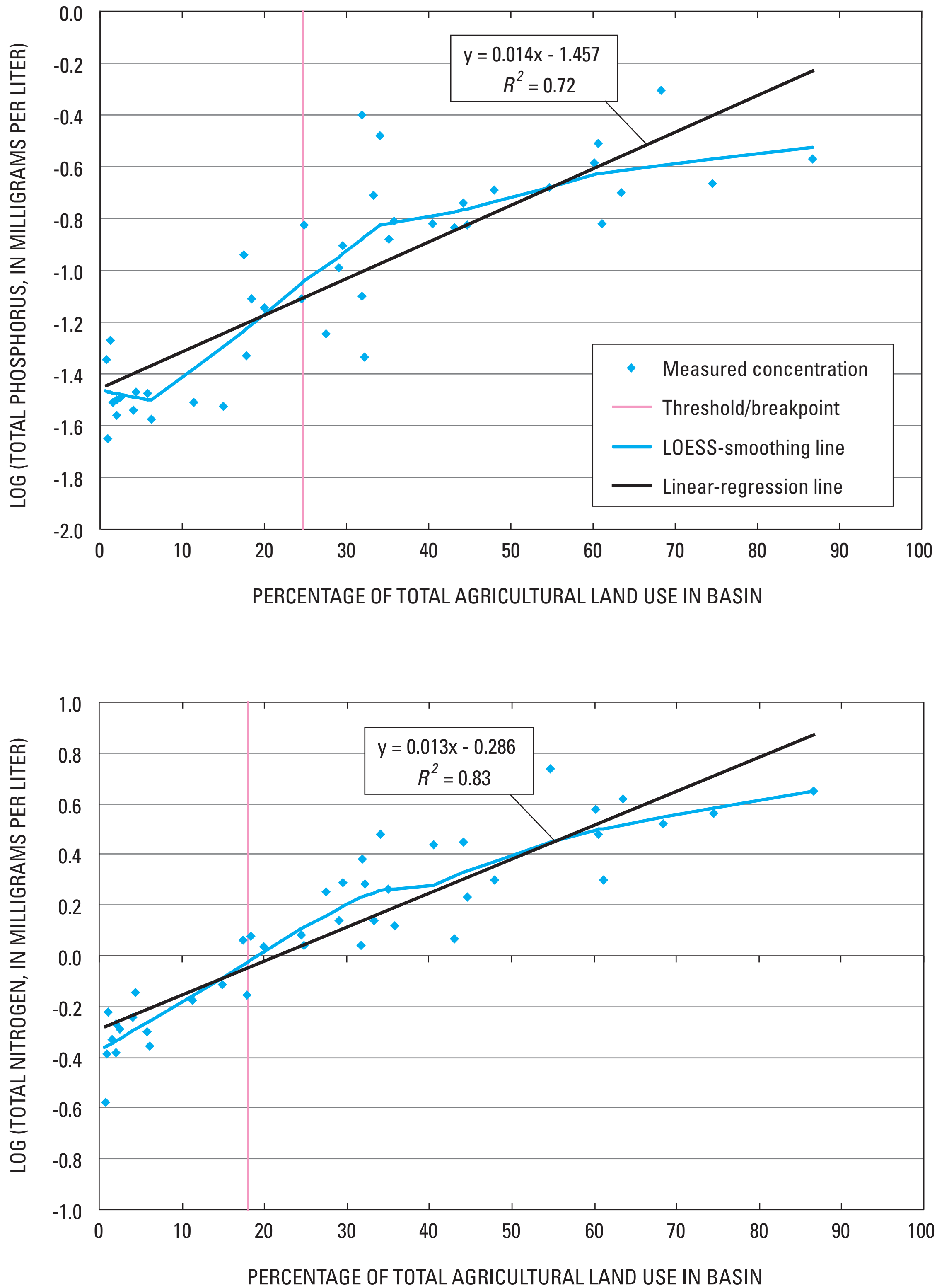

Figure 9. Logarithmically transformed total phosphorus (TP) and total nitrogen (TN) concentrations as a function of the percentage of total agriculture (Ag %) in the watersheds of the studied nonwadeable rivers in Wisconsin, 2003. Computed thresholds in the response are identified by vertical lines. LOESS-smoothing lines and linear-regression lines with the coefficients of determination (R^2) are given on each graph.

Table 8. Thresholds or breakpoints in the response in water quality to changes in the percentage of agricultural area in the basin for nonwadeable rivers in Wisconsin.

Constituent	Threshold percentage of agriculture
Total phosphorus	24.7
Dissolved phosphorus	24.7
Particulate phosphorus	31.8
Total nitrogen	18.1
Dissolved nitrite plus nitrate	8.8
Dissolved ammonia	43.7
Total Kjeldahl nitrogen	34.6
Suspended chlorophyll *a*	18.1
Secchi-tube depth	32.7
Suspended sediment	18.1

Concentrations of SCHL were significantly correlated with TP and TN concentrations (table 4). To better define these relations, Log SCHL concentrations were plotted against Log TP and Log TN concentrations (fig. 10), and regression-tree analyses were done. Log TP explained 45 percent of the variance in Log SCHL concentrations and Log TN explained 35 percent of the variance. If the sites with the highest SSC to SCHL ratios in drainage areas primarily in the Driftless Area ecoregion (southwest part of the State) were omitted, then Log TP and Log TN explained 79 and 59 percent of the variance in Log SCHL concentrations, respectively. Regression-tree results indicated that the best breakpoint in the response of SCHL to changes in TP concentrations was at 0.064 mg/L (Log TP = -1.19), and at 0.927 mg/L (Log TN = -0.03) for changes in TN concentrations; both breakpoints were statistically significant at $p < 0.001$. The relations between SCHL and TP and TN concentrations appear linear; however, the step-change response better defines these relations (on the basis of a mean-square-error criterion). A similar response was found between TP and SCHL in temperate streams throughout North America, with SCHL concentrations increasing most rapidly at TP concentrations less than 0.1 mg/L (Van Nieuwenhuyse and Jones, 1996).

To define the relations between SDs and TP and TN concentrations better, SDs were plotted against Log TP and Log TN concentrations (fig. 10) and regression-tree analyses were done. There was little apparent relation between SDs and TP and between SDs and TN at lower nutrient concentrations because of the limited length of the Secchi tube; however, as nutrient concentrations increased, SDs decreased. Overall concentrations of TP and TN explained 77 and 50 percent of the variance in SDs, respectively. Regression-tree results indicate that the best breakpoint in the response of SDs to changes in TP concentrations was at 0.091 mg/L (Log TP = -1.04) and to changes in TN concentrations was at 1.097 mg/L (Log TN = 0.04); both breakpoints were statistically significant at $p < 0.001$. A regression line defines the response for TP better than a step change; however, a step change defines the response better for TN (on the basis of a mean-square-error criterion). The reduction in SDs with increasing nutrient concentrations may have been caused by other factors that are correlated to TP and TN concentrations, such as the SSC concentrations (table 4).

Reference Water Quality

Several approaches have been used to define reference water quality (also referred to as background or potential water quality in some publications) for specific areas. In defining reference conditions for national nutrient criteria, the USEPA has suggested that the frequency distribution of the data available for a specific area could be used to define a reference condition: the lower (or best) 25^{th} percentile of all the data for a specific area or the upper (or worst) 75^{th} percentile of the data for a subset of streams thought to be minimally impacted (U.S. Environmental Protection Agency, 2000a). Because it is difficult to determine which sites are minimally impacted, the lower (the best) 25^{th} percentile of all the data is the more common approach and the one used by the USEPA to define their proposed water-quality criteria (table 1; U.S. Environmental Protection Agency, 2000b; and 2001).

The watersheds of the nonwadeable rivers in Wisconsin usually covered large areas of the State and extended across several ecoregions and environmental phosphorus zones (fig. 1); therefore, reference water-quality conditions were not examined for different areas or individual ecoregions in the State, but rather for the entire State. Reference TP and TN concentrations for the nonwadeable rivers of Wisconsin based on the 25^{th}-percentile approach were 0.034 and 0.670 mg/L, respectively (table 9). The reference conditions for SCHL and SD were 3.83 µg/L and greater than 120 cm, respectively. The reference conditions for the other constituents are also listed in table 9.

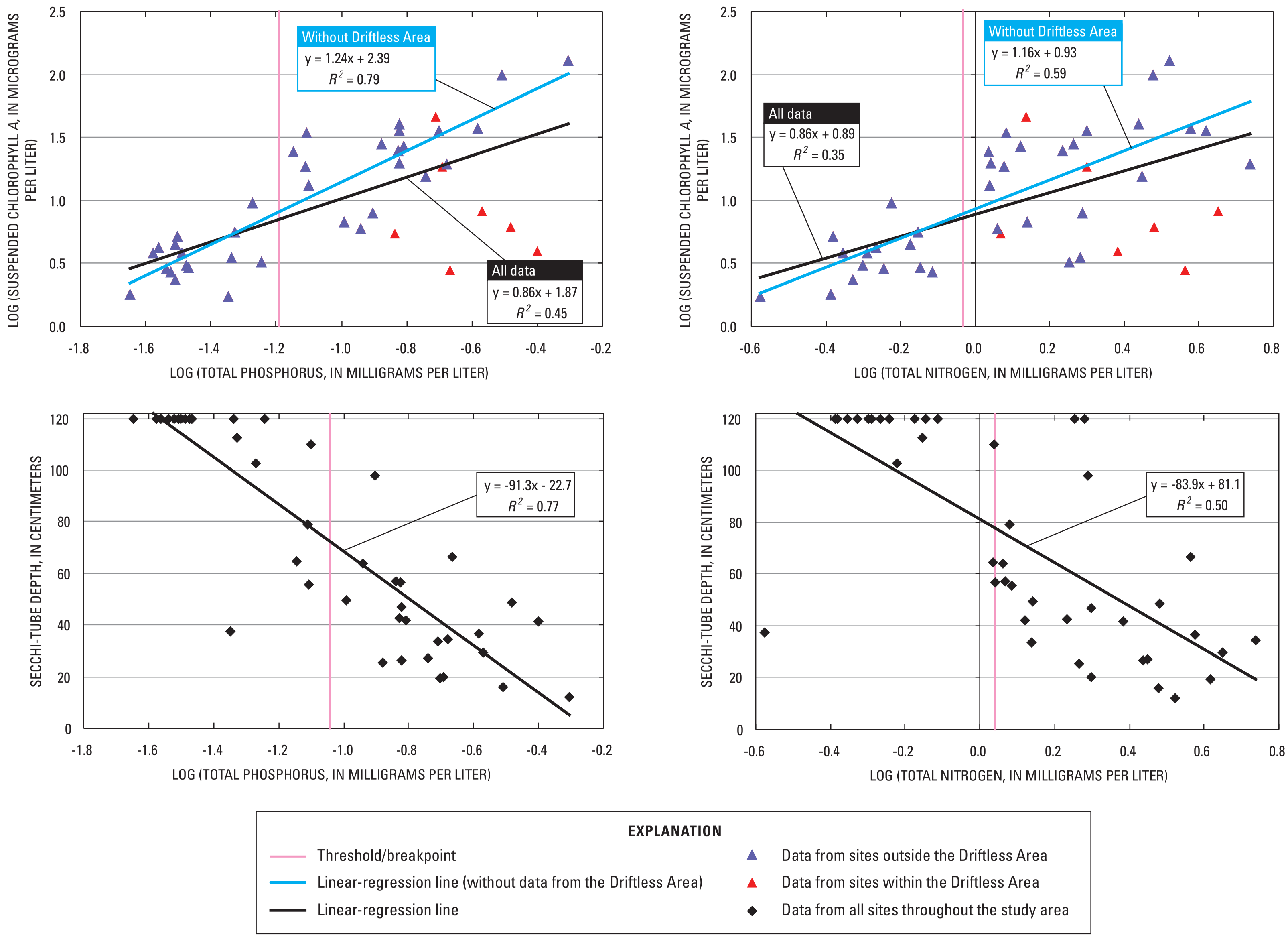

Figure 10. Logarithmically transformed suspended chlorophyll *a* (SCHL) concentrations and Secchi-tube depths (SDs) as a function of logarithmically transformed median total phosphorus and total nitrogen concentrations for the studied nonwadeable rivers in Wisconsin, 2003. Linear-regression lines and coefficients of determination (R^2) are given on each graph. Sites in the Driftless Area ecoregion (southwestern part of Wisconsin) are identified separately for SCHL.

Table 9. Reference conditions for water-quality constituents for nonwadeable rivers in Wisconsin. Median reference values, standard errors, and upper 95-percent confidence limits were estimated with the multiple linear-regression approach.

[mg/L, milligram per liter; cm, centimeter; µg/L, microgram per liter; >, greater than; --, insufficient data to estimate]

Constituent	Regression approach			Percentile approach	
	Median reference	Standard error	Upper 95-percent confidence limit	Best 25th percentile of all data	Worst 75th percentile of Reference sites
Total phosphorus (mg/L)	0.035	0.005	0.045	0.034	--
Dissolved phosphorus (mg/L)	.016	.002	.021	.017	--
Particulate phosphorus (mg/L)	.018	.003	.025	.018	--
Total nitrogen (mg/L)	.514	.043	.604	.670	--
Dissolved nitrite plus nitrate (mg/L)	.061	.020	.107	.132	--
Dissolved ammonia (mg/L)	.022	.000	.022	.019	--
Kjeldahl nitrogen (mg/L)	.434	.043	.524	.500	--
Suspended chlorophyll *a* (µg/L)	3.95	1.00	6.20	3.83	3.85
Secchi-tube depth (cm)	110	7	96[a]	>120	>120
Suspended sediment (mg/L)	3.2	.8	4.9	4.0	2.8

[a] A lower 95-percent confidence limit is given here because higher values represent better conditions for Secchi-tube depth.

Defining reference conditions based upon the percentile approach is arbitrary in nature because the percentages of agricultural and urban areas in the region can strongly affect the results for characteristics correlated with land use, such as the water-quality characteristics examined in this study. The 25th-percentile approach usually results in areas with extensive agriculture and urban development having relatively poor reference conditions; therefore, other approaches were examined.

Another approach to estimate reference concentrations is a multiple linear-regression model (regression approach) that relates water quality to anthropogenic characteristics of the watershed (Dodds and Oakes, 2004):

$$\text{Log } P_{\text{Predicted}} = a + b \text{ Ag \%} + c \text{ Urb \%}, \qquad (11)$$

where a, b, and c are empirical coefficients based on data for all nonwadeable rivers. After calibrating the model with data from a specific area, an estimate of reference conditions in the absence of anthropogenic activities can be obtained by setting the variables describing the anthropogenic characteristics to 0 (in this study, setting Ag % and Urb % to 0). The general form of this model is similar to that used to estimate residualized concentrations in equations 2, 4, 6, and 8 (page 25). These relations can also be used to place confidence intervals on the estimated reference concentrations. Because this type of model estimates the logarithm of the reference concentration (except for SD), the median reference concentrations were estimated as 10^a. The median reference condition, the standard error of the reference condition, and the upper bound of the 95-percent confidence interval of the reference condition for each water-quality constituent are given in table 9. A bias correction is typically applied to results for mean values obtained by logarithmic regression; however, the bias correction was not used here because median values were determined rather than mean values.

On the basis of the results of the regression approach, the reference concentration for TP was 0.035 mg/L, with an upper 95-percent confidence limit of 0.045 mg/L (table 9). The reference concentration for TN was 0.514 mg/L, with an upper 95-percent confidence limit of 0.604 mg/L. The reference concentration for SCHL was 3.95 µg/L, with an upper 95-percent confidence limit of 6.20 µg/L. The reference SD was 110 cm, with a lower 95-percent confidence limit of 96 cm (a lower limit is given because higher values represent better conditions). Reference conditions for the other water-quality characteristics are given in table 9.

A reference SCHL concentration was also estimated by examining the SCHL concentrations in sites with both reference TP and reference TN concentrations (considered to be minimally impacted sites). For this analysis, the 42 sites were divided into three categories: reference sites (Reference, fig. 11)—6 sites with both TP concentrations at or below the 0.035-mg/L reference concentration and TN concentrations at or below the 0.514-mg/L reference concentration; high nutrient-concentration sites (High)—29 sites with both TP and TN concentrations above their respective upper 95-percent confidence limits for reference concentrations (TP concentrations above

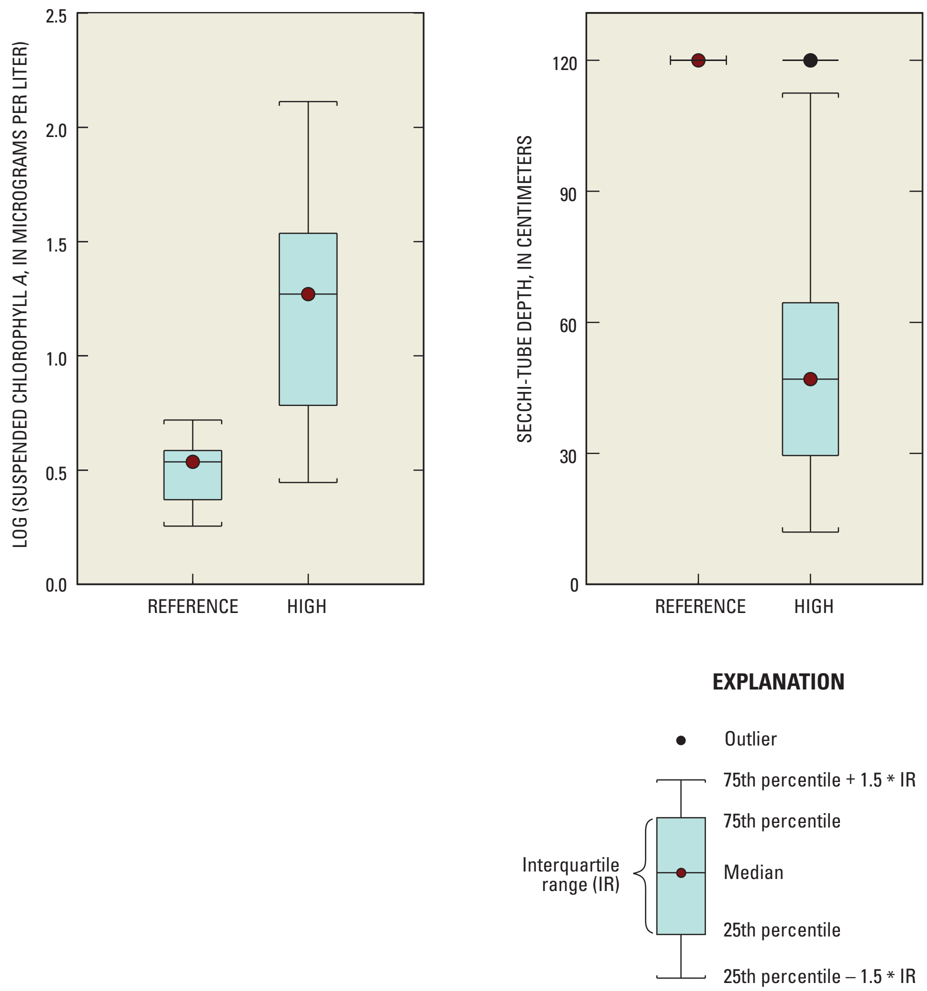

Figure 11. Suspended chlorophyll *a* (SCHL) concentrations and Secchi-tube depths (SDs) in Reference sites and High (nonreference) sites in the studied nonwadeable rivers in Wisconsin, 2003.

0.045 mg/L and TN concentrations above 0.604 mg/L); and nonclassified sites—7 sites with either TP or TN concentrations above their respective reference concentrations but below their upper 95-percent confidence limits (these sites were not included in this analysis and not included in fig. 11).

The median SCHL concentration of the Reference sites was 3.4 µg/L (Log (3.4) = 0.54), with the upper 75th percentile being 3.8 µg/L (Log (3.8) = 0.59), which was significantly less than the median concentration of 18.6 µg/L (Log (18.6) = 1.3) measured at the High sites (fig. 11). It has been suggested by the USEPA that the upper (worse) 75th percentile of a subset of streams thought to be minimally impacted (Reference sites) may represent the reference condition; therefore, an alternative reference SCHL concentration for the entire State would be 3.8 µg/L. The reference values (3.8 to 3.9 µg/L) estimated with the approaches used in this study are slightly less than those defined by the USEPA for nutrient ecoregions 7 and 8 (5.8 and 4.3 µg/L, respectively; values obtained when the trichromatic method is used for chlorophyll *a* analysis; table 1).

A reference SD was also determined by examining the sites at which both TP and TN concentrations were at or below their respective reference concentrations. The median SD measured at the Reference sites was greater than 120 cm, which was significantly greater than the median SD measured at the High sites (47 cm; fig. 11). The lower 25th percentile of SDs at the Reference sites (equivalent to the worst 75th percentile of the minimally impacted sites) was also greater than 120 cm; therefore, an alternative reference SD value for the entire State would be greater than 120 cm. Because this length exceeds the length of the Secchi tube, a specific reference condition was not able to be obtained.

Macroinvertebrate Assemblages and Their Relations with Water-Quality and Environmental Characteristics

Wisconsin Department of Natural Resources personnel preparing Hester-Dendy artificial substrate samplers for deployment and collection of macroinvertebrates. Macroinvertebrate photos provided by Stanley Szczytko (University of Wisconsin–Stevens Point).

Fourteen indices were used to describe the macroinvertebrate communities in the nonwadeable rivers in Wisconsin. These indices describe species richness (1 index: SPECIES), depositional substrate (1: %DEPOS) and pollution tolerance (2: MPTV and HBI), feeding ecology (3), and insect order (7) (table 10). SPECIES, HBI, MPTV, and both Ephemeroptera, Plecoptera, and Trichoptera (EPT) indices indicated that sites spanned from very poor to excellent conditions. The number of species (SPECIES) ranged from 10 to 51 (median = 32; table 10; fig. 12). The median percentage of individuals from EPT orders (%EPTN) was about 50 percent (ranged from 2.6 to 94.7 percent) and about 45 percent of the individuals were from the order Diptera (%DIPT; ranged from 3.3 to 92.7 percent). The percentage of individuals from the order Ephemeroptera (%EPHEM) ranged from 0.0 to 69.1 percent (table 10, fig. 12; median = 16.7 percent). Two indices described the assemblage's stress response to organic pollution: MPTV, which ranged from about 3.5 to 6.8; and HBI, which ranged from about 2.8 to 9.6 (table 10, fig. 12). For most macroinvertebrate indices, higher values are representative of better water quality except for MPTV and HBI for which lower index values are representative of better water quality. In general, macroinvertebrate communities in rivers in the southeast part of the State would normally be considered representative of poorer water quality, with fewer species, fewer EPT individuals and taxa (shown only for %EPHEM in fig. 12), and higher MPTV and HBI values than those of rivers in the rest of the State.

Table 10. Summary statistics for biotic indices for nonwadeable rivers in Wisconsin.

[Ephemeroptera, Plecoptera, and Trichoptera (EPT); %, percent; #, number]

Index	Abbreviation	Units	Count	Median	Mean	25th percentile of all data	75th percentile of all data	Standard deviation	Minimum	Maximum
Macroinvertebrates										
Species richness	SPECIES	#	41	32	32.2	28	38	8.7	10	51
Percentage of individuals from order Ephemeroptera	%EPHEM	%	41	16.7	21.1	5.6	31.4	18.1	.0	69.1
Percentage of individuals from order Plecoptera	%PLEC	%	41	.2	.9	.0	.8	1.9	.0	11.2
Percentage of individuals from order Trichoptera	%TRICHOP	%	41	24.0	26.0	9.4	35.3	20.7	.4	84.3
Percentage of individuals from order Diptera	%DIPT	%	41	44.7	46.6	27.2	65.8	23.3	3.3	92.7
Percentage of individuals from Diptera: Chironomidae	%CHIRON	%	41	41.9	44.3	25.6	59.4	23.0	3.3	92.7
Percentage of EPT number	%EPTN	%	41	49.4	48.0	28.4	62.9	24.6	2.6	94.7
Percentage of EPT taxa	%EPTTX	%	41	40.0	38.6	31.6	47.1	12.2	12.5	65.0
Percentage of individuals that are scrapers	%SCRAP	%	41	7.2	10.8	1.8	12.5	13.3	.0	55.9
Percentage of individuals that are shredders	%SHRED	%	41	8.6	10.7	4.8	14.8	9.0	.4	46.6
Percentage of individuals that are gatherers	%GATHER	%	41	23.3	28.9	12.3	37.9	22.3	3.2	94.8
Percentage of individuals tolerant to depositional habitat	%DEPOS	%	41	8.7	10.7	4.6	16.0	9.7	.6	58.0
Mean pollution tolerance value	MPTV	#	41	5.290	5.225	4.760	5.640	.740	3.520	6.769
Hilsenhoff Biotic Index	HBI	#	41	5.386	5.500	4.865	5.821	1.192	2.772	9.578
Fish										
Total number of native species (not exotic)	#NATIVESP	#	41	12	13.4	10	16	5.0	4	27
Number of river species	#RIVERSP	#	41	4	4.9	4	7	2.9	0	23
Number of sucker (Catostomidae) species	#SUCKER	#	41	4	4.0	3	5	1.6	1	8
Number of species considered intolerant of degradation	#INTOL	#	41	3	2.7	1	3	1.6	0	7
Weight of fish collected per unit effort	WPUE	kg	41	24.5	30.2	11.8	36.5	26.3	3.9	151
Percentage of individuals that are river species	%RIVERSP	%	41	26.4	28.3	14.3	39.4	19.5	.0	79.0
Percentage of individuals that are lithophilic spawners	%LITSPAWN	%	41	52.9	54.3	37.0	73.1	24.7	1.5	93.5
Percentage of suckers by weight	%SUCKER	%	41	32.0	41.9	15.7	71.8	31.4	.6	97.4
Percentage of insectivores by weight	%INSECT	%	41	40.5	49.1	29.1	76.6	29.7	.9	97.8
Percentage of fish with disease or deformities	%DISEASE	%	41	.0	.5	.0	.0	1.2	.0	6.7
Wisconsin large-river index of biotic integrity	IBI	#	41	75	67.1	50	90	27.1	5	100

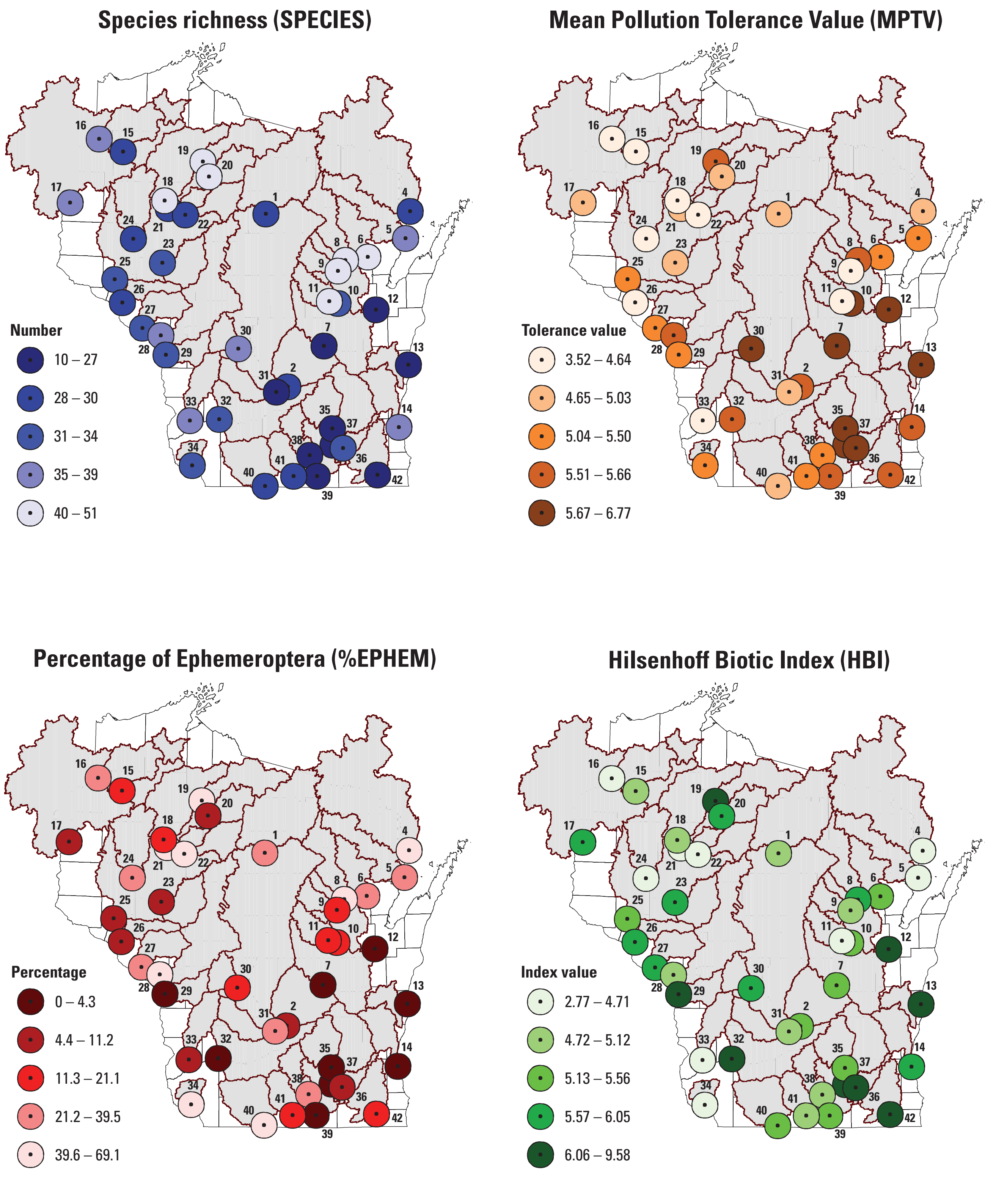

Figure 12. Distributions (quintiles) for four macroinvertebrate indices [species richness (SPECIES), mean pollution tolerance value (MPTV), percentage of individuals from the order Ephemeroptera (%EPHEM), and Hilsenhoff Biotic Index (HBI)] for the studied nonwadeable rivers in Wisconsin. Better macroinvertebrate communities are indicated by lower MPTV and HBI values.

Relations with Individual Characteristics

Correlations

Most of the macroinvertebrate indices were significantly correlated (r_s values) with several nutrient constituents except %DIPT, percentage of the individuals that are from the family chironomidae (%CHIRON), %EPTN, and gathers (%GATHER; table 11). Six of the indices were more strongly correlated with the nutrient constituents than the other indices [SPECIES, MPTV, %EPHEM, HBI, percentage of the individuals from the order Plecoptera (%PLEC), and percentage of the individuals that are scrapers (%SCRAP)]; these are listed in decreasing order of the strength of these relations. These indices were most strongly correlated with TP, TN, and TKN. SPECIES, %EPHEM, %PLEC, and %SCRAP were negatively correlated with most nutrient concentrations, although some of the correlations were not statistically significant; MPTV and HBI were positively correlated with all nutrient concentrations. Lower MPTV and HBI values are representative of better macroinvertebrate communities and better water quality. Unexpectedly, the percentage of individuals from the order Trichoptera (%TRICHOP) and percentage of individuals that are shredders (%SHRED) were positively correlated with all nutrient concentrations. There are, however, a few Trichoptera species, such as *Hydropsyche*, that are relatively pollution tolerant.

In general, the six indices most strongly correlated with the nutrient concentrations were also the indices most strongly correlated with SCHL, SD, SSC, water temperature, SC, pH, and the land-use characteristics, especially Ag %, percentage of row-crop agriculture (AgRow %), For %, and Urb %. Better macroinvertebrate index scores (higher SPECIES, %EPHEM, %PLEC, and %SCRAP scores and lower MPTV and HBI scores) generally were correlated with lower SCHL, SSC, SC, pH, Ag %, Urb %, and with higher SDs and For %. These six indices were also more strongly correlated with a few basin characteristics (air temperature, runoff, and basin slope) and the soil/surficial-deposit characteristics (clay content and soil slope) than were the other indices. In general, better macroinvertebrate assemblages were found in rivers in areas with cool air temperatures, high runoff, and soils with low clay content and steep slopes; these characteristics are generally found in the northern part of the State with mixed and mostly forested areas (figs. 1A and 12).

Response to Changes in Nutrient Concentrations

Responses of the four macroinvertebrate-assemblage indices most strongly correlated with nutrients (SPECIES, MPTV, EPHEM%, and HBI) are shown with respect to median TP and TN concentrations in figure 13 and the two indices most strongly related with nutrients (SPECIES and MPTV) are shown with respect to median DP, NO_3-N, NH_4-N, and TKN concentrations in figure 14. SPECIES and MPTV were chosen as the best macroinvertebrate measures and used for additional detailed investigation because correlation analyses and scatterplots indicated that they were the most responsive to differences in nutrient concentrations and they appeared to best represent the other macroinvertebrate indices. In general, SPECIES and EPHEM% decreased as nutrient concentrations increased, whereas MPTV and HBI values increased as nutrient concentrations increased. The strongest relations were found between SPECIES and MPTV values and TP, TN, and TKN concentrations. The other relations with nutrient concentrations ranged more widely; little relation was found with DP, NO_3-N, and NH_4-N concentrations for any of the indices. The wide range in biotic index values at any nutrient concentration in these graphs may indicate the effects of factors other than nutrient concentrations.

Table 11. Spearman rank correlation coefficients (r_s) between macroinvertebrate-assemblage indices and median water-quality, land-use, basin, and soil/surficial-deposit characteristics for the studied nonwadeable rivers in Wisconsin.

[All absolute values greater than 0.30 (Student's t-test; **bold black** values) and 0.48 (Bonferroni correction; **bold red** values) were significant at $p < 0.05$. All characteristics are defined in table 2 except macroinvertebrate indices, which are defined in table 10.]

Characteristic	SPECIES	%EPHEM	%PLEC	%TRICHOP	%DIPT	%CHIRON	%EPTN	%EPTTX	%SCRAP	%SHRED	%GATHER	%DEPOS	MPTV[a]	HBI[a]
						Water-quality characteristics								
TP	**-0.55**	**-0.39**	**-0.34**	0.27	0.00	0.06	-0.09	-0.25	-0.24	0.18	-0.06	-0.24	**0.42**	**0.39**
DP	**-.34**	-.23	-.26	.25	-.05	.01	-.04	-.20	-.06	.14	-.06	-.13	.26	.25
PP	**-.61**	**-.45**	**-.31**	.26	.04	.10	-.11	-.26	**-.33**	.20	-.05	-.28	**.47**	**.43**
TN	**-.44**	**-.33**	**-.37**	**.40**	-.09	-.04	.04	-.10	**-.31**	.27	-.03	-.16	**.36**	.27
NO_3-N	-.18	-.13	-.15	**.33**	-.06	-.03	.02	.03	-.10	.13	.04	-.02	.11	.13
NH_4-N	-.21	-.07	**-.45**	.24	-.16	-.13	.08	-.04	-.24	.22	.19	-.05	.28	.15
TKN	**-.58**	**-.59**	**-.69**	.34	-.01	.06	-.11	**-.42**	**-.56**	**.47**	.07	**-.31**	**.72**	**.51**
SCHL	**-.69**	**-.71**	**-.42**	.17	.22	.28	**-.31**	**-.45**	**-.64**	**.42**	.01	**-.39**	**.59**	**.51**
SD	**.66**	**.52**	**.44**	-.35	.05	-.01	.07	.26	**.38**	-.23	.04	**.34**	**-.58**	**-.45**
SSC	**-.52**	**-.46**	-.29	.29	-.02	.05	-.07	-.22	-.27	.16	-.08	-.27	**.43**	**.41**
WTemp	**-.53**	**-.42**	**-.44**	-.02	.10	.18	-.23	**-.40**	**-.34**	**.39**	.01	-.23	**.58**	**.43**
SC	**-.35**	**-.35**	**-.50**	**.34**	-.10	-.03	.00	-.22	**-.36**	.23	.12	-.11	**.45**	.27
pH	**-.39**	**-.48**	**-.34**	.07	.16	.19	-.21	**-.32**	**-.37**	**.33**	.03	-.16	**.38**	.29
Color	.24	.10	-.03	-.27	.05	.06	-.04	-.04	-.04	-.05	.06	-.03	.11	.01
						Land-use characteristics								
Urb %	**-.68**	**-.52**	**-.61**	.28	.03	.11	-.16	**-.38**	**-.50**	**.33**	.04	**-.31**	**.60**	**.46**
AgRow %	**-.52**	**-.55**	**-.45**	**.38**	.01	.06	-.09	**-.28**	**-.49**	**.31**	.02	-.26	**.48**	.33
AgOther %	**-.35**	**-.32**	-.26	**.33**	-.04	.01	-.01	-.18	-.17	.17	-.03	-.20	.25	.20
Ag %	**-.50**	**-.42**	**-.40**	**.40**	-.09	-.02	.01	-.20	**-.32**	.23	-.06	-.26	**.40**	.26
Grass %	-.21	**-.44**	-.19	.23	.14	.15	-.18	-.14	**-.31**	.17	.06	-.14	.14	.25
WetO %	-.13	-.22	-.28	-.19	.21	.22	-.23	-.24	**-.33**	.13	.07	-.14	**.35**	.25
WetF %	**.48**	.26	.15	-.25	.01	-.01	.03	.13	.15	-.03	.04	.16	-.18	-.20
Barren %	-.29	-.18	**-.37**	.01	.10	.13	-.18	**-.44**	**-.33**	**.36**	.35	.12	**.35**	.24
For %	**.55**	**.47**	**.47**	**-.37**	.00	-.06	.07	.26	**.43**	**-.30**	.01	.24	**-.44**	**-.33**

Table 11. Spearman rank correlation coefficients (r_s) between macroinvertebrate-assemblage indices and median water-quality, land-use, basin, and soil/surficial-deposit characteristics for the studied nonwadeable rivers in Wisconsin—Continued.

[All absolute values greater than 0.30 (Student's t-test; **bold black** values) and 0.48 (Bonferroni correction; **bold red** values) were significant at $p < 0.05$. All characteristics are defined in table 2 except macroinvertebrate indices, which are defined in table 10.]

Characteristic	SPECIES	%EPHEM	%PLEC	%TRICHOP	%DIPT	%CHIRON	%EPTN	%EPTTX	%SCRAP	%SHRED	%GATHER	%DEPOS	MPTV[a]	HBI[a]
							Basin characteristics							
Area	-0.15	-0.07	0.08	-0.01	0.05	0.07	0.00	-0.07	-0.11	0.21	-0.17	-0.12	0.11	-0.02
ATemp	**-.46**	**-.47**	**-.48**	**.37**	.01	.06	-.10	**-.32**	**-.37**	.27	.05	-.22	**.45**	**.36**
PPT	**-.34**	-.23	-.21	.19	.07	.10	-.09	-.18	-.17	.11	.09	-.06	.22	.26
Evap	**-.41**	**-.34**	-.27	**.38**	-.06	-.01	-.01	-.20	-.14	.13	-.08	-.21	.25	.23
Runoff	**.38**	**.49**	**.33**	-.17	-.17	-.22	.26	**.37**	**.35**	-.21	-.10	.19	**-.43**	**-.47**
Length	-.09	-.02	.11	-.09	.06	.07	-.02	-.02	-.05	.07	-.17	-.17	.06	-.06
BSlope	**.45**	**.32**	.28	-.20	-.06	-.10	.08	.26	**.42**	**-.30**	.04	.19	**-.33**	-.21
Flow	.14	.00	.20	-.03	.16	.12	-.08	.19	-.01	.04	-.09	-.01	-.27	-.19
							Soil/surficial-deposit characteristics							
SClay	**-.46**	**-.37**	**-.31**	.28	-.03	.03	-.04	-.21	-.23	.15	-.05	-.20	**.36**	.28
Erod	**-.43**	**-.31**	-.18	.16	.09	.11	-.11	-.13	-.24	.15	.00	-.15	.19	.17
OM	.20	.19	-.05	-.16	-.08	-.11	.09	.14	.01	-.08	.02	-.01	.00	-.11
Perm	**.34**	.11	.01	-.03	-.05	-.07	.02	.04	.02	-.01	.10	.10	-.03	-.07
SSlope	.22	**.39**	**.47**	.06	-.20	-.24	.23	**.39**	**.50**	**-.31**	-.10	.23	**-.45**	**-.31**
NonGlac	-.21	-.13	-.01	.11	.05	.11	-.10	-.21	.17	.02	-.19	-.10	.12	.23
SDClay	.05	-.13	**-.45**	.27	-.13	-.07	.06	-.16	-.21	.23	.23	-.02	**.43**	.22
Loam	.22	.16	.26	.12	-.19	-.18	.21	.15	.16	.10	-.16	.01	-.10	-.20
Peat	.15	.07	.16	**-.33**	.26	.23	-.25	-.07	.10	-.10	.01	-.10	-.15	.01
Sand	-.29	-.28	-.01	-.20	**.32**	.27	-.29	-.11	**-.37**	.09	.00	-.26	-.02	.08
SG	.12	.20	-.12	.04	-.23	-.22	.26	.21	-.08	.11	.05	.16	.06	-.12

[a] For MPTV and HBI, lower index values are typically thought to represent better water quality.

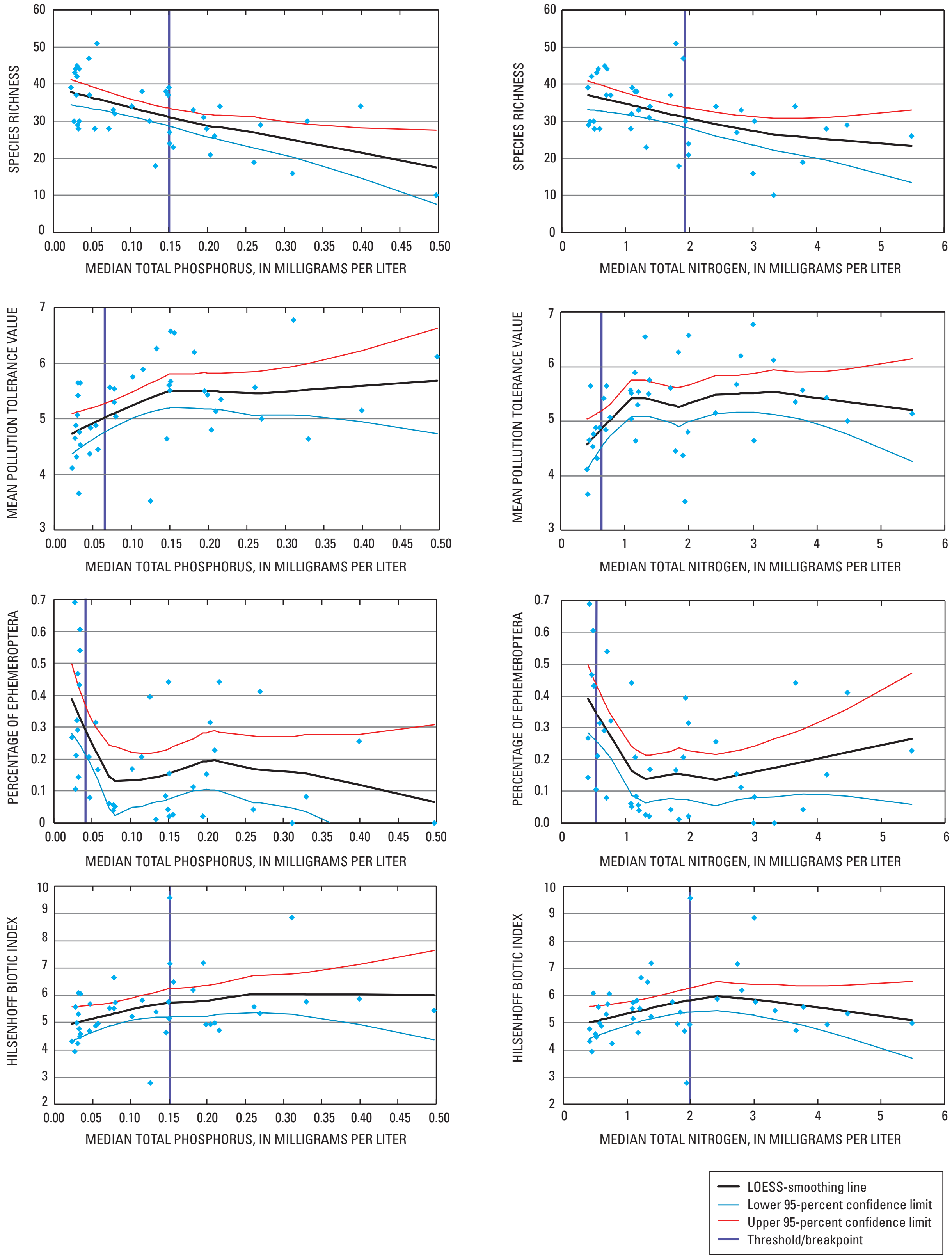

Figure 13. Species richness (SPECIES), mean pollution tolerance value (MPTV), percentage of individuals from the order Ephemeroptera (%EPHEM), and Hilsenhoff Biotic Index (HBI) values as a function of total phosphorus (TP) and total nitrogen (TN) concentration for the studied nonwadeable rivers in Wisconsin. LOESS-smoothing lines with 95-percent confidence limits and computed thresholds in the response, identified by vertical lines, are given on each graph.

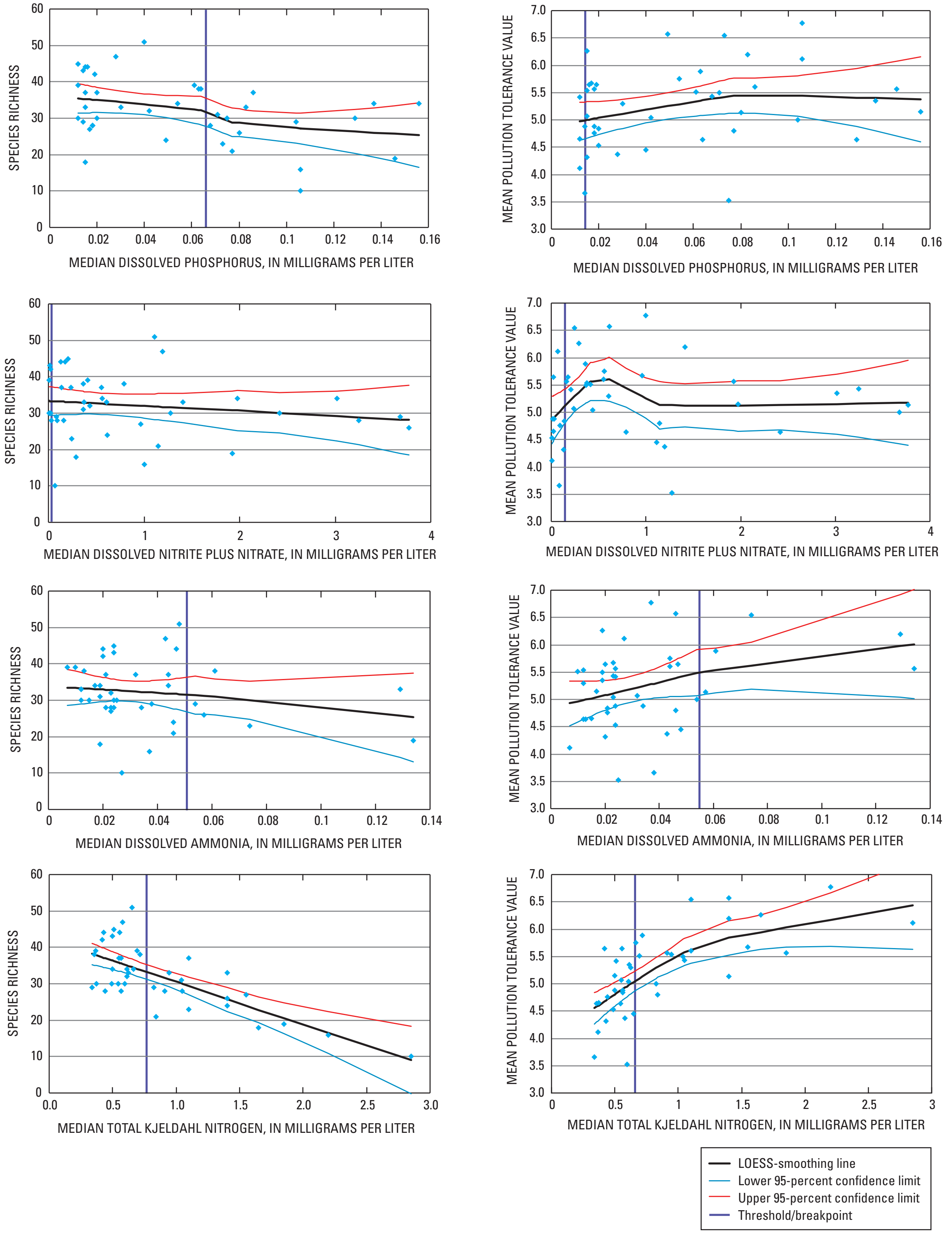

Figure 14. Species richness (SPECIES) and mean pollution tolerance value (MPTV) as a function of dissolved phosphorus (DP), dissolved nitrite plus nitrate nitrogen (NO_3-N), dissolved ammonia nitrogen (NH_4-N), and total Kjeldahl nitrogen (TKN) concentrations for the studied nonwadeable rivers in Wisconsin. LOESS-smoothing lines with 95-percent confidence limits and computed thresholds in the response, identified by vertical lines, are given on each graph.

The LOESS-smoothing lines on the scatterplots between nutrient concentrations and macroinvertebrate index values indicate that, for most nutrient constituents, there were nonlinear relations (figs. 13 and 14). Regression-tree analyses were then used to define the thresholds or largest breakpoints in the responses of the six macroinvertebrate indices most strongly correlated with nutrient concentrations (table 12). The ranges in the significant breakpoints in the responses to changes in nutrient concentrations were: 0.034 to 0.150 mg/L for TP; 0.014 to 0.066 mg/L for DP; 0.016 to 0.101 mg/L for PP; 0.527 to 1.990 mg/L for TN; 0.052 to 0.147 mg/L for NO_3-N; 0.035 to 0.055 mg/L for NH_4-N; and 0.603 to 0.928 mg/L for TKN. All of the breakpoints for TP and TN were statistically significant at $p < 0.05$ except those identified for %PLEC. These analyses indicated that the values of these six macroinvertebrate indices changed the most over a relatively narrow range in concentrations for each constituent. None of the breakpoints exceeded 0.15 mg/L for TP or 2.0 mg/L for TN. In general, for macroinvertebrate indices with relatively low breakpoint values (such as for MPTV and EPHEM%, with breakpoint values of about 0.03 and 0.04 mg/L for TP, respectively), the indices continue to degrade with increasing nutrient concentrations (fig. 13); however, there was little change in the index values with increasing nutrient concentrations above the relatively high breakpoint values (such as for HBI, with a breakpoint value for TP of 0.150 mg/L).

Effects of Multiple Characteristics on Macroinvertebrate Indices

Stepwise Regressions

Forward stepwise regressions were done with water-quality (median values) and environmental characteristics to determine which characteristics best described the variance in the six macroinvertebrate indices most strongly correlated with nutrient concentrations (table 13). Models with more than three variables did not substantially increase the amount of variance explained. For all of the indices, TKN was the first or second variable incorporated in the models. TKN concentrations alone explained 29 to 52 percent of the variance in the four indices most strongly correlated to nutrient concentrations. The percentage of clay deposits was the only other variable in more than one model. With three variables, the models explained between 26 and 66 percent of the variance in the indices. The models for HBI and %PLEC only explained 29 and 26 percent of the variance, respectively. Plecopterans were not commonly found in this study; they were absent at 17 sites and composed less than 4 percent of the abundance at 23 other sites.

Redundancy Analysis

One of the greatest impediments to understanding the relations between nutrient concentrations and biotic response is that the biota may respond to nutrient enrichment in the same way that they react to other stressors (Yoder and Rankin, 1995; Karr and Chu, 1999). In addition, environmental characteristics are often highly correlated, making it difficult to differentiate spurious correlations from cause-and-effect relations (Miltner and Rankin, 1998; Wang and others, 2003; Dodds and Oakes, 2004). The approach used for RDA in this study is similar to that of Wang and others (2003) and Weigel (2003) and had three main components. First, a forward-selection procedure in RDA was used to identify the most important (key) characteristics to include from each of three categories: nutrients (the seven nutrient constituents in table 2), other water-quality characteristics (the other seven water-quality characteristics in table 2), and environmental characteristics (all remaining land-use, basin, soil, and surficial-deposit characteristics). RDA was then used to determine the influence of each of these categories on the macroinvertebrate assemblages (the 14 macroinvertebrate indices). Finally, partial RDA was used to determine the relative importance of the nutrients, other water-quality characteristics, environmental characteristics, and interactions among categories (variability that could not be attributed to a specific category) in affecting the macroinvertebrate assemblages (the 14 macroinvertebrate indices). The same characteristics found with the forward variable-selection procedure were used to describe each category of characteristics.

Table 12. Thresholds or breakpoints in the responses in macroinvertebrate indices to changes in nutrient concentrations for nonwadeable rivers in Wisconsin.

[All concentrations in milligrams per liter; nss, not statistically significant at $p < 0.05$]

Index	Total phosphorus	Dissolved phosphorus	Particulate phosphorus	Total nitrogen	Dissolved nitrite plus nitrate	Dissolved ammonia	Total Kjeldahl nitrogen
Species richness (SPECIES)	0.150	0.066	0.101	1.925	0.030 (nss)	0.051 (nss)	0.770
Mean pollution tolerance value (MPTV)	.064	.014	.051	.634	.147	.055	.658
Percentage of individuals from order Ephemeroptera (%EPHEM)	.040	.024	.023	.527	.052	.055 (nss)	.875
Hilsenhoff Biotic Index (HBI)	.150	.014 (nss)	.082	1.990	.134	.035 (nss)	.928
Percentage of individuals from order Plecoptera (%PLEC)	.148 (nss)	.076 (nss)	.051 (nss)	1.965 (nss)	1.235 (nss)	.035	.603
Percentage of individuals that are scrapers (%SCRAP)	.034	.014 (nss)	.016	.527	.030 (nss)	.016 (nss)	.875

Table 13. Results from forward stepwise-regression analyses to explain variance in macroinvertebrate indices for the studied nonwadeable rivers in Wisconsin.

[r_s, Spearman correlation coefficient; R^2, coefficient of determination for the one-, two-, and three-variable models; no additional, no statistically significant ($p < 0.05$) terms]

Dependent variable	First variable	Second variable	Third variable
Species Richness (SPECIES)	Total Kjeldahl nitrogen	Clay deposits	Basin slope
r_s	-0.58	0.05	0.45
Accumulative R^2	.45	.58	.61
Mean pollution tolerance value (MPTV)	Total Kjeldahl nitrogen	Sand	Total nitrogen
r_s	.72	-.02	.36
Accumulative R^2	.52	.57	.66
Percent of individuals from order Ephemeroptera (% EPHEM)	Total Kjeldahl nitrogen	Grassland	Flow per unit area
r_s	-.59	-.44	.00
Accumulative R^2	.29	.38	.43
Hilsenhoff Biotic Index (HBI)	Total Kjeldahl nitrogen	(no additional)	(no additional)
r_s	.51		
Accumulative R^2	.29		
Percent of individuals from order Plecoptera (% PLEC)	Loam deposits	Total Kjeldahl nitrogen	Clay deposits
r_s	.26	-.69	-.45
Accumulative R^2	.14	.21	.26
Percent of individuals that are scrapers (% SCRAP)	Total Kjeldahl nitrogen	Permeability	Agriculture (row crop)
r_s	-.56	.02	-.49
Accumulative R^2	.16	.27	.65

RDA retained PP and TKN from the nutrient characteristics, SD and SCHL from the other water-quality characteristics, and AgRow % and basin slope from the environmental characteristics. RDA was then run again with only those key characteristics. RDA based on these six key characteristics and the use of multiple axes explained 61 percent of the variance in the macroinvertebrate indices ($p < 0.01$); however, the first axis alone accounted for 99 percent of the total explained variance. The strongest relations (farthest from a value of 0 on RDA axis 1) were between SPECIES and SD and basin slope (positive relations) and between SPECIES and PP, SCHL, TKN, and AgRow % (negative relations); strong but opposite relations were found between MPTV and HBI and these constituents (fig. 15). The percent forest (For %) had the opposite response as AgRow %. TP and TN, which were not included in the analysis, are displayed in a similar fashion to the selected variables on this figure without affecting the results: TP and TN corresponded strongly with the first RDA axis, similar to PP and TKN. Therefore, if these characteristics were used instead of PP and TKN, similar results would have been obtained.

Partial RDA was then used to determine the relative importance of nutrients, other water-quality characteristics, environmental characteristics, and interactions among categories in affecting the macroinvertebrate assemblages. These four categories explained 61 percent of the variance in the 14 macroinvertebrate-assemblage indices (fig. 16). Nearly all of the variance was explained by the interactions among variable categories (55 percent of the total variance or 89 percent of the explained variance). Environmental characteristics explained 4 percent of total variance (7 percent of the explained variance), and nutrients and other water-quality characteristics each explained only 1 percent of the total variance (2 percent of the explained variance for each). Therefore, nutrient concentrations by themselves explained only a small part of the total variance in the macroinvertebrate assemblages. About 39 percent of the total variance could not be explained by the characteristics in this study, and an additional 55 percent of the total variance could not be separated into a single category of characteristics.

Reference Values for the Macroinvertebrate Indices

The use of different approaches provides a range in estimated reference values for each of the macroinvertebrate indices. Reference values for the six macroinvertebrate indices most strongly related to nutrient concentrations (SPECIES, MPTV, %EPHEM, HBI, %PLEC, and %SCRAP) were determined by using the best 25th percentile based on data from all of the sites, the median value for sites considered minimally impacted (Reference sites with both TP and TN concentrations at or below the estimated reference concentrations), the worst 75th percentile for minimally impacted sites, and two variants of the regression approach (table 14). Six Reference sites had median TP concentrations at or below the 0.035-mg/L reference concentration and median TN concentrations at or below the 0.514-mg/L reference concentration (table 9).

The distributions of index values for the Reference sites are compared with the distribution of values for the 29 High sites (with median TP and TN concentrations above their respective upper 95-percent confidence limits for reference concentrations, 0.045 mg/L and 0.604 mg/L, respectively) (fig. 17 and tables 9 and 14). For all of these indices, there was a statistically significant ($p < 0.05$) difference between the medians from the Reference and High sites, except for %PLEC ($p = 0.08$) and SPECIES. Reference values based on the worst 75th percentile of the Reference sites were similar to the median of the Reference sites except for EPHEM%. The regression approach, based on the relation between water quality and Ag % and Urb %, gave estimated reference values similar to those from the 25th-percentile approach. %EPHEM and HBI, however, were only weakly related to changes in Ag % and Urb % ($0.06 > p < 0.08$) and %PLEC and %SCRAP were not significantly related ($p > 0.1$) to changes in Ag % and Urb %.

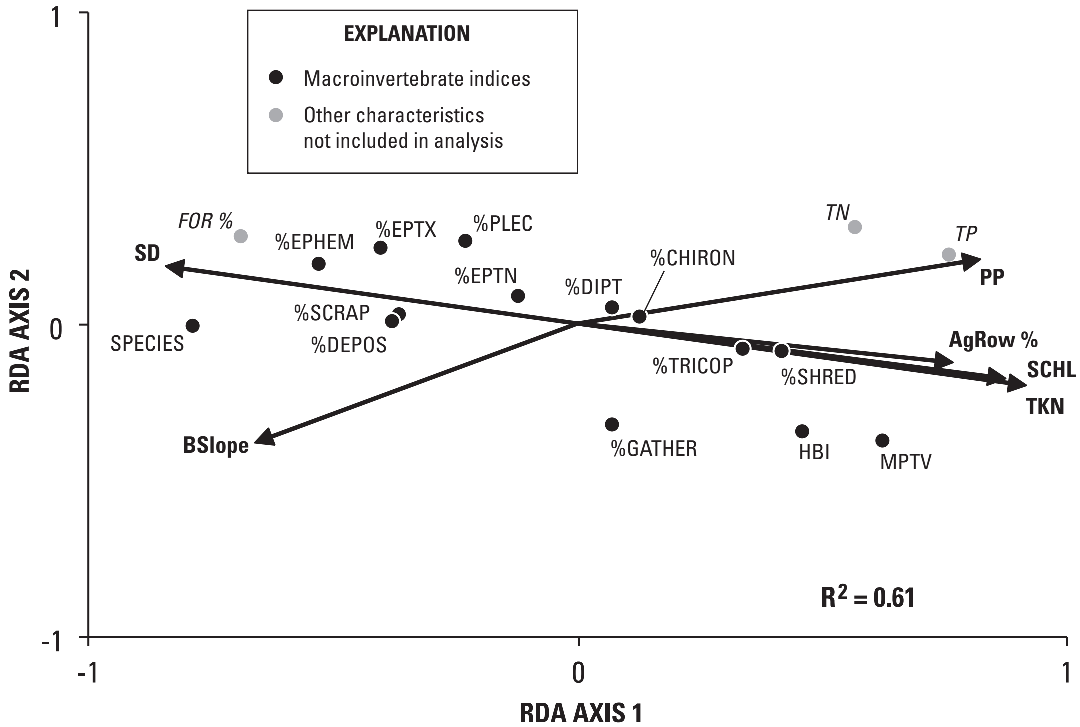

Figure 15. Redundancy analysis (RDA) results for macroinvertebrate indices and nutrients, other water-quality, and environmental characteristics: axis 2 scores are plotted as a function of axis 1 scores. Parameters describing each category were determined by forward-selection procedures in RDA. Water-quality and land-use abbreviations are defined in table 2 and macroinvertebrate abbreviations are defined in table 10.

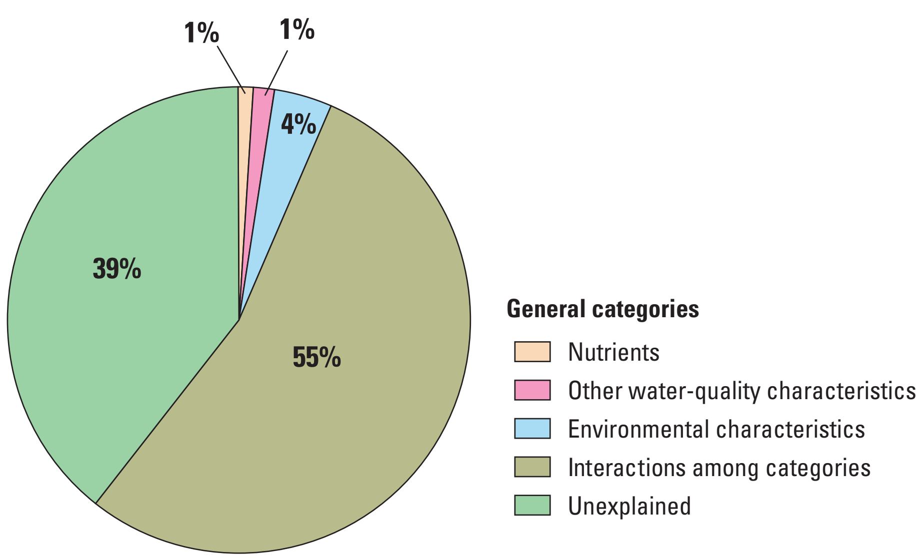

Figure 16. Percentages of variance in 14 macroinvertebrate-index values described by nutrients, other water-quality characteristics, environmental (land-use, soil, and surficial-deposit) characteristics, interactions (variance that cannot be explained by a single category), and unexplained variance for the studied nonwadeable rivers in Wisconsin. [%, percentage of total variance]

Table 14. Reference conditions for six macroinvertebrate indices for nonwadeable rivers in Wisconsin.

[nss, not statistically significant at $p < 0.1$]

Index	Abbreviation	Best 25th percentile for all data[a]	Median for Reference sites	Worst 75th percentile for Reference sites[a]	Regression approach (mean)	Regression approach (worst 95-percent confidence limit)[a]
Species richness	SPECIES	38	30 (nss)	29	38	34
Mean pollution tolerance value	MPTV	4.8	4.6	4.7	4.8	5.2
Percentage of individuals from order Ephemeroptera	%EPHEM	31.4	45.0	30.8	29.1 ($p = 0.08$)	20.0 ($p = 0.08$)
Hilsenhoff Biotic Index	HBI	4.9	4.5	4.7	5.0 ($p = 0.06$)	5.6 ($p = 0.06$)
Percentage of individuals from order Plecoptera	%PLEC	.8	.7 ($p = 0.08$)	.3	1.3 (nss)	.3 (nss)
Percentage of individuals that are scrapers	%SCRAP	12.5	17.4	11.8	13.7 (nss)	7.0 (nss)

[a] Higher values for all of these indices except for MPTV and HBI were shown to represent better water quality; therefore, for all indices except MPTV and HBI, the best 25th percentile for all of the data was obtained from the 75th percentile of all of the data, the worst 75th percentile for the Reference sites was obtained from the 25th percentile for the Reference sites, and the worst 95-percent confidence limit was obtained from the lower 95-percent confidence limits from the regression approach.

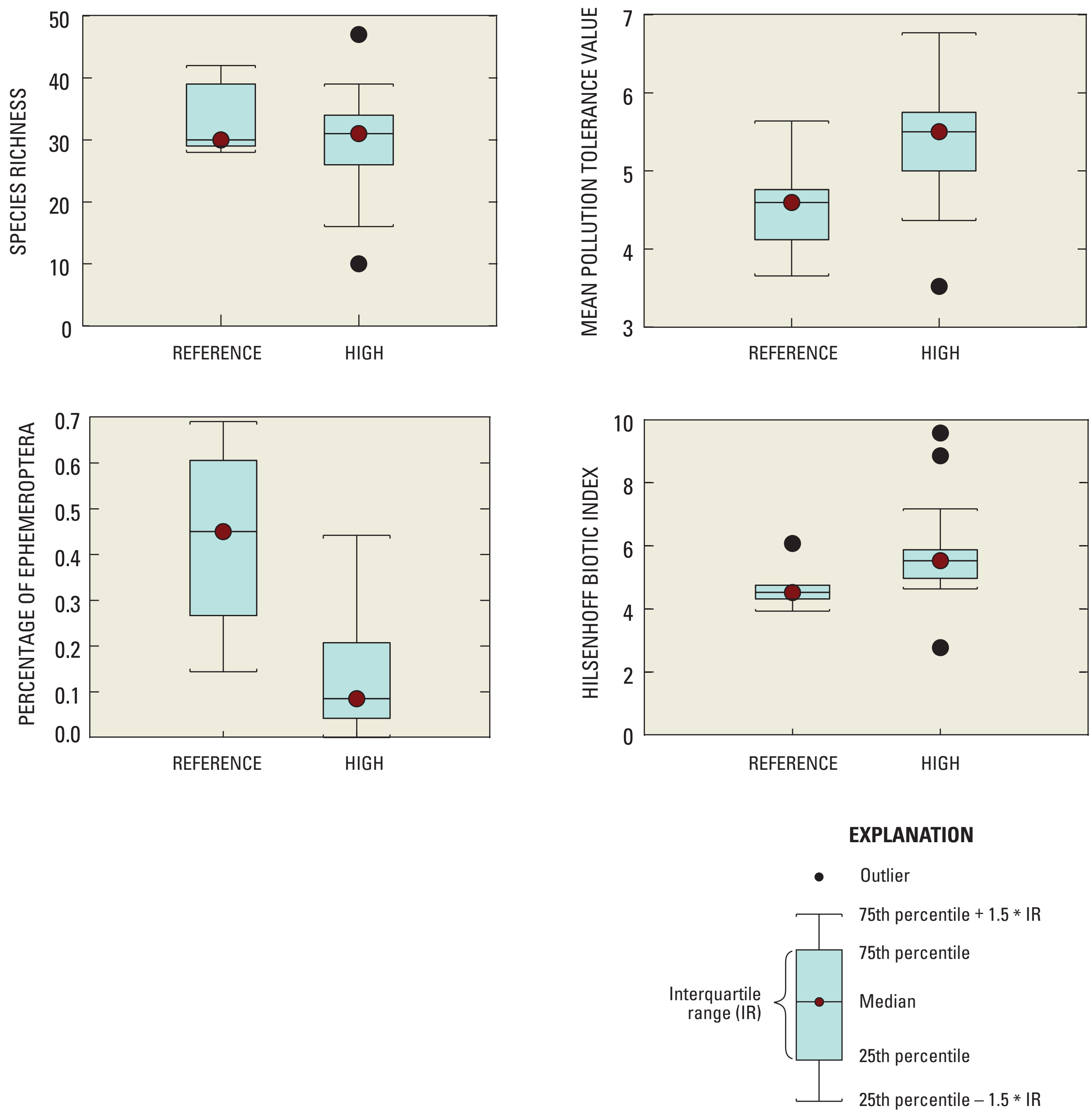

Figure 17. Species richness (SPECIES), mean pollution tolerance value (MPTV), percentage of individuals from the order Ephemeroptera (%EPHEM), and Hilsenhoff Biotic Index (HBI) values in Reference sites and High (nonreference) sites in the studied nonwadeable rivers in Wisconsin.

Fish Assemblages and Their Relations with Water-Quality and Environmental Characteristics





Wisconsin Department of Natural Resources personnel collecting fish with an electrofishing boat. Photos provided by John Lyons (Wisconsin Department of Natural Resources).

Eleven indices were computed to summarize the fish data, including indices describing the number of nonexotic species (1 index: #NATIVESP), number and percentage of riverine fish (2: #RIVERSP and %RIVERSP, respectively), number of sucker species and percentage by weight of round-bodied sucker species (2: #SUCKER and %SUCKER, respectively), number of species intolerant of degradation (1: #INTOL), weight per unit sampling effort (1: WPUE), percentage of fish that spawn over stony environments (1: %LITSPAWN), percentage of biomass that is composed of insectivores (1: %INSECT), percentage of fish with diseases or deformities (1: %DISEASE), and one overall index of biotic integrity for large rivers in Wisconsin (IBI; Lyons and others, 2001). The fish indices indicated a wide range of environmental quality for the 41 sites in the study (table 10, page 40). Fish IBI scores spanned a range from very poor to excellent (appendix 3), with most sites scoring good or better (IBI value of 60 or greater). All of the fish indices had a wide range of values; for example, #NATIVESP ranged from 4 to 27, #RIVERSP ranged from 0 to 23, WPUE ranged from 3.9 to 151 kg, and IBI ranged from 5 to 100. For all fish indices except %DISEASE, higher values are thought to represent better water quality. In general, fish assemblages in rivers in the southeast part of the State would normally be considered representative of poorer water quality than in rivers in other parts of the State; these rivers had lower IBI, %SUCKER, %RIVERSP, and #INTOL values (fig. 18). Many of the other fish indices did not exhibit strong regional patterns.

Relations with Individual Characteristics

Correlations

All of the fish indices were significantly correlated (r_s) with several nutrient constituents except WPUE (table 15). Six of the indices, however, were more strongly correlated with nutrient concentrations than the others (IBI, %SUCKER, #INTOL, %RIVERSP, #RIVERSP, and %LITSPAWN; listed in decreasing order of the strength of their relations). These indices were most significantly correlated with the three P constituents, TN, and TKN.

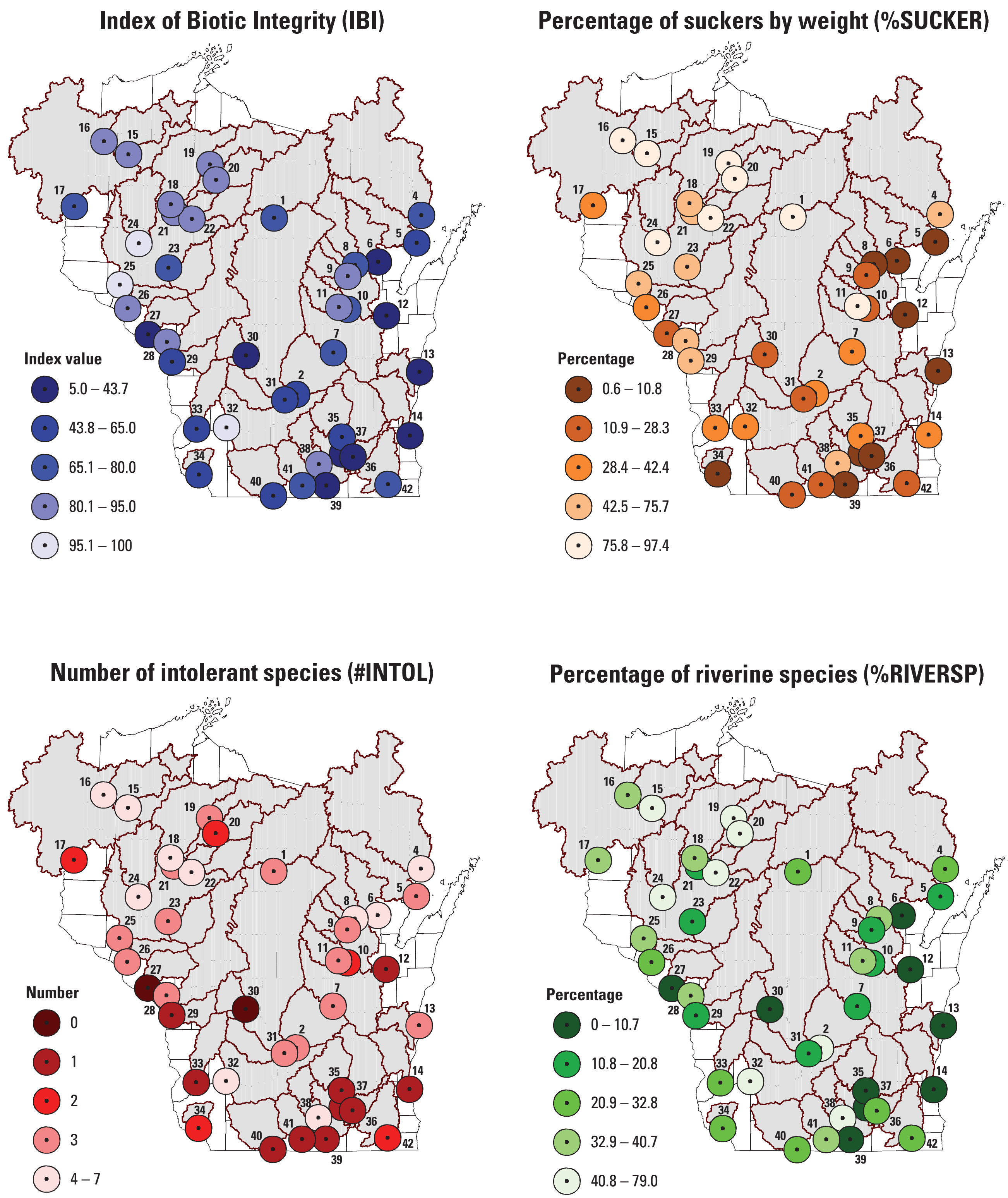

Figure 18. Distributions (quintiles) for four fish indices [Wisconsin large-river index of biotic integrity (IBI), percentage of suckers by weight (%SUCKER), number of intolerant fish species (#INTOL), and percentage of individuals that are riverine species (%RIVERSP)] for the studied nonwadeable rivers in Wisconsin.

Table 15. Spearman rank correlation coefficients (r_s) between fish-community indices and median water-quality, land-use, basin, and soil/surficial-deposit characteristics for the studied nonwadeable rivers in Wisconsin.

[All absolute values greater than 0.30 (Student's t-test; **bold black** values) and 0.48 (Bonferroni correction; **bold red** values) were significant at $p < 0.05$. All characteristics are defined in table 2 except fish indices, which are defined in table 10.]

Characteristic	#NATIVESP	#RIVERSP	#SUCKER	#INTOL	WPUE	%RIVERSP	%LITSPAWN	%SUCKER	%INSECT	%DISEASE[a]	IBI
Water-quality characteristics											
TP	-0.25	**-0.42**	**-0.32**	**-0.61**	-0.05	**-0.44**	**-0.43**	**-0.50**	**-0.41**	**0.30**	**-0.51**
DP	**-.30**	**-.36**	**-.33**	**-.63**	-.19	**-.39**	**-.30**	**-.41**	**-.35**	**.37**	**-.47**
PP	-.22	**-.40**	**-.30**	**-.56**	.00	**-.43**	**-.46**	**-.49**	**-.41**	.25	**-.50**
TN	-.09	**-.30**	-.20	**-.49**	-.08	**-.33**	**-.49**	**-.53**	**-.42**	.24	**-.43**
NO_3-N	.07	-.03	-.08	**-.36**	-.09	-.17	**-.35**	**-.46**	**-.37**	**.31**	-.28
NH_4-N	**-.31**	**-.44**	**-.45**	-.24	-.22	**-.31**	**-.37**	**-.43**	**-.44**	.13	**-.46**
TKN	-.20	**-.51**	**-.32**	**-.44**	.02	**-.43**	**-.68**	**-.51**	**-.45**	.20	**-.50**
SCHL	-.27	**-.44**	-.29	**-.41**	.22	**-.35**	**-.51**	-.28	-.24	.16	**-.37**
SD	.27	**.51**	**.31**	**.60**	.04	**.42**	**.52**	**.47**	**.38**	-.23	**.53**
SSC	-.15	**-.33**	-.23	**-.51**	-.05	**-.43**	**-.46**	**-.49**	**-.39**	.18	**-.46**
WTemp	**-.38**	**-.46**	-.28	**-.37**	.10	-.28	**-.40**	-.24	-.20	.19	**-.42**
SC	-.20	**-.42**	**-.31**	**-.49**	**-.34**	**-.45**	**-.60**	**-.63**	**-.54**	.18	**-.59**
pH	-.11	-.19	-.14	-.20	.12	-.25	**-.35**	-.23	-.11	.03	-.20
Color	-.07	-.13	-.02	.20	.04	-.01	.04	.14	.02	-.14	.08
Land-use characteristics											
Urb %	-.27	**-.48**	**-.33**	**-.46**	-.02	**-.34**	**-.53**	**-.39**	**-.37**	.17	**-.49**
AgRow %	-.22	**-.45**	**-.34**	**-.53**	-.14	**-.43**	**-.62**	**-.53**	**-.42**	.26	**-.55**
AgOther %	-.18	**-.32**	-.28	**-.52**	-.14	**-.41**	**-.52**	**-.54**	**-.44**	.28	**-.50**
Ag %	-.24	**-.42**	**-.30**	**-.55**	-.19	**-.43**	**-.62**	**-.58**	**-.48**	.24	**-.57**
Grass %	.06	-.12	-.16	-.29	.08	-.15	-.21	-.17	-.13	.27	-.14
WetO %	-.20	-.28	-.11	-.07	.16	.01	.03	.21	.13	.03	.04
WetF %	.15	.21	.14	**.38**	-.03	.16	.26	**.30**	.24	-.14	**.30**
Barren %	-.15	-.23	-.26	-.16	**.33**	.01	-.09	.05	.03	-.02	-.09
For %	.29	**.50**	**.36**	**.60**	.10	**.40**	**.55**	**.49**	**.41**	-.25	**.53**

Table 15. Spearman rank correlation coefficients (r_s) between fish-community indices and median water-quality, land-use, basin, and soil/surficial-deposit characteristics for the studied nonwadeable rivers in Wisconsin—Continued.

[All absolute values greater than 0.30 (Student's t-test; **bold black** values) and 0.48 (Bonferroni correction; **bold red** values) were significant at $p < 0.05$. All characteristics are defined in table 2 except fish indices, which are defined in table 10.]

Characteristic	#NATIVESP	#RIVERSP	#SUCKER	#INTOL	WPUE	%RIVERSP	%LITSPAWN	%SUCKER	%INSECT	%DISEASE[a]	IBI
Basin characteristics											
Area	0.11	0.18	0.24	0.17	-0.14	0.06	-0.01	0.05	0.05	0.06	0.11
ATemp	-.25	**-.45**	**-.34**	**-.58**	-.15	**-.44**	**-.59**	**-.51**	**-.45**	.20	**-.57**
PPT	-.14	-.26	-.17	**-.47**	.04	-.20	-.21	-.25	-.19	.09	**-.31**
Evap	-.18	**-.30**	-.24	**-.56**	-.03	-.29	**-.38**	**-.36**	-.29	.24	**-.42**
Runoff	.26	**.45**	**.36**	**.61**	.08	**.47**	**.51**	**.52**	**.49**	**-.32**	**.55**
Length	.15	.24	.24	**.31**	-.16	.07	-.01	.02	-.03	.03	.14
BSlope	.15	.18	.04	.23	-.06	-.03	.03	-.08	-.06	-.06	.05
Flow	**.30**	**.41**	.20	**.31**	-.08	.19	.26	.23	.23	-.06	**.33**
Soil/surficial-deposit characteristics											
SClay	-.27	**-.38**	-.24	**-.55**	-.14	**-.37**	**-.51**	**-.52**	**-.43**	.23	**-.53**
Erod	-.27	**-.31**	-.18	**-.42**	-.06	-.21	**-.33**	**-.34**	-.28	.15	**-.37**
OM	.02	.03	.12	.24	-.08	.17	.23	.25	.18	-.05	.17
Perm	.16	.16	.05	**.31**	.02	.20	.15	.29	.19	-.18	.24
SSlope	.16	**.38**	.25	.14	-.01	.24	**.37**	.17	.19	-.24	.23
NonGlac	-.08	-.03	.03	**-.44**	.06	-.09	-.11	-.17	-.13	.16	-.20
SDClay	.03	-.22	-.23	-.09	-.21	**-.33**	**-.59**	**-.49**	**-.50**	.02	**-.33**
Loam	.23	**.37**	**.31**	.27	.02	.17	-.22	-.07	-.04	.02	.16
Peat	-.06	.17	.22	.12	.23	**.38**	**.38**	**.46**	**.33**	.16	**.34**
Sand	-.17	-.11	.03	.14	.28	.22	.20	**.33**	.24	.19	.18
SG	.29	.20	.15	**.40**	-.12	.10	.01	-.04	-.01	-.12	.16

[a] For all of these fish indices except %DISEASE, higher index values are thought to represent better water quality than lower index values.

All of the indices (except %DISEASE) were negatively correlated with most nutrient constituents, although some of the correlations were not statistically significant. The #NATIVESP was only weakly correlated with the nutrient constituents, and WPUE was not statistically correlated with any nutrient constituent. Of the nutrient constituents, NO_3-N was significantly correlated with the fewest fish indices.

The six fish indices most strongly related to nutrient concentrations were also the indices most strongly correlated with SCHL, SD, and the land-use characteristics. These fish indices were most strongly correlated with Ag %, AgRow %, Urb %, and For %. High index values (better fish index values) were correlated with low Ag % and Urb % and high For %. IBI, #INTOL, %LITSPAWN, and %SUCKER were more strongly correlated with several basin characteristics (air temperature, runoff, and evaporation) and soil characteristics (clay content in the soils and surficial deposits) than were the other indices. In general, rivers with better fish index values had cool air temperatures, high runoff, and soils with low clay content; these characteristics are generally found in the northern part of the State with mixed and mostly forested areas (figs. 1A and 18).

Responses to Changes in Nutrient Concentrations

Responses of the four fish-assemblage indices most strongly correlated with nutrients (IBI, %SUCKER, #INTOL, %RIVERSP) are shown with respect to median TP and TN concentrations in figure 19, and the responses of the two indices most strongly related with nutrients (IBI and %SUCKER) are shown with respect to median DP, NO_3-N, NH_4-N, and TKN concentrations in figure 20. IBI and %SUCKER were chosen as the best fish indices and used for additional detailed investigation because correlation analyses indicated that they were the most responsive to differences in nutrient concentrations, and because they appeared to be most representative of the other fish indices. The LOESS-smoothing lines indicate a consistent decrease in all of these indices as nutrient concentrations increased (except for TN). All of the graphs show a wide range in index values at any nutrient concentration; however, at low nutrient concentrations, the measured indices had a slightly larger range in values than at higher nutrient concentrations. The variability at any nutrient concentration may indicate that factors in addition to nutrients are affecting the fish assemblages.

The LOESS-smoothing lines on the scatterplots between nutrient concentrations and fish-index values (figs. 19 and 20) indicate that, for some nutrient constituents, there is a nonlinear relation. To define the thresholds or largest breakpoints in these responses, regression-tree analyses were done (table 16). The ranges in the breakpoints in response to changes in nutrient concentrations were: 0.055 to 0.147 mg/L for TP, 0.035 to 0.081 mg/L for DP, 0.010 to 0.064 mg/L for PP, 0.634 to 1.965 mg/L for TN, 0.030 to 0.241 mg/L for NO_3-N, 0.016 to 0.051 mg/L NH_4-N, and 0.505 to 1.075 mg/L for TKN. All of the breakpoints for TP and TN were statistically significant at $p < 0.05$. These analyses indicated that the values of these six fish indices changed the most over a relatively narrow range in concentrations for each constituent. None of the breakpoints exceeded 0.15 mg/L for TP or 2.0 mg/L for TN, similar to the results for the macroinvertebrate indices. However, unlike the trends in the macroinvertebrate indices, at concentrations above even the highest threshold or breakpoint values, the biotic indices usually continued to degrade.

Effects of Multiple Characteristics on Fish Indices

Stepwise Regressions

Forward stepwise regressions were done with the median water-quality and environmental characteristics to determine which three characteristics best described the variance in the six fish indices most strongly correlated with nutrient concentrations (table 17). Models with more than three variables did not significantly increase the amount of variance explained. TP, TKN, air temperature, and runoff were the first variables incorporated into these models. Runoff is highly correlated with all of the nutrient constituents; when runoff was omitted from the analyses, TP or Ag % were the first variables incorporated into the models. Several other variables, mostly describing nutrient concentrations, land use, or soil type, were incorporated into the models as the second and third variables. With three variables, the models explained between 37 and 63 percent of the variance in the indices.

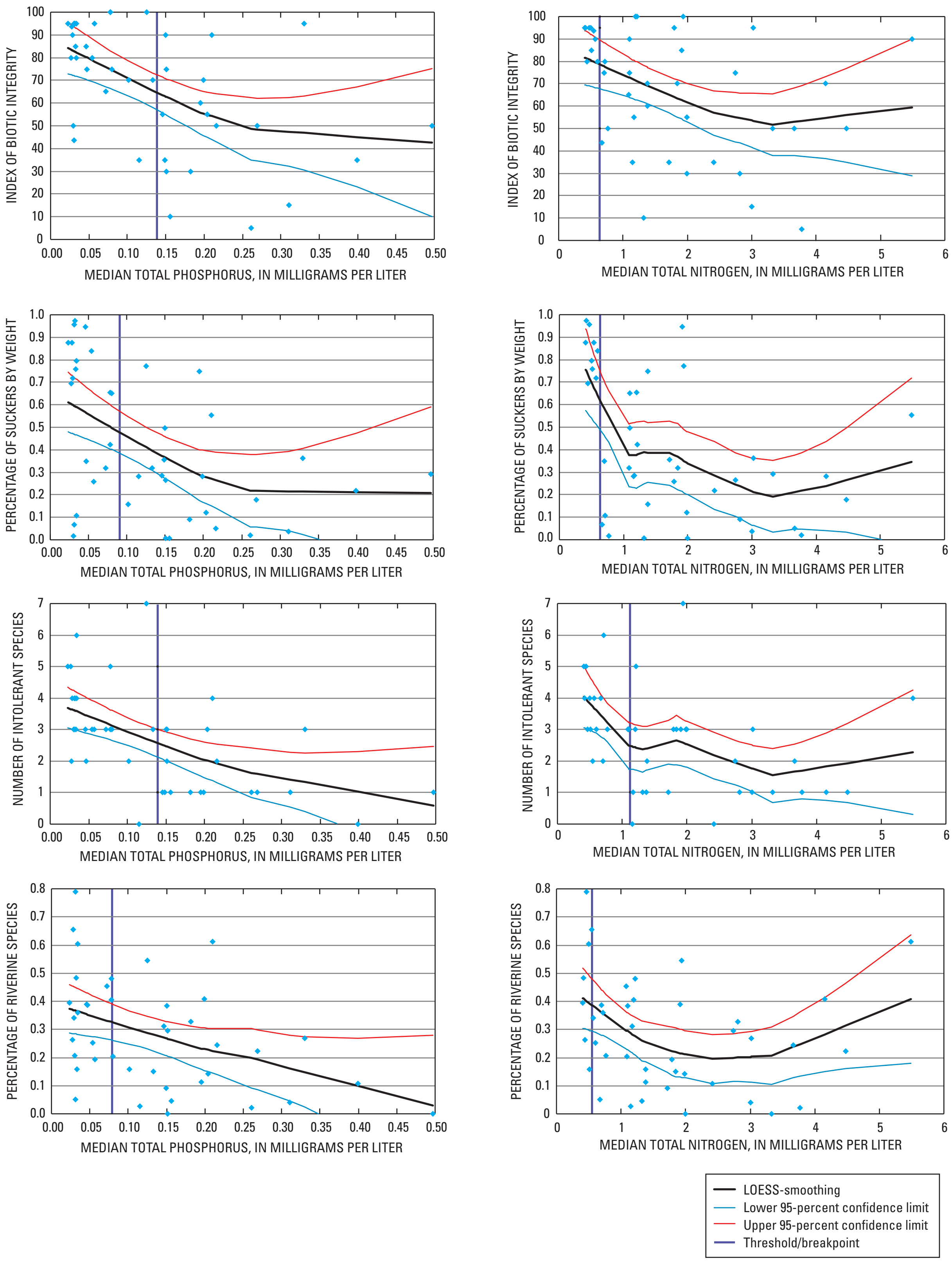

Figure 19. Wisconsin large-river index of biotic integrity (IBI), percentage of suckers by weight (%SUCKER), number of intolerant fish species (#INTOL), and percentage of individuals that are riverine species (%RIVERSP) as a function of total phosphorus (TP) and total nitrogen (TN) concentration for the studied nonwadeable rivers in Wisconsin. LOESS-smoothing lines with 95-percent confidence limits and computed thresholds in the response, identified by vertical lines, are given on each graph. The default parameter for the LOESS-smoothing lines was changed to 0.6 for the relation between TN and %SUCKER.

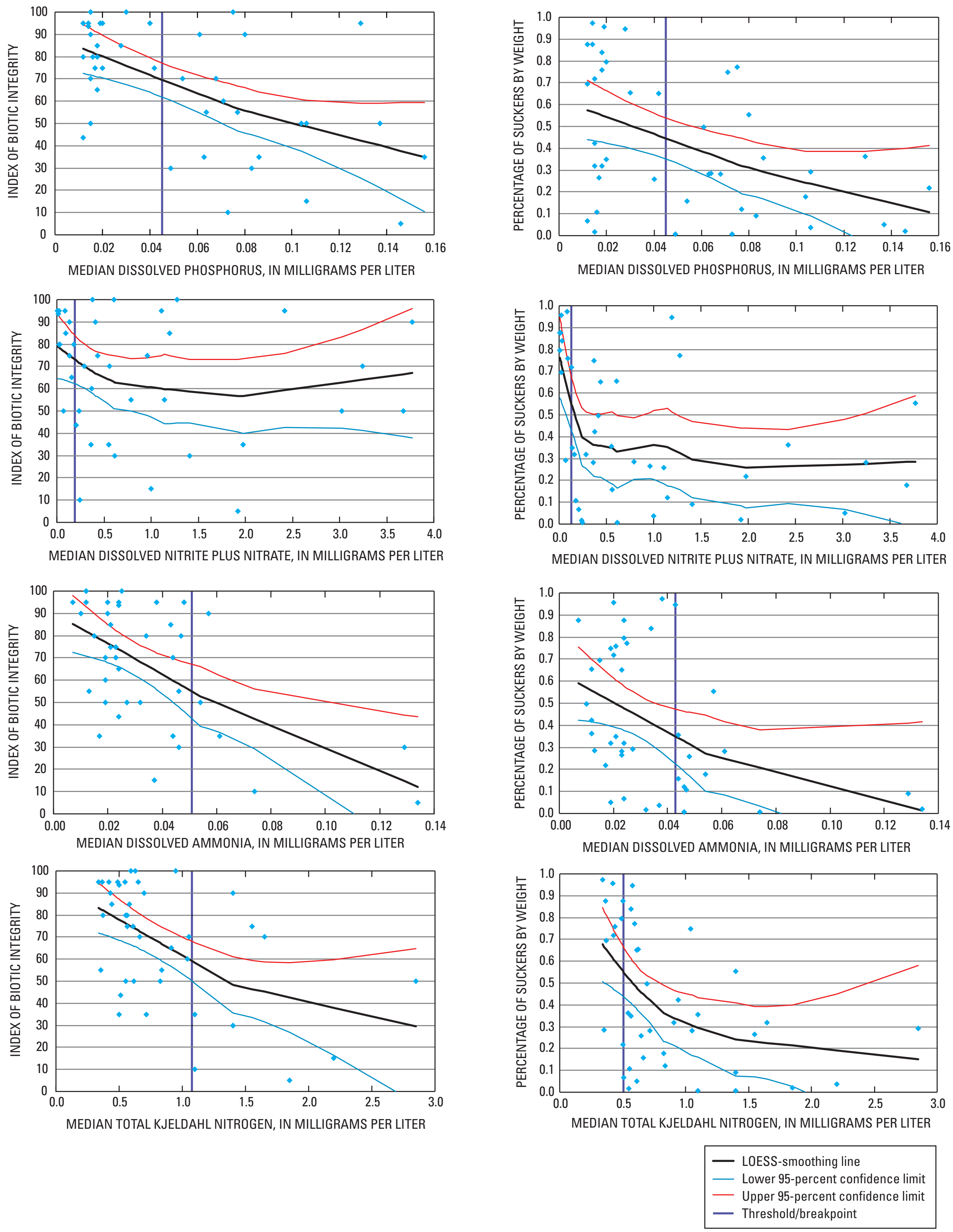

Figure 20. Wisconsin large-river index of biotic integrity (IBI) and percentage of suckers by weight (%SUCKER) as a function of dissolved phosphorus (DP), dissolved nitrite plus nitrate nitrogen (NO_3-N), dissolved ammonia nitrogen (NH_4-N), and total Kjeldahl nitrogen (TKN) concentrations for the studied nonwadeable rivers in Wisconsin. LOESS-smoothing lines with 95-percent confidence limits and computed thresholds in the response, identified by vertical lines, are given on each graph. The default parameter was changed to 0.6 for the LOESS-smoothing lines for the relation between dissolved nitrite plus nitrate (NO_3-N) and %SUCKER.

Table 16. Thresholds or breakpoints in the responses of fish indices to changes in nutrient concentrations for nonwadeable rivers in Wisconsin.

[All concentrations in milligrams per liter; nss, not statistically significant at $p < 0.05$]

Index	Total phosphorus	Dissolved phosphorus	Particulate phosphorus	Total nitrogen	Dissolved nitrite plus nitrate	Dissolved ammonia	Total Kjeldahl nitrogen
Wisconsin large-river index of biotic integrity (IBI)	0.139	0.045	0.051	0.635	0.191	0.051	1.075
Percentage of suckers by weight (%SUCKER)	.091	.045	.051	.634	.134	.043	.505
Number of intolerant species (#INTOL)	.139	.051	.051	1.125	.241	.051	.613
Percentage of individuals that are river species (%RIVERSP)	.079	.035	.015	.556	.030	.026	.505
Number of river species (#RIVERSP)	.147	.081	.064	1.965	.078 (nss)	.016	.993
Percentage of individuals that are lithophilic spawners (%LITSPAWN)	.055	.035	.019	.634	.147	.043	.505

Table 17. Results from forward stepwise-regression analyses to explain variance in fish indices for the studied nonwadeable rivers in Wisconsin.

[r_s, Spearman correlation coefficient; R^2, coefficient of determination for the one-, two-, and three-variable models]

Dependent variable	First variable	Second variable	Third variable
Wisconsin large-river index of biotic integrity (IBI)	Air temperature	Ammonia	Total nitrogen
r_s	-0.57	-0.46	-0.43
Accumulative R^2	.34	.51	.59
Percentage of suckers by weight (%SUCKER)	Runoff	Sand and gravel deposits	Grassland
r_s	.36	.03	-.16
Accumulative R^2	.35	.48	.57
Number of species considered intolerant of degradation (%INTOL)	Total phosphorus	Wetland (open)	Nonglacial deposits
r_s	-.61	-.07	-.44
Accumulative R^2	.34	.41	.49
Percentage of individuals that are river species (%RIVERSP)	Runoff	Wetland (forested)	Particulate phosphorus
r_s	.47	.16	-.43
Accumulative R^2	.22	.32	.37
Number of river species (#RIVERSP)	Total Kjeldahl nitrogen	Loam deposits	Flow per unit area
r_s	-.51	.37	.41
Accumulative R^2	.28	.35	.42
Percentage of individuals that are lithophilic spawners (%LITSPAWN)	Total Kjeldahl nitrogen	Clay deposits	Total agriculture
r_s	-.68	-.59	-.62
Accumulative R^2	.38	.54	.63

Redundancy Analysis

The forward-selection procedures in RDA were used to identify the key characteristics to include from each of three categories: nutrients, other water-quality characteristics, and environmental characteristics. The RDA analysis retained DP and TKN from the nutrient category; SC, SD, SCHL, and water color from the other water-quality category; and For % from the environmental-characteristics category. RDA was then run again with only these seven key characteristics. RDA based on these key characteristics and the use of multiple axes explained 44 percent of the variance in the fish indices ($p < 0.01$). The first two RDA axes accounted for 91 percent of the variance explained by the full model (all of the axes). The first axis alone accounted for 72 percent of the total explained variance. On RDA axis 1, almost all of the fish indices (except %DISEASE) were related positively with SD and For % and negatively with SCHL, TKN, DP, and SC (fig. 21). RDA axis 2 accounted for 19 percent of the total explained variance. On RDA axis 2, WPUE was related positively with SCHL, TKN, and water color. The other nutrient characteristics and AgRow %, when plotted in a similar manner, corresponded strongly with RDA axis 1 and were related to the fish indices similarly to DP, SCHL, SC, and TKN. Therefore, if these characteristics were used instead of DP, SCHL, and TKN, the results would have been similar.

Partial RDA was then used to determine the relative importance of nutrients, other water-quality characteristics, environmental characteristics, and interactions among categories in affecting the fish assemblages (11 fish indices). The same characteristics found with the forward variable-selection procedure were used to describe each category of characteristics. These 4 categories explained 45 percent of the variance in the 11 fish indices (fig. 22). Of the total variance, 11 percent was described by the nutrients alone (25 percent of the explained variance), 17 percent by the other water-quality characteristics alone, and 3 percent by the environmental characteristics alone. About 55 percent of the total variance could not be explained with the characteristics in this study, and an additional 14 percent of the total variance could not be separated into a single category of characteristics. Although the selected characteristics explained less of the variance in the fish indices than in the macroinvertebrate indices, nutrients by themselves explained much more of the total variance in the fish indices than the macroinvertebrate indices.

Reference Values for the Fish Indices

The use of different approaches provides a range in estimated reference conditions for the fish indices. Reference values for the six fish indices most related to nutrient concentrations (IBI, %SUCKER, #INTOL, %RIVERSP, #RIVERSP, and %LITSPAWN; table 18) were determined by using the same approaches that were used to determine reference conditions for the macroinvertebrate indices. Median values for the Reference sites or minimally impacted sites (sites with both reference TP and reference TN concentrations) were higher than those estimated by the other approaches for all of the indices except for #RIVERSP. The distributions of index values for the 6 Reference sites are compared with the distribution of values for the 29 High sites for the 4 fish indices most strongly related to nutrient concentrations in figure 23. For each of these indices, the median values for the Reference sites were significantly higher than those for the High sites. The other approaches provided similar reference conditions for all of the fish indices (table 18).

Multiparameter Biotic Indices to Estimate Nutrient Concentrations in Nonwadeable Rivers

One goal of this study was to estimate nutrient concentrations in rivers from the biotic data. Individual relations between specific biotic indices and TP and TN concentrations explained 45 and 35 percent of the variance or less, respectively (SCHL explained the most variance in TP and TN concentrations). Combining several biotic indices, however, was expected to improve these relations. To develop multiparameter indices to estimate TP and TN concentrations in nonwadeable rivers, the biotic indices found to be most strongly related to differences in nutrient concentrations were input into forward stepwise-regression analyses. Thirteen biotic indices were included in this analysis: one describing the amount of suspended algae in the stream (chlorophyll *a* concentrations: Log SCHL), six describing the macroinvertebrate assemblages (table 12), and six describing the fish assemblages (table 16).

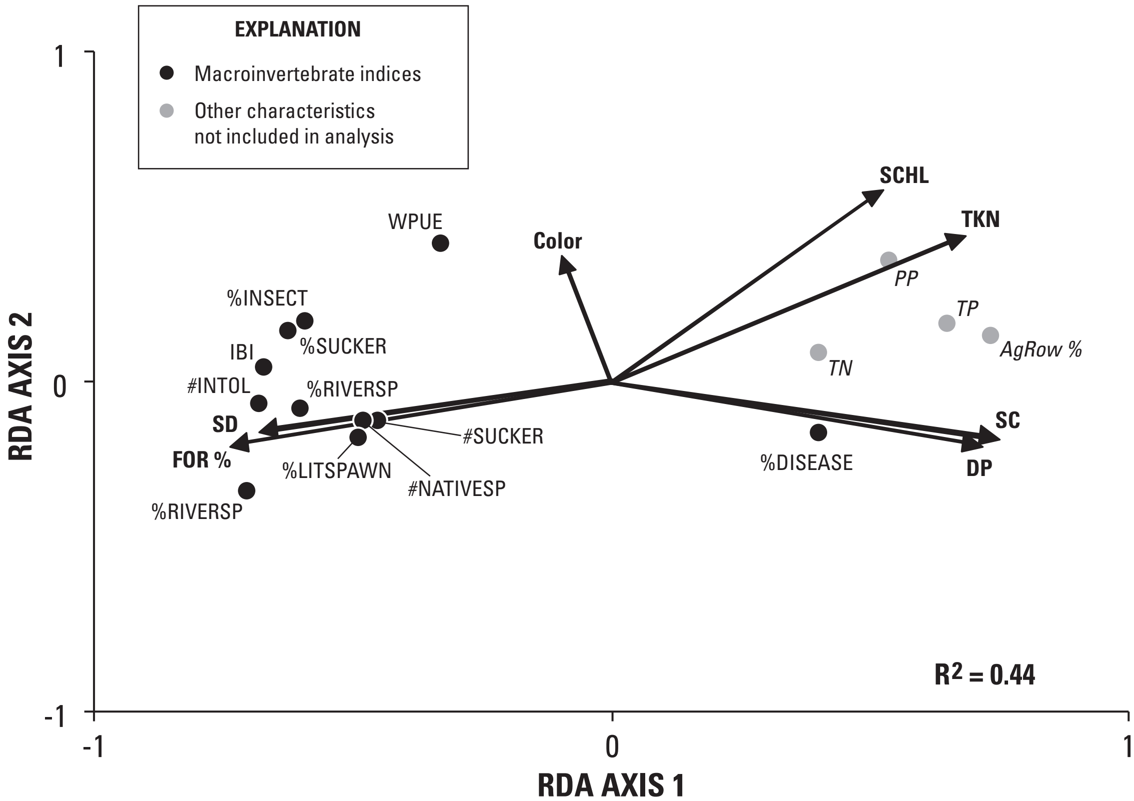

Figure 21. Redundancy analysis (RDA) results for fish indices and nutrients, other water-quality, and environmental characteristics: axis 2 scores are plotted as a function of axis 1 scores. Parameters describing each category were determined by forward-selection procedures in RDA. Water-quality and land-use abbreviations are defined in table 2 and fish abbreviations are defined in table 10.

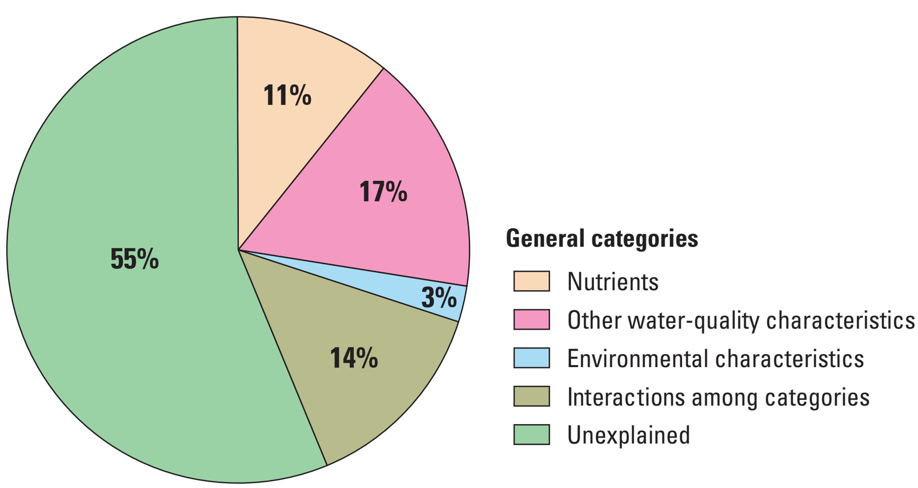

Figure 22. Percentages of variance in 11 fish-index values described by nutrients, other water-quality characteristics, environmental (land-use, soil, and surficial-deposit) characteristics, interactions (variance that cannot be explained by a single category), and unexplained variance for the studied nonwadeable rivers in Wisconsin. [%, percentage of total variance]

Table 18. Reference conditions for six fish indices for nonwadeable rivers in Wisconsin.

[nss, not statistically significant at $p < 0.1$]

Index	Abbreviation	Best 25th percentile for all data[a]	Median of Reference sites	Worst 75th percentile for Reference sites[a]	Regression approach (mean)	Regression approach (worst 95-percent confidence limit)[a]
Wisconsin large-river index of biotic integrity	IBI	90.0	95.0	87.5	87.0	74.9
Percentage of suckers by weight	%SUCKER	71.8	83.5	76.6	66.6	53.1
Number of species considered intolerant of degradation	#INTOL	3	4	3.3	3.7	3.0
Percentage of individuals that are river species	%RIVERSP	39.4	43.8	29.7	38.9	29.4
Number of river species	#RIVERSP	7	6 (nss)	4.3	6.4	5.0
Percentage of individuals that are lithophilic spawners	%LITSPAWN	73.1	87.6	84.5	73.5	62.7

[a] Higher values for all of these indices were shown to represent better water quality; therefore, the best 25th percentile for all of the data was obtained from the 75th percentile of all of the data, and the worst 75th percentile for the Reference sites was obtained from the 25th percentile for the Reference sites, and the worst 95-percent confidence limits was obtained from the lower 95-percent confidence limits from the regression approach.

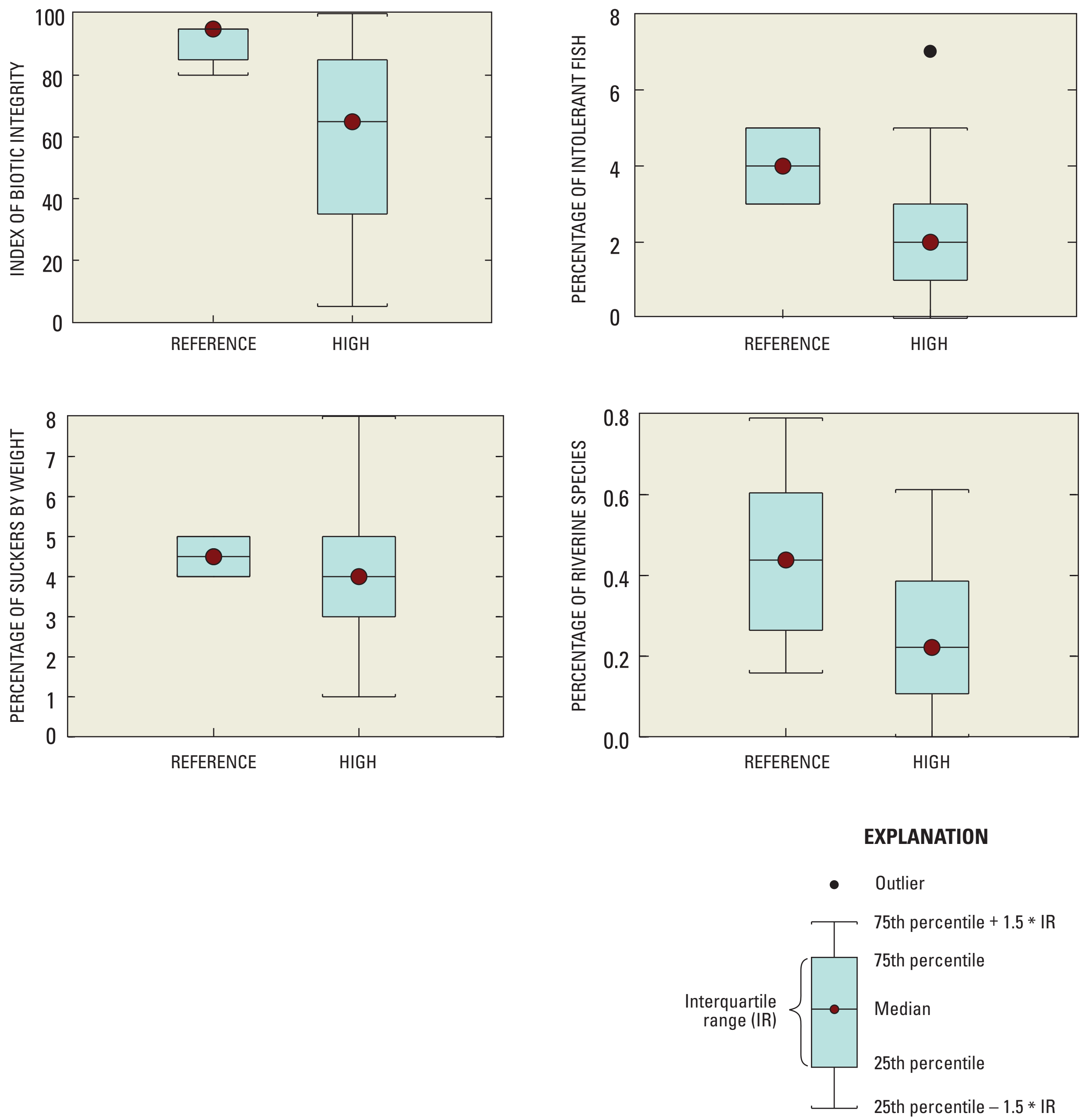

Figure 23. Wisconsin large-river index of biotic integrity (IBI), percentage of suckers by weight (%SUCKER), number of intolerant fish species (#INTOL), and percentage of individuals that are riverine species (%RIVERSP) in Reference sites and High (nonreference) sites in the studied nonwadeable rivers in Wisconsin.

The three-parameter model to estimate TP concentrations in nonwadeable rivers included indices describing the amount of suspended algae (Log SCHL), the fish assemblage (#INTOL), and the macroinvertebrate assemblage (%PLEC). This model explained 63 percent of the variance in TP concentrations (table 19, fig. 24A). The three-parameter model to estimate TN concentrations included indices describing the amount of suspended algae (Log SCHL), the fish assemblage (%SUCKER), and the macroinvertebrate assemblage (MPTV). This model explained 51 percent of the variance in TN concentrations (table 19, fig. 24B). Models with more than three variables did not significantly increase the amount of explained variance.

The regression equations described in table 19 were then used to develop multiparameter biotic indices to estimate TP and TN concentrations in nonwadeable rivers. The indices were developed (by including additional coefficients) to provide values ranging from 1 to 10, with 1 representing the lowest TP and TN concentrations and 10 representing the highest concentrations.

The Biotic Index of total P (BIP) is computed as

$$\text{BIP} = 5.0 \times (-1.167 + 0.428 \text{ Log SCHL} - 0.125 \text{ \#INTOL} + 5.601 \text{ \%PLEC}) + 10.0, \text{ and} \tag{12}$$

the Biotic Index of total N (BIN) is computed as

$$\text{BIN} = 6.67 \times (0.764 + 0.394 \text{ Log SCHL} - 0.578 \text{ \%SUCKER} - 0.151 \text{ MPTV}) + 4.0. \tag{13}$$

BIP and BIN estimated the measured Log median TP and TN concentrations equally well over the range of concentrations measured in this study (fig. 24); however, BIP estimated TP concentrations better than the BIN estimated TN concentrations (63 percent of the variance in TP explained by the BIP compared to 51 percent of the variance in TN explained by the BIN). The difference in the predictability of these indices was consistent with most of the biotic indices being more strongly correlated with TP concentrations than with TN concentrations. This difference in predictability indicates that TP concentrations are more important than TN concentrations in affecting the biotic communities over the range in nutrient concentrations measured in this study.

Summary of Results for Nonwadeable Rivers and Wadeable Streams

Excessive nutrient input from point and nonpoint sources into streams and rivers is frequently associated with degraded water quality. Point-source discharges of nutrients are fairly constant and are controlled by the U.S. Environmental Protection Agency's (USEPA's) National Pollutant Discharge Elimination System. To reduce input from agricultural areas, performance standards and regulations for croplands and livestock operations are being proposed by various States. In addition, the USEPA is establishing regionally based nutrient criteria that can be refined by each State to determine whether actions are needed to improve water quality. More confidence in the environmental benefits of the proposed standards and nutrient criteria would be possible with an improved understanding of the biotic responses to a range of nutrient concentrations in different environmental settings.

Table 19. Results from forward stepwise-regression models to explain variances in total phosphorus and total nitrogen concentrations with biotic indices for the studied nonwadeable rivers in Wisconsin.

[All regressions were on log-transformed concentrations; *r*, Pearson correlation coefficient; R^2, coefficient of determination for the one-, two-, and three-variable models; SCHL, suspended chlorophyll *a* concentration; see table 10 for definitions of abbreviations and units for each biotic index]

	Constant	First variable	Second variable	Third variable
Total phosphorus (TP)		Log SCHL	#INTOL	%PLEC
Coefficient	-1.167	0.428	-0.125	5.601
r		.67	-.58	-.08
Accumulative R^2		.44	.58	.63
Total nitrogen (TN)		Log SCHL	%SUCKER	MPTV
Coefficient	0.764	.394	-.578	-.151
r		.59	-.55	.37
Accumulative R^2		.32	.46	.51

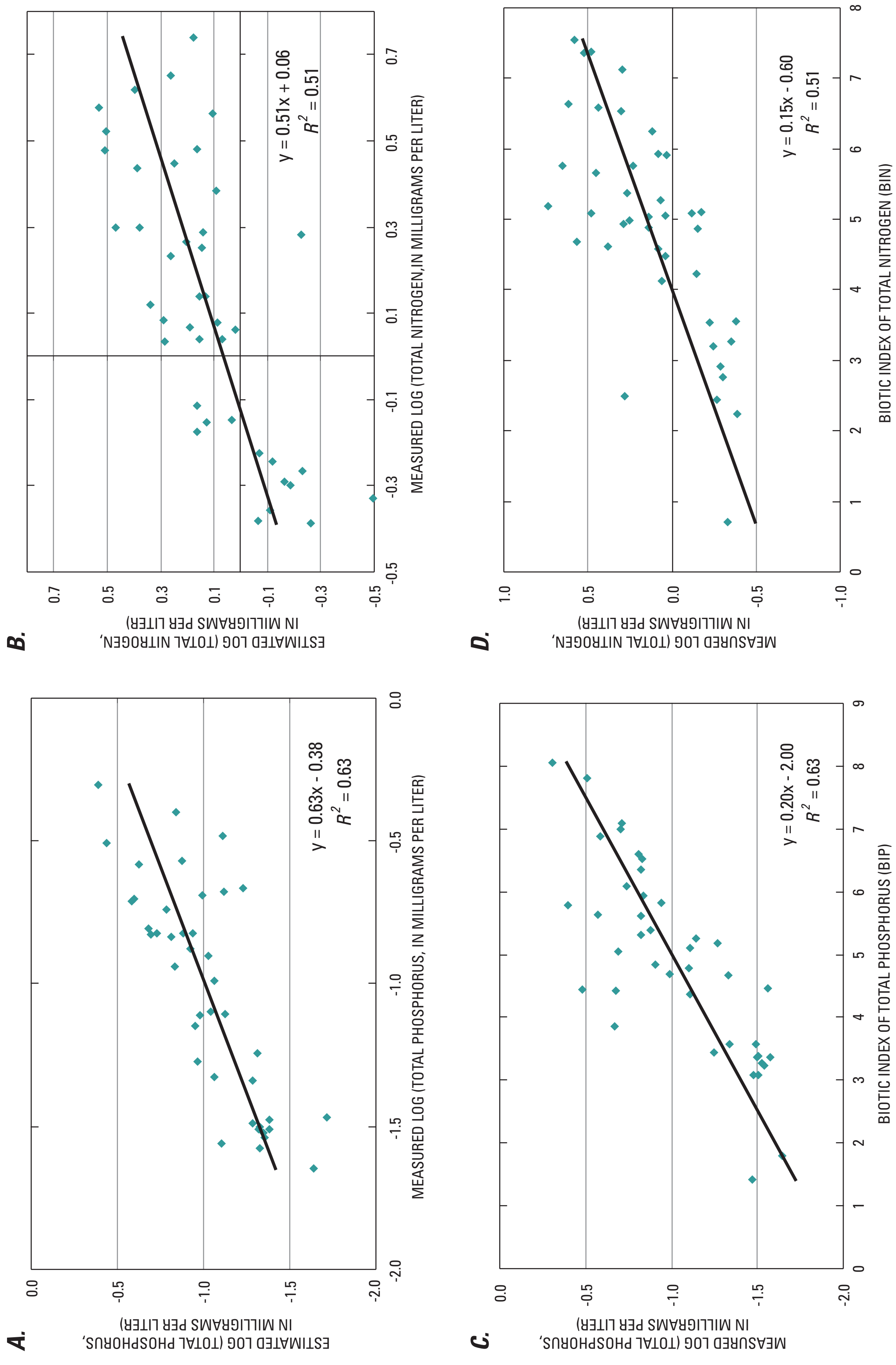

Figure 24. Measured and estimated ***A***, total phosphorus and ***B***, total nitrogen concentrations (logarithm to base 10 transformation) for the three-parameter regression models; ***C***, measured phosphorus concentrations as a function of Biotic Index of total Phosphorus (BIP) values; and ***D***, measured nitrogen concentrations as a function of Biotic Index of total Nitrogen (BIN) values, with regression equations and coefficients of determination (R^2) for the studied nonwadeable rivers in Wisconsin.

To provide the information needed by water-resource managers to develop regionally based nutrient criteria for Wisconsin's streams and rivers, the U.S. Geological Survey (USGS) and Wisconsin Department of Natural Resources (WDNR) collected water-quality and biotic data in 240 wadeable streams and 42 nonwadeable rivers throughout Wisconsin to (1) describe how nutrient concentrations and biotic-community structure differ throughout the State, (2) determine which environmental characteristics are most strongly related to the distribution of nutrient concentrations, (3) determine reference water-quality and biotic conditions for streams and rivers throughout the State, (4) determine how biotic communities respond to differences in nutrient concentrations, (5) determine the best regionalization scheme to describe the patterns in reference conditions and the corresponding responses in water quality and the biotic communities (primarily for smaller streams), and (6) develop new indices to estimate nutrient concentrations from a combination of biotic indices. This report primarily describes the results for the nonwadeable rivers, but in this section, the results are compared with the results for the wadeable streams.

Water Quality

Nutrient concentrations were consistently highest in the nonwadeable rivers in the southern and western parts of the State. In general, nutrient concentrations in the wadeable streams also had this same pattern; however, the wadeable streams, such as those in the central part of the State, had many isolated areas with high concentrations. Nutrient concentrations in the nonwadeable rivers typically had a smaller range in concentrations (primarily because of lower maximum values) than the wadeable rivers. These differences reflect the fact that the basins of the larger rivers integrate many small streams and the more predominant land uses, and that wadeable streams are more likely to be affected by local point sources. The basins of the nonwadeable rivers may have included more point sources, but their effects were diluted by inflow from many other tributaries in the basin.

The overall median and mean TP and TN concentrations were similar in the nonwadeable rivers and wadeable streams. The median TP and TN concentrations in the nonwadeable rivers were 0.109 and 1.268 mg/L, respectively (table 2), compared to 0.085 and 1.695 mg/L in the wadeable streams (table 2 in Robertson and others, 2006a). The proportion in different forms of the nutrients was quite different. In the nonwadeable rivers, most of the nutrients were in particulate forms (approximately 55–75 percent for P and 45–80 percent for N), whereas in wadeable streams approximately 30–45 percent of the P and 20–45 percent of the N were in particulate forms. In nonwadeable rivers, N was about equally partitioned between NO_3-N and TKN; however, in the wadeable streams, there was about twice as much NO_3-N as TKN.

The nonwadeable rivers had much higher SCHL concentrations and lower clarity than the wadeable streams. The overall median and mean SCHL concentrations in the nonwadeable rivers were 7.31 and 18.47 μg/L (table 2), respectively, compared to only 2.27 and 3.23 μg/L, respectively, in the wadeable streams (table 2 in Robertson and others, 2006a). The overall median and mean SDs in the nonwadeable rivers were 60.5 and 71.8 cm, respectively, compared to 112 and 97.3 cm, respectively, in the wadeable streams. The lower concentrations of dissolved nutrients in the nonwadeable streams, higher SCHL, and lower clarity indicate that more of the nutrients are taken up by the algae in the larger rivers.

The watersheds of the nonwadeable rivers usually continued across several areas defined by the regionalization schemes evaluated for the wadeable streams (environmental phosphorus zones and Omernik ecoregions); for this reason, regional differences in the relations between nutrient concentrations and biotic responses were not examined in detail. The only regional response found in large rivers was for SCHL. Concentrations of SCHL in rivers in the southwestern part of the State (Driftless Area ecoregion) were lower than would be expected given the nutrient concentrations, possibly because of the relatively high SSC concentrations in rivers in that area.

The water quality of the nonwadeable rivers was significantly related to many of the same environmental characteristics as the water quality in the wadeable streams; however, in general, the correlations were much stronger for the nonwadeable rivers. The water quality of small streams and large rivers was strongly related to the land use/land cover in the basin [percentage of forested (For %) and agricultural (Ag %) land], runoff from the basin, and the clay content, erodibility, and permeability of the soil.

More variability in the water-quality characteristics (TP, TN, SCHL, and SD) was explained by the environmental characteristics for the nonwadeable rivers (74 percent; fig. 7) than for the wadeable streams (43 percent). Concentrations of TP and TN in the nonwadeable rivers explained 45 and 35 percent, respectively, of the variance in Log SCHL concentrations and 77 and 50 percent of the variance in SDs, respectively (fig. 10). These percentages are higher than the percentages explained for small streams (10 to 28 percent for SCHL and SD; fig. 7 in Robertson and others, 2006a). Although nutrient concentrations by themselves explained only a small part of the variance in SCHL concentrations and SDs in the nonwadeable rivers and wadeable streams (based on redundancy analyses), nutrients by themselves explained more of the total variance in the nonwadeable rivers (about 20 percent of the variance in SCHL and SDs; fig. 8) than in the wadeable streams (only about 12 percent). This, again, indicates that nutrients are more important for larger nonwadeable rivers than for smaller wadeable streams.

The reference water-quality conditions in the nonwadeable rivers (table 20) are similar to those in the wadeable streams (table 22 in Robertson and others, 2006a). The best estimates of median reference TP and TN concentrations in the nonwadeable rivers (regression approach) were 0.035 and 0.514 mg/L, respectively, compared to 0.03–0.04 and 0.4–0.7, respectively, for the wadeable streams. The range in reference concentrations for the wadeable streams was the result of subdividing the State into areas with high clay-content soils (Zone 3; fig. 1) and the rest of the State. The best estimate of a median reference SCHL concentration for the nonwadeable rivers was 3.95 μg/L, which is higher than that for the wadeable streams (1.0 to 1.7 μg/L). The best estimate of a median reference SD for rivers and streams was about 110 cm.

Reference values from this study are similar to those defined by the USEPA for nutrient ecoregion 7 (southern part of the State), but higher than those defined for nutrient ecoregion 8 (northern part of the State). The USEPA defined reference TP and TN concentrations for nutrient ecoregion 7 as 0.033 and 0.54 mg/L, respectively, and for nutrient ecoregion 8 as 0.010 and 0.20–0.38 mg/L, respectively. The lower values defined by the USEPA for the northern part of the State were probably a result of their values being estimated with the 25^{th}-percentile approach, whereas most of the watersheds of streams and rivers in those areas are dominated by forest, and probably less than 25 percent of the streams and rivers were substantially affected by anthropogenic factors. The reference SCHL concentration found in this study (3.8–3.9 μg/L) is close to those defined by the USEPA using the Trichromatic Method of analysis: 5.8 μg/L for the southern part of the State and 4.3 μg/L for the northern part of the State.

Response in the Biotic Communities

In general, biotic communities in wadeable streams and nonwadeable rivers in the southern half of the State, especially the southeastern part, were representative of poorer water quality than in the streams and rivers throughout the rest of the State. Nutrient concentrations and probably other factors associated with agriculture and urbanization that affect the biotic community were highest in these southern streams and rivers.

Reference conditions for the macroinvertebrate and fish indices that were most strongly related to differences in nutrient concentrations are listed in table 20. For each biotic category, the indices are listed in decreasing order of the strength of the relation with differences in nutrient concentrations [for macroinvertebrates, species richness (SPECIES) was most strongly related to nutrients, and for fish, the IBI was the most strongly related]. It has also been suggested that best 25^{th} percentile of all data or the upper 75^{th} percentile for a subset of sites thought to be minimally impacted for a defined area may represent the reference condition (U.S. Environmental Protection Agency, 2000a). The values for each biotic index at the best 25^{th} percentile of all the data and the worst 75^{th} percentile of the subset of streams thought to be minimally impacted are listed in table 20.

Changes in biotic indices as nutrient concentrations increase or decrease indicate that nutrients in wadeable streams and nonwadeable rivers have direct or indirect effects on the composition of the biotic community. The responses of most biotic indices to increases in nutrient concentrations in large nonwadeable rivers were nonlinear, with a broad range of values at all nutrient concentrations. The ranges of values for most macroinvertebrate indices were broad and of similar magnitude at all nutrient concentrations; in contrast, the ranges of values of many fish indices were broader at low nutrient concentrations and narrower, indicative of consistently poor conditions, at high nutrient concentrations (wedge-shaped response). In comparison, the responses of most biotic indices in small wadeable streams were broad at low nutrient concentrations and narrow at high nutrient concentrations.

Table 20. Reference conditions for water-quality constituents and biotic indices for nonwadeable rivers in Wisconsin.

[nss, not statistically significant at $p < 0.1$; mg/L, milligram per liter; µg/L, microgram per liter; cm, centimeter; >, greater than]

Constituent/index	Abbreviations	Regression approach		Percentile approach	
		Median reference	Worst 95-percent confidence limit	Best 25th percentile for all data	Worst 75th percentile for Reference sites
Water-quality constituents					
Total phosphorus (mg/L)	TP	0.035	0.045	0.034	--
Dissolved phosphorus (mg/L)	DP	.016	.021	.017	--
Particulate phosphorus (mg/L)	PP	.018	.025	.018	--
Total nitrogen (mg/L)	TN	.514	.604	.670	--
Dissolved nitrite plus nitrate (mg/L)	NO_3-N	.061	.107	.132	--
Dissolved ammonia (mg/L)	NH_4-N	.022	.022	.019	--
Total Kjeldahl nitrogen (mg/L)	TKN	.434	.524	.500	--
Suspended chlorophyll *a* (µg/L)	SCHL	3.9	6.2	3.8	3.8
Secchi-tube depth (cm)[a]	SD	110	96	>120	>120
Suspended sediment (mg/L)	SSC	3.2	4.9	4.0	2.8
Macroinvertebrate indices					
Species richness[a]	SPECIES	38	34	38	29
Mean pollution tolerance value	MPTV	4.8	5.2	4.8	4.7
Percentage of individuals from order Ephemeroptera[a]	%EPHEM	29.1 ($p = 0.08$)	20.0 ($p = 0.08$)	31.4	30.8
Hilsenhoff Biotic Index	HBI	5.0 ($p = 0.06$)	5.6 ($p = 0.06$)	4.9	4.7
Percentage of individuals from order Plecoptera[a]	%PLEC	1.3 (nss)	.3 (nss)	.8	.3
Percentage of individuals that are scrapers[a]	%SCRAP	13.7 (nss)	7.0 (nss)	12.5	11.8
Fish indices					
Wisconsin large-river index of biotic integrity[a]	IBI	87.0	74.9	90.0	87.5
Percentage of suckers by weight[a]	%SUCKER	66.6	53.1	71.8	76.6
Number of species considered intolerant of degradation[a]	#INTOL	3.7	3.0	3.0	3.3
Percentage of individuals that are river species[a]	%RIVERSP	38.9	29.4	39.4	29.7
Number of river species[a]	#RIVERSP	6.4	5.0	7.0	4.3
Percentage of individuals that are lithophilic spawners[a]	%LITSPAWN	73.5	62.7	73.1	84.5

[a] Higher values for this constituent/index was shown to represent better water quality; therefore, the worst 95-percent confidence limit was obtained from the lower 95-percent confidence limit from the regression approach, the best 25th percentile for all of the data was obtained from the 75th percentile of all of the data, and the worst 75th percentile for the Reference sites was obtained from the 25th percentile for the Reference sites.

This wedge-shaped response to increases in nutrient concentrations is common for relations between biotic indices and anthropogenic factors, such as the percentage of urban land use in an area (Wang and others, 2001; Wang, 2003). The wedge-shaped response indicates that at low nutrient concentrations, factors in addition to nutrients limit the health of the biotic communities, whereas in streams and rivers with high nutrient concentrations, nutrients or factors correlated with high nutrient concentrations may be the predominant factors affecting the biotic communities (Cade and others, 1999).

Changes in the biotic communities of wadeable streams and nonwadeable rivers were more strongly related to changes in TP concentrations than to changes in TN concentrations. Because the relations between nutrient concentrations and most biotic indices were nonlinear, thresholds or breakpoints in the responses were determined (table 21). A threshold represents the concentration at which the biotic community changes most rapidly and, therefore, represents a critical concentration with ecological significance. The thresholds in the responses to changes in TP concentrations were at slightly higher concentrations for nonwadeable rivers (0.034–0.150 mg/L) than for wadeable streams (0.04–0.09 mg/L; table 23 in Robertson and others, 2006a). These thresholds are only slightly higher than the estimated reference TP concentrations. The thresholds in the responses to TN concentrations ranged more widely for nonwadeable rivers (0.527–1.990 mg/L) and wadeable streams (0.5–1.2 mg/L). Thresholds for macroinvertebrate and fish indices fluctuated over these ranges.

The biotic communities in a river reflect the overall ecological integrity (physical, chemical, and biological integrity). The biotic communities integrate the effects of many different stressors (such as extreme hydrologic conditions, extreme sedimentation rates, pesticides, and nutrients) over timespans of days to years and thus provide a broad measure of their aggregate effect. In addition, the geomorphologic and geochemical regimes, and land use/land cover in the watershed control the physicochemical habitat in which the biota live. Results of redundancy analyses for nonwadeable rivers and wadeable streams indicated that nutrient concentrations alone explained only a small part of the variance in the biotic indices. For small streams and large rivers, nutrient concentrations by themselves explained only about 1 to 11 percent of the total variance in the biotic indices (about 2 to 25 percent of the explained variance), and were most strongly related to SCHL concentrations.

Through a combination of the biotic indices, two new multiparameter indices (BIP and BIN) were developed for streams and rivers. For both streams and rivers, the BIP estimated TP concentrations better than the BIN estimated TN concentrations. Both of these indices, however, predicted the nutrient concentrations better for nonwadeable rivers than for wadeable streams. The BIP explained 63 percent of the variance in TP concentrations for the rivers, but only 54 percent for the streams. The BIN explained 51 percent of the variance in TN concentrations for the rivers, but only 41 percent for the streams. The differences in the predictability of these indices are consistent with the stronger correlations between the biotic indices and TP concentrations than between the biotic indices and TN concentrations. These differences indicate that TP concentrations are more important than TN concentrations in affecting the biotic communities.

Table 21. Summary of thresholds or breakpoints in the responses of water quality and biotic indices to changes in nutrient concentrations for nonwadeable rivers in Wisconsin.

[All concentrations given in milligrams per liter]

Biotic indices	Abbreviation	Total phosphorus	Dissolved phosphorus	Particulate phosphorus	Total nitrogen	Dissolved nitrite plus nitrate	Dissolved ammonia	Total Kjeldahl nitrogen
Water quality								
Secchi-tube depth	SD	0.091	0.045	0.048	1.097	0.241	0.051	0.658
Suspended chlorophyll *a* (log)	SCHL	.064	.041	.064	.927	.030	.026 (nss)	.833
Macroinvertebrates								
Species richness	SPECIES	.150	.066	.101	1.925	.030 (nss)	.051 (nss)	.770
Mean pollution tolerance index	MPTV	.064	.014	.051	.634	.147	.055	.658
Percentage of individuals from order Ephemeroptera	%EPHEM	.040	.024	.023	.527	.052	.055 (nss)	.875
Hilsenhoff Biotic Index	HBI	.150	.014 (nss)	.082	1.990	.134	.035 (nss)	.928
Percentage of individuals from order Plecoptera	%PLEC	.148 (nss)	.076 (nss)	.051 (nss)	1.965 (nss)	1.235 (nss)	.035 (nss)	.603
Percentage of individuals that are scrapers	%SCRAP	.034	.014	.016	.527	.030 (nss)	.016	.875
Fish								
Wisconsin large-river index of biotic integrity	IBI	.139	.045	.051	.635	.191	.051	1.075
Percentage of suckers by weight	%SUCKER	.091	.045	.051	.634	.134	.043	.505
Number of intolerant species	#INTOL	.139	.051	.051	1.125	.241	.051	.613
Percentage of individuals that are river species	%RIVERSP	.079	.035	.015	.556	.030	.026	.505
Number of river species	#RIVERSP	.147	.081	.064	1.965	.078 (nss)	.016	.993
Percentage of individuals that are lithophilic spawners	%LITSPAWN	.055	.035	.019	.634	.147	.043	.505

Conclusions

The ultimate goal of this study was to provide the information needed by water resource managers to develop regionally based nutrient criteria for rivers and streams in Wisconsin by refining reference or background nutrient and biotic conditions and describing the responses (thresholds) of the biotic community to differences in nutrient concentrations. Meaningful changes in macroinvertebrate and fish assemblages were correlated with changes in nutrient concentrations, and most of the assemblages changed at concentrations only slightly above the refined reference concentrations. Nutrient concentrations in many streams and rivers, especially those in agricultural areas, are well above the response thresholds for many biotic indices; therefore, large reductions in nutrient concentrations in these systems would be needed to have large effects on the biotic community. Differences in nutrient concentrations alone explained only a small part of the variability in the biotic community because the biotic community represents the overall ecological integrity of the river or stream. Therefore, it is difficult to predict the exact result of reducing nutrient concentrations without also modifying the factors typically associated with high nutrient concentrations. Actions taken to reduce the input of nutrients from the watersheds will likely not only reduce nutrient concentrations, but could also mitigate the effects of many other correlated stressors on the biotic community, such as suspended sediment and siltation. Reductions in nutrient input from the watersheds as a result of the establishment and implementation of nutrient criteria and standards would not only reduce nutrient concentrations in streams and rivers, but would also improve riparian habitat, the ecological functioning of streams and rivers, and the quality of downstream nutrient-limited receiving waters.

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Appendix 1. Stream identification, location information, and summary statistics for flow and water-quality data collected in 2003 for each of the 42 studied nonwadeable rivers in Wisconsin.

[ID, stream identifier; USGS, U.S. Geological Survey; km², square kilometer; (m³/s)/km², (cubic meter per second) per square kilometer; mg/L, milligram per liter; µg/L, microgram per liter; cm, centimeter; °C, degrees Celsius; µS/cm, microsiemen per centimeter. Location of stream sites shown on figure 2.]

ID	Stream name	USGS site number	Ecoregion ID	Longitude (decimal degrees)	Latitude (decimal degrees)	Water-shed area (km²)	Flow per unit area [(m³/s)/km²)]	Total phos-phorus (mg/L)
1	Wisconsin River at Grandfather Dam	05393705	3-NLF-09	89.7761	45.3233	5,870	0.010	0.054
2	Wisconsin River at Portage	05404024	3-NCHF-10	89.4733	43.5361	21,100	.011	.072
3	White River near Ashland	04027500	3-NLF-04	90.9042	46.4972	781	.010	.045
4	Menominee River near McAllister	04067500	3-NLF-12	87.6633	45.3258	10,100	.008	.027
5	Peshtigo River at Peshtigo	04069500	3-NLF-11	87.7444	45.0469	2,870	.009	.030
6	Oconto River near Gillett	04071000	3-NLF-10	88.3000	44.8647	1,770	.009	.031
7	Fox River at Berlin	04073500	3-SWTP-10	88.9522	43.9539	3,450	.008	.133
8	Wolf River near Shawano	04077400	3-NCHF-18	88.6250	44.8358	2,190	.008	.034
9	Embarrass River near Embarrass	04078500	3-NCHF-16	88.7361	44.7247	1,010	.008	.057
10	Wolf River at New London	04079000	3-NCHF-12	88.7403	44.3922	5,870	.020	.102
11	Little Wolf River at Royalton	04080000	3-NCHF-13	88.8653	44.4125	1,330	.010	.046
12	Fox River near Wrightstown	04084500	3-NCHF-14	88.1972	44.3175	15,600	.023	.156
13	Sheboygan River at Sheboygan	04086000	3-SWTP-09	87.7539	43.7417	1,110	.004	.151
14	Milwaukee River at Milwaukee	04087000	3-SWTP-08	87.9089	43.1000	1,810	.003	.149
15	Namekagon River at Trego	05332500	3-NLF-03	91.8881	45.9481	1,260	.012	.032
16	St. Croix River near Danbury	05333500	3-NLF-02	92.2472	46.0750	4,000	.014	.023
17	St. Croix River at St. Croix Falls	05340500	3-NLF-01	92.6469	45.4069	16,200	.012	.047
18	Chippewa River near Bruce	05356500	3-NLF-08	91.2608	45.4522	4,250	.012	.029
19	North Fork Flambeau River at Oxbo	05358330	3-NLF-05	90.7081	45.8592	2,480	.007	.031
20	South Fork Flambeau River near Phillips	05359500	3-NLF-06	90.6153	45.7042	1,600	.010	.028
21	Flambeau River near Bruce	05360500	3-NLF-07	91.2094	45.3725	4,900	.051	.033
22	Jump River at Sheldon	05362000	3-NCHF-01	90.9564	45.3081	1,490	.005	.034
23	Eau Claire River near Fall Creek	05366500	3-NCHF-03	91.2806	44.8097	1,970	.004	.080
24	Red Cedar River at Colfax	05367500	3-NCHF-02	91.7111	45.0525	2,840	.009	.125
25	Chippewa River at Durand	05369500	3-NCHF-04	91.9689	44.6283	23,400	.016	.078
26	Buffalo River near Tell	05372000	3-DFA-01	91.8492	44.3917	1,050	.009	.330
27	Trempealeau River at Dodge	05379500	3-DFA-02	91.5533	44.1317	1,670	.007	.399
28	Black River near Galesville	05382000	3-NCHF-05	91.2872	44.0603	5,390	.006	.150
29	La Crosse River at La Crosse	05383075	3-DFA-03	91.2103	43.8608	1,230	.006	.195
30	Lemonweir River near New Lisbon	05403500	3-NCHF-08	90.1775	43.9292	1,110	.008	.115
31	Baraboo River near Baraboo	05405000	3-DFA-07	89.6358	43.4808	1,570	.006	.204
32	Wisconsin River at Muscoda	05407000	3-NCHF-09	90.4433	43.1981	27,000	.044	.078
33	Kickapoo River at Steuben	05410490	3-DFA-04	90.8583	43.1828	1,780	.006	.146
34	Grant River at Burton	05413500	3-DFA-05	90.8192	42.7203	710	.004	.216
35	Crawfish River at Milford	05426000	3-SWTP-04	88.8494	43.1000	1,960	.003	.497
36	Bark River at State Highway D	05426460	3-SWTP-06	88.7014	42.8942	655	.004	.182
37	Rock River at Fort Atkinson	05427085	3-SWTP-05	88.8428	42.9275	5,810	.007	.311
38	Yahara River near Fulton	05430175	3-SWTP-01	89.1719	42.8264	1,280	.007	.210
39	Rock River at Afton	05430500	3-SWTP-03	89.0706	42.6092	8,650	.024	.261
40	Pecatonica River at Martintown	05434500	3-DFA-06	89.7994	42.5094	2,680	.004	.269
41	Sugar River near Brodhead	05436500	3-SWTP-02	89.3981	42.6117	1,360	.005	.199
42	Fox River near New Munster	05545750	3-SWTP-07	88.2258	42.6108	2,020	.004	.151

Dissolved phosphorus (mg/L)	Total nitrogen (mg/L)	Dissolved nitrite plus nitrate (mg/L)	Dissolved ammonia (mg/L)	Total Kjeldahl nitrogen (mg/L)	Suspended sediment (mg/L)	Suspended chlorophyll *a* (µg/L)	Secchi-tube depth (cm)	Average water temperature (°C)	Specific conductance (µS/cm)	pH	Color (standard units)	Dissolved oxygen minimum (mg/L)
0.018	0.598	0.033	0.034	0.565	4.0	9.6	103	19.6	90.5	7.75	77.5	5.10
.018	1.085	.159	.024	.910	11.0	24.4	64.5	19.9	154	7.80	40.0	8.00
.017	.266	.011	.025	.255	13.5	1.7	37.5	16.8	180	7.95	23.8	8.30
.012	.441	.026	.015	.370	4.0	3.9	120	19.6	280	8.15	40.0	8.10
.015	.769	.239	.032	.550	1.0	2.7	120	19.3	274	8.00	52.5	6.60
.012	.670	.204	.024	.510	7.0	4.5	120	19.2	280	7.95	75.0	8.30
.015	1.841	.288	.019	1.650	53.0	28.3	25.5	20.4	362	8.30	45.0	6.40
.016	.714	.178	.047	.555	5.5	3.0	120	19.2	261	8.10	38.8	5.30
.040	1.790	1.110	.048	.650	6.0	3.3	120	17.6	417	8.20	52.5	6.30
.054	1.382	.559	.044	.665	22.5	6.7	49.5	19.3	421	8.15	37.5	5.70
.028	1.910	1.195	.043	.580	4.0	3.5	120	19.4	428	8.20	32.5	8.80
.073	1.321	.244	.074	1.100	22.5	27.2	42.0	20.9	403	8.50	16.3	8.30
.049	1.991	.615	.046	1.400	36.5	35.7	47.0	20.4	651	8.60	47.5	8.70
.086	1.713	.555	.044	1.100	14.5	24.8	42.5	21.5	844	8.50	33.8	7.20
.014	.416	.085	.038	.340	2.0	5.2	120	19.4	170	8.30	23.8	7.40
.012	.411	.011	.007	.365	1.0	1.8	120	17.6	135	8.00	32.5	8.00
.020	.701	.136	.021	.565	5.5	5.7	113	19.8	190	7.80	60.0	7.30
.015	.571	.132	.020	.430	2.5	2.9	120	18.8	106	7.90	55.0	8.10
.019	.470	.021	.020	.420	2.0	2.4	120	18.7	135	7.55	60.0	7.20
.014	.542	.018	.024	.500	3.0	4.2	120	17.7	78.0	7.60	72.5	7.90
.018	.513	.092	.021	.440	3.0	3.8	120	19.4	108	7.75	67.5	8.10
.020	.501	.011	.024	.490	1.0	3.1	120	20.6	150	8.30	62.5	5.30
.042	1.095	.435	.023	.610	6.0	13.3	110	20.2	110	8.15	32.5	8.60
.075	1.940	1.275	.025	.595	11.0	7.9	98.0	17.3	175	7.70	12.5	7.00
.030	1.198	.609	.012	.625	11.5	18.6	79.0	19.2	181	8.35	32.5	8.60
.129	3.020	2.420	.012	.545	74.0	6.1	48.5	18.4	345	8.10	8.8	8.20
.156	2.415	1.975	.017	.500	53.0	3.9	41.5	19.6	311	8.05	8.8	8.20
.061	1.099	.413	.010	.695	15.5	19.8	56.5	21.3	157	8.65	27.5	9.10
.071	1.376	.368	.019	1.040	54.5	45.8	33.5	22.2	341	8.75	7.5	8.70
.063	1.152	.362	.061	.715	4.5	6.0	64.0	18.8	191	7.55	80.0	6.50
.077	1.990	1.145	.046	.840	69.0	18.3	20.0	18.0	382	7.95	15.0	6.80
.015	1.215	.376	.012	.945	20.0	34.4	55.5	20.6	243	8.35	35.0	8.80
.064	1.170	.791	.013	.355	51.0	5.5	57.0	17.8	486	8.20	8.8	7.60
.137	3.665	3.020	.019	.615	22.0	2.8	66.5	19.9	638	8.20	5.0	8.10
.106	3.326	.070	.027	2.850	87.5	129.7	12.0	22.0	664	8.60	40.0	5.60
.083	2.810	1.410	.129	1.400	43.0	15.5	27.0	20.5	673	8.10	42.5	6.50
.106	3.000	1.000	.037	2.200	51.5	98.5	16.0	21.8	700	8.50	36.3	6.10
.080	5.485	3.770	.057	1.400	48.0	19.6	34.5	20.2	680	8.40	15.0	8.80
.146	3.775	1.925	.134	1.850	22.5	37.0	36.5	20.4	769	8.30	18.8	8.20
.104	4.475	3.680	.054	.825	74.0	8.1	29.5	20.1	634	8.20	8.8	7.60
.068	4.150	3.245	.023	1.050	39.5	36.1	19.5	20.6	596	8.25	15.0	8.10
.017	2.740	.963	.023	1.550	29.5	40.5	26.5	21.9	904	8.45	30.0	9.40

Appendix 2. Macroinvertebrate indices for each of the 41 studied nonwadeable rivers in Wisconsin. (All data were collected by the Wisconsin Department of Natural Resources.)

[ID, stream identifier; EPT, Ephemeroptera, Plecoptera, Trichoptera. Location of sites shown on figure 2.]

ID	Stream name	Species richness	Percent Ephemeroptera	Percent Plecoptera	Percent Trichoptera	Percent Diptera	Percent Chironomidae
1	Wisconsin River at Grandfather Dam	28	31.4	2.9	19.0	43.6	38.1
2	Wisconsin River at Portage	28	6.0	.0	43.5	44.7	44.1
4	Menominee River near McAllister	30	69.1	3.9	8.2	15.9	15.5
5	Peshtigo River at Peshtigo	37	32.1	.2	45.8	20.4	19.3
6	Oconto River near Gillett	45	29.2	1.2	4.5	45.8	45.8
7	Fox River at Berlin	18	1.1	.0	84.3	13.8	13.6
8	Wolf River near Shawano	44	54.0	.0	8.4	36.4	36.4
9	Embarrass River near Embarrass	51	16.7	.2	33.2	47.6	41.9
10	Wolf River at New London	34	16.9	.0	67.3	13.2	13.0
11	Little Wolf River at Royalton	47	20.7	.4	41.8	36.5	35.8
12	Fox River near Wrightstown	23	2.5	.0	24.0	53.9	53.5
13	Sheboygan River at Sheboygan	24	2.2	.0	.4	92.7	92.7
14	Milwaukee River at Milwaukee	37	4.3	.0	28.2	56.4	54.4
15	Namekagon River at Trego	29	14.4	.6	24.8	59.9	43.9
16	St. Croix River near Danbury	39	26.7	.8	29.3	41.9	25.6
17	St. Croix River at St. Croix Falls	37	7.9	.5	23.3	67.0	66.3
18	Chippewa River near Bruce	44	21.1	2.1	5.3	70.8	58.8
19	North Fork Flambeau River at Oxbo	42	46.8	.0	2.6	41.0	40.7
20	South Fork Flambeau River near Phillips	43	10.5	.8	8.6	79.5	73.9
21	Flambeau River near Bruce	28	43.2	.2	11.9	44.7	42.9
22	Jump River at Sheldon	30	60.6	1.3	.6	35.4	35.4
23	Eau Claire River near Fall Creek	32	5.2	.3	9.1	84.5	83.6
24	Red Cedar River at Colfax	30	39.5	11.2	31.3	17.4	12.4
25	Chippewa River at Durand	33	5.6	.4	24.5	68.9	64.8
26	Buffalo River near Tell	30	8.2	1.7	3.8	85.5	84.8
27	Trempealeau River at Dodge	34	25.5	1.3	10.6	60.8	57.4
28	Black River near Galesville	39	44.1	.9	14.7	27.2	27.2
29	La Crosse River at La Crosse	31	2.1	.0	10.5	61.3	59.4
30	Lemonweir River near New Lisbon	38	20.7	.0	25.8	40.2	39.7
31	Baraboo River near Baraboo	21	31.4	.2	63.2	3.3	3.3
32	Wisconsin River at Muscoda	33	4.1	.2	12.1	76.5	75.2
33	Kickapoo River at Steuben	38	8.5	3.0	33.6	51.0	49.9
34	Grant River at Burton	34	44.2	.0	37.6	15.8	15.0
35	Crawfish River at Milford	10	.0	.0	69.7	30.1	29.9
36	Bark River at State Highway D	33	11.2	.0	31.0	23.1	22.9
37	Rock River at Fort Atkinson	16	.0	.0	9.4	78.8	78.8
38	Yahara River near Fulton	26	22.8	.0	36.0	39.1	36.8
39	Rock River at Afton	19	4.2	.0	23.4	65.8	65.7
40	Pecatonica River at Martintown	29	41.0	.6	35.3	22.5	22.5
41	Sugar River near Brodhead	28	15.2	.5	61.0	23.3	22.7
42	Fox River near New Munster	27	15.6	.0	9.7	74.3	74.1

Percent EPT number	Percent EPT taxa	Percent scrapers	Percent shredders	Percent gatherers	Percent from a depositional habitat	Mean pollution tolerance value	Hilsenhoff Biotic Index
53.3	48.0	4.2	19.0	28.8	17.7	4.88	4.86
49.4	23.1	1.9	9.2	18.8	4.6	5.57	5.53
81.2	46.2	55.9	4.0	7.9	6.8	4.65	3.93
78.1	54.3	15.7	14.6	12.3	8.6	5.06	4.22
34.8	39.0	5.7	5.1	71.7	58.0	5.42	5.31
85.4	41.2	.6	8.8	3.8	.8	6.27	5.39
62.4	29.3	10.3	6.0	62.1	23.1	5.65	6.05
50.1	46.7	8.3	17.6	23.3	8.7	4.45	4.96
84.3	46.9	8.5	3.4	14.4	6.3	5.75	5.22
62.9	48.8	6.7	11.6	26.5	16.2	4.37	4.70
26.5	30.4	1.7	15.6	34.0	9.5	6.55	6.47
2.6	21.7	.0	2.0	94.8	4.4	6.57	9.58
32.5	21.2	.8	27.2	27.8	14.1	5.60	5.75
39.7	50.0	5.3	2.5	16.8	3.2	3.65	4.76
56.8	47.1	10.7	2.1	34.2	9.0	4.12	4.31
31.7	34.4	7.4	8.1	6.1	4.4	4.84	5.67
28.4	46.2	9.9	15.6	43.2	16.0	4.32	4.98
49.4	31.6	15.1	.7	53.9	21.5	5.64	6.07
19.9	40.0	1.6	10.9	22.2	20.7	4.88	5.58
55.3	56.0	19.8	7.0	6.1	3.0	4.76	4.47
62.5	48.1	55.1	1.0	10.1	8.9	4.54	4.59
14.6	26.7	4.6	8.6	13.7	4.4	5.03	5.73
82.0	50.0	7.2	10.3	37.9	9.1	3.52	2.77
30.5	40.0	4.6	7.6	9.7	9.7	5.29	5.51
13.7	39.3	7.6	2.8	5.7	3.8	4.64	5.77
37.4	41.2	12.6	3.8	27.6	18.4	5.15	5.87
59.7	36.1	33.0	13.2	20.3	9.0	5.52	5.13
12.6	17.2	12.5	19.8	51.5	11.1	5.50	7.17
46.5	14.3	11.2	4.8	41.0	7.5	5.89	5.82
94.7	65.0	27.9	.4	6.0	1.4	4.80	4.94
16.4	33.3	1.8	17.7	30.8	4.8	5.53	6.66
45.1	37.8	6.2	7.5	31.2	11.8	4.64	4.64
81.8	40.0	18.3	4.8	23.2	17.2	5.35	4.71
69.7	33.3	.0	26.9	3.2	.6	6.11	5.43
42.2	40.6	7.5	9.3	43.9	5.5	6.19	6.18
9.4	12.5	1.1	6.5	80.7	1.1	6.77	8.85
58.8	40.0	1.0	21.5	33.3	18.6	5.14	4.97
27.6	22.2	.0	46.6	15.6	6.3	5.56	5.57
76.9	59.3	35.6	12.3	11.3	7.8	5.00	5.32
76.7	50.0	5.8	5.6	12.3	7.4	5.42	4.92
25.4	32.0	.2	14.8	67.7	16.4	5.67	7.15

Appendix 3. Fish indices and ratings for each of the 41 studied nonwadeable rivers in Wisconsin. (All data were collected by the Wisconsin Department of Natural Resources.)

[ID, stream identifier; kg, kilogram. Location of sites shown on figure 2.]

ID	Stream name	Number of native species	Number of riverine species	Number of sucker species	Number of intolerant species	Weight per unit effort (kg)	Percent river species
1	Wisconsin River at Grandfather Dam	8	3	3	3	151,000	25.3
2	Wisconsin River at Portage	11	4	4	3	22,300	45.5
4	Menominee River near McAllister	17	7	5	5	7,340	26.4
5	Peshtigo River at Peshtigo	18	6	3	3	4,830	20.8
6	Oconto River near Gillett	8	2	1	4	14,400	5.1
7	Fox River at Berlin	19	4	4	3	25,700	15.1
8	Wolf River near Shawano	27	8	6	6	21,100	35.9
9	Embarrass River near Embarrass	25	7	6	3	32,100	19.4
10	Wolf River at New London	18	5	4	2	10,800	15.8
11	Little Wolf River at Royalton	14	7	4	3	32,000	38.8
12	Fox River near Wrightstown	5	1	2	1	3,890	4.5
13	Sheboygan River at Sheboygan	10	0	2	3	33,600	.0
14	Milwaukee River at Milwaukee	4	1	3	1	4,010	9.1
15	Namekagon River at Trego	12	7	4	4	57,800	48.2
16	St. Croix River near Danbury	15	5	5	5	11,800	39.4
17	St. Croix River at St. Croix Falls	12	7	7	2	24,500	38.6
18	Chippewa River near Bruce	16	7	5	4	21,600	34.2
19	North Fork Flambeau River at Oxbo	9	4	5	3	38,000	79.0
20	South Fork Flambeau River near Phillips	11	6	5	2	33,300	65.5
21	Flambeau River near Bruce	18	7	4	3	15,900	15.8
22	Jump River at Sheldon	10	4	4	4	52,000	60.5
23	Eau Claire River near Fall Creek	14	4	3	3	79,300	20.4
24	Red Cedar River at Colfax	23	13	5	7	32,700	54.5
25	Chippewa River at Durand	16	10	7	3	47,900	40.6
26	Buffalo River near Tell	19	7	5	3	41,300	26.8
27	Trempealeau River at Dodge	10	4	3	0	10,200	10.7
28	Black River near Galesville	15	8	6	3	34,900	38.4
29	La Crosse River at La Crosse	12	4	2	1	61,100	11.4
30	Lemonweir River near New Lisbon	11	1	1	0	17,100	2.7
31	Baraboo River near Baraboo	14	4	3	3	21,900	14.3
32	Wisconsin River at Muscoda	16	10	8	5	58,900	48.0
33	Kickapoo River at Steuben	11	6	4	1	7,720	31.1
34	Grant River at Burton	14	5	4	2	8,230	24.3
35	Crawfish River at Milford	8	0	3	1	30,800	.0
36	Bark River at State Highway D	8	3	4	1	11,700	32.8
37	Rock River at Fort Atkinson	12	1	2	1	13,000	3.9
38	Yahara River near Fulton	16	6	3	4	36,500	61.3
39	Rock River at Afton	9	1	2	1	9,800	2.2
40	Pecatonica River at Martintown	8	4	5	1	20,000	22.2
41	Sugar River near Brodhead	11	4	5	1	35,900	40.7
42	Fox River near New Munster	15	4	5	2	42,500	29.4

Percent lithophilic spawners	Percent suckers by weight	Percent insectivores by weight	Percent with disease	Large-river index of biotic integrity	Fish rating based on the large-river index of biotic integrity
91.9	83.8	84.1	0.0	80	Excellent
41.4	32.0	32.7	.0	65	Good
59.8	69.5	72.6	.0	80	Excellent
24.8	1.6	3.7	.0	50	Fair
6.1	6.7	6.7	.0	44	Fair
19.8	32.0	42.3	.0	70	Good
73.1	10.8	11.8	.0	80	Excellent
50.4	26.0	40.5	.0	95	Excellent
43.9	15.7	40.0	.0	70	Good
74.1	94.7	97.8	1.7	85	Excellent
22.7	.6	10.4	.0	10	Very poor
1.5	.8	.9	.0	30	Poor
36.4	35.5	35.5	.0	35	Poor
91.2	97.4	97.5	.0	95	Excellent
83.9	87.6	91.3	.0	95	Excellent
64.4	34.8	36.5	2.0	75	Good
83.6	71.8	72.4	.0	90	Excellent
93.5	95.8	95.9	.0	95	Excellent
91.4	87.7	87.8	.0	94	Excellent
88.8	75.7	81.8	.0	85	Excellent
86.4	79.4	79.4	.0	95	Excellent
47.3	65.1	67.0	.6	75	Good
53.3	77.2	79.6	.0	100	Excellent
69.2	65.4	76.6	.0	100	Excellent
72.2	36.2	38.4	.0	95	Excellent
76.8	21.9	37.6	1.8	35	Poor
60.5	49.5	56.7	1.2	90	Excellent
64.0	74.7	88.2	.0	60	Good
32.4	28.3	29.1	.0	35	Poor
47.6	12.1	25.3	1.2	55	Fair
43.9	42.4	63.7	.0	100	Excellent
62.2	28.5	38.7	.0	55	Fair
37.8	5.2	21.3	.0	50	Fair
37.0	29.3	46.2	.0	50	Fair
34.3	8.9	9.0	1.5	30	Poor
23.5	3.7	14.6	2.0	15	Very poor
70.4	55.3	57.7	.0	90	Excellent
28.9	2.1	6.9	6.7	5	Very poor
40.0	17.8	36.7	.0	50	Fair
42.4	28.3	60.3	.0	70	Good
52.9	26.6	36.9	.0	75	Good

USGS

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