National Water-Quality Assessment Program

The Effects of Urbanization and Other Environmental Gradients on Algal Assemblages in Nine Metropolitan Areas across the United States

Scientific Investigations Report 2009–5022

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

Cover. **Dense algal growth in the Kewaunee River tributary at Lowell Road near Luxemburg, Wisconsin** *(photograph taken by Jana Stewart, U.S. Geological Survey)*.

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By James F. Coles, Amanda H. Bell, Barbara C. Scudder, and Kurt D. Carpenter

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Foreword

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

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

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

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

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

Matthew C. Larsen
Associate Director for Water

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Abstract

The U.S. Geological Survey conducted studies from 2000 to 2004 to determine the effects of urbanization on stream ecosystems in nine major metropolitan study areas across the United States. Biological, chemical, and physical components of streams were assessed at 28 to 30 sites in each study area. Benthic algae were sampled to compare the degree to which algal assemblages correlated to urbanization, as characterized by an urban intensity index (UII), relative to other environmental gradients that function at either the watershed or reach scales. Ordination site scores were derived from principal components analyses of the environmental data to define environmental gradients at two spatial scales: (1) watershed-scale gradients that summarized (a) landscape modifications and (b) socioeconomic factors, and (2) reach-scale gradients that characterized (a) physical habitat and (b) water chemistry. Algal response was initially quantified by site scores derived from nonmetric multi-dimensional scaling ordinations of the algal assemblage data. The site scores were then correlated with a set of algal metrics of structure and function to help select specific indicators that would best represent changes in the algal assemblages and would infer ecological condition. The selected metrics were correlated to the UII and other environmental gradients. The results indicated that diatom-taxa in the assemblages were distinctly different across the nine study areas, likely due to physiographic differences across the country, but nevertheless, some algal metrics were applicable to all areas. Overall, the study results indicated that although the UII represented various landscape changes associated with urbanization across the country, the algal response was more strongly related to more specific factors generally associated with water quality measured within the stream reach.

Introduction

Increasing urban development throughout the United States often has led to biological impairment in the receiving streams of watersheds. Urbanizing areas are associated with flow alterations and increased loads of nutrients, ions, heavy metals, pesticides, and other chemicals to waterways that generally have contributed to the decreased quality of aquatic biological communities including the introduction of non-native, pollution tolerant, invasive, or nuisance species, and the extirpation of native, pollution sensitive, or rare species (Paul and Meyer, 2001; Walsh and others, 2005). Benthic algae are critical to stream ecosystems, acting as primary producers, food sources, and components of biofilms; therefore, changes in benthic algal assemblages may be related to changes in resident benthic invertebrates and fish assemblages (McCormick and Cairns, 1997). Accordingly, benthic algae are sensitive indicators of physical, chemical, and biological changes in stream ecosystems.

The amount of time a biological assemblage takes to respond to a disturbance is an important consideration when assessing stream condition. The National Water-Quality Assessment (NAWQA) Program, conducted by the U.S. Geological Survey (USGS) over the past two decades, has relied in part on surveys of algae, invertebrates, and fish assemblages to help assess the condition of the Nation's waters (Gurtz, 1994). In using biological assemblages to assess stream condition, the spatial and temporal scales of ecological change are critical (Barbour and others, 1999). Changes in fish assemblages may be related to alterations in streamflow and connectivity in the watershed that are often associated with water management features such as impoundments and diversions (Brown and others, 2009). The response of fish to these alternations can occur over a period of years, because of their long generation time and the relative permanence of alterations, such as dams. Changes to aquatic invertebrate assemblages often are related to inclusive changes at the watershed scale (T.F. Cuffney and others, U.S. Geological Survey, written commun., 2009). Complex life cycles with moderate generation times, the ability to move into a stream segment with suitable conditions, and taxa with wide-ranging sensitivities allow invertebrates to respond to multiple disturbances integrated at the watershed scale.

The period over which environmental conditions are integrated by algae typically is shorter than those of invertebrates and fish, because of the quick response and recovery of algal assemblages, on the order of weeks (Hoagland and others, 1982; Lowe and Pan, 1996; Duong and others, 2007). Furthermore, diatoms can be better indicators of nutrient enrichment even where invertebrates are more sensitive to watershed-scale disturbance (Sonneman and others, 2001). In comparing algae, invertebrates, and fish in stream bioassessments, Barbour and others (1999) suggest that algae are valuable indicators of short-term impacts and environmental changes but they do not integrate environmental effects over entire seasons or years. These characteristics were exemplified in a study by Walker and Pan (2006) that investigated differences in diatom assemblages that occurred over a 1-year period at an urban and a rural site. Benthic diatom assemblages were sampled at least monthly at both sites, and very high seasonal variation was shown over the duration of the study. However, the variation was distinctly different between the two sites and was most strongly related to water chemistry differences between the urban and rural watersheds.

Historically, the focus of environmental assessments with algae has been on diatoms because of the extent of knowledge about the environmental preferences of this group. Also fewer issues are associated with identification and enumeration of diatoms compared to other algal groups (Stevenson and Pan, 1999). Algal measures have included basic metrics similar to those used for invertebrates and fish including overall abundance, taxa richness, diversity, and indicator taxa, such as pollution-sensitive taxa. Additional metrics or indices specific to algae also are commonly used such as abundance or percentage of nondiatom taxa, number of algal groups, nitrogen-fixing taxa, motile taxa, eutraphentic taxa, and indices of biotic integrity (Lange-Bertalot, 1979; Bahls, 1993; Kentucky Division of Water, 1993; van Dam and others, 1994; Kelly and others, 1995; Pan and others, 1996; Hill and others, 2000; Fore and Grafe, 2002; Potapova and Charles, 2007; Porter, 2008). Benthic algae have been used in stream assessments for decades in Europe and more recently in Australia, New Zealand, and North America, including national monitoring programs of the USGS (Porter and others, 1993; Moulton and others, 2002; Hambrook-Berkman and Porter, 2004) and U.S. Environmental Protection Agency (USEPA; Barbour and others, 1999; Fore, 2003).

Purpose and Scope

As part of the NAWQA Program, studies were conducted from 2000 to 2004 to determine the effects of urbanization on riverine ecosystems in nine major metropolitan study areas in varying physiographic settings across the United States (Couch and Hamilton, 2002; Tate and others, 2005): Portland (POR), Oregon; Salt Lake City (SLC), Utah; Denver (DEN), Colorado; Dallas-Fort Worth (DFW), Texas; Milwaukee-Green Bay (MGB), Wisconsin; Birmingham (BIR), Alabama; Atlanta (ATL), Georgia; Raleigh (RAL), North Carolina; and Boston (BOS), Massachusetts (fig. 1). These studies were designated collectively as the Effects of Urbanization on Stream Ecosystems study or EUSE. In each study area, 28–30 watersheds with relatively consistent key environmental factors, such as watershed size, soil characteristics, and stream geomorphology, were selected to characterize a gradient of urbanization from low to high. Depending on the study area, urbanization generally was associated with the loss of either agriculture or naturally vegetated land (typically forest).

The urban intensity of each watershed was assigned a value from an urban intensity index (UII) derived from three watershed-scale variables: housing density, road density, and percentage of urban land cover (Cuffney and Falcone, 2008). These variables, respectively, represent the extent of population, infrastructure, and developed land cover that function together in the UII, which in turn acts as a proxy for many changes associated with urbanization. The expectation of the UII was that changes in biological assemblages would relate more strongly to a measure of urbanization that was comprehensive rather than to a single environmental variable. Algal, invertebrate, and fish assemblage data were collected

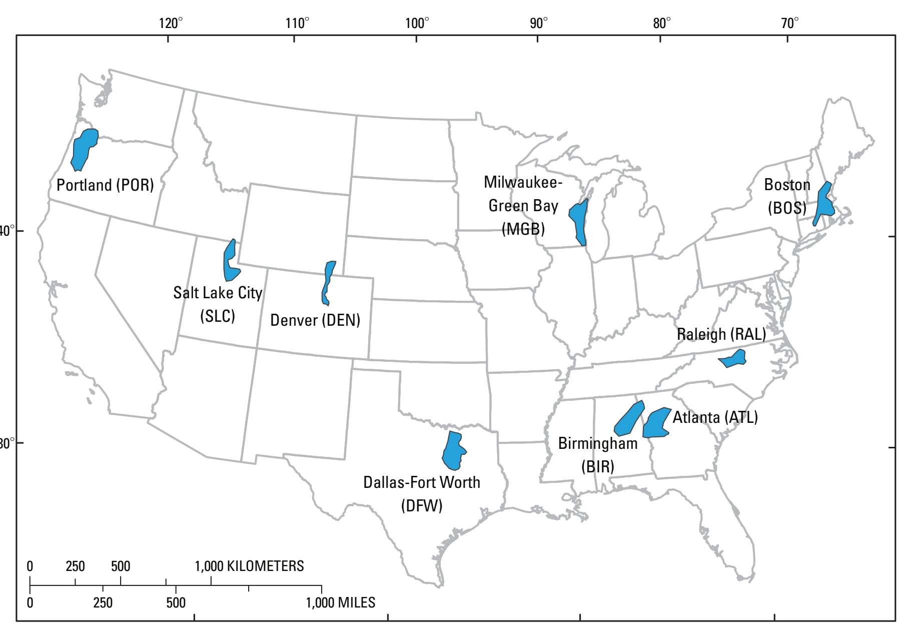

Figure 1. Locations of the nine metropolitan study areas where urban studies were conducted as part of the U.S. Geological Survey National Water-Quality Assessment Program. The shaded areas show the spatial extent of each metropolitan study area.

from a sampling reach within each stream. In addition to the UII value for each site, multiple physical and chemical variables were measured at two distinct scales: in the sampling reach (proximal) and over the watershed (distal).

This report specifically addresses analysis of the algal data collected for the EUSE studies. We defined the algal response as variation in algal assemblages across sites in a study area. Our goal was to determine if the algal response could be directly related to urbanization as characterized by the UII, or if the algal response was more clearly related to other environmental gradients functioning at either the watershed or reach scales. The UII was the prime example of a watershed gradient; however, the other gradients investigated were based on changes in landscape features, derived primarily from GIS variables, and socioeconomic factors, derived primarily from census statistics. Environmental gradients at the reach scale were derived from water-chemistry and physical-habitat data that were collected in the reach at each site. Accordingly, the reach-scale gradients characterized physical habitat and water quality.

A conceptual model was used to help explain how the response of algal assemblages may be related to environmental gradients at the watershed or the reach scales (fig. 2). The algal response is characterized primarily with a group of indicator metrics that were relatively consistent across the nine study areas in signifying changes to the structure and function of algal assemblages (Stevenson, 1996). The objective was first to determine how strongly the algal response was related to environmental gradients that function at the watershed scale (fig. 2, red arrow). In addition to the UII as a generalized characterization of urbanization, other watershed-scale gradients were derived to specifically characterize landscape features, such as land use, and socioeconomic factors, such as human presence. The second objective was to determine if the algal response was more strongly related to environmental gradients that function at the reach scale (fig. 2, blue arrow). These reach-scale gradients characterized variation in physical habitat features and in water chemistry.

Although the reach gradients are depicted as being distinct from the watershed gradients, there is likely an effective link between the two (fig. 2, dashed arrow). This link indicates that watershed changes that occur from urbanization may ultimately affect the environmental conditions at the reach scale. The purpose of this report, however, is to determine if the algal response can be directly related to the UII, which integrates many factors associated with urbanization over time, or if the algal response is more strongly related to proximal variables that measure reach conditions on a shorter time scale. The results will help determine the relative effects of urbanization and other environmental gradients on algal assemblages in different areas and physiographic settings across the country. Additionally, the algal metrics found to be most effective at characterizing the algal response should have applications in different regions as likely indicators of disturbance that affect the algal assemblages.

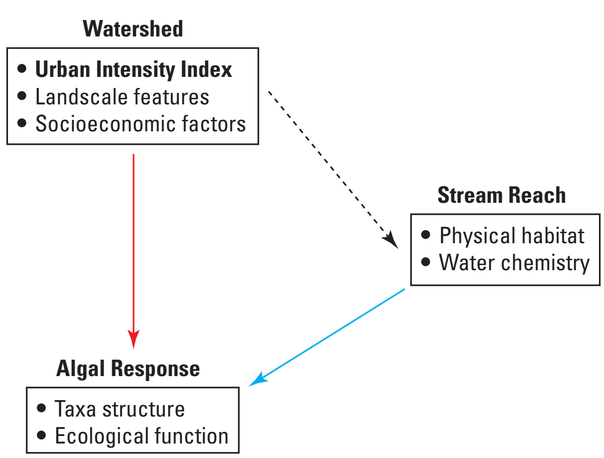

Figure 2. Conceptual model used to determine if variation in algal assemblages (algal response) were more strongly related to environmental gradients at the watershed scale (red arrow) or at the stream-reach scale (blue arrow) for urban studies conducted as part of the U.S. Geological Survey National Water-Quality Assessment Program. Gradients at the reach scale were not considered independent from the watershed scale, however, and this link is indicated by the dashed arrow. The urban intensity index was associated with comprehensive human-related changes in watersheds to characterize urbanization.

Methods

The procedures used for data collection, processing, and analysis are divided into four sections that each characterize the benthic algal assemblages, the watershed environmental gradients, and the reach environmental gradients, and describe the analysis procedures. USGS standard protocols were used for these procedures and the details describing each of these processes follow.

Characterizing Benthic Algal Assemblages

Benthic algal assemblages were represented by two types of samples collected at each site using NAWQA sampling protocols (Porter and others, 1993; Moulton and others, 2002; Potapova and Charles, 2005): a richest-targeted habitat (RTH) sample was collected from cobble substrate in riffle areas of the stream or woody snags in streams without riffle areas because these areas are generally considered the biologically richest substrates (although not always true for benthic algae), and a depositional-targeted habitat (DTH) sample was collected from depositional areas of the stream.

The RTH samples were collected as a composite from typically five cobble-sized rocks at riffle-dominated streams in seven of the nine study areas. In the DFW and ATL areas, riffle areas were not common, and the streambed substrate did not allow for cobble samples; therefore, woody snags were collected for the RTH sample. Aliquots from the RTH samples were prepared for analysis of chlorophyll *a* (Chl *a*) and ash-free dry mass. The DTH samples were a composite of depositional sediment from five locations in a stream reach with little to no flow. The DTH samples are more likely than the RTH to contain epipelic and episammic taxa, as well as taxa that had drifted downstream from other microhabitats. Both the RTH and DTH samples were preserved with 3–5 percent buffered formalin and sent to Academy of Natural Sciences of Philadelphia for taxonomic identification and calculations of density and biovolume. Additional details of the algal sampling procedures are described in Giddings and others (in press).

The RTH samples were collected at 258 sites, and 555 of 680 taxa were diatoms (~82 percent). The DTH samples were collected at 256 sites among all study areas, and 674 of 779 taxa were diatoms (~87 percent). Both RTH and DTH diatom data were collected at 255 sites (a few sites had only one or the other), and these data were combined into a qualitative dataset (diatom presence/absence taxa) identified as "QUAL." The QUAL dataset did not include diatom taxa if highly ambiguous (for example, identified only as "diatom") or seen only at one site, resulting in 572 diatom taxa. The RTH and DTH datasets were expressed in relative abundance and each was evaluated by all taxa (diatoms + soft taxa) and by diatoms only. Previous algal studies using NAWQA data (Potapova and Charles, 2005) as well as other data (Pan and others, 1996) have shown that algal assemblages from both RTH and DTH substrates are indicative of environmental conditions. The greatest emphasis was placed on the RTH diatom assemblages because, in part, the RTH samples are currently (2009) the standard algal sample in the NAWQA Program, and many of the algal metrics used to assess ecological condition are based on diatoms. A greater reliance was placed on the QUAL dataset, however, to assess the degree that taxa composition changed across the country by study area.

The algal response was characterized separately for each study area in two conceptually different ways: (1) multivariate ordination site scores and (2) indicator metrics of structure and function. Ordination site scores were first used to characterize the overall structure of the diatom assemblages. A two-dimensional nonmetric Multi-Dimensional Scaling (nMDS) analysis was used to derive the site scores from the first and second ordination axes of the diatom data. Although site scores effectively indicate the relative differences across assemblages, the actual values are mainly intrinsic to the dataset from which they are derived and do not indicate actual ecological condition. To address this limitation, the nMDS site scores were directly correlated with 29 indicator metrics to help identify specific metrics that best characterized algal assemblages and ecological condition across the nine study areas. The preliminary set of indicator metrics selected from Lange-Bertalot (1979), Bahls (1993), and van Dam and others (1994) and were summarized in Porter (2008), and are associated with various algal attributes that are sensitive to ecological condition (table 1). Of these metrics, those having the strongest correlations to site scores (Spearman |rho|) across study areas were considered reliable indicators of algal assemblages in the different regions and were used in the subsequent correlations with environmental gradients.

The UII was developed to characterize urbanization with a multimetric approach to determine the simplest set of variables that represented urbanizing watersheds most consistently across the Nation (Cuffney and Falcone, 2008). These variables (housing density, road density, and urban land-cover percentage) were equally weighted for the UII, which was then derived as two versions that differ in how urban intensity is scaled. The first version is a metropolitan area-national urban intensity index (MA-NUII) that was derived for each of the nine metropolitan study areas and scaled 0 to 100 to represent the extremes of urban intensity for the particular study area. The second version is the national urban intensity index (NUII) that was scaled 0 to 100 to represent the extremes of urban intensity among all sites in all nine metropolitan study areas combined.

A result of the inclusive scaling for the NUII was that urban intensity was generally higher in the western study areas than in the eastern study areas. For example, each study area had sites representing the urban extremes with MA-NUII values 0 and 100, but the urban extremes of 0 and 100 with the NUII were sites in the SLC study area, whereas sites in the BOS study area had NUII values ranging from 2.7 to only 52.2. The relative difference in high urban intensity between these two study areas is almost twofold, which is important when comparing responses across regions (Cuffney and Falcone, 2008). This difference in urbanization, however, may have been related to the age of the city; many of the older, highly urbanized areas in the East lacked above-ground streams because they apparently have been buried as part of the storm-sewer system.

In addition to the UII, environmental gradients were derived to characterize watersheds by landscape features and socioeconomic factors (fig. 2). Data for characterizing landscape features included land-cover data from the National Land Cover Database 2001 (NLCD01; U.S. Geological Survey, 2005), roadway density from the Census 2000 TIGER/Line (GeoLytics, 2001), point-source discharge and toxic-release inventory locations from USEPA inventory databases (U.S. Environmental Protection Agency, 2005a, b), and water-management features from the National Inventory of Dams (U.S. Army Corps of Engineers, 1996). Watershed gradients derived from these data are identified in this report by "GIS" to indicate the close association of the data with geographic information systems. Data used in characterizing socioeconomic factors included population, housing, energy use, and income statistics from U.S. census datasets (U.S. Census Bureau, 2003). Watershed gradients derived from these data are identified in this report by "CEN" to indicate the association with census data. Additional information about the datasets used to derive these gradients is available in Falcone and others (2007).

Table 1. Algal metrics that were investigated initially as response variables but in the subsequent analyses the metrics in **bold** would have the greatest potential as response variables that could be associated with the environmental gradients investigated in urban studies conducted as part of the U.S. Geological Survey National Water-Quality Assessment Program.

[Adapted from Porter, 2008; Indicator metric code is an abbreviation of the response characteristic; g/m^2, grams per square meter; cells/m^2, cells per square meter]

Indicator metric code	Group	Unit	Response characteristic
			Taxa composition
TAXA_RICH	all taxa	count	Number of algal taxa
DIAT_RICH	diatoms	count	Number of diatom taxa
DVS_SW_ALL	all taxa	index	Shannon Wiener Diversity Index
DVS_SW_DTM	diatoms	index	Shannon Wiener Diversity Index for diatoms
ACHMIN_P	**diatoms**	**percentage**	**Relative abundance of *Achnanthidium minutissimum***
			Salinity tolerance
SL_FR	diatoms	percentage	Salinity: fresh water
SL_BR	diatoms	percentage	Salinity: brackish water
SL_HB	**diatoms**	**percentage**	**Salinity: halobiontic diatoms (sum of brackish-fresh and brackish)**
			Trophic state
TR_E	**diatoms**	**percentage**	**Eutraphentic diatoms**
ON_NH	diatoms	percentage	Organic nitrogen index: nitrogen heterotrophs
SP_OL	diatoms	percentage	Oligosaprobic
SP_BM	**diatoms**	**percentage**	**Beta-mesosaprobic**
SP_AM	diatoms	percentage	Alpha-mesosaprobic
SP_AP	diatoms	percentage	Alpha-mesosaprobic – polysaprobic
SP_AM_AP	diatoms	percentage	Total of *SP_AM* + *SP_AP*
			Pollution tolerance
PT_TA	diatoms	percentage	Pollution tolerance (Lange-Bertalot, 1979): tolerant (2a)
PT_TB	diatoms	percentage	Pollution tolerance (Lange-Bertalot, 1979): tolerant (2b)
PT_TA_TB	diatoms	percentage	Pollution tolerance (Lange-Bertalot, 1979): sum of tolerant 2a and 2b
PC_MT	diatoms	percentage	Pollution class (Bahls, 1993): most tolerant
PC_SN	**diatoms**	**percentage**	**Pollution class (Bahls, 1993): sensitive**
			Oxygen requirements
OT_LW	diatoms	percentage	Oxygen requirement: dissolved oxygen low
OT_VL	diatoms	percentage	Oxygen requirement: dissolved oxygen very low
OT_LW_VL	diatoms	percentage	Oxygen requirement: sum of low and very low
OT_AH	**diatoms**	**percentage**	**Oxygen requirement: dissolved oxygen always high**
OT_FH	diatoms	percentage	Oxygen requirement: dissolved oxygen fairly high
			Motility
MT_YS	**all taxa**	**percentage**	**Motile algae**
SILTIDX	**diatoms**	**percentage**	**Motile diatoms**
			Biomass
Chl a	all taxa	g/m^2	Chlorophyll *a* concentration
CELLDENS	all taxa	cells/m^2	Total cell density

The watershed gradients were derived from principal components analysis (PCA) of separate ordinations with the GIS and CEN data. These gradients were represented by the site scores from the first three axes of each ordination: GIS 1–3 to characterize three gradients of change in landscape features and CEN 1–3 to characterize three gradients of change in socioeconomic factors. For each study area, stepwise regression analysis was also used to identify GIS variables closely related to variation in algal assemblages as represented by a similarity matrix of the RTH diatom data. The results indicated that watershed variables most consistently related to algal assemblages across study areas were the number of dams, percentage of watershed area upstream from dams, percentage of impervious surfaces in both the watershed and the riparian zone, and total length of artificial flow paths (for example, ditches and pipelines). These variables were used in separate PCA ordinations to derive three additional watershed gradients designated as HYD 1–3 that directly characterized hydrologic disturbances. Details on these datasets and associated watershed gradients are described in Giddings and others (in press).

Characterizing Reach Environmental Gradients

Environmental gradients were derived to characterize stream reaches in terms of physical habitat and water chemistry (fig. 2). The principal area sampled at a site was the stream reach, which was defined as approximately 20 times the mean stream width (reach length typically 150 to 300 meters [about 500 to 1,000 feet]); habitat variables were measured at 11 equally spaced transects by using NAWQA protocols (Fitzpatrick and others, 1998), and included channel dimensions, flow velocity and aspect, substrate, habitat cover, canopy closure, and bank characteristics. Habitat features measured over the length of the reach included stream gradient, frequency of geomorphic channel units (pools, riffles, runs), and continuous stream-stage and water-temperature data monitored with in situ instruments at typically hourly intervals. Reach gradients were represented by site scores derived from the first three axes of PCA ordinations with habitat data; these were designated HAB 1–3 to indicate three gradients that characterized changes to habitat features of the reach.

Water chemistry was sampled in stream reaches for routine field properties (dissolved oxygen, pH, specific conductance, alkalinity, water temperature), nutrients, and pesticides typically twice over the study to characterize high- and low-flow conditions. High-flow samples were not collected in the BIR area because of a drought in that region at the time. Typically one of the water chemistry samples was collected (usually low flow) to coincide with the algae sampling to characterize the ambient water-quality conditions for the algae. Reach gradients were represented by site scores derived from the first three axes of PCA ordinations of the water chemistry data (low and high flow combined); these were designated CWQ 1–3 to indicate three gradients characterizing the comprehensive water quality of the reach. Stepwise regression was used to identify reach-scale variables closely related to algal assemblages. The strongest correlations across study areas were with several water chemistry variables sampled near the time that algae were collected: specific conductivity, total phosphorus, total nitrogen, pH, and alkalinity (alkalinity was not sampled in RAL, ATL, DEN). These variables were used in PCA ordinations to derive three additional sets of reach-scale gradients designated as AWQ 1–3 to characterize conditions of ambient water quality most closely associated with the algal assemblages.

Analysis Procedures

Data were analyzed with the use of multivariate, multimetric, and correlation methods to determine how strongly the watershed and reach-scale gradients were associated with the algal-response variables (nMDS site scores and indicator metrics) in each of the nine study areas. Environmental data were standardized over their range prior to analyses. PCA site scores that characterized environmental gradients were scaled over their range from 0 to 100 percent, and slopes were reversed if necessary so that values indicated increasing human disturbance. Spearman correlation analysis was primarily used to relate algal-response variables with the environmental gradients. Several hundred variables were available for analyses; to limit spurious correlations, absolute rho values ($|rho|$) > 0.500 and $p < 0.05$ were considered ecologically relevant. A rho value meeting these criteria could explain 25 percent or more variability in a correlation, which was deemed a credible relation between disturbance and response.

The statistical software SYSTAT (SPSS, 2007) was used for the correlation analysis between the UII, other environmental gradients, and the algal-response variables. PRIMER software (Clarke and Gorley, 2006) was used for all multivariate analyses. Routines in PRIMER included nMDS to derive algal assemblage site scores; PCA to derive site scores for watershed and reach gradients; BEST (biological-environmental stepwise) to determine environmental variables most strongly related to algal assemblages; and ANOSIM (analysis of similarity) to determine the extent algal assemblages varied across study areas (for example, regional differences). Probability values were based on 1,000 random permutations that were used to develop a nonparametric probability distribution. Algal data were processed with the USGS Algal Data Analysis System, a program modified from the USGS Invertebrate Data Analysis System that is used to process data collected routinely in the NAWQA Program (Cuffney, 2003). The Algal Data Analysis System categorized algal taxa to the lowest taxonomic level (typically species) and calculated indicator-metric values for each site on the basis of attributes of structure and function.

Results

The results of comparing the relations of urbanization and other environmental gradients to algal assemblages are summarized into four sections that discern variation among algal assemblages, identify algal indicator metrics, relate urban intensity to other watershed gradients, and quantify the algal response to environmental gradients. Details of these sections follow.

Discerning Variation Among Algal Assemblages

Results from the ANOSIM procedure indicated that algal assemblages were significantly different across study areas, even though considerable variation in assemblages also existed within each study area. The result was indicative of the region effect, where algal assemblages were most strongly affected by physiographic factors inherent to the respective study areas. The region effect appeared slightly stronger with the DTH than with the RTH diatom data (rho = 0.782 and 0.719, respectively), but was strongest with qualitative RTH plus DTH diatom data in the QUAL dataset (rho = 0.956). Except for four sites, variation in algal assemblages within a study area (presumably from environmental gradients operating within the study area) was not so great that a site could not be linked to its respective study area (fig. 3). Subsequent analyses were done by study area because if all sites were combined, regional differences could obscure the response of human-disturbance gradients (for example, UII) on algal assemblages. Furthermore, the RTH dataset was selected for subsequent analyses because a preliminary survey of the data indicated that variation in the RTH diatoms could most likely be characterized by the indicator metrics that were used.

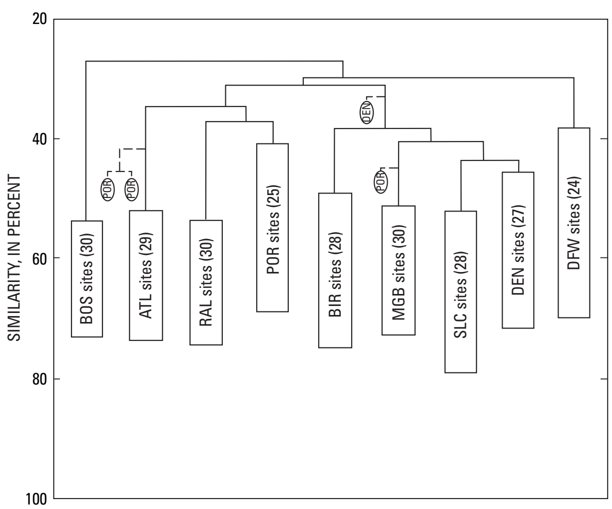

Figure 3. Dendogram derived from the similarity matrix of QUAL data (diatom presence/absence), which indicates that the algal assemblages are relatively distinct among study areas where urban studies were conducted as part of the U.S. Geological Survey National Water-Quality Assessment Program. Each box represents the number of sites (n) clustered for a study area; vertical scale of the box indicates the range in percent similarity among the sites (Y-axis). The nodes of the branches indicate the similarity among study areas. Each of the four ovals linked with a dashed branch (---) represents a site that was not associated with its respective study area (outlier).

Identifying Algal Indicator Metrics

For each study area, the RTH data were used to derive nMDS site scores from ordination axes 1 and 2 (denoted as nMDS-1 and nMDS-2) and values for 29 indicator metrics (table 1). Correlations between the nMDS scores and the metrics resulted in relevant relations ($|rho| > 0.500$, $p < 0.05$) in every study area (table 2), which indicated that the overall assemblage structure (characterized by nMDS scores) was related to some common measures of algal attributes (characterized by indicator metrics). The number of relevant correlations varied greatly among study areas; 26 of 29 metrics were strongly correlated with the nMDS site scores in the BOS compared to only 6 metrics in the SLC area. In addition, the BOS, SLC, and MGB had relevant correlations only with nMDS-1, whereas the other study areas had relevant correlations with both axes nMDS-1 and 2.

For each of the 29 metrics, values of $|rho| > 0.500$ were summed to derive a correlation rank (table 2) to help identify metrics that may be most applicable across study areas. All attribute categories except “Biomass” had metrics with relevant correlations in at least seven study areas. Of these, the eight metrics had the highest correlation ranks for their respective attribute category (table 2, in bold); among the 29 metrics, these eight metrics subsequently were shown to have the strongest correlations with the environmental gradients across the study areas.

Relating Urban Intensity to Other Watershed Gradients

Correlations between the MA-NUII and GIS 1–3 and CEN 1–3 axes indicated that urbanization was most strongly related to either the first or second axis (table 3). GIS-1 was most strongly correlated across all study areas ($|rho|$ = 0.887–0.987), indicating that the predominant landscape changes specific to each study area were well represented by the UII (scaled either as the MA-NUII or NUII) as a common measure of urbanization. For example, the variable with the highest loading on GIS-1 axis for the ATL was landscape fragmentation from an increase in patches of urban areas; therefore, urbanization can be characterized by this fragmentation process in the ATL. In the MGB, conversely, the GIS-1 axis loaded highest and with a negative value on patches of agricultural land, and urbanization was inversely correlated with loss of agricultural land cover.

Compared to the GIS gradients, CEN gradients characterizing socioeconomic factors were less strongly correlated

Table 2. Correlation coefficients (|rho|) of nonmetric multi-dimensional scaling axes (nMDS-1 and nMDS-2) scores to indicator metrics from urban studies conducted as part of the U.S. Geological Survey National Water-Quality Assessment Program. Values of |rho| > 0.500 were considered relevant (shaded) and were summed for each metric to derive a correlation rank to help identify metrics that may be most applicable across metropolitan study areas (identified in top row). All attribute categories except "Biomass" had metrics with relevant correlations in at least seven study areas. Of these, the eight metrics shown in **bold** have the highest correlation ranks for their respective attribute categories and were used in subsequent analyses with the environmental gradients.

[Indicator metrics are defined in table 1; POR, Portland, Oregon; SLC, Salt Lake City, Utah; DEN, Denver, Colorado; DFW, Dallas-Fort Worth, Texas; MGB, Milwaukee-Green Bay, Wisconsin; BIR, Birmingham, Alabama; ATL, Atlanta, Georgia; RAL, Raleigh, North Carolina; BOS, Boston, Massachusetts; nMDS-1, nonmetric multi-dimensional scaling axis 1; nMDS-2; nonmetric multi-dimensional scaling axis 2]

Indicator metric	Correlation rank	POR		SLC		DEN		DFW		MGB		BIR		ATL		RAL		BOS	
		nMDS-1	nMDS-2	nMDS-1	nMDS-2	nMDS-1	nMDS-2	nMDS-1	nMDS-2	nMDS-1	nMDS-2	nMDS-1	nMDS-2	nMDS-1	nMDS-2	nMDS-1	nMDS-2	nMDS-1	nMDS-2
Taxa composition																			
TAXA_RICH	0.7	0.216	0.432	0.398	0.286	0.115	0.108	0.164	0.197	0.162	0.110	0.402	0.166	0.375	0.478	0.282	0.430	0.710	0.123
DIAT_RICH	0.7	0.277	0.377	0.454	0.218	0.076	0.142	0.221	0.205	0.187	0.072	0.437	0.180	0.422	0.467	0.251	0.459	0.736	0.127
DVS_SW_ALL	1.4	0.218	0.579	0.327	0.189	0.193	0.047	0.237	0.086	0.124	0.003	0.053	0.048	0.415	0.060	0.191	0.245	0.795	0.141
DVS_SW_DIA	3.3	0.095	0.299	0.712	0.189	0.070	0.069	0.291	0.305	0.078	0.146	0.557	0.139	0.547	0.227	0.672	0.248	0.858	0.201
ACHMIN_P	**6.8**	0.753	0.010	0.896	0.184	0.889	0.013	0.725	0.334	0.147	0.459	0.958	0.060	0.899	0.119	0.412	0.708	0.976	0.006
Salinity tolerance																			
SL_FR	0.7	0.148	0.204	0.122	0.028	0.421	0.448	0.440	0.673	0.197	0.074	0.067	0.167	0.161	0.462	0.113	0.106	0.004	0.419
SL_BF	4.5	0.147	0.637	0.360	0.299	0.727	0.116	0.417	0.590	0.934	0.201	0.284	0.268	0.249	0.461	0.799	0.332	0.817	0.138
SL_HB	**5.1**	0.113	0.645	0.369	0.303	0.732	0.155	0.427	0.577	0.937	0.202	0.274	0.310	0.396	0.603	0.803	0.328	0.830	0.120
Trophic state																			
TR_E	**5.9**	0.797	0.073	0.854	0.145	0.993	0.054	0.377	0.786	0.242	0.335	0.758	0.454	0.477	0.218	0.835	0.151	0.854	0.468
ON_NH	5.3	0.291	0.832	0.022	0.250	0.220	0.851	0.013	0.122	0.925	0.190	0.429	0.579	0.403	0.640	0.657	0.163	0.781	0.040
SP_OL	2.4	0.204	0.354	0.014	0.262	0.468	0.299	0.555	0.622	0.140	0.034	0.037	0.287	0.481	0.693	0.044	0.223	0.569	0.107
SP_BM	**5.8**	0.181	0.761	0.107	0.467	0.097	0.595	0.626	0.258	0.942	0.277	0.360	0.507	0.713	0.287	0.807	0.205	0.845	0.086
SP_AM	2.9	0.256	0.671	0.200	0.212	0.021	0.030	0.013	0.355	0.934	0.167	0.161	0.183	0.077	0.180	0.412	0.508	0.790	0.019
SP_AP	3.5	0.311	0.584	0.079	0.239	0.048	0.813	0.499	0.356	0.393	0.408	0.347	0.534	0.293	0.397	0.736	0.173	0.800	0.019
SP_AM_AP	4.6	0.255	0.743	0.106	0.295	0.013	0.581	0.392	0.728	0.948	0.267	0.392	0.483	0.169	0.348	0.800	0.191	0.817	0.030
Pollution tolerance																			
PT_TA	2.1	0.316	0.712	0.215	0.116	0.184	0.356	0.198	0.118	0.380	0.103	0.295	0.429	0.080	0.033	0.444	0.615	0.795	0.076
PT_TBP	2.0	0.200	0.413	0.311	0.240	0.252	0.126	0.325	0.121	0.040	0.168	0.407	0.486	0.639	0.395	0.060	0.572	0.771	0.070
PT_TA_TB	2.2	0.300	0.765	0.226	0.120	0.091	0.174	0.284	0.104	0.382	0.121	0.339	0.458	0.238	0.024	0.407	0.612	0.827	0.009
PC_MT	4.8	0.299	0.617	0.159	0.171	0.020	0.777	0.751	0.033	0.419	0.348	0.361	0.644	0.396	0.522	0.786	0.210	0.690	0.195
PC_SN	**6.0**	0.248	0.789	0.112	0.378	0.027	0.637	0.768	0.375	0.947	0.271	0.300	0.558	0.805	0.333	0.695	0.224	0.832	0.126
Oxygen requirements																			
OT_LW	5.5	0.254	0.623	0.107	0.201	0.187	0.762	0.794	0.053	0.521	0.392	0.320	0.541	0.407	0.634	0.795	0.038	0.828	0.013
OT_VL	0.7	0.256	0.240	0.145	0.116	0.351	0.321	0.030	0.288	0.054	0.059	0.146	0.253	0.226	0.131	0.239	0.094	0.699	0.173
OT_LW_VL	5.5	0.254	0.623	0.125	0.214	0.271	0.816	0.748	0.096	0.512	0.435	0.301	0.550	0.399	0.637	0.792	0.033	0.844	0.007
OT_AH	**5.7**	0.796	0.014	0.877	0.097	0.928	0.138	0.194	0.344	0.179	0.310	0.886	0.275	0.519	0.404	0.770	0.378	0.921	0.316
OT_FH	4.3	0.703	0.064	0.841	0.279	0.691	0.056	0.030	0.112	0.929	0.301	0.531	0.092	0.467	0.148	0.392	0.296	0.575	0.137
Motility																			
MT_YS	**5.1**	0.118	0.692	0.431	0.055	0.340	0.462	0.720	0.248	0.917	0.236	0.204	0.662	0.621	0.483	0.803	0.249	0.705	0.046
SILTIDX	**6.4**	0.369	0.614	0.527	0.054	0.297	0.670	0.696	0.293	0.840	0.303	0.239	0.676	0.507	0.765	0.919	0.069	0.720	0.133
Biomass																			
CHLA	1.2	0.276	0.603	0.054	0.088	0.547	0.134	0.257	0.252	0.067	0.225	0.322	0.025	0.016	0.334	0.032	0.340	0.082	0.086
CELLDENS	0.0	0.073	0.183	0.134	0.346	0.494	0.221	0.273	0.199	0.007	0.272	0.062	0.021	0.145	0.201	0.070	0.249	0.420	0.111

Table 3. Correlation coefficients (|rho|) between the metropolitan area-national urban intensity index (MA-NUII) and the principal components analysis (PCA) derived watershed environmental gradients for each metropolitan study area where urban studies were conducted as part of the U.S. Geological Survey National Water-Quality Assessment Program. GIS 1–3 represents the first three axes from the PCA ordination of landscape features and CEN 1–3 represents the first three axes from the PCA ordination of human socioeconomic and census demography factors. **Bold** values indicate the specific GIS and CEN axes with the strongest correlations to the MA-NUII.

[GIS-1, GIS-2, and GIS-3, geographic information system principal components analysis, axes 1–3; CEN-1, CEN-2, and CEN-3, socioeconomic/census data principal components analysis, axes 1–3; POR, Portland, Oregon; SLC, Salt Lake City, Utah; DEN, Denver, Colorado; DFW, Dallas-Fort Worth, Texas; MGB, Milwaukee-Green Bay, Wisconsin; BIR, Birmingham, Alabama; ATL, Atlanta, Georgia; RAL, Raleigh, North Carolina; BOS, Boston, Massachusetts]

Metropolitan study area	GIS-1	GIS-2	GIS-3	CEN-1	CEN-2	CEN-3
POR	**0.966**	0.013	0.033	**0.710**	0.568	0.057
SLC	**0.852**	0.109	0.419	**0.520**	0.152	0.444
DEN	**0.887**	0.617	0.577	0.328	**0.744**	0.682
DFW	**0.932**	0.053	0.018	0.284	**0.801**	0.129
MGB	**0.934**	0.123	0.157	**0.935**	0.073	0.115
BIR	**0.909**	0.603	0.330	0.202	**0.747**	0.244
ATL	**0.987**	0.014	0.033	0.421	**0.853**	0.319
RAL	**0.971**	0.054	0.203	0.444	**0.634**	0.297
BOS	**0.937**	0.283	0.232	0.194	**0.884**	0.157

to the MA-NUII (rho = 0.520 – 0.855), except for the MGB (rho = 0.935 with CEN-1). The MA-NUII was correlated most strongly to CEN-1 for SLC, POR, and MGB studies, but most strongly to CEN-2 for the other study areas. As with the GIS-1 axis, the variables with the highest loadings on the CEN axis varied with study area. However, whether CEN-1 or CEN-2 was correlated most strongly with the MA-NUII, that particular axis generally was more highly loaded with population-related variables, whereas the opposing axis was more highly loaded with socioeconomic variables, indicating "wealth" may be less important.

Quantifying the Algal Response to Environmental Gradients

The 29 indicator metrics were correlated individually with urbanization (MA-NUII) and the watershed (GIS 1–3, CEN 1–3, HYD 1–3) and reach scale (CWQ 1–3, AWQ 1–3, HAB 1–3) environmental gradients. For each indicator metric, a tally was made of the number of study areas that had at least one relevant correlation (|rho| > 0.500) between the metric and any of the watershed or reach-scale gradients. These tallies indicated that eight metrics had relevant correlations in most study areas: *SL_HB* (diatoms tolerant to halobiontic systems; six study areas), *TR_E* (diatoms tolerant of eutrophic conditions; seven study areas), *SP_BM* (diatoms sensitive to conditions of organic-load oxidation; seven study areas), *PC_SN* (diatoms sensitive to pollution; eight study areas), *OT_AH* (diatoms requiring high dissolved oxygen; six study areas), *MT_YS* (motile taxa; five study areas), *SILTIDX* (diatoms tolerant of silt deposition; seven study areas), and *ACHMIN_P* (relative abundance of *Achnanthidium minutissimum*; five study areas). Not unexpectedly, these were the same metrics that were correlated most strongly with the nMDS scores (table 2), and consequently these eight were selected for subsequent analyses (table 4).

A relevant correlation resulted between the MA-NUII and at least one of the algal metrics in five study areas (SLC, DEN, MGB, RAL, and BOS); however, in all study areas, correlations were stronger or more numerous with environmental gradients other than the MA-NUII (table 4). Among the watershed gradients, indicator metrics were not correlated more strongly to CEN gradients (socioeconomic factors) than to either GIS gradients or the MA-NUII; therefore, correlations with CEN gradients are not presented.

Although taxa richness was not one of the selected indicator algal metrics, its relation to urbanization was notable. No study area showed a significant decline in richness across the full gradient of urban intensity. However, in four study areas (POR, SLC, RAL, BOS), richness increased significantly over a low (0) to moderate (50) range of urban intensity as measured by the NUII (fig. 4). The NUII (scaled for the entire United States) was used instead of the MA-NUII (scaled for each study area) so that urban intensity could be equally scaled across study areas for this comparison. In part, this increase in taxa richness may be related to nutrient enrichment of streams that can occur during the early stages of urbanization; compared to the relation between NUII and taxa richness (fig. 4), the derived water-quality gradient for each study area

Table 4. Correlation coefficients (|rho|) between the algal indicator metrics and environmental gradients; relevant correlations (|rho| > 0.500, p < 0.05) are in **bold**, for urban studies conducted as part of the U.S. Geological Survey National Water-Quality Assessment Program. The primary environmental gradient for each metropolitan study area is identified in **bold** as having the strongest correlations overall; two are identified for the Salt Lake City area (SLC; GIS-1, CWQ-1). If no relevant correlation was found for a particular gradient in a study area, all cells for that gradient are indicated with dashes (---). The |rho| values for the metropolitan area-national urban intensity index (MA-NUII), however, are shown regardless (shaded cells). The signs below each metric (+/–) indicate if the values increased or decreased with disturbance.

[Indicator metrics are defined in table 1; POR, Portland, Oregon; SLC, Salt Lake City, Utah; DEN, Denver, Colorado; DFW, Dallas-Fort Worth, Texas; MGB, Milwaukee-Green Bay, Wisconsin; BIR, Birmingham, Alabama; ATL, Atlanta, Georgia; RAL, Raleigh, North Carolina; BOS, Boston, Massachusetts; MA-NUII, metropolitan area-national urban intensity index; GIS, geographic information system principal components analysis; HYD, hydrologic modifications principal components analysis; HAB, in-stream habitat principal components analysis; GIS-1 and GIS-2, geographic information system principal components analysis, axes 1–2; HYD-1, HYD-2, and HYD-3, hydrologic modifications principal components analysis, axes 1–3; AWQ-1, AWQ-2, and AWQ-3, ambient water-quality principal components analysis, axes 1–3; HAB-1, HAB-2, and HAB-3, in-stream habitat principal components analysis, axes 1–3; HYD-1, HYD-2, and HYD-3, hydrologic modifications principal components analysis, axes 1–3; CWQ-1, comprehensive water-quality principal components analysis, axis 1]

Metro-politan study area	Environ-mental gradient	Indicator metrics							
		SL_HB (+)	*TR_E* (+)	*SP_BM* (–)	*PC_SN* (–)	*OT_AH* (–)	*MT_YS* (+)	*SILTIDX* (+)	*ACHMIN_P* (–)
POR	MA-NUII	0.120	0.406	0.436	0.444	0.396	0.232	0.360	0.349
	GIS-1	0.123	**0.516**	0.425	0.466	0.498	0.257	0.391	0.433
	HYD-3	0.298	0.001	**0.544**	0.443	0.002	0.367	**0.534**	0.054
	AWQ-1	0.240	**0.654**	**0.507**	**0.571**	**0.691**	0.400	**0.602**	**0.568**
	HAB	---	---	---	---	---	---	---	---
SLC	MA-NUII	0.472	0.291	0.440	0.429	0.284	**0.515**	0.436	0.233
	GIS-1	**0.563**	**0.563**	**0.544**	**0.537**	**0.555**	**0.522**	0.477	0.497
	HYD	---	---	---	---	---	---	---	---
	CWQ-1	**0.623**	0.365	**0.566**	**0.585**	0.409	0.488	0.385	0.372
	HAB-2	0.332	**0.649**	0.418	0.374	**0.595**	0.404	0.379	**0.576**
DEN	MA-NUII	0.244	0.082	0.352	0.395	0.125	0.436	**0.556**	0.027
	GIS-1	0.100	0.041	0.357	0.420	0.085	0.421	**0.548**	0.036
	HYD-1	0.060	0.165	0.459	**0.522**	0.106	0.426	**0.554**	0.232
	AWQ-1	**0.749**	**0.733**	0.000	0.143	**0.633**	0.369	0.319	0.485
	HAB	---	---	---	---	---	---	---	---
DFW	MA-NUII	0.003	0.056	0.029	0.050	0.001	0.218	0.097	0.149
	GIS	---	---	---	---	---	---	---	---
	HYD	---	---	---	---	---	---	---	---
	AWQ-2	**0.589**	0.490	0.341	**0.618**	0.171	**0.510**	0.421	**0.621**
	HAB-3	0.414	0.301	**0.519**	0.420	0.314	0.491	**0.546**	0.463
MGB	MA-NUII	**0.655**	0.185	**0.567**	**0.563**	0.119	**0.541**	0.387	0.188
	GIS-1	**0.539**	0.261	0.444	0.418	0.264	0.412	0.216	0.254
	HYD-1	**0.601**	0.307	**0.557**	**0.532**	0.294	**0.531**	0.396	0.229
	AWQ-1	**0.592**	0.273	**0.574**	**0.608**	0.290	**0.576**	**0.652**	0.302
	HAB	---	---	---	---	---	---	---	---
BIR	MA-NUII	0.409	0.160	0.104	0.126	0.039	0.311	0.284	0.211
	GIS	---	---	---	---	---	---	---	---
	HYD	---	---	---	---	---	---	---	---
	AWQ-1	0.144	**0.543**	0.084	0.225	0.397	0.128	0.112	0.167
	HAB	---	---	---	---	---	---	---	---
ATL	MA-NUII	0.232	0.216	0.288	0.160	0.430	0.239	0.469	0.112
	GIS-2	0.048	0.427	**0.602**	**0.603**	0.469	0.296	0.075	**0.748**
	HYD-2	0.170	0.408	0.018	0.163	**0.617**	0.339	**0.537**	0.198
	AWQ-2	0.176	**0.505**	**0.691**	**0.658**	0.390	0.392	0.275	**0.575**
	HAB-1	0.026	0.325	0.385	**0.587**	0.330	0.360	0.062	**0.544**
RAL	MA-NUII	0.478	**0.520**	0.423	0.373	0.364	**0.599**	**0.719**	0.139
	GIS-1	**0.523**	**0.516**	0.436	0.389	0.367	**0.614**	**0.728**	0.129
	HYD-2	**0.664**	**0.582**	0.450	0.417	0.374	**0.723**	**0.764**	0.051
	AWQ-3	**0.523**	**0.747**	**0.729**	**0.630**	**0.725**	**0.665**	**0.666**	0.483
	HAB-1	0.405	0.497	0.383	0.353	0.368	**0.547**	**0.616**	0.041
BOS	MA-NUII	**0.759**	**0.614**	**0.820**	**0.834**	**0.622**	**0.798**	**0.820**	**0.685**
	GIS-1	**0.631**	**0.544**	**0.744**	**0.781**	**0.595**	**0.750**	**0.773**	**0.592**
	HYD-1	**0.739**	**0.567**	**0.736**	**0.721**	**0.595**	**0.712**	**0.689**	**0.598**
	AWQ-1	**0.834**	**0.701**	**0.838**	**0.865**	**0.752**	**0.823**	**0.840**	**0.785**
	HAB-1	**0.680**	0.496	**0.652**	**0.615**	0.499	**0.661**	**0.642**	0.495

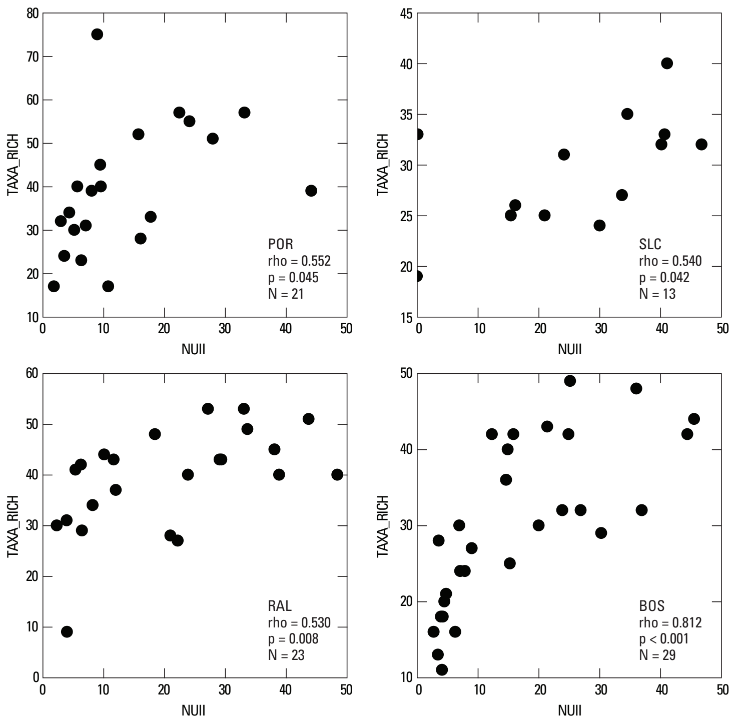

Figure 4. Correlations between algal taxa richness (TAXA_RICH) and the NUII that indicate a positive relation over the early stages of urbanization in four study areas where urban studies were conducted as part of the U.S. Geological Survey National Water-Quality Assessment Program. To emphasize the initial stages of urbanization, the range of urban intensity values is limited to NUII < 50. No study areas had a significant decline in taxa richness with increasing urban intensity over this range.

(table 4) was related to taxa richness slightly more strongly (POR, AWQ-1: rho = 0.622; SLC, CWQ-1: rho = 0.571; RAL, AWQ-3: rho = 0.566; BOS, AWQ-1: rho = 0.822). In addition, it is also notable that the biomass metrics (Chl *a* and cell density) were related to urbanization only in the BOS (Chl *a*; rho = 0.573), and very few relevant correlations resulted from the biomass metrics with any other environmental gradient. The strongest of these occurred in the DFW between cell density and HAB-1 (rho = 0.703), where the primary variables defining the HAB-1 gradient in the DFW were increases in channel size and frequency of pools.

In general, the indicator metrics were consistent in how they related to environmental variables across study areas in that they responded predictably to declining ecological conditions (for example, sensitive taxa declined with degraded water quality). The relations between the indicator metrics and the environment gradients (table 4) are described below for the individual study areas.

Portland (POR), Oregon

The algal response in POR was related most strongly with ambient water quality as represented by AWQ-1 (table 4). Relevant correlations resulted with six indicator metrics (*TR_E, SP_BM, PC_SN, OT_AH, SILTIDX, ACHMIN_P*). The primary variables contributing to the AWQ-1 gradient were increases in specific conductance, total phosphorus, and alkalinity. The relation between AWQ-1 and the MA-NUII was relatively strong (rho = 0.689; table 5), which suggests a linked association where urbanization affects water quality that, in turn, can cause an algal response.

Salt Lake City (SLC), Utah

The algal response in SLC was somewhat more mixed with environmental gradients than in the other study areas. GIS-1 had relevant correlations with all metrics except *SILTIDX* and *ACHMIN_P*; however, *SL_HB, SP_BM,* and *PC_SN* were correlated more strongly with CWQ-1 (table 4). The primary variables contributing to the GIS-1 gradient were fragmentation and loss of natural land cover (shrubland, forest, grassland) and a decrease stream gradient (a natural factor); primary variables contributing to the CWQ-1 gradient were related mainly to increased pesticide concentrations. Correlations of MA-NUII to GIS-1 and CWQ-1 were relatively strong (table 5; rho = 0.852 and 0.660, respectively). In addition, the algal response in the SLC may have been affected by factors related to configuration of sites. Unlike the other study areas, the SLC sites were nested in only a few watersheds that originated in the mountains (Wasatch Range) and extended to highly developed areas at lower elevations (Wasatch Front). The consequences of this layout are that sites were situated from upstream to downstream to represent increasing urban intensity, but concurrently, watershed size increased and stream gradient decreased.

Denver (DEN), Colorado

The algal response in DEN was related most strongly to AWQ-1, which had relevant correlations with three metrics *(SL_HB, TR_E, OT_AH),* although the watershed gradients (MA-NUII, GIS-1, HYD-1) had relevant correlations to *SILTIDX* (table 4). The primary variables contributing to the AWQ-1 gradient were an increase in total nitrogen and specific conductance, and secondarily, an increase in pH and total phosphorus. The MA-NUII was not correlated strongly with AWQ-1 (rho = 0.104) but was with GIS-1 (rho = 0.887) and HYD-1 (rho = 0.868; table 5). Results indicate that water-quality factors that explained variability in the algal assemblages were not directly related to urbanization as characterized by the MA-NUII, but diatoms associated with *SILTIDX* may have been more directly affected by urbanization.

Table 5. Correlation coefficients (rho) between metropolitan area-national urban intensity index (MA-NUII) and environmental gradients identified in table 4 as the most relevant in each metropolitan study area where urban studies were conducted as part of the U.S. Geological Survey National Water-Quality Assessment Program. The Environmental gradients that are shaded (typically, ambient water quality) were related most strongly to indicator metrics in each metropolitan study area. Rho values that are **bold** are statistically significant ($|rho| \geq 0.500$, $p < 0.05$).

[MA-NUII, metropolitan area-national urban intensity index; POR, Portland, Oregon; SLC, Salt Lake City, Utah; DEN, Denver, Colorado; DFW, Dallas-Fort Worth, Texas; MGB, Milwaukee-Green Bay, Wisconsin; BIR, Birmingham, Alabama; ATL, Atlanta, Georgia; RAL, Raleigh, North Carolina; BOS, Boston, Massachusetts; GIS-1 and GIS-2, geographic information system principal components analysis, axes 1–2; HYD-1, HYD-2, and HYD-3, hydrologic modifications principal components analysis, axes 1–3; AWQ-1, AWQ-2, and AWQ-3, ambient water-quality principal components analysis, axes 1–3; CWQ-1, comprehensive water-quality principal components analysis, axis 1; HAB-1, HAB-2, and HAB-3, in-stream habitat principal components analysis, axes 1–3]

Metropolitan study area	Environmental gradient	Correlation with MA-NUII (rho)
POR	GIS-1	**0.966**
	HYD-3	0.395
	AWQ-1	**0.689**
SLC	GIS-1	**0.852**
	HYD-2	0.075
	CWQ-1	**0.660**
	HAB-2	0.394
DEN	GIS-1	**0.887**
	HYD-1	**0.868**
	AWQ-1	0.104
DFW	AWQ-2	0.033
	HAB-3	0.319
MGB	GIS-1	**0.934**
	HYD-1	**0.859**
	AWQ-1	0.311
BIR	AWQ-1	0.431
ATL	GIS-2	0.014
	HYD-2	**0.808**
	AWQ-2	0.028
	HAB-1	0.235
RAL	GIS-1	**0.971**
	HYD-2	**0.831**
	AWQ-3	0.437
	HAB-1	**0.653**
BOS	GIS-1	**0.937**
	HYD-1	**0.879**
	AWQ-1	**0.885**
	HAB-1	**0.763**

Dallas-Fort Worth (DFW), Texas

The algal response in DFW was related most strongly to AWQ-2, which had relevant correlations with four metrics *(SL_HB, PC_SN, MT_YS, ACHMIN_P),* although *SP_BM* and *SILTIDX* had relevant correlations with HAB-3 (table 4). The primary variables contributing to the AWQ-2 gradient were an increase in total phosphorus and specific conductance, and a decrease in pH. The primary factor contributing to the HAB-3 gradient was variation in stream velocity within a reach; it is likely this factor was related to natural variability in the region, because it did not appear related to anthropogenic activity in the watershed. Neither the AWQ-2 nor HAB-3 gradients were correlated significantly to the MA-NUII (table 5), which indicates that algal assemblages were not directly responding to urbanization as characterized by the MA-NUII.

Milwaukee-Green Bay (MGB), Wisconsin

The algal response in MGB was related most strongly to AWQ-1, which had relevant correlations with five metrics *(SL_HB, SP_BM, PC_SN, MT_YS, SILTIDX;* table 4). The primary variables contributing to the AWQ-1 gradient were increases in alkalinity, total nitrogen, and specific conductance. Except for *SILTIDX,* the same metrics had relevant correlations with the MA-NUII, but all were weaker. Based on the similarity in how the metrics were correlated to AWQ-1 and the MA-NUII, a strong relation between these two environmental gradients might be expected; however, the correlation (rho = 0.311) was not significant (table 5).

Birmingham (BIR), Alabama

Among the study areas, the algal responses in the BIR were correlated least strongly to any environmental gradient. The BIR region was experiencing a severe drought at the time of the study that may have had a prevailing effect on the algal response. The single relevant correlation resulted between AWQ-1 and *TR_E* (table 4). The variables contributing to the AWQ-1 gradient were increases in specific conductance, total nitrogen, and total phosphorus. The relation between the MA-NUII and AWQ-1 was relatively weak (rho = 0.431) but significant (table 5). An effect of the drought was that high concentrations of fish frequently were found in pools at the BIR sites, and the algae-grazing largescale stoneroller (*Campostoma oligolepis*) was often dominant (Brown and others, 2009). BIR fish data were used subsequently to derive fish nMDS scores that were correlated with the algal nMDS-1 scores. The relation was very strong ($|rho| = 0.758$), which suggests that high fish density caused by the drought may have affected algal assemblages (Power and Matthews, 1983).

Atlanta (ATL), Georgia

The algal response in the ATL was related most strongly to AWQ-2, which had relevant correlations with four metrics *(TR_E, SP_BM, PC_SN, ACHMIN_P),* although *OT_AH* and *SILTIDX* had relevant correlations with HYD-2 (table 4). The primary variable contributing to the AWQ-2 gradient was an increase in total phosphorus. The primary variables contributing to the HYD-2 gradient were increases in impervious surface in the watershed and along the riparian zone. The correlation between MA-NUII and AWQ-2 indicated no direct association (rho = 0.028). Conversely, a strong relation between MA-NUII and HYD-2 (rho = 0.808) suggested that impervious surface was closely associated with urbanization in that region (table 5).

Raleigh (RAL), North Carolina

The algal response in the RAL was related most strongly to AWQ-3, which had relevant correlations with all metrics except *ACHMIN_P* (table 4). The primary variable contributing to the AWQ-3 gradient was an increase in total phosphorus, and secondary variables were increases in pH and specific conductance. It was notable that both *MT_YS* and *SILTIDX* had relevant correlations to all environmental gradients (MA-NUII, GIS-1, HYD-2, HAB-1) and that *TR_E* had relevant correlations to all environmental gradients except HAB-1 (rho = 0.497). The primary contributing variables to the GIS-1 gradient were fragmentation of the landscape and increase in road density, whereas the contributing variable to the HYD-2 gradient was increase in impervious surface; predictably, the relation between GIS-1 and HYD-2 was strong (rho = 0.857). Furthermore, the MA-NUII was weakly correlated to AWQ-3 (rho = 0.437), but was correlated much more strongly to GIS-1 (rho = 0.971) and to HYD-2 (rho = 0.831; table 5).

Boston (BOS), Massachusetts

The algal response in the BOS was related most strongly to AWQ-1, which had relevant correlations with all metrics; relations were relatively strong, with all values of rho > 0.700 (table 4). Except for HAB-1, relevant (but somewhat weaker) correlations also resulted between the other environmental gradients (MA-NUII, GIS-1, HYD-1) and all metrics. However, only three metrics *(TR_E, OT_AH, ACHMIN_P)* did not have a relevant correction with HAB-1; even among these three, the lowest value of rho = 0.495 (table 4). The variables contributing to the AWQ-1 gradient included all factors used in the ordination (total nitrogen, total phosphorus, specific conductance, alkalinity, pH). Primary variables contributing to each of the other environmental gradients are for HAB-1, increase in channel depth; for GIS-1, loss of forest land; and for HYD-1, increase in impervious surface. The MA-NUII was strongly correlated to all environmental gradients in the BOS (rho = 0.763; table 5). The BOS results indicate a strong co-variance (linking) among the environmental gradients and are a clear example of a model where predictable changes occurring in the watershed are related to urbanization; these changes then affect stream-reach conditions of water quality and habitat, which in turn affect algal assemblages.

Discussion

The results of the study were consistent with the assertions that algae respond relatively quickly to changing conditions and that they are often most sensitive to a localized or recent disturbance. For the EUSE study, algal, invertebrate, and fish samples were collected once and water chemistry was sampled usually twice to characterize conditions for each site, including one sample typically within 4 weeks of biological sampling. While the point-in-time sampling was not optimal for understanding specific details of the algal response to urbanization, the data were suited to investigating whether the algal response was stronger with urban intensity (MA-NUII) or with other environmental gradients at the watershed or reach scales, and how responses varied across the nine regions.

In some study areas, the endeavor to relate the algal response to urbanization was likely confounded by effects from agricultural land use (Porter and others, 2001; Foster and others, 2003). For example, urban intensity increased in the BOS with the concurrent loss of primarily forested land, whereas urban intensity increased in the MGB, DFW, and DEN areas with the conversion of primarily agricultural land. A major consequence of this land-use conversion was that stream condition had already been degraded before the onset of urbanization in the MGB, DFW, and DEN areas (T.F. Cuffney and others, U.S. Geological Survey, written commun., 2009). Conversely, the consistency in the BOS area where all environmental gradients were strongly related to the indicator metrics (table 4) suggests fewer confounding variables were present in the BOS area and that stream condition was linked most directly to the loss of forests with encroaching urban development.

Algal assemblages were structurally different (for example, species composition) between study areas, even though the assemblages were responding to environmental gradients operating within each study area (fig. 3). These differences across study areas indicated a regional effect, which was likely related to physiographic variation across the country that contributed to regionally different algal assemblages. This regional effect has also been described in the ecoregion concept, which uses primarily natural landscape features to explain natural variation in biological communities (Omernik, 1987). Recognizing that algal assemblages vary naturally by ecoregion is a critical point to consider when evaluating algal data across regions (Stevenson, 1997; Potapova and Charles, 2002; Potapova and others, 2005; Charles and others, 2006). Therefore, in addressing the primary objective of this study—Is the algal response more directly related to urbanization as characterized by derived urban indexes or to other environmental gradients?—variation in algal assemblages was evaluated by individual study area.

Algal taxa richness increased significantly in four study areas (RAL, SLC, BOS, POR) with increasing urbanization from the initial stages to mid-level intensities (NUII 0–50), whereas no study area showed a significant decrease in algal richness over the urban gradient. This response of richness to urbanization may be indicative of the intermediate disturbance concept, where complexity in biological assemblages can increase over the initial stages of a disturbance (Connell, 1978). Conversely, in evaluating the response of invertebrate assemblages in nine study areas, T.F. Cuffney and others (U.S. Geological Survey, written commun., 2009) reported that a decrease in invertebrate richness over the entire gradient of increasing urbanization was one of the most reliable metrics across the regions. Carlisle and others (2009) used qualitative assemblage data for the Appalachian region to infer stream condition by comparing taxa observed at a study site against taxa expected at reference sites; the taxa ratio of observed/expected (O/E) is, therefore, a measure of impairment relative to reference conditions. Their results for algae indicated an "apparently counterintuitive finding that diatom assemblage impairment was associated with less urbanization." Furthermore, they acknowledged the model for algae was less precise than for fish or invertebrates, which might be due to a greater sensitivity of diatom assemblages to short-term variation in conditions within a reach. Overall, the results of the current study are best explained by such short-term variation, and accordingly, it is emphasized that time and spatial scales are crucial when evaluating the algal response.

Across the study areas, the algal response was associated weakly with urbanization as characterized by the MA-NUII; the MA-NUII was not the primary environmental gradient related to algal assemblages in any study area, and it had relevant correlations only to one or more indicator metrics in five study areas (SLC, DEN, MGB, RAL, BOS; table 4). Of the other watershed-scale gradients, GIS had relevant correlations to algal assemblages in seven study areas, and HYD had relevant correlations in six study areas. Only in the SLC area, however, was a watershed gradient most strongly related to algal assemblages (GIS-1 to six indicator metrics). Of the reach-scale gradients, HAB had relevant correlations in five study areas. The habitat gradients were not strongly associated with the algal response likely because stream-reach characteristics were standardized among sites within a particular study area as part of the study design. Beyond the restrictions of this study, however, variation in reach habitat can be an important environmental gradient that affects algal assemblages (Kutka and Richards, 1996).

The algal response was most strongly related overall to reach-scale gradients of water quality. In all study areas, the indicator metrics were generally most strongly correlated to one of the water-quality gradients (table 4). Of the water-quality gradients CWQ (high and low flow measurements combined) and AWQ (measurement concurrent with algal sampling), AWQ was most relevant to the algal response in every study area except the SLC area (CWQ was most relevant), which suggests that the algae are responding directly to recent water-quality conditions. The metrics *SL_HB, TR_E, SP_BM,* and *PC_SN* each had relevant correlations to a water-quality gradient in six or more study areas, but all study areas had relevant correlations with some combination of these metrics (for example, *TR_E* or *PC_SN* relevant in all study areas). As a group, these indicator metrics—increase in specific conductance *(SL_HB),* change in trophic state *(TR_E, SP_BM),* and loss of sensitive species *(PC_SN)*—are characteristic of water-quality changes that often are related to urbanization and that reflect conditions important from a resource management perspective (Potapova and Charles, 2002, 2003, 2007).

Environmental gradients to which algae responded generally were not associated with urbanization in a way that was characterized by the MA-NUII. The MA-NUII characterized urbanization in terms of the prevailing changes of land use and human presence to the landscape (table 3), but reach-scale gradients were not always linked in a way that could be considered transparent when describing the effects of urbanization on the algal response. An example of this "uncoupling" of responses is seen when comparing the MA-NUII and water-quality gradients in the MGB and BOS areas; each had algal responses that related strongly to an AWQ gradient, but AWQ was linked strongest to the MA-NUII in the BOS (table 5; fig. 5). The strong relation in the BOS area between AWQ and the MA-NUII, however, is perhaps an exception rather than the rule, since ecological interactions typically defy simplifications to a general scale. In a study of this size, it is also likely that some important environmental factors were not fully characterized (for example, latent variables). Aside from conjecture, this study showed that certain indicator metrics were applicable across the country even though algal assemblage structure was different and that the response of the metrics was most closely associated with reach-scale rather than watershed-scale environmental gradients.

Summary and Conclusions

Algal assemblages varied greatly across study areas, regardless of human-based environmental gradients that might have been affecting the algal assemblages within a study area. Even though watershed and reach-scale environmental gradients were related to changes in taxa structure in every study area, algal assemblages were still relatively distinct in each study area. These results suggest a strong effect of ecoregion on algal assemblages. Consequently, when evaluating the responses of algae, the physiographic scale of the study area should be scrutinized so that the responses to disturbances are not inadvertently obscured by natural environmental variation that occurs across the country.

Algal richness did not show a significant decrease over the urban gradient in any study area. In four study areas, taxa richness increased significantly over the low to mid-levels of urban intensity (from 0 to 50) as characterized by the NUII, which indicated that the response was comparable among the study areas. This increase in algal richness with initial urbanization differed from the response typically expected for invertebrate richness, which often decreases with increasing urbanization. The response of algal richness to urbanization may be the result of an increase in nutrient enrichment or hydrologic modifications and is indicative of the intermediate disturbance concept, where complexity in assemblages increases over the initial stages of a disturbance.

Compared to all taxa (diatoms plus soft taxa), the diatoms proved to be the most reliable for measuring a response to environmental gradients, and diatoms from the RTH samples had better results than from the DTH samples. To provide a comprehensive numerical characterization of the algal assemblages, multivariate-ordination site scores were derived from nonmetric multi-dimensional scaling (nMDS). The nMDS site scores (axis 1 and axis 2) were then correlated with 29 conventional algal metrics for each study area to identify measures of assemblage structure and function that would be reliable indicators of the ecological condition of a stream. Spearman correlations indicated eight metrics were strongly associated with the nMDS site scores: (1) diatoms tolerant to halobiontic systems; (2) diatoms tolerant of eutrophic conditions; (3) diatoms sensitive to conditions of organic-load oxidation; (4) diatoms sensitive to pollution; (5) diatoms requiring continuously high dissolved oxygen; (6) diatoms tolerant of silt deposition; (7) taxa capable of motility; and (8) relative abundance of *Achnanthidium minutissimum*. These eight metrics were used as indicators to determine how strongly algal assemblages respond to urbanization and other environmental gradients at the watershed and reach scales. A Spearman rho value > $|0.5|$ and $p < 0.05$ was deemed a relevant correlation.

Across the study areas, the algal metrics were weakly associated with urbanization as characterized by the urban intensity index (UII). Spearman rank correlations with any of the eight algal metrics exceeded 0.50 in only five study areas (SLC, DEN, MGB, RAL, and BOS), and these correlations were fewer than the number of relevant correlations with other environmental gradients. Overall, the algal metrics were most strongly related to water quality (a reach-scale environmental gradient). Correlations greater than 0.50 between a water-quality gradient and algal metrics occurred in all study areas. Of the eight algal metrics listed above, the first four each had relevant correlations to water quality in at least six study areas, and all study areas had relevant correlations between water quality and some combination of these four metrics. As a group, these four metrics are characteristic of water-quality changes related to disturbances. An increase in specific conductance (metric 1), change in trophic state (metrics 2 and 3), and loss of sensitive species (metric 4) indicate conditions important from a resource management perspective.

The ambient water quality—the water-quality gradient derived from data collected closest in time to when the algae were sampled—was the most relevant to the algal response in every study area except the SLC area. The ambient water-quality gradient included specific conductivity, total nitrogen, total phosphorus, pH, and alkalinity. In the SLC area, the strongest algal responses occurred to the comprehensive water-quality gradient, which was derived from water-chemistry data collected in both the low and high flow seasons. Overall, the stronger relations of the algal response metrics to water quality, compared to other watershed- or reach-level environmental gradients, indicate that algal assemblages are most closely associated with water-quality conditions that occur over a relatively short time-scale.

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