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Algal bioassessment metrics for wadeable streams and rivers of Maine, USA

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Abstract. Many state water-quality agencies use biological assessment methods based on lotic fish and macroinvertebrate communities, but relatively few states have incorporated algal multimetric indices into monitoring programs. Algae are good indicators for monitoring water quality because they are sensitive to many environmental stressors. We evaluated benthic algal community attributes along a landuse gradient affecting wadeable streams and rivers in Maine, USA, to identify potential bioassessment metrics. We collected epilithic algal samples from 193 locations across the state. We computed weighted-average optima for common taxa for total P, total N, specific conductance, % impervious cover, and % developed watershed, which included all land use that is no longer forest or wetland. We assigned Maine stream tolerance values and categories (sensitive, intermediate, tolerant) to taxa based on their optima and responses to watershed disturbance. We evaluated performance of algal community metrics used in multimetric indices from other regions and novel metrics based on Maine data. Metrics specific to Maine data, such as the relative richness of species characterized as being sensitive in Maine, were more correlated with % developed watershed than most metrics used in other regions. Few community-structure attributes (e.g., species richness) were useful metrics in Maine. Performance of algal bioassessment models would be improved if metrics were evaluated with attributes of local data before inclusion in multimetric indices or statistical models.

Key words: algae, diatoms, metrics, biological assessments, streams, tolerance values, optima, Biological Condition Gradient, reference conditions.

Human population growth and concurrent changes in land use, pollution, water supply, and fluvial geomorphology threaten water quality of streams and rivers. In the USA, the Federal Water Pollution Control Act or Clean Water Act (US Code title 13, sections 1251–1387) requires states to adopt water-quality standards and criteria to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters." Many state water-quality agencies use bioassessment methods based on fish and benthic macroinvertebrate communities to determine if lotic systems attain biological criteria in their water-quality standards (USEPA 2002). Biological assessments are increasingly important parts of water-quality monitoring programs because they can be used to evaluate the condition of aquatic resources, determine attainment of water-quality standards, and inform management decisions that maintain and restore water quality (Karr 1991, Courtemanch 1995, Yoder and Rankin 1998). Relatively few states have identified algal metrics for flowing waters and integrated them into multimetric indices of biotic integrity (Bahls 1993, Fore and Grafe 2002, KDEP 2002, Fore 2003, Passy and Bode 2004, Wang et al. 2005).

Algae are good indicators for monitoring and assessing the condition of rivers and streams because they are sensitive to many environmental stressors (Patrick 1949, McCormick and Cairns 1994, Stevenson

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and Bahls 1999). Algal metrics and inference models have been used to measure effects of sedimentation (Bahls 1993, Kutka and Richards 1996, Cuffney et al. 1997, Detenbeck et al. 2000, Fore and Grafe 2002, Fore 2003), nutrient enrichment (Cuffney et al. 1997, Leland and Porter 2000, Fore 2003, Wang et al. 2005, Ponader et al. 2007, 2008, Porter et al. 2008, Stevenson et al. 2008a), increased salinity or specific conductance (Fore 2003, Potapova and Charles 2003, Stevenson et al. 2008b), organic enrichment (Fore and Grafe 2002, Fore 2003, Kelly et al. 2008), and acidification (Hill et al. 2000, 2003, Stevenson et al. 2008b). Combinations of diagnostic metrics have been used to distinguish the effects of agricultural land use from urban land use (Fore 2003) and mining (Pan et al. 1996) and to distinguish effects of organic and inorganic effluents on diatom communities (Kelly 1998a, b, Rott et al. 1998, Leland and Porter 2000). Water-quality agencies should critically evaluate potential metrics to confirm that they perform as expected before using them in bioassessments (Karr and Chu 1999).

Algal bioassessments are a good alternative or supplement to macroinvertebrate and fish bioassessments. Benthic algae are comparatively less motile than fish and macroinvertebrates, so they are particularly well suited for local-scale or upstream–downstream studies (McCormick and Cairns 1994). Algae may be better indicators than macroinvertebrates of water chemistry, nutrient enrichment, and land uses that alter water quality, whereas macroinvertebrates may be better indicators than algae of O_2 depletion, changes to hydrology and habitat, and some toxic substances (Passy et al. 2004, Hering et al. 2006, Johnson et al. 2006). Benthic algae are more useful than fish in fish-depauperate systems, such as small or oligotrophic streams in formerly glaciated regions. In addition, community structure of benthic algae may be less responsive than that of than fish and macroinvertebrates to increasing stream size (McCormick and Cairns 1994).

The Biological Condition Gradient (BCG) can expedite development of new bioassessment methods. The BCG is a conceptual framework that describes changes in 10 ecological attributes along an environmental-stress gradient represented by 6 tiers of condition, ranging from natural condition (Tier 1) to severe alteration of structure and function (Tier 6) (Davies and Jackson 2006). The BCG originally was applied to fish and macroinvertebrate communities of permanent, hard-bottomed streams, but the BCG framework has great potential for application to a broad range of ecosystems and taxonomic groups. Incorporating the BCG into algal bioassessment requires identification of taxa that are sensitive to disturbance (attributes II and III), of intermediate tolerance (attribute IV), or tolerant to disturbance (attribute V) (Davies and Jackson 2006). The biological condition of a site can be determined by comparing metrics based on the tolerance groupings to regional reference conditions (Stoddard et al. 2006).

We evaluated effectiveness of benthic algal community metrics for detecting a general disturbance gradient and identified those metrics particularly sensitive to conditions in Maine's streams and rivers. First, we determined major patterns in algal species composition that reflected a general disturbance gradient. Second, we tested predicted response of metrics from existing algal multimetric indices to determine if they were transferable to Maine. We computed Maine stream tolerance values for algae, assigned algal taxa to BCG attributes II to V, and generated metrics based on these attributes. Last, we evaluated algal community attributes to identify metrics that respond to environmental stress and indicate deviation from Maine reference conditions.

Methods

Study site description

We collected samples from 193 locations distributed across Maine and spanning Level-III ecoregions in the Acadian Plains and Hills ($n = 136$), Northeastern Highlands ($n = 35$), and Northeastern Coastal Zone ($n = 22$) (Omernik 1987, Griffith et al. 2009; Fig. 1). We randomly selected 1 sample from sites that were sampled in >1 y. The Acadian Plains and Hills is a mixture of rolling hills and glacial deposits with coniferous and deciduous forests, fields, pasture, tilled agriculture, and blueberry barrens. Much of the state's population is in the southern and central area of this region. The northern portion of the Acadian Plains and Hills has a concentration of tilled agriculture on Ca-rich soils. The Northeastern Highlands is mountainous, has primarily acidic soils, and contains a mosaic of forests and small towns. Areas in the Northeastern Coastal Zone are flat, dominated by glacial or marine deposits, and well populated.

Our study sites represented a gradient of disturbance ranging from streams with entirely forested watersheds to streams in urban watersheds. Watershed area ranged from 0.17 to 3660 km^2. Minimally disturbed sites represented reference conditions (Stoddard et al. 2006), where >95% of upstream watersheds consisted of forest or wetland and no upstream dams, significant discharges such as wastewater treatment plants, or isolated sources of pollution were present. Many of the state's streams and rivers are still recovering from 19^{th}- to 20^{th}-century logging practices and channel alteration to transport

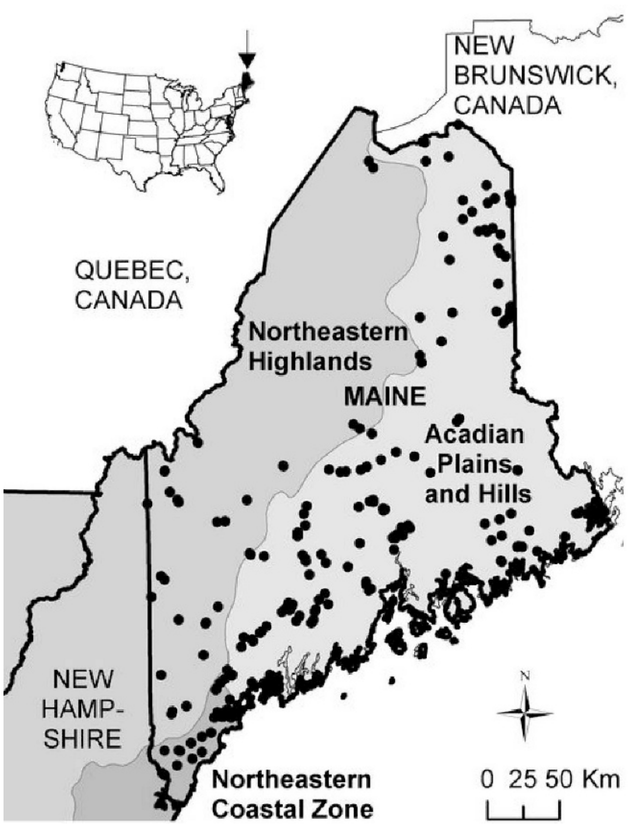

FIG. 1. Sampling locations and ecoregions in Maine, USA (Omernik 1987, Griffith et al. 2009).

logs. Current disturbances include atmospheric deposition of chemicals and diverse effects from urbanization, logging, agriculture, and point-source discharges of pollutants.

Sampling procedures

We collected algal samples during late June or July 1999–2006 from cobbles or small boulders ($n = 18$ rocks) in riffles or runs along 6 transects established within a stream reach and perpendicular to stream flow. Study reaches had open to partly open canopy cover, and reach length (10–50 m) varied according to stream width and availability of suitable substrate. We avoided eddies, pools, back waters, and areas along the bank where benthos could be exposed by fluctuating water levels. We used a stiff brush to scrape epilithic algae from a 2.54-cm diameter area on each rock and combined all scrapings in the reach into a single sample preserved with 1 mL M^3/50 mL of sample (APHA 2005). Diatoms and soft algae were identified and enumerated (Michigan State University and The Patrick Center for Environmental Research of The Academy of Natural Sciences, Philadelphia, Pennsylvania) following protocols given by Charles et al. (2002). Algae were identified to the lowest practical level with most diatoms identified to the species level and some nondiatoms identified to genus level. Results were reported as cell densities (cells/cm^2 substrate) and cell biovolumes (mm^3 cells/cm^2 substrate) (Charles et al. 2002, Charles 2010).

We collected water samples and analyzed them for alkalinity (Alk) (Standard Method 2320B, APHA 2005), dissolved organic C (DOC) (USEPA 2009), total P (TP) and soluble reactive P (SRP) (Method 365.1, USEPA 1993), $NO_3 + NO_2$-N (NO_x) (Method 300.0, USEPA 1993), and total Kjeldahl N (TKN) (Method 351.2, USEPA 1993). Dissolved O_2 (DO) was measured with a HANNA™ HI 9142 probe (Hanna Instruments, Inc., Woonsocket, Rhode Island), and temperature (Temp), pH, and specific conductance (Cond) were collected with a HANNA HI 991300 probe (Hanna Instruments). We measured water velocity with a Global FP 101 meter (Global Water Instrumentation, Gold River, California). We captured spatial coordinates with a Garmin eTrex® (Garmin International, Olathe, Kansas). We digitized watersheds upstream of sample locations from elevation contours on US Geological Survey 1:24,000-scale quadrangles with ArcMap™ (9.2/2007; Environmental Systems Research Institute, Redlands, California). We calculated % land area within watersheds consisting of impervious surfaces (Imp) (MeGIS 2004a) and forest, wetland, and tilled agriculture (MeGIS 2004b). Percent developed watershed (Dev) included urban, agricultural, and residential land uses and was estimated by subtracting % forest and % wetland from 100.

Data analysis

Diatom assemblage patterns.—We identified major patterns of diatom species composition with nonmetric multidimensional scaling (NMDS) using PC-ORD (5.0/2005; MjM Software, Gleneden Beach, Oregon). NMDS is an unconstrained ordination technique that is particularly useful for ecological data with a large number of taxa and many 0 counts (McCune and Grace 2002). We reduced our data set to include only sites with complete environmental variables ($n = 81$) (Table 1). The data included 182 species occurring in $\geq$7 samples ($\sim$5%) in a set of 150 samples used to build a multimetric model (Danielson 2010). We reduced the influence of numerically abundant, ubiquitous taxa, such as *Achnanthidium minutissimum* (Kützing) Czarnecki and *Gomphonema parvulum* (Kützing) Kützing, by transforming all taxon abundances to $\sqrt{(\%\ \text{abundance})}$. We used the Sørenson

TABLE 1. Mean values of environmental variables measured at 190 locations in Maine during 1999–2006. Min = minimum, DL = detection limit, max = maximum, quart = quartile.

Variable	Min (DL)	Max	Mean	1st quart	Median	3rd quart
Watershed area (km^2)	0.7	3,660	217	18	82	211
% forest and wetland	6	100	80	74	88	96
% wetland	0	45	7	3	6	10
% developed	0	94	20	4	12	26
% impervious	0	46	5	0	2	4
% tilled agriculture	0	71	6	0	2	5
Latitude (N) NAD83/WGS84 (m)	43°15′3.853″	47°20′21.620″	44°54′57.758″	44°17′9.005″	44°48′45.337″	45°21′17.398″
Longitude (W) NAD83/WGS84 (m)	70°59′43.484″	67°0′18.123″	69°22′26.129″	70°17′49.537″	69°10′26.469″	68°15′32.930″
Elevation (m)	3	713	134	32	79	134
% canopy cover	0	66	22	9	19	33
Width (m)	0	282	16	3	9	20
Depth (cm)	1	140	32	18	29	40
Velocity (cm/s)	0	120	36	20	35	61
% fines	0	98	32	18	5	10
Dissolved O_2 (mg/L)	3.9 (1)	14.1	8.8	8.1	8.8	9.4
Temperature (°C)	9.8 (0)	27.8	21.1	18.8	21.5	23.9
pH	5.9 (0.1)	9.0	7.1	6.8	7.1	7.5
Specific conductance (µS/cm)	7 (0)	2,930	164	29	59	164
Alkalinity as $CaCO_3$ (mg/L)	2 (0)	206	37	8	16	45
Dissolved organic C (mg/L)	1.4 (1)	20.0	6.5	4.6	6.2	8.2
Total P (µg/L)	3 (1)	870	28	11	18	30
Soluble reactive P (µg/L)	1 (1)	130	6	1	2	5
Total N (mg/L)	0.13 (0.11)	3.99	0.56	0.31	0.45	0.72
$NO_3 + NO_2$-N (mg/L)	0.01 (0.01)	1.6	0.16	0.02	0.06	0.22
Total Kjeldahl N (mg/L)	0.1 (0.1)	3.7	0.4	0.3	0.4	0.5

distance measure, random starting configurations, 250 runs with real data, and 250 runs with randomized data. We rotated resulting NMDS graphs to the most correlated environmental variable to improve interpretation. Last, we determined correlations of environmental variables with the 3 NMDS axes.

Maine stream tolerance values.—We calculated Maine stream tolerance (MST) values of common taxa. First, we calculated weighted-average optima using C^2 (1.5.0/2007; Newcastle University, Newcastle upon Tyne, UK) for 195 diatoms and 41 soft algal taxa for a subset of environmental variables strongly correlated with the primary NMDS axis (TP: n = 167, TN: n = 166, Cond: n = 166, Dev: n = 186, Imp: n = 186). We chose this subset of environmental variables because they represented the major stressors affecting Maine streams. We calculated diatom optima based on $\sqrt{}$(% abundance) to down-weight the influence of ubiquitous, dominant taxa. We calculated optima for soft algal taxa separately from the diatoms and based on $\log_{10}$(density) to avoid distortion of relative abundances by large densities of cyanobacteria. We identified major patterns of the taxa optima for TP, TN, Cond, Dev, and Imp with Principle Components Analysis (PCA) (PC-ORD). We computed MST values by rescaling the taxon scores for PCA axis 1 from 1 (most sensitive) to 100 (most tolerant) to simplify application and communication of the tolerance values.

We categorized algal taxa as sensitive, intermediate, or tolerant based on: 1) their MST values, 2) their range of occurrence with respect to Dev, and 3) general linear models of Dev and taxon $\sqrt{}$(% abundances) with CanoDraw (4.1.4/2002; Microcomputer Power, Ithaca, New York). We used Dev as a surrogate for overall disturbance because it was strongly correlated with PCA axis 1, TP, TN, Cond, and Imp, and it was more easily communicated to stakeholders than PCA axis 1. We selected the best-fitting general linear models (GLM) of 3 types (quadratic [2nd-order], linear [1st-order], and null model [flat line]) with Akaike's Information Criteria (AIC) (Hastie and Tibshirani 1990). We established an MST threshold of 32.2 to separate sensitive and intermediate taxa. Sensitive taxa were most abundant in sites with low Dev and had either decreasing response curves or narrow unimodal curves centered at low Dev values. We also established an MST threshold of 60.0 to separate intermediate and tolerant taxa. Tolerant taxa were most abundant at sites with high Dev and either increasing response curves or narrow unimodal curves centered at high Dev values. We assigned taxa with central Dev optima and not categorized as sensitive or tolerant ($32.2 < MST < 60.0$) to the intermediate group. We grouped BCG attributes

II (sensitive rare taxa) and III (sensitive ubiquitous taxa) because some of the most sensitive taxa were not rare. Intermediate and tolerant taxa correspond with BCG attributes IV and V, respectively. We did not identify any attribute-I taxa (historically documented, sensitive, long-lived, or regionally endemic).

Metric calculation and selection.—We tested algal community attributes from multimetric indices developed elsewhere to determine if they had predictable and empirical relationships with Dev. Three sites with watersheds extending outside of Maine were excluded from analysis because we could not compute Dev, resulting in 190 samples. We used a combination of 3 methods to determine attribute suitability as metrics (Karr and Chu 1999) with empirical and predictable responses to Dev. First, we plotted and examined attribute response to Dev with locally weighted regression lines using R (2.6.2/2008; R Foundation for Statistical Computing, Vienna, Austria). The scatterplots displayed relationships of attributes with watershed condition and deviation from reference conditions (Van Sickle 2003). Second, we determined strength of relationships between attributes and Dev with Spearman rank correlations in R (R Project for Statistical Computing, Vienna, Austria). Last, we identified significant differences between median attribute values of reference ($n = 42$) and nonreference ($n = 148$) sites with Mann–Whitney *U* tests in SYSTAT (11.0/2005; SYSTAT Software, Richmond, Virginia). We used a p-value of 0.001 to determine significance to account for the large number of attributes in the analysis. We grouped attributes into the categories of community composition, tolerance/intolerance, nutrient and organic enrichment, and individual condition (Karr and Chu 1999), and summarized predicted responses of metrics used in previous studies for comparison.

We calculated attributes from previous multimetric indices, including Pollution Tolerance Index (Bahls 1973) relative richness and relative abundance of erect, prostrate, stalked, and unattached diatom growth forms (Fore 2003, Wang et al. 2005), and relative richness and relative abundance of motile diatoms (Fore and Grafe 2002, Wang et al. 2005). Attributes of diatom autecology were expressed in terms of richness, relative richness, and relative abundance and were based on van Dam et al. (1994) species indicator values for pH, salinity (Sal), N-uptake metabolism, DO requirements, saprobity (Sap), trophic state (T), and moisture (M). We used the following cutoffs to define diatom guilds based on the species indicator values: alkalibiontic ($pH \geq 4$), acidobiontic ($pH = 1$), require high O_2 concentrations ($O = 1$), tolerate low O_2 concentrations ($O \geq 3$), salt-tolerant ($Sal \geq 3$), tolerant of dry conditions ($M \geq 4$), N-autotrophic ($N = 1$), N-heterotrophic ($N \geq 3$), oligosaprobic ($Sap = 1$), polysaprobic ($Sap \geq 4$), oligotraphentic ($T = 1$), eutraphentic ($T \geq 5$). Diatoms with $T = 7$ and $pH = 6$ were excluded from trophic and pH computations, respectively. Diatoms without autecological values were excluded from both the numerator and denominator of computations.

We also computed and tested attributes based on Maine tolerance groups, including the richness, relative richness, relative abundance, and relative biovolume of sensitive ($MST < 32.2$), intermediate, and tolerant ($MST > 60.0$) diatoms and nondiatoms. We created triplots of sensitive, intermediate, and tolerant attributes to display concurrent patterns in the related attributes and deviation from reference conditions. We developed novel metrics for families associated with streams in disturbed watersheds in Maine (relative richness and abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae; BCRS) and genera associated with minimally disturbed sites in Maine (relative richness and abundance of *Brachysira*, *Eunotia*, *Tabellaria*, and *Anomoeoneis*; BETA). We also computed the relative biovolume of sensitive soft algae associated with low Dev, which included *Batrachosperma* (Rhodophyta), *Mougeotia* (Chlorophyta), *Ulothrix* (Chlorophyta), *Zygnema* (Chlorophyta), *Tolypothrix* (Cyanophyta), and Rivulaceae (Cyanophyta).

Results

Diatom assemblage patterns

NMDS analysis resulted in a 3-dimensional solution ($p < 0.001$) explaining 77% of the variation with final instability <0.0001 and final stress = 16.6 (within the 10–20 range considered suitable for ecological data; McCune and Grace 2002). Axis 3 (37% of variation) was correlated with watershed land-cover percentages and water-chemistry variables (Fig. 2), whereas axis 2 (17% of variation) was correlated with *A. minutissimum* ($r = -0.74$), *Achnanthidium deflexum* (Rabenhorst) Lange-Bertalot et Ruppel ($r = 0.65$), *Cymbella tumida* (Brébisson ex Kützing) Van Heurck ($r = 0.53$), and *Nitzschia frustulum* (Kützing) Grunow ($r = 0.57$). Axis 1 (23% of variation) represented a latitudinal gradient. Most Northeastern Coastal Zone samples were on the disturbed ½ of Axis 3 (<0), and none was categorized as minimally disturbed using the landuse criteria. In contrast, most samples in the sparsely populated Northeastern Highlands were aggregated on the less-disturbed ½ of Axis 3 (>0). Longitude, watershed area, channel width, average water depth, water velocity, % canopy cover, % fine

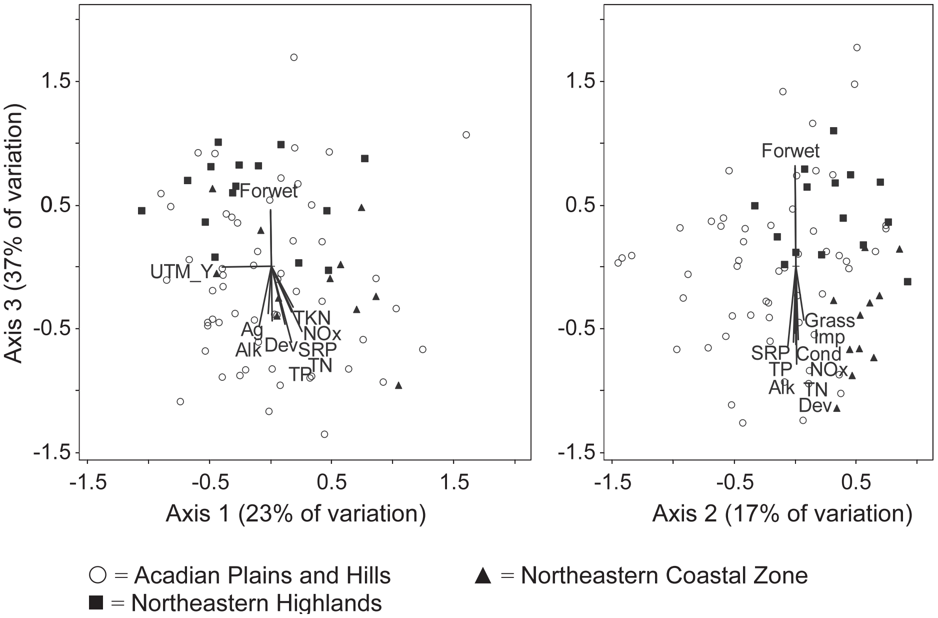

FIG. 2. Nonmetric multidimensional scaling (NMDS) biplots of species composition with correlated environmental variables shown as vectors. Samples are grouped by ecoregion. Ag = % tilled agriculture, Alk = alkalinity, Dev = % developed, Forwet = % forest and wetland, Grass = % grass, Imp = % impervious surfaces, NOx = $NO_3 + NO_2$-N, Cond = specific conductance, SRP = soluble reactive P, TN = total N, TP = total P, UTM_Y = Universal Transverse Mercator latitude.

substrate, pH, TKN, and % wetlands in watersheds were not correlated with a major pattern in species composition.

MST values

Weighted-average optima for TP, TN, Cond, Dev, and Imp for individual taxa were correlated ($r > 0.70$, $p < 0.001$) with each other and were correlated ($r > 0.90$, $p < 0.001$) with the major pattern in taxa optima (PCA 1-axis solution, 80% of variation, eigenvalue = 4.28, $p = 0.001$). Approximately 25% of taxa were classified as sensitive with MST < 32.2 (Table S1; available online from: http://dx.doi.org/10.1899/10-162.1.s1). Sensitive taxa were most abundant at sites with low Dev, were restricted in occurrence to less-disturbed sites, and had either linearly declining response to Dev or narrow unimodal response centered at low Dev values. For example, *Tabellaria flocculosa* (Roth) Kützing (TAfloccu) had a tolerance value of 18.76, a decreasing response, and was classified as sensitive (Fig. 3A). Sensitive taxa included many diatoms and the green algae *Bulbochaete, Mougeotia, Tetraedron minimum* (Braun) Hansgirg, *Ulothrix*, and *Zygnema*, the red algae *Audouinella* (including unidentified chantransia stage Floridophyceae) and *Batrachospermum*, and the cyanobacteria *Calothrix* and *Chroococcus minor* (Kützing) Nägeli. Approximately 25% of taxa, including 0 nondiatom taxa, were classified as tolerant because they were most abundant at disturbed sites, had MST > 60.0, and had either increasing response to Dev or narrow unimodal response curves centered at large Dev values (Table S1). For example, *Gomphonema kobayasii* Kociolek et Kingston (GOkobaya) had a tolerance value of 71.24 and an increasing response ($p < 0.05$) to Dev (Fig. 3A). *Cladophora* spp. and *Cladophora glomerata* (Linnaeus) Kützing were not sufficiently common to compute MST values. However, they were dominant in several polluted, urban streams and were assigned MST = 80. The remaining taxa had MST ranging from 32.2 to 60.0, exhibited a variety of response curves, and were classified as intermediate. For example, *Reimeria sinuata* (Gregory) Kociolek et Stoermer (REsinuat) had a unimodal response (Fig. 3B). In contrast, *Fragilaria vaucheriae* (Kützing) Petersen (FRvauche) did not have a significant quadratic or linear response and the best fitting model was the null model (Fig. 3A).

Algal community metrics

Algal community attributes that qualified as metrics met our criteria of having strong responses to Dev (Spearman rank correlations, $|\rho| \geq 0.50$, $p < 0.001$) and distinguishing reference and nonreference sites (Mann–Whitney U tests, $p < 0.001$). Many algal community attributes used in previous bioassessments were not responsive metrics in Maine (Appendix). Only the relative richness of erect diatoms and

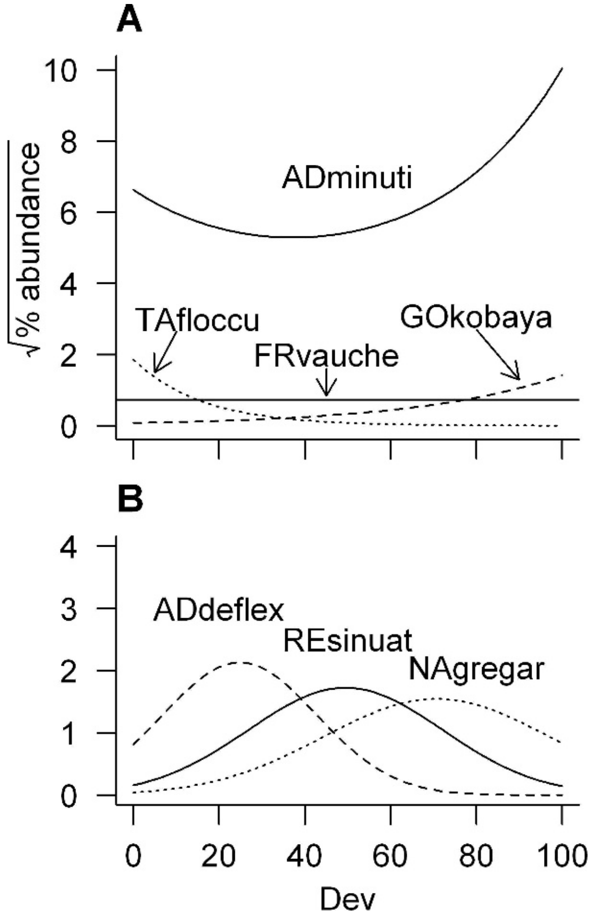

FIG. 3. Examples of diatom species with different general linear model curves in response to increasing % watershed development (Dev). A.—*Tabellaria flocculosa* (TAfloccu) decreased and *Gomphonema kobayasii* (GOkobaya) increased in response to Dev. *Fragilaria vaucheriae* (FRvauche) was indifferent and *Achnanthidium minutissimum* (ADminuti) had an inverse quadratic response to Dev. B.—*Achnanthidium deflexum* (ADdeflex), *Reimeria sinuata* (REsinuat), and *Navicula gregaria* (NAgregar) had unimodal responses with different optima.

relative richness of prostrate diatoms qualified as metrics in the community structure category. No attributes related to the richness, relative richness, diversity, density, or biovolume of the entire algal community (e.g., total richness) or major groups (e.g., diatoms, green algae) were responsive metrics in Maine. Many attributes in the tolerance/intolerance category were responsive metrics (Appendix), including the strongest metrics ($|\rho| \geq 0.70$), such as relative abundance of BCRS; relative richness and relative abundance of BETA; relative richness of sensitive taxa; and richness, relative richness, relative abundance, and relative biovolume of tolerant taxa. Other metrics in the nutrient and organic enrichment category (relative richness of N-autotrophic diatoms; richness and relative richness of eutraphentic diatoms) had the strongest response ($|\rho| \geq 0.70$).

Many attributes were correlated and some responded nonlinearly to Dev. For example, the relative abundance of sensitive (SEN_RR), intermediate (INT_RR), and tolerant (TOL_RR) algae had nonlinear decreasing, unimodal, and increasing response curves to Dev, respectively (Fig. 4A–C). Triplots of sensitive, intermediate, and tolerant attributes, such as SEN_RR,

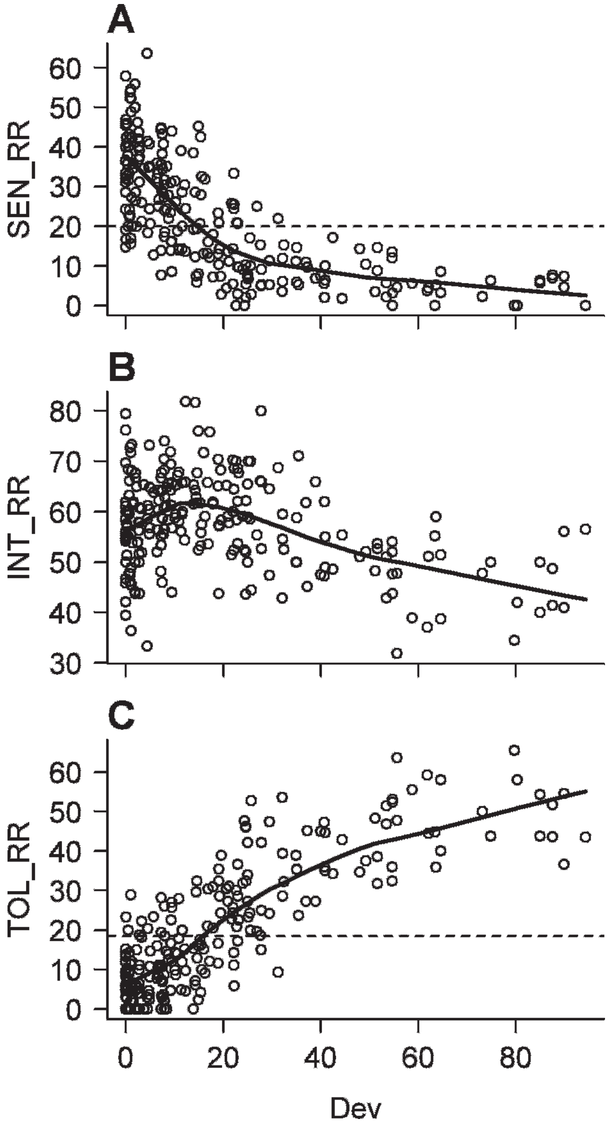

FIG. 4. Examples of attributes with decreasing (relative richness of sensitive taxa [SEN_RR]) (A), unimodal (relative richness of intermediate taxa [INT_RR]) (B), and increasing (relative richness of tolerant taxa [TOL_RR]) (C) responses to % watershed development (Dev). The trend lines are locally weighted regression (LOWESS) lines. Horizontal dashed lines indicate the 90th percentile of reference sites.

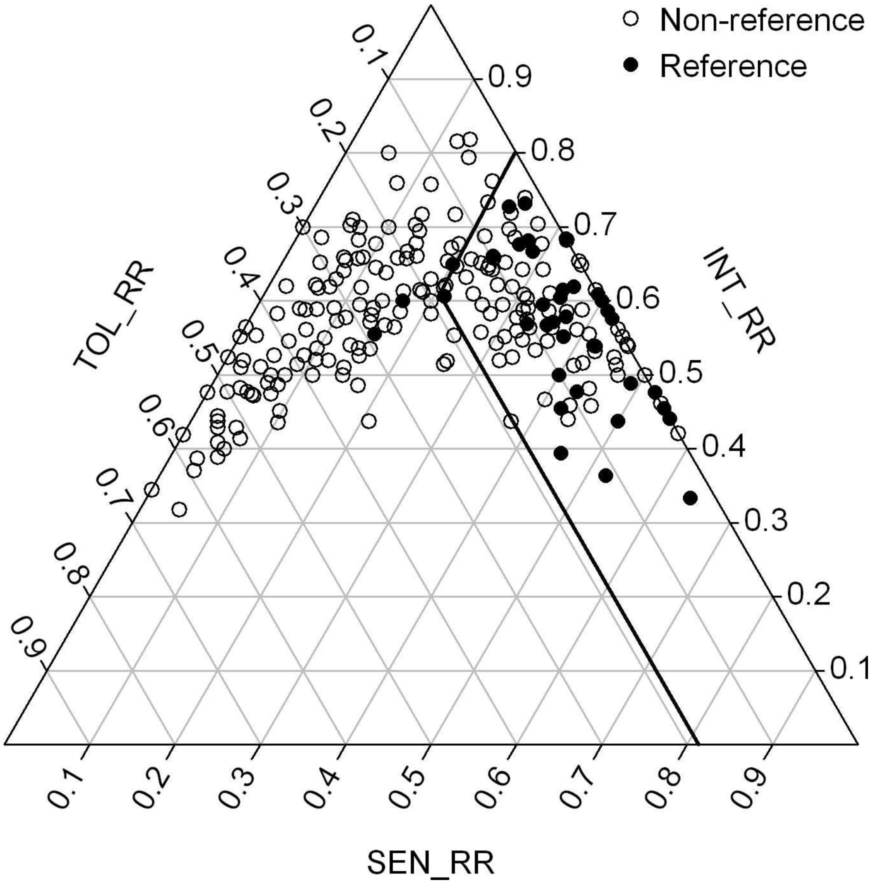

FIG. 5. Relative richness of sensitive (SEN_RR), intermediate (INT_RR), and tolerant (TOL_RR) taxa collected from reference (n = 42) and nonreference (n = 148) sites. Bold lines show the 90th percentile of reference sites and define the range of natural condition.

INT_RR, and TOL_RR (Fig. 5), revealed simultaneous changes in these related variables.

Discussion

Algal taxa tolerance to environmental disturbance

The major pattern in species composition reflected a generalized gradient of disturbance associated with land use and increases in nutrients, specific conductance, agriculture, and impervious surfaces. Width, depth, velocity, and canopy cover were not correlated with major patterns in species composition, presumably because the standardized site selection and sampling protocols succeeded in yielding similar samples. We were surprised that pH was not correlated with major patterns in diatom composition. Algal communities are shaped by pH (Hustedt 1939, Nygaard 1956, Patrick and Reimer 1966, Planas 1996). However, most streams in Maine are somewhat acidic to circumneutral during the summer (Table 1). The ordination of taxon optima for TP, TN, Cond, Dev, and Imp successfully distilled the major pattern in taxon tolerance to multiple, correlated environmental variables and was an objective approach to assign MST values. Our approach could be an alternative to assigning tolerance values with best professional judgment or could verify those tolerance values.

We grouped sensitive taxa (MST < 32.2) because we had difficulty distinguishing attribute II (sensitive-rare) and attribute III (sensitive-ubiquitous) taxa. Taxa with the smallest MST values were not always rare in terms of presence or abundance in samples. Fixed counts of 600 diatom valves used in our study could hamper separation of attribute II and III taxa by omitting rare taxa. Historic practices of identifying $\geq$8000 valves (Patrick et al. 1954, Patrick 1961) might increase detections of rare attribute-II taxa. An alternative approach would be to base attributes II and III on sensitivity and not on rarity, which would reduce a potential influence of sample size on categorization of the stream condition and reduce effort required to discriminate sites at the less-disturbed ½ of the BCG.

Attributes II and III taxa could be distinguished based on occurrence restricted to less-disturbed sites vs occurrence across the full disturbance gradient. For example, *Brachysira brebissonii* has MST = 16.29, is restricted in occurrence to watersheds with little watershed disturbance, and could be categorized as an attribute-II taxon. Although *B. microcephala* has an MST = 18.00, it occurs in streams with a greater range of watershed disturbance and could be categorized as an attribute-III taxon. Assignments could be reevaluated periodically with additions of new samples to confirm patterns of occurrence.

We grouped 2 ecologically different groups into the intermediate category. Taxa in the 1st group responded unimodally to watershed development, nutrient enrichment, and specific conductance. Taxa in the 2nd group were apparently indifferent to watershed development and the range of water-quality conditions observed in our study, indicated by flat response curves. Indifferent taxa are not good indicators when used individually, and their removal from analyses potentially could improve assessment of overall community condition. We decided to retain the indifferent taxa because they were common and abundant and their exclusion would greatly alter community attributes based on relative richness, relative abundance, or relative biovolume.

Several patterns of tolerance appeared with multispecies diatom genera. *Brachysira, Cymbella, Eunotia, Fragilaria*, *Frustulia*, *Psammothidium*, and *Synedra* species included many sensitive and intermediate species with MST < 50. Many species in these genera are associated with oligotrophic to mesotrophic, oligosaprobic, and well oxygenated streams that range from mildly acidic to circumneutral (e.g., Maine reference sites). In addition, most species in these genera are not motile and are susceptible to sedimentation observed in degraded streams in urban and agricultural settings. Many species in the genera *Achnanthidium*, *Gomphonema*, *Karayevia*, *Meridion*, and *Staurosira* were classified as intermediate tolerance in Maine and have broad tolerance ranges in the literature (van Dam et al. 1994, Porter 2008). Most species in the genera *Amphora, Cocconeis, Encyonema, Mayamaea, Navicula, Nitzschia, Planothidium,* and *Surirella* were generally classified as tolerant or intermediate in Maine, and demonstrate tolerance to increased nutrient enrichment, Cl^- concentrations, and Cond (van Dam et al. 1994, Potapova and Charles 2003, Porter 2008). Maine's most impaired streams are in urban and agricultural settings, which could favor species tolerant of Cl^- (Rott et al. 1998, Leland and Porter 2000, Fore 2003) and sedimentation (Bahls 1993, Kutka and Richards 1996). As a group, the genera *Achnanthes, Diatoma*, and *Pinnularia* would not be reliable indicators of stream condition because species within the genera were distributed equally among sensitive, intermediate, and tolerant categories.

Metric evaluation

A formal process of evaluating metrics is necessary to confirm that attributes actually respond as theorized. Developers of some algal multimetric indices apparently did not test predicted attribute response (e.g., Bahls 1993, Hill et al. 2000, 2003, KDEP 2002), which could confound assessments of resource condition. For example, the Shannon–Weiner Diversity Index was expected to decrease with disturbance in Kentucky (KDEP 2002), but increased when evaluated empirically (Wang et al. 2005). Similarly, diatom species richness was expected to decrease with human disturbance in the Mid-Atlantic ecoregion (Hill et al. 2000, 2003), but increased when evaluated empirically (Fore 2003). Most community-structure attributes based on richness (e.g., total richness, diatom richness, *Cymbella* richness), diversity, and relative abundances of algal groups were not metrics in Maine, presumably because of species replacements by more-tolerant species.

Sample collection should include sites representing a complete disturbance gradient with the most refined selection of minimally disturbed reference sites possible. Metrics developed in regions that lack minimally disturbed reference conditions (i.e., best sites are BCG Tier 3) could possibly contribute to distinguishing BCG Tiers 3 to 6, but would not provide a reference condition to detect changes in Tiers 1 to 3 and could undervalue less-disturbed water bodies. Developers of water-quality programs could reduce confusion in bioassessment results by clearly describing reference conditions in terms of BCG Tiers and indicating the range of BCG Tiers covered by the metrics and overall bioassessment.

A formal process of evaluating metrics also is necessary to confirm that metrics developed in other regions actually respond as theorized in the region in which they are to be applied. The relative abundance of dominant diatoms, for example, was expected to increase with disturbance in the Mid-Atlantic region (Hill et al. 2000, 2003, Fore 2003), decreased with disturbance in Kentucky (Wang et al. 2005), and was not a responsive metric in Maine. Regional variation in metric response could be caused by differences in region-specific reference conditions (Grenier et al. 2006). Although predicted to decrease in some regions (KDEP 2002, Hill et al. 2003), diatom richness can increase in response to disturbance in regions with naturally oligotrophic reference sites (Fore 2003). In addition, differences of regional land use and human activities can impose different stressors that could cause regional variation in metric response (Griffith et al. 2002, Wang et al. 2005). For example, relative abundance of acidobiontic diatoms increased in the Mid-Atlantic region where mining activities caused acidification (Hill et al. 2000, 2003). However, this metric was not reliable in Maine where reference streams are somewhat acidic to circumneutral in the summer and impaired streams have higher pH because of geologic and anthropogenic alkalinity. Locally derived tolerance values and metrics often provide more accurate assessments than application of tolerance values and metrics developed in other parts of the world (e.g., van Dam et al. 1994, Potapova et al. 2005, Newall et al. 2006). Most metrics that were strongly correlated ($r \geq 0.70$) with Maine's disturbance gradient were developed with Maine data, such as the MST values. The large number of minimally disturbed reference sites in Maine presumably contributed to the development of more sensitive metrics.

Combining multiple metrics for assessment

Simultaneous evaluation of multiple metrics can improve assessments of resource condition (Gerritsen 1995, Karr and Chu 1999). For example, plotting the relative richness of sensitive, intermediate, and tolerant taxa in a triplot (Fig. 5) clearly illustrates departure of a particular sample from reference conditions. Attributes with unimodal responses can be valuable for interpreting the BCG when combined with other metrics. For example, the relative richness of intermediates (INT_RR) had a unimodal, subsidy–stress response (Odum et al. 1979) to disturbance in Maine (Fig. 4B). Conventional development of most multimetric indices would not include INT_RR as a metric, because it is not strongly correlated with Dev, and a small INT_RR value could be associated with a reference site or an impaired site, but would be scored equally (e.g., 1). However, biologists can apply professional judgment and nonadditive multivariate models to interpret INT_RR in context with other metrics.

Bioassessment programs often use landuse indicators as primary disturbance gradients, but assessment accuracy could be improved by also evaluating attribute response to other disturbance gradients, most notably when point-source discharges and isolated pollution sources confound metric response to landuse disturbance. For example, the relative abundance of acidobiontic and polysaprobic diatoms was not identified as metrics in Maine. However, atypically large values could indicate damaged conditions, such as those created by mining or isolated sources of organic pollution (Griffith et al. 2002). Sensitivity of bioassessment programs may be enhanced by adding stressor-specific metrics as components of expert judgment when evaluating attainment of water-quality standards. Stressor-specific metrics also serve a critical role in diagnosing sources of impairment. Multimetric indices provide an assessment of overall condition, whereas personnel implementing water-quality programs can use stressor-specific metrics and autecological indices to prioritize and target actions to restore water quality and monitor incremental improvements of resource condition.

Algal bioassessments improve water-quality programs because algae are reliable indicators of water quality. We developed an empirical method of assigning tolerance values based on local data, rather than using professional judgment or tolerance values from other regions. Maine's tolerance values are transferable to similar ecoregions, but our process of determining tolerance values is widely transferable to other regions or taxonomic groups. We found that metrics based on local tolerance values outperformed metrics that used tolerance values or autecological traits from other parts of the world. We also found that many metrics used in other algal bioassessments were not useful indicators in Maine, presumably because of regional differences in climate, geology, and predominant anthropogenic stressors. Metrics developed in Maine may have been more sensitive to early degradation because of the number of minimally disturbed reference sites in the data set. Our next step is to build a statistical model with the best-performing metrics to evaluate algal community condition relative to the BCG and to predict attainment of Maine's tiered aquatic life classes. The finished model should be applicable to a range of smaller streams and wadeable segments of larger rivers because the major patterns in algal community composition were not strongly correlated with width and depth. Also, we are developing stressor-specific inference models to improve management response to impaired waters. Adding an algal bioassessment model to the existing macroinvertebrate model will greatly improve our ability to detect water-quality impairment from multiple stressors, detect some impairments that are not apparent using only macroinvertebrates, and diagnose causes of impairment.

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Literature Cited

APHA (American Public Health Association). 2005. Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

Bahls, L. L. 1973. Diatom community response to primary waste water effluent. Journal of the Water Pollution Control Federation 45:134–144.

Bahls, L. L. 1993. Periphyton bioassessment methods for Montana streams. Montana Water Quality Bureau, Department of Health and Environmental Sciences, Helena, Montana. (Available from: http://openlibrary.org/books/OL7147294M/Periphyton_bioassessment_methods_for_Montana_streams)

Charles, D. F. 2010. Algal biovolumes for USGS NAWQA samples. Academy of Natural Sciences, Philadelphia, Pennsylvania. (Available from: http://diatom.ansp.org/nawqa/Protocols.aspx)

Charles, D. F., C. Knowles, and R. S. Davis. 2002. Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program. 02–06. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, Pennsylvania. (Available from: http://diatom.ansp.org/nawqa/Protocols.aspx)

Courtemanch, D. L. 1995. Merging the science of biological monitoring with water resource management policy: criteria development. Pages 315–325 *in* W. P. Davis and T. P. Simon (editors). Biological assessment and criteria: tools for water resource planning and decision making. CRC Press, Boca Raton, Florida.

Cuffney, T. F., M. R. Meador, S. D. Porter, and M. E. Gurtz. 1997. Distribution of fish, benthic invertebrate, and algal communities in relation to physical and chemical conditions, Yakima River Basin, Washington, 1990. Water Resources Investigations Report 96-4280. US Geological Survey, Raleigh, North Carolina.

Danielson, T. J. 2010. Assessing the biological condition of Maine streams and rivers using benthic algal communities. PhD Dissertation, University of Maine, Orono, Maine. (Available from: http://www.library.umaine.edu/theses/theses.asp?highlight=1&Cmd=abstract&ID=EES2010-007)

Davies, S. P., and S. K. Jackson. 2006. The biological condition gradient: a descriptive model for interpreting change in aquatic ecosystems. Ecological Applications 16:1251–1266.

Detenbeck, N. E., S. L. Batterman, V. J. Brady, J. C. Brazner, V. M. Snarski, D. L. Taylor, J. A. Thompson, and J. W. Arthur. 2000. A test of watershed classification systems for ecological risk assessment. Environmental Toxicology and Chemistry 19:1174–1181.

Fore, L. A. 2003. Response of diatom assemblages to human disturbance: development and testing of a multimetric index for the Mid-Atlantic Region (USA). Pages 445–480 *in* T. P. Simon (editor). Biological response signatures: indicator patterns using aquatic communities. CRC Press, New York.

Fore, L. S., and C. Grafe. 2002. Using diatoms to assess the biological condition of large rivers in Idaho (USA). Freshwater Biology 47:2015–2037.

Gerritsen, J. 1995. Additive biological indices for resource management. Journal of the North American Benthological Society 14:451–457.

Grenier, M., S. Campeau, I. Lavoie, Y. S. Park, and S. Lek. 2006. Diatom reference communities in Quebec (Canada) streams based on Kohonen self-organizing maps and multivariate analyses. Canadian Journal of Fisheries and Aquatic Sciences 63:2087–2106.

Griffith, G. E., J. M. Omernik, S. A. Bryce, J. Royte, W. D. Hoar, J. W. Homer, D. Keirstead, K. J. Metzler, and G. Hellyer. 2009. Ecoregions of New England (color poster with map, descriptive text, summary tables, and photographs; map scale 1:1,325,000). US Geological Survey, Reston, Virginia.

Griffith, M. B., B. H. Hill, A. Herlihy, and P. R. Kaufmann. 2002. Multivariate analysis of periphyton assemblages in relation to environmental gradients in Colorado Rocky Mountain streams. Journal of Phycology 38: 83–95.

Hastie, T. J., and R. J. Tibshirani. 1990. General additive models. Chapman and Hall, London, UK.

Hering, D., R. K. Johnson, S. Kramm, S. Schmutz, K. Szoszkiewicz, and P. F. M. Verdonschot. 2006. Assessment of European streams with diatoms, macrophytes, macroinvertebrates, and fish: a comparative metric-based analysis of organism response to stress. Freshwater Biology 51:1757–1785.

Hill, B. H., A. Herlihy, P. R. Kaufmann, S. J. DeCelles, and M. A. Vander Borgh. 2003. Assessment of streams of the eastern United States using a periphyton index of biotic integrity. Ecological Indicators 2:325–338.

Hill, B. H., A. Herlihy, P. R. Kaufmann, R. J. Stevenson, F. H. McCormick, and C. B. Johnson. 2000. Use of periphyton assemblage data as an index of biotic integrity. Journal of the North American Benthological Society 19:50–67.

HUSTEDT, F. 1939. Systematische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali, und Sumatra nach dem Material der Deutschen Limnologischen Sundaexpedition III. Die okologischen faktoren in und ihr Einfluss auf die Diatomeen-Flora. Archiv für Hydrobiologie (Supplement)16:274–394.

JOHNSON, R. K., D. HERING, M. T. FURSE, AND R. T. CLARKE. 2006. Detection of ecological change using multiple organism groups: metrics and uncertainty. Hydrobiologia 566:115–137.

KARR, J. R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecological Applications 1:66–84.

KARR, J. R., AND E. W. CHU. 1999. Restoring life in running waters: better biological monitoring. Island Press, Washington, DC.

KDEP (KENTUCKY DEPARTMENT OF ENVIRONMENTAL PROTECTION). 2002. Methods for assessing biological integrity of surface waters. Kentucky Department of Environmental Protection, Division of Water, Frankfort, Kentucky. (Available from: http://water.ky.gov/Documents/QA/Surface%20Water%20SOPs/Historic%20SOPs/BiologicalSOPMainDocument03_08.pdf)

KELLY, M., S. JUGGINS, R. GUTHRIE, S. PRITCHARD, J. JAMIESON, B. RIPPEY, H. HIRST, AND M. YALLOP. 2008. Assessment of ecological status in UK rivers using diatoms. Freshwater Biology 53:403–422.

KELLY, M. G. 1998a. Use of community-based indices to monitor eutrophication in European rivers. Environmental Conservation 25:22–29.

KELLY, M. G. 1998b. Use of the trophic diatom index to monitor eutrophication in rivers. Water Research 32: 236–242.

KUTKA, F. J., AND C. RICHARDS. 1996. Relating diatom assemblage structure to stream habitat quality. Journal of the North American Benthological Society 15: 469–480.

LELAND, H. V., AND S. D. PORTER. 2000. Distribution of benthic algae in the upper Illinois River basin in relation to geology and land use. Freshwater Biology 44:279–301.

MCCORMICK, P. V., AND J. CAIRNS. 1994. Algae as indicators of environmental change. Journal of Applied Phycology 6: 509–526.

MCCUNE, B., AND J. B. GRACE. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, Oregon.

MEGIS. 2004a. Maine Imperviousness (IMPERV). Maine Office of Geographic Information Systems, Augusta, Maine. (Available from: http://megis.maine.gov/)

MEGIS. 2004b. Maine Land Cover Dataset (MELCD). Maine Office of Geographic Information Systems, Augusta, Maine. (Available from: http://megis.maine.gov/)

NEWALL, P., N. BATE, AND L. METZELING. 2006. A comparison of diatom and macroinvertebrate classification of sites in the Kiewa River system, Australia. Hydrobiologia 572: 131–149.

NYGAARD, G. 1956. Ancient and recent flora of diatoms in the Chrysophyceae in Lake Gribbso. Folia Limnologica Scandinavia 8:32–94.

ODUM, E. P., J. T. FINN, AND E. H. FRANZ. 1979. Perturbation theory and the subsidy-stress gradient. BioScience 29: 349–352.

OMERNIK, J. M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77:118–125.

PAN, Y., R. J. STEVENSON, B. H. HILL, A. T. HERLIHY, AND G. B. COLLINS. 1996. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. Journal of the North American Benthological Society 15:481–495.

PASSY, S. I., AND R. W. BODE. 2004. Diatom model affinity (DMA), a new index for water quality assessment. Hydrobiologia 524:241–251.

PASSY, S. I., R. W. BODE, D. M. CARLSON, AND M. A. NOVAK. 2004. Comparative environmental assessment in the studies of benthic diatom, macroinvertebrate, and fish communities. International Review of Hydrobiology 89: 121–138.

PATRICK, R. 1949. A proposed biological measure of stream conditions based on a survey of the Conestoga Basin, Lancaster County, Pennsylvania. Proceedings of the Academy of Natural Sciences of Philadelphia 101: 277–341.

PATRICK, R. 1961. A study of the numbers and kinds of species found in rivers in eastern United States. Proceedings of the Academy of Natural Sciences of Philadelphia 113:215–258.

PATRICK, R., M. H. HOHN, AND J. H. WALLACE. 1954. A new method for determining the pattern of the diatom flora. Notulae Naturae of the Academy of Natural Sciences of Philadelphia 259:12.

PATRICK, R., AND C. W. REIMER. 1966. The diatoms of the United States, exclusive of Alaska and Hawaii. Volume 1. Academy of Natural Sciences, Philadelphia, Pennsylvania.

PLANAS, D. 1996. Acidification effects. Pages 497–530 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe (editors). Algal ecology. Freshwater benthic ecosystems. Academic Press, Boston, Massachusetts.

PONADER, K. C., D. F. CHARLES, AND T. J. BELTON. 2007. Diatom-based TP and TN inference models and indices for monitoring nutrient enrichment of New Jersey streams. Ecological Indicators 7:79–93.

PONADER, K. C., D. F. CHARLES, T. J. BELTON, AND D. M. WINTER. 2008. Total phosphorus inference models and indices for coastal plain streams based on diatom assemblages from artificial substrates. Hydrobiologia 610:139–152.

PORTER, D. E., D. K. MUELLER, N. E. SPAHR, M. D. MUNN, AND N. M. DUBROVSKY. 2008. Efficacy of algal metrics for assessing nutrient and organic enrichment in flowing waters. Freshwater Biology 53:1036–1054.

PORTER, S. D. 2008. Algal attributes: an autecological classification of algal taxa collected by the National Water-Quality Assessment Program. Data Series 329. US Geological Survey, Reston, Virginia.

POTAPOVA, M., AND D. F. CHARLES. 2003. Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. Freshwater Biology 48: 1311–1328.

POTAPOVA, M., J. COLES, E. GIDDINGS, AND H. ZAPPIA. 2005. A comparison of the influences of urbanization in contrasting environmental settings on stream benthic algal assemblages. Pages 333–359 *in* L. R. Brown, R. H. Gray, R. M. Hughes, and M. R. Meador (editors). Symposium on the Effects of Urbanization on Aquatic Ecosystems, Quebec City, Quebec (Canada), 11–12 Aug 2003. American Fisheries Society, Bethesda, Maryland.

ROTT, E., H. C. DUTHIE, AND E. PIPP. 1998. Monitoring organic pollution and eutrophication in the Grand River, Ontario, by means of diatoms. Canadian Journal of Fisheries and Aquatic Sciences 55:1443–1453.

STEVENSON, R. J., AND L. L. BAHLS. 1999. Periphyton protocols. Pages 6.1–6.22 *in* M. T. Barbour, J. Gerritsen, B. D. Snyder, and J. B. Stribling (editors). Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish. 2nd edition. EPA 841-B-99-002. Office of Water, US Environmental Protection Agency, Washington, DC.

STEVENSON, R. J., B. H. HILL, A. T. HERLIHY, L. L. YUAN, AND S. B. NORTON. 2008a. Algae–P relationships, thresholds, and frequency distributions guide nutrient criterion development. Journal of the North American Benthological Society 27:783–799.

STEVENSON, R. J., Y. PAN, K. MANOYLOV, C. A. PARKER, D. P. LARSEN, AND A. HERLIHY. 2008b. Development of diatom indicators of ecological condition for streams in the western US. Journal of the North American Benthological Society 27:1000–1016.

STODDARD, J. L., D. P. LARSEN, C. P. HAWKINS, R. K. JOHNSON, AND R. H. NORRIS. 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecological Applications 16:1267–1276.

USEPA (US ENVIRONMENTAL PROTECTION AGENCY). 1993. Methods for the determination of inorganic substances in environmental samples. EPA/600/R-93/100. Environmental Monitoring Systems Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio.

USEPA (US ENVIRONMENTAL PROTECTION AGENCY). 2002. Summary of biological assessment programs and biocriteria development for States, Tribes, Territories, and Interstate Commissions: streams and wadeable rivers. EPA-822-R-02-048. US Environmental Protection Agency, Washington, DC.

USEPA (US ENVIRONMENTAL PROTECTION AGENCY). 2009. Method 415.3: determination of total organic carbon and specific UV Absorbance at 254 nm in source water and drinking water. Revision 1.2. EPA/600/R-09/122. US Environmental Protection Agency, Cincinnati, Ohio.

VAN DAM, H., A. MERTENS, AND J. SINKELDAM. 1994. A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. Netherlands Journal of Aquatic Ecology 28:117–133.

VAN SICKLE, J. 2003. Analyzing correlations between stream and watershed attributes. Journal of the American Water Resources Association 39:717–726.

WANG, Y. K., R. J. STEVENSON, AND L. METZMEIER. 2005. Development and evaluation of a diatom-based Index of Biotic Integrity for the Interior Plateau Ecoregion, USA. Journal of the North American Benthological Society 24: 990–1008.

YODER, C., AND E. RANKIN. 1998. The role of biological indicators in a state water quality management process. Journal of Environmental Monitoring and Assessment 51:61–88.

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APPENDIX. Algal attributes tested to determine response to increasing % developed land in the watershed (Dev) using Maine data. Mann-Whitney *U* tests were for algal attributes grouped by reference ($n = 42$) and nonreference ($n = 148$) sites. Footnotes for algal community attributes indicate the attribute source. Arrows indicate predicted direction of response. Bold indicates the metric meets Spearman rank correlation ($|\rho| = 0.50$, $p < 0.001$) and Mann-Whitney *U* test ($p < 0.001$) criteria. BCRS = Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae, BETA = Brachysira, Eunotia, Tabellaria, and Anomoneis, R = richness, RR = relative richness (% of species), RA = relative abundance, RB = relative biovolume, − = not assessed.

Algal community attribute	Predicted response	Spearman rank correlation		Mann–Whitney *U* test		
		ρ	p	U	$\chi^2_{1\mathrm{df}}$	p
Community structure category						
Total species R		0.11	0.135	3,735	4.0	0.046
Diatom species R	↓^{e,g}	0.14	0.051	3,971	7.5	0.006
Diatom RA	↓^{c}	0.15	0.046	3,078	0.0	0.924
Diatom genera R	↓^{e}	–	–	–	–	–
Generic R/expected R	↓^{c}	–	–	–	–	–
Diatom divisions R	↓^{e}	–	–	–	–	–
Green algae R		−0.13	0.084	2,881	0.5	0.466
Green algae RA		−0.08	0.300	3,164	0.0	0.859
Red algae R		−0.06	0.396	3,282	0.4	0.538
Red algae RA		−0.01	0.874	3,856	0.9	0.345
Cyanobacteria R		−0.07	0.370	3,103	0.0	0.985
Cyanobacteria RA		−0.14	0.065	2,915	0.4	0.539
Shannon diversity index	↓^{a,e}	0.17	0.018	3,332	0.5	0.476
% dominant diatom		−0.14	0.047	2,187	8.6	0.003
% Achnanthidium minutissimum		−0.14	0.064	2,255	7.4	0.007
Diatom similarity index^{a} and average similarity to reference^{h}	↓^{a,h}	–	–	–	–	–
Erect diatoms^{f,h} R		−0.42	<0.001	2,400	5.1	0.024
Erect diatoms^{f,h} RR		**−0.57**	**<0.001**	1,916	14.4	**<0.001**
Erect diatoms^{f,h} RA		−0.51	<0.001	2,253	7.4	0.007
Prostrate diatoms^{f,h} R		0.45	<0.001	4,587	22.1	<0.001
Prostrate diatoms^{f,h} RR		**0.59**	**<0.001**	4,769	27.9	**<0.001**
Prostrate diatoms^{f,h} RA		0.39	<0.001	3,984	7.8	0.005
Stalked diatoms^{f,h} R		0.11	0.130	3,443	1.1	0.285
Stalked diatoms^{f,h} RR		−0.03	0.732	2,734	1.4	0.234
Stalked diatoms^{f,h} RA		0.11	0.128	3,923	6.7	0.010
Unattached diatoms^{f,h} R		−0.08	0.306	2,870	0.6	0.437
Unattached diatoms^{f,h} RR		−0.23	0.002	2,261	7.3	0.007
Unattached diatoms^{f,h} RA		−0.30	<0.001	2,030	11.8	0.001
Tolerance/intolerance category						
Pollution tolerance index^{a}	↓^{a,e,h}	−0.25	<0.001	1,623	22.3	<0.001
Fragilaria group R	↓^{e}	−0.19	0.009	2,913	0.4	0.532
Cymbella group R	↓^{e}	0.13	0.089	3,552	2.0	0.153
Cymbella group RR	↓^{h}	0.09	0.228	3,252	0.2	0.647
***Navicula* species RR**	↑^{h}	**0.64**	**<0.001**	4,933	33.7	**<0.001**
***% Achnanthes*/(*Achnanthes* + *Navicula*)**	↓^{a,h}	**−0.51**	**<0.001**	1,353	31.1	**<0.001**
Alkalibiontic diatom^{b} R	↓^{d} ↑^{f}	**0.58**	**<0.001**	4,781	28.4	**<0.001**
Alkalibiontic diatom^{b} RA	↓^{g}	**0.52**	**<0.001**	4,458	18.4	**<0.001**
Acidobiontic diatom^{b} R		−0.35	<0.001	2,267	9.5	0.002
Acidobiontic diatom^{b} RR		−0.39	<0.001	2,134	12.6	<0.001
Acidobiontic diatom^{b} RA	↑^{c}	−0.37	<0.001	2,193	11.1	0.001
High O_2 diatom^{b} R		−0.28	<0.001	2,685	1.8	0.178
High O_2 diatom^{b} RR		**−0.67**	**<0.001**	1,519	25.5	**<0.001**
High O_2 diatom^{b} RA		−0.45	<0.001	1,334	31.8	<0.001
Low O_2 diatom^{b} R		**0.57**	**<0.001**	4,602	23.2	**<0.001**
Low O_2 diatom^{b} RR		**0.54**	**<0.001**	4,510	19.9	**<0.001**
Low O_2 diatom^{b} RA		0.33	<0.001	4,555	21.1	<0.001
Salt-tolerant diatom^{b} R		**0.66**	**<0.001**	4,749	28.0	**<0.001**
Salt-tolerant diatom^{b} RR		**0.66**	**<0.001**	4,603	22.8	**<0.001**

APPENDIX. Continued.

Algal community attribute	Predicted response	Spearman rank correlation		Mann–Whitney *U* test		
		ρ	*p*	*U*	$\chi^2_{1\mathrm{df}}$	*p*
Salt-tolerant diatom[b] RA		**0.64**	**<0.001**	4,790	28.9	**<0.001**
Dry tolerant diatom[b] R		−0.14	0.051	2,934	0.3	0.567
Dry tolerant diatom[b] RR		−0.27	<0.001	2,530	3.4	0.065
Dry tolerant diatom[b] RA		−0.13	0.076	3,032	0.1	0.808
Motile diatom[d,h] R		**0.50**	**<0.001**	4,606	22.7	**<0.001**
Motile diatom[d,h] RR		**0.63**	**<0.001**	4,772	28.0	**<0.001**
Motile diatom[d,h] RA	↑[a,c,d,f,g]	**0.53**	**<0.001**	4,760	27.6	**<0.001**
Sensitive soft algae RB		−0.39	<0.001	2,312	7.4	0.007
BCRS R		**0.63**	**<0.001**	5,023	37.5	**<0.001**
BCRS RR		**0.69**	**<0.001**	5,070	38.9	**<0.001**
BCRS RA		**0.70**	**<0.001**	5,220	45.1	**<0.001**
BETA R		**−0.63**	**<0.001**	1,697	20.4	**<0.001**
BETA RR		**−0.74**	**<0.001**	1,249	35.1	**<0.001**
BETA RA		**−0.70**	**<0.001**	1,292	33.5	**<0.001**
Sensitive taxa[i] R	↓[h]	**−0.69**	**<0.001**	1,340	31.7	**<0.001**
Sensitive taxa[i] RR	↓[h]	**−0.81**	**<0.001**	791	54.3	**<0.001**
Sensitive taxa[i] RA	↓[d,f]	**−0.62**	**<0.001**	1,621	22.3	**<0.001**
Sensitive taxa RB		**−0.64**	**<0.001**	1,296	33.2	**<0.001**
Intermediate tolerance taxa R		0.16	0.034	4,219	12.5	<0.001
Intermediate tolerance taxa RR		−0.03	0.699	3,936	6.9	0.009
Intermediate tolerance taxa RA		0.04	0.558	3,671	3.2	0.073
Tolerant taxa R		**0.77**	**<0.001**	5,200	44.4	**<0.001**
Tolerant taxa RR		**0.80**	**<0.001**	5,226	45.4	**<0.001**
Tolerant taxa[i] RA	↑[d]	**0.76**	**<0.001**	5,145	42.0	**<0.001**
Tolerant taxa RB		**0.77**	**<0.001**	5,067	38.8	**<0.001**
Nutrient and organic-enrichment category						
Chlorophyll *a*	↑[c,e]	–	–	–	–	–
Ash-free dry mass	↑[c,e,g]	–	–	–	–	–
Total biovolume		0.09	0.233	3,597	2.4	0.120
Diatom biovolume		0.13	0.074	3,553	2.0	0.157
Soft algae biovolume		0.03	0.655	3,591	2.4	0.125
Total density		0.09	0.219	3,474	1.4	0.245
Diatom density		0.20	0.005	3,642	2.9	0.090
Soft algae density		<0.01	0.992	3,359	0.6	0.425
N-autotrophic diatom[b] R		−0.43	<0.001	2,150	9.3	0.002
N-autotrophic diatom[b] RR	↓[d,f]	**−0.72**	**<0.001**	1,163	38.3	**<0.001**
N-autotrophic diatom[b] RA		**−0.53**	**<0.001**	1,754	18.5	**<0.001**
N-heterotrophic diatom[b] R		**0.60**	**<0.001**	4,853	31.5	**<0.001**
N-heterotrophic diatom[b] RR		**0.58**	**<0.001**	4,824	29.8	**<0.001**
N-heterotrophic diatom[b] RA		0.36	<0.001	4,703	25.7	<0.001
Oligosaprobic diatom[b] R		−0.43	<0.001	2,256	7.4	0.007
Oligosaprobic diatom[b] RR		**−0.69**	**<0.001**	1,360	30.9	**<0.001**
Oligosaprobic diatom[b] RA		−0.46	<0.001	2,107	10.1	0.001
Polysaprobic diatom[b] R		**0.50**	**<0.001**	4,510	20.4	**<0.001**
Polysaprobic diatom[b] RR		0.39	<0.001	4,080	9.5	0.002
Polysaprobic diatom[b] RA		0.19	0.008	4,227	12.7	<0.001
Oligotraphentic diatom[b] R		−0.44	<0.001	2,443	4.6	0.033
Oligotraphentic diatom[b] RR		**−0.58**	**<0.001**	1,931	14.0	**<0.001**
Oligotraphentic diatom[b] RA		−0.42	<0.001	2,223	7.9	0.005
Eutraphentic diatom[b] R		**0.71**	**<0.001**	5,045	38.1	**<0.001**
Eutraphentic diatom[b] RR		**0.74**	**<0.001**	4,951	34.3	**<0.001**
Eutraphentic diatom[b] RA	↑[c,f,g]	**0.63**	**<0.001**	4,962	34.7	**<0.001**
Individual condition category						
% deformed diatom valves	↑[d]	–	–	–	–	–

Appendix. Continued.

Algal community attribute	Predicted response	Spearman rank correlation		Mann–Whitney U test		
		ρ	p	U	χ^2_{1df}	p
Other ecological attributes category						
Phosphatase production	↑[c,g]	–	–	–	–	–

[a] Bahls 1993
[b] van Dam et al. 1994
[c] Hill et al. 2000
[d] Fore and Grafe 2002
[e] KDEP 2002
[f] Fore 2003
[g] Hill et al. 2003
[h] Wang et al. 2005
[i] Studies used different sets of sensitive and tolerant taxa