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An algal model for predicting attainment of tiered biological criteria of Maine's streams and rivers

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Abstract. State water-quality professionals developing new biological assessment methods often have difficulty relating assessment results to narrative criteria in water-quality standards. An alternative to selecting index thresholds arbitrarily is to include the Biological Condition Gradient (BCG) in the development of the assessment method. The BCG describes tiers of biological community condition to help identify and communicate the position of a water body along a gradient of water quality ranging from natural to degraded. Although originally developed for fish and macroinvertebrate communities of streams and rivers, the BCG is easily adapted to other habitats and taxonomic groups. We developed a discriminant analysis model with stream algal data to predict attainment of tiered aquatic-life uses in Maine's water-quality standards. We modified the BCG framework for Maine stream algae, related the BCG tiers to Maine's tiered aquatic-life uses, and identified appropriate algal metrics for describing BCG tiers. Using a modified Delphi method, 5 aquatic biologists independently evaluated algal community metrics for 230 samples from streams and rivers across the state and assigned a BCG tier (1–6) and Maine water quality class (AA/A, B, C, nonattainment of any class) to each sample. We used minimally disturbed reference sites to approximate natural conditions (Tier 1). Biologist class assignments were unanimous for 53% of samples, and 42% of samples differed by 1 class. The biologists debated and developed consensus class assignments. A linear discriminant model built to replicate a priori class assignments correctly classified 95% of 150 samples in the model training set and 91% of 80 samples in the model validation set. Locally derived metrics based on BCG taxon tolerance groupings (e.g., sensitive, intermediate, tolerant) were more effective than were metrics developed in other regions. Adding the algal discriminant model to Maine's existing macroinvertebrate discriminant model will broaden detection of biological impairment and further diagnose sources of impairment. The algal discriminant model is specific to Maine, but our approach of explicitly tying an assessment tool to tiered aquatic-life goals is widely transferrable to other regions, taxonomic groups, and waterbody types.

Key words: biological assessment, discriminant analysis, tiered aquatic life uses, Biological Condition Gradient, metrics, algae, reference conditions, diatoms, water quality, expert judgment, Delphi method.

The objective of the US Federal Water Pollution Control Act (Clean Water Act) is to "restore and maintain the chemical, physical and biological integrity of the Nation's waters" (Public Law 92-500, Section 101). In response to this goal, state water-quality programs emphasize the use of fish, macroinvertebrate, and algal communities as water-quality indicators (USEPA 2002). The Biological Condition Gradient (BCG) is a mechanism to "(1) assess aquatic

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resources more uniformly and directly and (2) communicate more clearly to the public the current status of aquatic resources and their potential for restoration'' (Davies and Jackson 2006b, p. 1251). The BCG describes changes in 10 ecological attributes of aquatic life along an environmental-stress gradient, and 6 tiers of biological assemblage condition ranging from natural condition (Tier 1) to severe alteration of structure and function (Tier 6). The BCG was originally applied to fish and macroinvertebrate communities of permanent, hard-bottomed streams exposed to increasing temperature, nutrients, and fine sediments (Davies and Jackson 2006b, Snook et al. 2007). Applications of the BCG framework to other ecosystems and biological assemblages are incomplete, but the BCG framework has great potential for application to a broad range of ecosystems and taxonomic groups.

A natural extension of the BCG is to integrate tiers of biological condition into water-quality standards in the form of Tiered Aquatic Life Uses (Courtemanch 1995, Yoder and Rankin 1998, State of Maine 2004b, Davies and Jackson 2006b, Yoder and Barbour 2009). Maine's water-quality standards recognize 4 classes of streams and rivers with narrative biological criteria describing the aquatic-life goals of each class (Courtemanch et al. 1989, Courtemanch 1995). Class AA and A waters have the same biological expectations, but Class AA allows fewer activities that require permits, such as dams (Appendix 1). Both may be represented as BCG Tiers 1 and 2. Class B corresponds most closely to BCG Tier 3 and Class C most closely with BCG Tier 4. Class C is Maine's interpretation of the interim goal of the US Clean Water Act (US Code title 33, sections 1251–1387), which is to attain water quality sufficient to provide for the protection and propagation of fish, shellfish, and wildlife, and for recreation in and on the water. Streams that do not attain Class C are called nonattainment (NA) and correspond to BCG Tiers 5 and 6. In Maine, Class AA and A water-quality standards are interpreted as fully attaining the physical, chemical, and biological integrity goals expressed in the Clean Water Act, whereas Class C standards are interpreted as attaining the interim fishable/swimmable goals of the Act [Section 101(a)(2)]. The Maine Department of Environmental Protection (MDEP) predicts class attainment with a discriminant analysis model of benthic macroinvertebrate data (Davies et al. 1995, Davies and Tsomides 2002, State of Maine 2003, Shelton and Blocksom 2004). The tiered classes provide flexibility to manage streams and rivers at multiple levels of risk and condition (Courtemanch et al. 1989, State of Maine 2004b).

Monitoring multiple taxonomic assemblages improves the ability to detect environmental degradation and diagnose stressors (Patrick 1949, Yoder and DeShon 2003, Hering et al. 2006, Yoder and Barbour 2009). Most states use fish or macroinvertebrates to assess the biological condition of rivers and streams, but algae also are good indicators of water quality (Stevenson and Bahls 1999). Algal indices have been developed for nutrient and organic pollution (Kolkwitz and Marsson 1908, Pantle and Buck 1955, Watanabe 1962, Palmer 1969, Descy 1979, Lange-Bertalot 1979, Kelly et al. 1995, Kelly 1998a, b, Potapova and Charles 2007), and inference models have been developed to estimate levels of nutrients and other water-quality variables (Pan et al. 1996, Winter and Duthie 2000, Potapova et al. 2004, Ponader et al. 2007, 2008, Stevenson et al. 2008b). These assessments often lack a direct link to minimally disturbed reference conditions and emphasize single water-chemistry or enrichment gradients. Several states and regions of the USA have developed algal multimetric indices (MMIs) of biotic integrity to quantify the condition of algal communities with respect to reference conditions (Bahls 1973, Fore and Grafe 2002, KYDEP 2002, Fore 2003, Passy and Bode 2004, Wang et al. 2005). Algal indices also have been developed in Australia (Chessman et al. 1999, 2007), Canada (Belore et al. 2002, Lavoie et al. 2006), and Europe (Kelly et al. 2008).

The objectives of our study were to: 1) develop a model with benthic algal community data to predict the probability of a stream attaining biological criteria of its assigned Maine water classification (i.e., AA/A, B, C), 2) determine the response of BCG tiers to watershed development, and 3) compare the water-quality class predictions of the algal and macroinvertebrate discriminant models and examine patterns in model agreement. An algal model was attractive because the major patterns in algal species composition in samples collected across the state reflected a gradient of human disturbance, and MDEP previously identified many potential community metrics (Danielson et al. 2011).

Methods

We collected samples from 193 locations distributed across Maine in June or July 1999 to 2006 (Danielson et al. 2011). Some locations ($n = 37$) were sampled in 2 y with different climatic conditions and a minimum of 2 y between samples, resulting in 230 samples. Sampling locations spanned 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, Danielson et al. 2011). The Acadian Plains and Hills cover the eastern 53% of the state, the Northeastern Highlands cover the western 43% of the state, and the Northeastern Coastal Zone covers the southern 4% of Maine. Our study sites represented a gradient of disturbance ranging from streams with entirely forested watersheds to streams in urban watersheds. 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 recovering from 19th- and 20th-century logging practices and channel alteration to transport logs. Current disturbances include atmospheric deposition of chemicals and urbanization, logging, agriculture, and point-source discharges of pollutants.

We established 3 transects at each location and collected 6 rocks/transect (cobble or small boulder) for a total of 18 rocks/location (Danielson 2006). We removed algae with a stiff-bristled brush from a 2.54-cm-diameter circle on the top of each rock. We composited the algae removed from the 18 rocks into a single sample representing the stream reach, preserved the sample with M3 (APHA 2005), and stored it in a brown Nalgene™ bottle. Diatoms and soft algae were identified to the lowest practical taxonomic level and enumerated at Michigan State University and the Patrick Center for Environmental Research (Academy of Natural Sciences, Philadelphia, Pennsylvania) following methods published by Charles et al. (2002). Most diatoms were identified to species, and most soft algae were identified to species or genus. We expressed algal counts as cell densities (cells/cm^2 substrate) and cell biovolumes (mm^3 cells/cm^2 substrate) (Charles 2010). We collected water-quality samples at the same time as the algal samples and analyzed them for total P (TP), soluble reactive P, NO_3 + NO_2-N, total Kjeldahl N, alkalinity, and dissolved organic C (Danielson et al. 2011). Total N (TN) was estimated by adding NO_3 + NO_2-N and total Kjeldahl N. Specific conductance (Cond), pH, and temperature were measured with a HANNA HI 991300 probe (Hanna Instruments, Inc., Woonsocket, Rhode Island). We used ArcMap™ (9.2/2007; Environmental Systems Research Institute, Redlands, California) to calculate landcover percentages in watersheds upstream of sampling locations and estimated % developed watershed (Dev) by subtracting % forest and % wetland from 100 (Danielson et al. 2011). We also used ArcMap to calculate % impervious surface (Imp) in the watersheds.

Ecoregion effects

We analyzed spatial patterns in algal species composition at 42 minimally disturbed reference sites to determine if a single, statewide discriminant model was appropriate or if ecoregion-specific models would be necessary. We were unable to sample reference sites in the Northeastern Coastal Zone (southern 4% of state) because of widespread development and agriculture. We converted taxon counts to $\sqrt{(\%\ \text{abundance})}$ to reduce the influence of dominant, ubiquitous taxa, such as *Achnanthidium minutissimum* (Kützing) Czarnecki and *Gomphonema parvulum* (Kützing) Kützing. Species occurring in <7 training-set samples (~5% of training-set samples) were considered rare and excluded. First, we identified major patterns in species with nonmetric multidimensional scaling (NMDS; Kruskal 1964, Mather 1976) using PC-ORD (5.0/2005; MjM Software, Gleneden Beach, Oregon). We used the Sørenson distance measure, random starting configurations, 250 runs with real data, and 250 runs with randomized data. We determined if samples clustered by ecoregion by examining NMDS plots. We determined if species composition varied by ecoregion with multiresponse permutation procedures (MRPP, Mielke and Berry 2001) via the Sørenson (Bray–Curtis) distance measure in PC-ORD.

BCG framework for Maine stream algae

We modified the original BCG framework to apply to Maine stream algae (Appendix 2). We focused on attributes relating to taxon sensitivities, including Attribute II (sensitive-rare taxa), III (sensitive-ubiquitous taxa), IV (taxa of intermediate tolerance), and V (tolerant taxa) (Davies and Jackson 2006b). We combined attributes II and III into a single category; identified sensitive, intermediate, and tolerant taxa with Maine stream-tolerance values; and developed metrics based on sensitive, intermediate, and tolerant taxa (Danielson et al. 2011). We included Attribute VI (nonnative or intentionally introduced taxa), but the only known potential species was the diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt which occurs in nearby states (Vermont and New Hampshire) and Canadian provinces (Quebec and New Brunswick) and could colonize many Maine oligotrophic and mesotrophic rivers (MDEP 2010, VTDEC 2010). We lacked adequate data to include Attribute 1 (historically documented, sensitive, long-lived, or regionally endemic taxa), VII (organism condition), VIII (ecosystem functions), IX (spatial and temporal extent of detrimental effects), and X (ecosystem connectance).

TABLE 1. Metrics supplied to Maine Department of Environmental Protection (MDEP) biologists for assigning Maine water-quality groups and Biological Condition Gradient (BCG) tiers via expert judgment. Table includes range of expected values for reference sites, overall range of values observed in all study sites, and predicted response to watershed development (↓ = decrease, ↑ increase). LWD indicates metrics of low watershed disturbance.

Metric	Code	Range of values for reference sites	Overall range of values	Predicted response to watershed development
Community structure				
Relative richness of erect diatoms[a,b]	EREC_RR	>12%[LWD]	0–42%	↓
Relative abundance of erect diatoms[a,b]	EREC_RA	>9%[LWD]	0–88%	↓
Tolerance/intolerance				
Relative richness of sensitive taxa[c]	SEN_RR	>20%	0–63%	↓
Relative richness of tolerant taxa[c]	TOL_RR	<18.5%	0–64%	↑
Relative abundance of sensitive taxa[c]	SEN_RA	>5%[LWD]	0–92%	↓
Relative abundance of tolerant taxa[c]	TOL_RA	<2%	0–62%	↑
Relative biovolume of sensitive taxa[c]	SEN_RB	>10%	0–98%	↓
Relative biovolume of tolerant taxa[c]	TOL_RB	<4%	0–97%	↑
Relative richness of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae[c]	BCRS_RR	<10%	0–41%	↑
Relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae[c]	BCRS_RA	<4%	0–42%	↑
Relative biovolume of sensitive soft algae[c]	SNSFT_RR	>3%[LWD]	0–94%	↓
Relative richness of *Brachysira*, *Eunotia*, *Tabellaria*, and *Anomeoneis*[c]	BETA_RR	>7%	0–45%	↓
Relative abundance of *Brachysira*, *Eunotia*, *Tabellaria*, and *Anomeoneis*[c]	BETA_RA	>2%	0–96%	↓
Nutrient and organic enrichment				
Relative richness of diatoms that require high dissolved O_2 concentrations[d]	HIGH_RR	>42%	6–72%	↓
Relative richness of polysaprobic diatoms[d]	PSAP_RR	<18%	0–44%	↑
Relative abundance of polysaprobic diatoms[d]	PSAP_RA	<17%	0–49%	↑
Relative richness of N autotrophic diatoms[d]	NAUT_RR	>37%	0–75%	↓
Relative richness of eutraphentic diatoms[d]	EUTR_RR	<40%	0–92%	↑
Relative abundance of eutraphentic diatoms[d]	EUTR_RA	<33%	0–97%	↑
Diatom total P index	DTPI	<18	4–73	↑
Specific conductance				
Relative richness of salt-tolerant diatoms[d]	SALT_RR	<10%	0–26%	↑
Diatom specific conductance index	DSCI	<100	4–1772	↑
Watershed disturbance				
Diatom watershed disturbance index	DWDI	<20	0–81	↑
Relative richness of motile diatoms[b,e]	MOT_RR	<38%	4–70%	↑
Relative abundance of motile diatoms[b,e]	MOT_RA	<18%	0–69%	↑

[a] (Fore 2003)
[b] (Wang et al. 2005)
[c] (Danielson et al. 2011)
[d] (van Dam et al. 1994)
[e] (Fore and Grafe 2002)

Metric calculation and selection

The set of metrics (Table 1) used to interpret Maine water-quality classes and BCG tiers included species traits obtained from literature sources and metrics empirically derived from Maine data. We based literature metrics on diatom motility ratings (Fore and Grafe 2002, Wang et al. 2005), diatom growth forms (Fore 2003, Wang et al. 2005), and diatom preferences for organic enrichment, N uptake, eutrophication, salinity, and O_2 requirements (van Dam et al. 1994). We generated Maine tolerance metrics, such as relative abundance of sensitive taxa, after: 1) calculating tolerance values for 195 diatoms and 41 soft algal taxa based on a principal components analysis (PCA) ordination of taxon optima for TP, TN, Cond, Dev, and Imp, and 2) categorizing taxa as sensitive, intermediate, or tolerant based on their Maine tolerance values, range of occurrence with respect to Dev, and response to Dev (Danielson et al. 2011). We screened metrics to ensure that they had strong correlation with Dev and that they could distinguish reference sites from nonreference sites (Danielson et al. 2011). We retained some metrics that were correlated with each other for the process of assigning Maine water-quality groups and BCG tiers to allow biologists flexibility to determine which metrics were the most important for making assignments. We also retained metrics that were correlated with each other with the expectation that redundant variables would be removed in the process of developing a statistical model.

We generated the Diatom Total Phosphorus Index (DTPI), Diatom Specific Conductance Index (DSCI), and Diatom Watershed Development Index (DWDI) with C^2 software (1.5.0/2007; New Castle University, Newcastle upon Tyne, UK) with Weighted Average – Partial Least Squares (ter Braak 1995). The indices were designed to estimate TP, Cond, and Dev based on the species composition of algal samples. The DTPI, DSCI, and DWDI were built with $\sqrt{(\%}$ abundances) of 209 diatom species that occurred in ≥ 7 samples and with 167, 166, and 186 samples, respectively, because of missing data for some samples. TP and Cond were $\log(x)$-transformed prior to analysis. The DTPI, DSCI, and DWDI were added to the set of metrics used by biologists to assign BCG tiers and Maine's water-quality classes.

We grouped metrics into the following categories: community structure, tolerance/intolerance, nutrient and organic enrichment, specific conductance, and watershed disturbance (Table 1). We produced quantile plots for each metric in R (2.6.2/2008; R Foundation for Statistical Computing, Vienna, Austria) with samples grouped as reference and nonreference to display ranges of values for the 2 groups of samples. We used locally weighted regression (LOWESS) to produce plots of metrics and Dev (Fig. 1). Metric plots also included lines to indicate the limit of natural conditions to facilitate interpretation of the Class AA/A requirement of biological communities being "as naturally occurs". For metrics that increased with watershed disturbance, the upper limit of natural conditions was defined by using expert judgment to select a value between the 90th and 95th percentiles of minimally disturbed reference samples (Table 1, Fig. 1). Similarly, for metrics expected to decrease with watershed disturbance, the lower limit of natural conditions was defined by selecting a value between the 5th and 10th percentiles of minimally disturbed reference samples (Table 1, Fig. 1). This approach was not appropriate for several metrics with widely ranging values for reference sites, but most of the samples with large values for these metrics, such as relative richness (proportion of species) of diatoms with erect growth form or the relative abundance (proportion of individuals) of sensitive algae, were from locations with little watershed disturbance (Fig. 1). These metrics were retained as indicators of low watershed disturbance (LWD). Greater values of LWD indicators indicated better watershed conditions, but smaller values were not reliable indicators of poor conditions.

Assignment of Maine water-quality classes and BCG tiers

Five MDEP biologists (BC, TJD, JLDF, Caitlin Kersten, and LT) independently assigned Maine water-quality groups and BCG tiers to 230 samples over a period of several weeks in 2008. The 4 water-quality groups were Class AA/A, Class B, Class C, and NA. We used random numbers to identify and sort samples. No study site information was provided to facilitate blind assignments based only on biological information. Biologists were provided with the narrative descriptions of BCG tiers adapted to Maine stream algae (Appendix 1; adapted from Davies and Jackson 2006a, b), Maine's narrative biological criteria, sample taxon lists, computed metrics for each sample, and a report summarizing each metric's response to changes in Dev. The original stream algal BCG framework (Appendix 2) used qualitative wording instead of percentage ranges, which were later revised with project results. Taxon lists for each sample included taxon densities (number of cells/cm^2), relative abundances, biovolumes, relative biovolumes, Maine tolerance values, diatom motility ratings (Fore and Grafe 2002, Wang et al. 2005), diatom growth forms (Fore 2003, Wang et al. 2005), and diatom preferences for organic enrichment, N uptake, eutrophication, salinity, and O_2 requirements (van Dam et al. 1994). At the end of the process, each biologist assigned a water-quality group (i.e., AA/A, B, C, NA) and BCG tier (e.g., 1, 2, 3) to each sample. After individually assigning water-quality groups and BCG tiers to samples, the biologists met for 2 d to compare results. Biologists conferred to reach consensus on water-quality group assignments with a modified Delphi approach (Bakus et al. 1982). Samples with unanimous agreement were reviewed quickly, but samples without unanimous agreement required discussion to reach consensus assignments (Biologist Classifications). Instead of establishing consensus BCG tiers, the biologists calculated average BCG tier assignments for each sample. Last, the panel of biologists recommended additional metrics for inclusion in the linear discriminant model (Table 2).

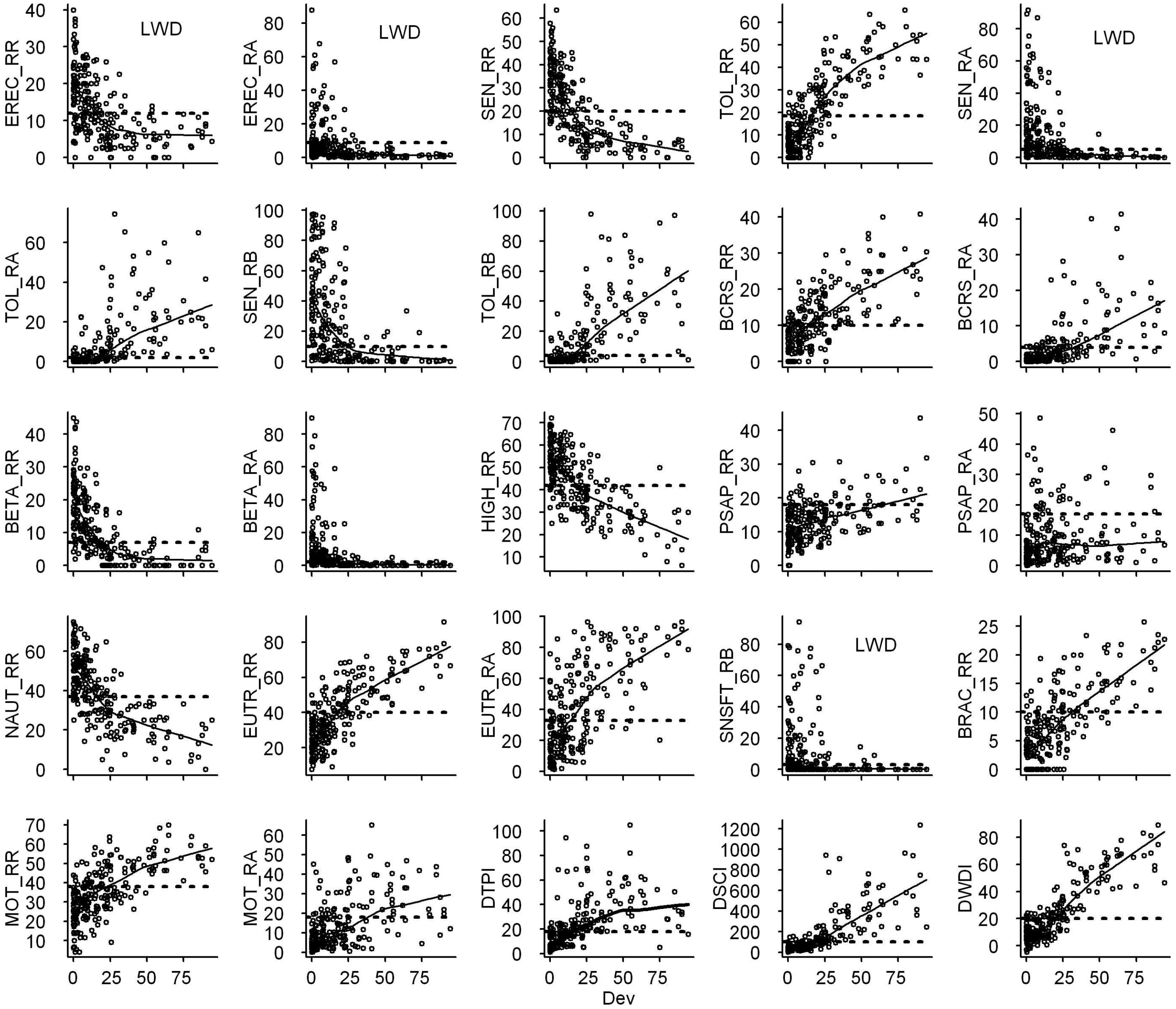

FIG. 1. Plots of metrics supplied to Maine Department of Environmental Protection biologists for assigning Maine water-quality groups (i.e., AA/A, B, C, and NA) and Biological Condition Gradient tiers with land cover that is developed (Dev) (i.e., no longer forest and wetland). Metric codes are defined in Table 1. Dashed horizontal lines indicate limit of expected reference conditions. Solid curves are locally weighted regression (LOWESS) lines. LWD indicates metrics that are indicators of low watershed disturbance.

Representatives from the Academy of Natural Sciences (D. Charles and M. Potapova) replicated the process and assigned Maine water-quality groups and BCG tiers to a subset of 40 samples selected in a stratified random design with 10 samples from each water-quality group as defined by consensus Biologist Classifications.

Algal discriminant analysis model

We transformed metrics in Tables 1 and 2 (e.g., $\sqrt{}$, $\sqrt[4]{}$, arcsine, arcsine$\sqrt{}$, log) to normalize variance or improve homoscedacity. We created a preliminary discriminant model for a sample training set ($n = 150$) with automatic backward stepwise selection of metrics with a probability of 0.05 and tolerance of 0.001 using the DISCRIM function in SYSTAT (version 13; SYSTAT, Inc., Evanston, Illinois). The stepwise selection process excludes some metrics that are redundant with ≥ 1 metrics already in the model. However, individually these metrics may predict groups better than other metrics included in the model. We removed discriminant analysis metrics with large within-pool correlations ($|r| > 0.70$) and iteratively

TABLE 2. Additional metrics for potential inclusion in the algal discriminant analysis model at the recommendation of Maine Department of Environmental Protection (MDEP) biologists after making a priori Maine water-quality-group (i.e., AA/A, B, C, NA) and Biological Condition Gradient tier (e.g., 1–6) assignments. ↑ = increase, ↓ = decrease, ∩ = unimodal.

Metric (formula)	Code	Predicted response to watershed development
Relative richness of diatoms that tolerate low dissolved O$_2$[a]	LOW_RR	↑
Sensitive:tolerant richness ratio	SENTOL	↓
Intermediate:tolerant richness ratio	INTTOL	↓
Relative richness of intermediate taxa[b]	INT_RR	∩
Relative abundance of intermediate taxa[b]	INT_RA	∩
Relative biovolume of intermediate taxa[b]	INT_RB	∩
Richness of erect diatoms[c,d]	EREC_R	↓
Richness of sensitive taxa[b]	SEN_R	↓
Richness of intermediate diatoms[b]	INTD_R	∩
Richness of tolerant diatoms[b]	TOLD_R	↑
Richness of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae[b]	BCRS_R	↑
Richness of polysaprobic diatoms[a]	PSAP_R	↑
Richness of nitrogen autotrophic diatoms[a]	NAUT_R	↓
Richness of eutraphentic diatoms[a]	EUTR_R	↑
Richness of salt-tolerant diatoms[a]	BRAC_R	↑
Richness of motile diatoms[d,e]	MOT_R	↑

[a] (van Dam et al. 1994)
[b] (Danielson et al. 2011)
[c] (Fore 2003)
[d] (Wang et al. 2005)
[e] (Fore and Grafe 2002)

added and dropped additional metrics to improve model performance. We plotted box-and-whisker plots of metrics in the final model with samples grouped by predicted water-quality group (i.e., AA/A, B, C, NA) in R. Average BCG tier assignments were plotted against Dev with locally weighted regression lines and samples grouped by predicted water-quality group in R.

We used analysis of variance (ANOVA) with Tukey's post hoc pairwise comparisons between groups to test the ability of metrics in the final discriminant model to distinguish Biologist Classifications (i.e., AA/A, B, C, NA) (SYSTAT). Samples in the validation set ($n = 80$) were not used to build the model, but the model predicted their group membership. We compared model predictions of training and validation samples to Biologist Classifications to calculate % correct predictions. We excluded 1 sample in the validation set from calculations because it had atypically small taxon richness ($n = 11$). We produced tables of agreement between the algal discriminant model and Biologist Classifications for the training and validation data sets. Strength of agreement between the discriminant model and Biologist Classifications was measured with Cohen's κ (Cohen 1960) and Kendall's τ_B (Kendall 1938, Kruskal 1958). Cohen's κ values >0.75 indicate strong agreement, and Kendall's τ_B is a measure of association of 2 ranked, ordinal variables similar to a correlation. Symmetry of disagreements above and below the diagonal line of agreement was tested with McNemar's χ^2 test of symmetry (McNemar 1947, Wilkinson 1990). Algal discriminant-model predictions were compared to paired macroinvertebrate discriminant-model predictions ($n = 137$) for the same sites with 2-way tables. We compared water-quality groups produced by the algal discriminant models to the aquatic-life use goals assigned to streams by Maine's water-quality standards (i.e., statutory goals) to compute the percentage of streams that attained their statutory goals. A stream attained its statutory goal if the algal discriminant model result was the same as or of a higher class (i.e., better quality) than the statutory goal. For example, a stream with a statutory goal of Class B would attain its goal if the discriminant model prediction were AA/A or B. However, this stream would not attain its goal if the discriminant model prediction were C or NA. We made a similar comparison between the macroinvertebrate discriminant model predictions and statutory goals to compute the percentage of streams that attained their statutory goals based on macroinvertebrate sample composition.

Results

Ecoregion effects

Major patterns in species composition of minimally disturbed reference sites were not strongly related to ecoregions. The NMDS 3-axis solution described 83% of variation (final stress = 14.9, instability < 0.00001, $p < 0.001$), and samples from the Northeastern Highlands and Acadian Plains and Hills ecoregions broadly overlapped. None of the sites we sampled in the Northeastern Coastal Zone (southern 4% of state) met our reference-site criteria. The species composition of reference sites in the Northeastern Highlands was statistically different from the species composition of reference sites in the Acadian Plains and Hills (MRPP, $T = -2.55$, $p = 0.017$). However, the effect size was very small ($A = 0.013$), indicating that the difference is of questionable ecological importance. Therefore, we developed a single, statewide bioassessment model with data compiled across the ecoregions.

Diatom community inference models

The 3 Weighted Average – Partial Least Squares (WA) models that inferred environmental conditions based on diatom species composition performed well. All 3 inference models were 2nd-component models, which exploit patterns in WA model residuals to reduce model error and bias (ter Braak and Juggins 1993). The DWDI inferred Dev ($p = 0.001$) with R^2 and RMSE $= 0.88$ and 7.2, respectively ($R^2_{\text{boot}} = 0.75$, $\text{RMSE}_{\text{boot}} = 10.9$). The DSCI inferred Cond ($p = 0.007$) with R^2 and RMSE $= 0.87$ and 0.166, respectively ($R^2_{\text{boot}} = 0.71$, $\text{RMSE}_{\text{boot}} = 0.266$). The DTPI inferred TP ($p = 0.01$) with R^2 and RMSE $= 0.80$ and 0.103, respectively ($R^2_{\text{boot}} = 0.59$, $\text{RMSE}_{\text{boot}} = 0.193$).

Biologist Classifications

The Biologist Classifications were 105 Class AA/A, 46 Class B, 46 Class C, and 33 NA. Few samples, typically the best- and worst-quality samples, fit the descriptions of the Maine aquatic-life uses and BCG tiers exactly. Many samples had metrics that provided mixed signals and required biologists to evaluate the combination of metric values critically to determine the most appropriate water-quality groups and BCG tiers. Fifty-three percent of the 230 samples in the training and test sets had unanimous group assignments, 42% differed by 1 group by at least 1 biologist, and 5% differed by >1 group. Assignments by the national experts differed between experts and the Biologist Classifications. Assignments by 1 expert agreed with MDEP biologists for 35 of 40 (87.5%) samples. MDEP biologists had reached consensus assignment with difficulty for the 5 samples that did not match the expert assignments. Assignments by the 2nd expert agreed with MDEP biologists for 15 of 45 (37.5%) samples. The 2nd expert consistently assigned the samples to a better water-quality group than did MDEP biologists, and made more AA/A and B assignments and fewer C and NA assignments than did the MDEP biologists. The average of the BCG tiers assigned by the biologists decreased in relation to Dev (Fig. 2), with a steep decline from BCG Tier 2 to Tier 4.

Algal discriminant model

The algal discriminant model based on automatic backward selection process included 11 metrics: DSCI, relative abundance of erect diatoms (EREC_RA), richness of diatoms that require high O_2 concentrations (HIGH_R), relative richness of intermediate taxa (INT_RR), relative biovolume of intermediate taxa (INT_RB), relative richness of sensitive taxa (SEN_RR), relative biovolume of sensitive taxa (SEN_RB), intermediate:tolerant richness ratio (INTTOL), sensitive:tolerant richness ratio (SENTOL), relative richness of tolerant taxa (TOL_RR), and relative biovolume of tolerant taxa (TOL_RB). Metric calculations are described in Tables 1 and 2. The relative richness and relative biovolume of tolerant taxa were removed because of large within-pool correlations ($|r| > 0.80$) with other metrics. The DSCI was removed and replaced with the relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA) to avoid including an inference model in the discriminant model. The algal discriminant model included an equation, called a discriminant function, for each of the 4 water-quality classes (AA/A, B, C, NA). The 4 discriminant functions were similar to an additive MMI, but included a constant and metric coefficients (Appendix 3). The algal discriminant model used the 4 discriminant functions to compute the probabilities of a sample belonging to the 4 water-quality class groups (AA/A, B, C, NA) (Appendix 3). The algal discriminant model classified samples based on the water-quality group with the greatest probability. For example, a sample with probabilities of belonging to the AA/A, B, C, and NA groups of 0.22, 0.88, 0.00, and 0.00 would be classified as B. The greatest probabilities were always >0.51 and 79% of samples ($n = 150$) had probabilities >0.90.

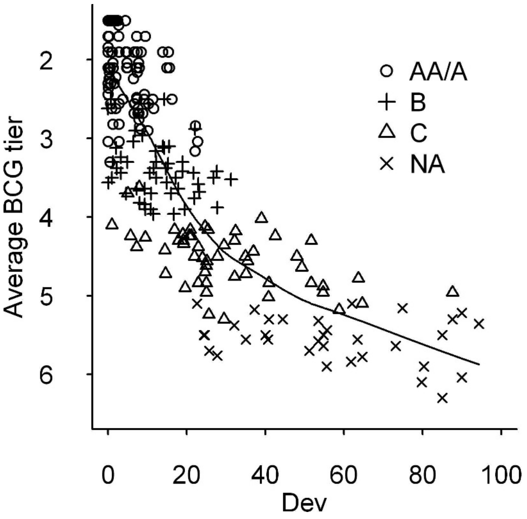

FIG. 2. Relationship between % developed watershed (Dev) and the average value of the Biological Condition Gradient (BCG) tier assigned by the 5 biologists. Samples are identified by algal discriminant model predictions of water-quality classes AA/A, B, C, and nonattainment (NA).

Each of the 9 metrics in the final algal discriminant model effectively distinguished ≥1 Biologist

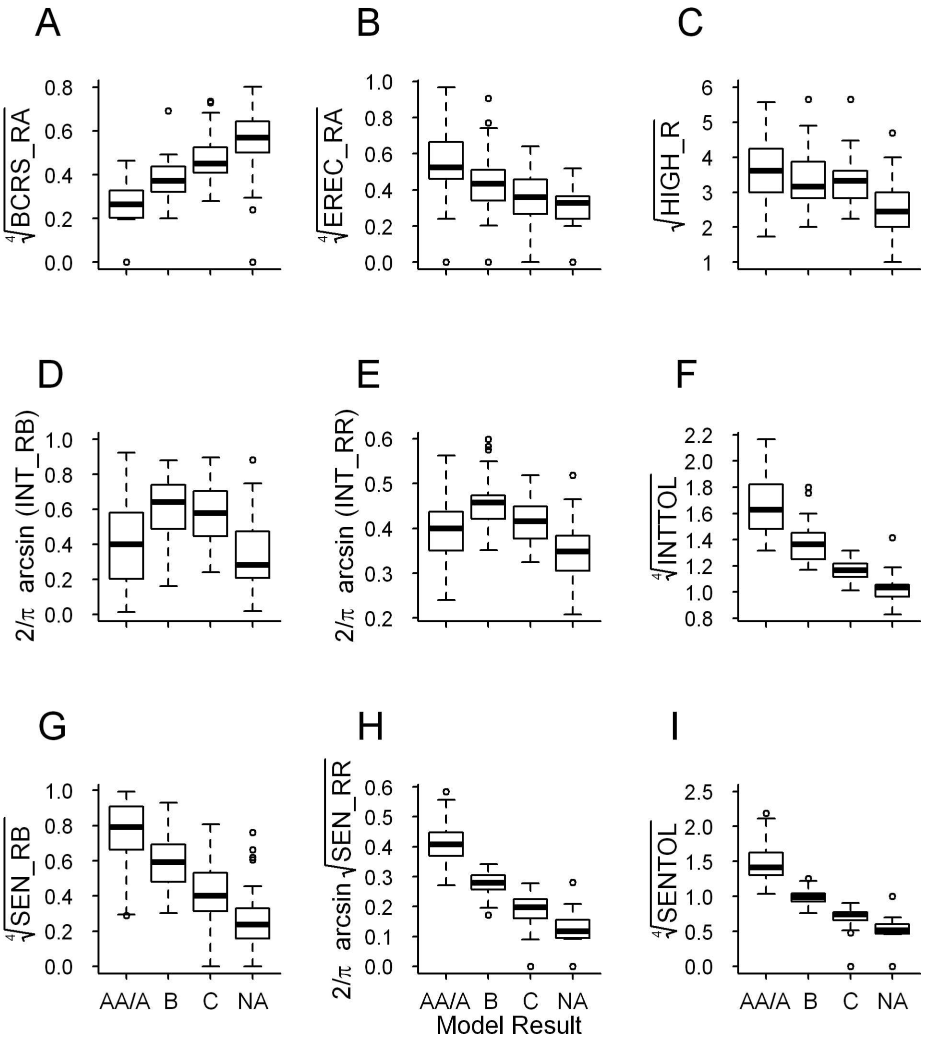

FIG. 3. Box-and-whisker plots for 9 metrics of the 4-way algal discriminant model with samples grouped by Biologist Classifications (AA/A, B, C, NA). The 9 metrics are relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA) (A), relative abundance of erect diatoms (EREC_RA) (B), richness of diatoms that require high O_2 concentrations (HIGH_R) (C), relative biovolume of intermediate taxa (INT_RB) (D), relative richness of intermediate taxa (INT_RR) (E), intermediate-tolerant richness ratio (INTTOL) (F), relative biovolume of sensitive taxa (SEN_RB) (G), relative richness of sensitive taxa (SEN_RR) (H), and sensitive-tolerant richness ratio (SENTOL) (I).

Classifications (1-way ANOVA, $F_{3,226} > 18.494$, $p < 0.001$, and ≥1 Tukey pairwise comparison $p < 0.05$; Fig. 3A–I). All Tukey pairwise comparisons of BCRS_RA (Fig. 3A), INTTOL (Fig. 3F), SEN_RB (Fig. 3G), SEN_RR (Fig. 3H), and SENTOL (Fig. 3I) were significant ($p < 0.001$). HIGH_R distinguished NA from A, B, and C ($p < 0.001$; Fig. 3C). INT_RB distinguished A and NA from B and C ($p < 0.001$; Fig. 3D). All pairwise comparisons of EREC_RA and INT_RR were significant ($p < 0.01$) except for the C and NA groups for EREC_RA (Fig. B) and the A and C groups for INT_RR (Fig. 3E).

The algal discriminant model correctly classified 95% of training samples (discriminant analysis, Wilk's $\lambda = 0.032$, approximate $F_{27,403} = 33.6$, $p < 0.001$; Table 3). The algal discriminant model classified 97% of the best-quality (Class A) and 100% of the worst quality (NA) samples correctly (Table 3). The algal discriminant model correctly classified 90% of Class B samples and 93% of Class C samples. Agreement between the model and Biologist Classifications was strong (Cohen's $\kappa = 0.932$, SE = 0.025; Kendall's $\tau_B = 0.968$, SE = 0.012). The algal discriminant model did not consistently assign samples to water-quality groups of greater or poorer quality than the Biologist Classifications (McNemar's $\chi^2 = 4.33$, df = 6, $p = 0.632$). Canonical factor scores of training samples formed distinct clusters with little overlap, a result

TABLE 3. Performance of algal linear discriminant model compared to consensus class assignments of biologists with the training data set ($n = 150$), jackknife analysis of training data set, and validation data set ($n = 80$). Cell values are % classification of samples with numbers of samples in parentheses.

		Algal discriminant model			
Data set	Biologist classifications	AA/A	B	C	NA
Training data (95% correct)	AA/A	97% (67)	3% (2)	–	–
	B	3% (1)	90% (27)	7% (2)	–
	C	–	–	93% (28)	7% (2)
	NA	–	–	–	100% (21)
Jackknife analysis of training data (93% correct)	AA/A	96% (66)	4% (3)	–	–
	B	3% (1)	90% (27)	7% (2)	–
	C	–	–	93% (28)	7% (2)
	NA	–	–	10% (2)	90% (19)
Validation data (91% correct)	AA/A	97% (35)	3% (1)	–	–
	B	13% (2)	81% (13)	6% (1)	–
	C	–	13% (2)	88% (14)	–
	NA	–	– (1[a])	9% (1)	91% (10)

[a] Sample excluded from calculations because of atypical total richness ($n = 11$)

showing that the algal model effectively distinguished samples from different groups. The jackknife analysis estimated the precision of the model by randomly selecting subsets of data and yielded correct classification of 93% of the training data (Table 3). Discriminant analysis factor 1 explained 92% of dispersion in the data, was the primary factor separating water-quality groups, and was most influenced by SEN_RR, SENTOL, and INTTOL.

Classification of validation samples ($n = 80$) yielded 91% correct classification (Table 3). Agreements between the model with validation data and Biologist Classifications were strong (Cohen's $\kappa = 0.855$, SE $= 0.048$; Kendall's $\tau_B = 0.919$, SE $= 0.029$). Disagreements between the model and Biologist Classifications were symmetrical (McNemar's $\chi^2 = 2.667$, df $= 6$, $p = 0.849$). One sample was excluded because it had atypically small taxon richness ($n = 11$). This sample had a Biologist Classification of NA (3 biologists assigned NA and 2 biologists assigned C), but the model predicted it was Class B.

The classifications of sampling locations based on the algal and macroinvertebrate discriminant models had moderate agreement (Cohen's $\kappa = 0.441$, SE $= 0.055$; Kendall's $\tau_B = 0.569$, SE $= 0.058$) and matched 58% of the time (Table 4). Disagreements between the 2 models were not strongly asymmetrical (McNemar's $\chi^2 = 7.444$, df $= 6$, $p = 0.282$). The macroinvertebrate model predicted a better water-quality group than the algal model for 25% of the samples and predicted poorer water-quality groups than algal groups for 17% of the samples. Based on the algal discriminant model results, 60% of samples attained or exceeded their statutory goals assigned by Maine's water-quality standards. In contrast, 75% of samples attained or exceeded their statutory goals based on the macroinvertebrate discriminant model results. Only 47% of samples attained or exceeded their statutory goals with both algae and macroinvertebrate models.

Discussion

Algal discriminant model

We developed an algae-based bioassessment model, in the form of a linear discriminant analysis model that computes the probabilities of samples belonging to water-quality groups (AA/A, B, C, and NA). The model integrates professional judgment based on interpretation of narrative biocriteria found in Maine state water-quality law, the BCG adapted for stream algae, and the degree of departure of algal metrics from regional reference conditions. The model is specific to the tiered aquatic-life uses in Maine's water-quality standards. However, we incorporated the BCG by empirically developing tolerance values for algal taxa (Danielson et al. 2011), computing

TABLE 4. Comparison of sample assignments generated by the algal and macroinvertebrate discriminant analysis models. Cell values are % classification of samples with numbers of samples in parentheses.

	Macroinvertebrate model			
Algal model	AA/A	B	C	NA
AA/A	77% (45)	12% (7)	9% (5)	2% (1)
B	21% (5)	58% (14)	13% (3)	8% (2)
C	30% (10)	27% (9)	27% (9)	15% (5)
NA	18% (4)	18% (4)	14% (3)	50% (11)

metrics based on tolerance groups (i.e., sensitive, tolerant), and interpreting a BCG framework adapted to Maine algal communities.

The discriminant model, with stepwise selection and weighting of metrics, replicated Biologist Classifications with both the training and validation data. The discriminant model's performance would have been weaker had disagreement been greater in the a priori assignments made by individual biologists. The algal discriminant model included some metrics that were correlated (Danielson et al. 2011), but not greatly correlated when separated among the water-quality groups (i.e., small within-group correlations). The algal discriminant model also included multiple metrics related to sensitive, intermediate, and tolerant taxon groups. The metrics were used by biologists to distinguish different water-quality groups. For example, SEN_RR was particularly used by biologists to distinguish Class AA/A samples from Class B samples. Similarly, SENTOL was important for distinguishing Class B from Class C and INTTOL was important for distinguishing Class C from NA. Inclusion of multiple metrics related to tolerance groups does not mean that other metrics were not valuable for separating water-quality groups. Other metrics could have been excluded during the stepwise selection process because they were highly correlated with metrics that were included. The metrics in the model were simply the best combination of metrics for replicating Biologist Classifications.

Most of the algal community attributes that responded predictably to a disturbance gradient in Maine and served as metrics (Karr and Chu 1999) were locally derived (Danielson et al. 2011). Locally derived metrics and indices better estimate the deviation of algal communities from local reference conditions than attributes developed in other parts of the country or world (Kelly et al. 1998, Pipp 2002, Rott et al. 2003, DeNicola et al. 2004, Potapova et al. 2005, Newall et al. 2006, Potapova and Charles 2007). Better metric performance in some regions may reflect regional differences in climate and topography, which partly determine the algal species at reference sites (Grenier et al. 2006). Regional variation in metric performance also may reflect regional differences in the type, magnitude, duration, timing, and spatial distribution of anthropogenic stressors that degrade algal assemblages. For example, algal communities from streams damaged by acid mine drainage differ from those in streams damaged by agricultural activities (Pan et al. 2000). Streams affected by acid mine drainage and agriculture could be equally damaged in terms of departure from their regional reference conditions, but they may have differing biological responses to acidification and eutrophication that would be expressed in their metric values.

Differences in class assignments by the national experts and MDEP biologists could reflect either contrasting reliance on local metrics to inform the assignments or differing expectations based on past experience with algal communities from different parts of the country. The national expert with most agreement with Biologist Classifications relied more on MDEP-specific metrics, and the expert that disagreed most with Biologist Classifications placed greater emphasis on literature-based metrics and expectations of other regions of the country. Algal communities of Maine streams with mild-to-moderate stress could be measurably different than algae in Maine's minimally disturbed streams, but similar to algal communities in the best remaining sites (i.e., least disturbed) in other regions. Knowledge of local algal communities and availability of locally derived metrics can improve the development and performance of algal bioassessment models. In some cases, knowledge of chemical and physical characteristics can also aid BCG tier assignments. Biologists participating in a study of New England macroinvertebrates found it difficult to distinguish Tier 1 and 2 communities without supporting chemical and physical information (Snook et al. 2007).

We developed a single statewide model because the algal communities from minimally disturbed sites in different ecoregions were similar. The major patterns of diatom community composition of Maine streams were most influenced by nutrient enrichment, increased specific conductance, and sedimentation caused by human activities at the regional and watershed scales (Danielson et al. 2011). Anthropogenic stressors can impose filters (Poff 1997) that overwhelm the influence of natural constraints on algal communities (Leland and Porter 2000, Pan et al. 2000, Fore 2003). For example, the algal composition of streams with primarily forested watersheds in the Washington Yakima River basin varied with basin geology, but the species composition of agricultural streams was shaped primarily by anthropogenic stressors affecting local conditions, such as enrichment, turbidity, and embeddedness (Leland and Porter 2000). In New Zealand and the Mid-Atlantic region of the USA, human activities at the watershed scale (e.g., urbanization) and reach scale (e.g., riparian alteration) influenced algae more than regional conditions, such as climate, geology, soil, and vegetation (Pan et al. 1999, 2000, Biggs 2000). In Maine, the Northeastern Highlands ecoregion had few degraded algal communities and the Northeast Coastal Zone ecoregion had few healthy algal communities, a pattern reflecting unequal distribution of development and agricultural activities. Algal bioassessments in other regions might warrant ecoregion-specific models if differences in natural ecological conditions among ecoregions are more pronounced than observed in Maine.

BCG framework for Maine stream algae

We adjusted the BCG framework originally applied and calibrated to stream fish and macroinvertebrate communities (Davies and Jackson 2006a, b) to Maine stream algal communities (Appendix 2). The adjustment reflects that BCG Tier 1 and 2 samples from Maine typically have a large relative abundance of sensitive macroinvertebrates, but the same locations often do not have an abundance of sensitive algae. Minimally disturbed sites in Maine typically have a diverse assemblage of sensitive algae, but they are not necessarily abundant. The relative abundance of ubiquitous, eurytopic taxa that tolerate a wide range of ecological conditions (e.g., *Achnanthidium minutissimum*) is >80% in some Maine reference sites. Therefore, the algal BCG emphasizes the relative richness of sensitive algae, which was strongly correlated with Dev (Danielson et al. 2011).

The relationship between the BCG and stressor gradients has been conceptualized with hypothetical lines or sigmoidal curves with little change between Tiers 1 and 2, rapid change between Tiers 3 and 5, and little change between Tiers 5 and 6 (e.g., Davies and Jackson 2006b). The conceptual responses (i.e., line, sigmoidal curve) were not meant to represent all possible responses, and empirical relationships based on sampling data may reveal different responses. When calibrated with Maine algal data and minimally disturbed reference conditions, we found that average BCG tiers decreased rapidly from Tiers 1 to 4 before leveling to Tier 6 (Fig. 2). The transitions between Tiers 2 and 3 (~10% developed) and Tiers 3 and 4 (~20% developed) occur at relatively low levels of watershed disturbance. Our results highlight the need for water-quality programs to focus management activities on protecting watersheds of high-quality streams and rivers where small changes in development can lead to large changes in BCG tiers. Water-quality programs that focus criteria, water-quality goals, and management activities at the transition between Tiers 4 and 5 could fail to detect and prevent rapid and substantial degradation of streams and rivers.

Quality of regional reference sites may differ (Stoddard et al. 2006), and the BCG tiers can help communicate the ecological condition of regional reference sites. Some regions may have minimally disturbed reference sites that approximate BCG Tiers 1 and 2. Other regions with widespread watershed disturbance may have few or no minimally disturbed reference sites (Brown et al. 2009, Cuffney et al. 2010). The best-quality sites in those regions may be considered least-impaired reference sites that approximate BCG Tiers 3 or 4 (Snook et al. 2007). Clear articulation of reference-site condition can improve public communication, interpretation of assessment results, and water-quality management.

Comparison of algal discriminant model and MMI approaches

Our algal discriminant model is similar to an MMI. Both approaches rely on experienced biologists with knowledge of regional algal communities to evaluate potential metrics critically and to identify those that respond along a disturbance gradient. Both approaches typically combine metrics by adding them, but discriminant analysis includes metric coefficients and a constant (Appendix 3). In addition, both approaches incorporate professional judgment. For MMIs, biologists use professional judgment when selecting metrics, selecting a method of scoring individual metrics with discrete values or ranges, determining how to combine the metrics into an index, and establishing condition categories (e.g., good, fair, poor) or numeric index thresholds related to biological criteria in water-quality standards (Karr 1981, Barbour et al. 1995, Gerritsen 1995). Both approaches effectively evaluate the condition of streams and manage water quality.

The 2 approaches differ in several ways. In the MMI approach, biologists build an index and allow the model to determine the condition of a sample by producing an index value. In contrast, our approach was to let the biologists determine the condition of a sample by interpreting the interrelationships of metrics with respect to biological goals articulated in narrative aquatic-life criteria and assigning samples to a water-quality group (AA/A, B, C, or NA). Then we built a model to replicate professional judgment. Maine's macroinvertebrate models were similarly constructed (Davies et al. 1995). A key advantage is that Maine's bioassessment models are seamlessly integrated with Maine's tiered aquatic-life uses, which provides transparency in the interpretation of stream condition and management of water quality (Courtemanch et al. 1989, Courtemanch 1995, Barbour et al. 2000, Davies and Jackson 2006b). MMIs have been criticized for having arbitrarily set scoring thresholds, combinations of metrics that can change at different rates, and a single index value generated by arbitrarily adding metrics (Suter 1993, Norris 1995). In contrast, discriminant analysis is a robust statistical method that can accommodate the advantages of expert judgment to assess attainment of water-quality goals while permitting objective identification and weighting of the best combination of metrics for classifying samples. Another advantage is that our algal discriminant model describes the uncertainty of a new sample belonging to one class or another by computing probabilities of class attainment (Appendix 3).

Comparison of the algal and macroinvertebrate discriminant models

Disagreements between classifications based on the algal and macroinvertebrate discriminant models reflect different sensitivities to environmental degradation. Algae may be influenced more by water chemistry, nutrient enrichment, and land uses that alter water quality, whereas, macroinvertebrates may be more sensitive to organic enrichment, O_2 depletion, changes to hydrology and habitat, and some toxic substances (Passy et al. 2004, Hering et al. 2006, Johnson et al. 2006). Sites found to be in better condition with the macroinvertebrate model (i.e., better water-quality group) than indicated by the algal model had moderate nutrient enrichment that caused a functional replacement of sensitive algae adapted to low nutrient concentrations by intermediate algae. Nutrients increased stream productivity at those sites, but abundant dissolved O_2 may have prevented negative effects to macroinvertebrate communities (Odum et al. 1979). Many sites that supported better quality algal than macroinvertebrate communities were in urban watersheds or downstream of lake outlets or fish hatcheries (TJD, unpublished data). Some of the urban streams have pollution or altered temperature or hydrogeomorphology that could affect sensitive macroinvertebrates more than algae. Algae can be expected to recolonize more quickly after disturbances than many sensitive and intermediate macroinvertebrates because of rapid reproduction and recolonization (Peterson 1996). Some sites downstream of lake outlets or fish hatcheries had extremely high abundances of caddisflies (e.g., *Hydropsychidae*) and midges (e.g., *Rheotanytarsus*) that filter feed on plankton, zooplankton, and particulate organic matter, which are sources of nutrients not readily available to benthic algae (TJD). Some high-quality sites were classified differently by the algal and macroinvertebrate discriminant models. Streams and rivers may not provide reference-quality conditions for every taxonomic assemblage, because the same set of geology, climate, topography, reach characteristics, water chemistry, and local habitat conditions could impose different constraints on different taxonomic groups (Poff 1997). A river might provide reference-quality conditions for algae, but would not automatically provide reference conditions for macroinvertebrates or fish. Responses of algae and macroinvertebrates to environmental degradation need additional study.

Management implications

Biological monitoring data can be the foundation of water-quality management if assessment tools are coordinated with water-quality standards and criteria (Courtemanch et al. 1989, Karr 1991, Courtemanch 1995, Yoder and Rankin 1998, Barbour et al. 2000, Yoder and Barbour 2009). MDEP could incorporate the algal discriminant model into water-quality standards as numeric biocriteria, in a manner similar to that used with the discriminant model for stream macroinvertebrates (State of Maine 2003). MDEP uses bioassessment results to identify impaired water bodies in need of restoration (MDEP 2010), target high-quality waters for conservation (State of Maine 2004b), and improve management of dams, point-source discharges, stormwater, and nonpoint-source pollution (Davies et al. 1999). Biological assessments also can improve decision making required by Total Maximum Daily Load (TMDL) analyses (Karr and Yoder 2004). MDEP has used attainment of aquatic-life criteria as the endpoint of concern in several urban stream TMDLs (Meidel and MDEP 2003a, b, Meidel and Evers 2007). All of these management activities would be improved with the addition of the algal bioassessment model.

Evaluations of waterbody condition based on both algae and macroinvertebrates can be more comprehensive and can detect effects of a broader range of stressors than evaluations based on only 1 assemblage (Barbour et al. 1999, Passy et al. 2004, Griffith et al. 2005, Hering et al. 2006, Johnson et al. 2006, Yoder and Barbour 2009). We found that the percentage of impaired streams increased from 35 to 40% when one taxonomic assemblage was evaluated to 53% when both algae and macroinvertebrates were assessed. Under Maine's water-quality standards, algal and macroinvertebrate discriminant models are independently applicable, and a water body could be listed as impaired if either model indicates that a sample does not attain its statutory goal. In a study of multiple assemblages in Appalachian streams, Carlisle et al. (2008) found that 45% of streams (n = 108) were impaired when the assessment was based on diatoms, 66% were impaired when macroinvertebrates were used, and only 17% were not impaired when the assessment included both assemblages. (Carlisle et al. 2008)

Including multiple biological assemblages in a biological monitoring program can improve diagnostic capabilities (Patrick 1949, Paavola et al. 2003, Yoder and DeShon 2003, Passy et al. 2004), especially when incorporated into a formal process such as the US Environmental Protection Agency's Stressor Identification and Evaluation process (Cormier et al. 2003). Individual algal metrics and inference models that might not be included in overall assessments of resource condition can help diagnose 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 algal 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 the effects of organic and inorganic effluents on diatom communities (Kelly 1998a, b, Rott et al. 1998, Leland and Porter 2000). MDEP can improve diagnosis of stressors damaging a stream or river by simultaneously evaluating algal and macroinvertebrate diagnostic metrics and indices.

Bioassessment programs could benefit from adding expert judgment review of bioassessment models when determining if streams and rivers have impaired water quality. Maine includes professional judgment in interpreting the results of its macroinvertebrate discriminant model (State of Maine 2003). Biologists use expert judgment to determine class attainment or, in cases where the model outcome is suspect, to require resampling for samples that either: 1) do not meet minimum requirements for using the macroinvertebrate discriminant model (e.g., richness < 15, total abundance < 50), or 2) have probabilities of attaining a class between 0.40 and 0.60. Similar provisions could be applied to the algal discriminant model. Also, it could be beneficial to include expert review of key diagnostic metrics that are not included in an algal discriminant model or MMI when determining attainment of aquatic life use goals. Our model and MMIs that emphasize metric response to a generalized disturbance gradient potentially can lead us to overlook effects of stressors that are not correlated with the general disturbance gradient (Wang et al. 2005). For example, large relative abundances of polysaprobic or acidophilic diatoms could indicate damaged algal communities, but neither attribute was correlated with Dev in Maine and infrequent problems with these stressors usually occurred in mostly forested watersheds. As a result, the algal discriminant model might not detect impairment caused by the effects of localized sources of acidification (e.g., mines) or organic pollution (e.g., poorly managed agriculture, hatcheries, and wastewater discharges). For example, the algal discriminant model predicted that Blood Brook in Katahdin Ironwork Township attained Class A because of a predominance of taxa sensitive to increased Dev, TP, TN, and Cond. Blood Brook is oligotrophic and its watershed is almost entirely forested, but it is damaged by acidification from historical mining activities resulting in atypically low diatom richness and large relative abundance of acidophilic diatoms. The brook also does not support a viable benthic macroinvertebrate assemblage (TJD, unpublished data). Bioassessment programs could benefit from adding expert review of diagnostic metrics to the process of reviewing algal bioassessment results and determining if streams attain biological criteria. No model is perfect and one must consider the assumptions around which a model has been constructed and weaknesses that may be present when making final decisions about attainment of water-quality standards.

The addition of algal discriminant model, inference models (e.g., DTPI), and diagnostic metrics (e.g., relative richness of motile diatoms) will improve the management of water quality in Maine's streams and rivers. The metrics and model are specific to Maine streams and adjacent areas within the same ecoregions, but our approach of using the BCG to help develop a model to predict attainment of tiered water-quality classes is widely transferable to other waterbody types and taxonomic groups in other regions.

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APPENDIX 1. Maine's water-quality classes for rivers and streams, management goals, narrative habitat and aquatic-life criteria, and definitions of key terms.

CLASS	Management goals	Narrative habitat and aquatic life criteria
AA[a]	Highest quality water, minimal human interference. No discharges allowed. No impoundment allowed.	Habitat shall be characterized as free-flowing and *natural*[b]. *Aquatic life*[c] shall be *as naturally occurs*[d].
A[a]	High quality water with limited human interference. Discharges limited to noncontact process water or highly treated wastewater of quality equal to or better than the receiving water. Impoundments allowed.	Habitat shall be characterized as natural. Aquatic life shall be as naturally occurs.
B	Good water quality. Discharge of well treated effluent with ample dilution permitted. Impoundments allowed.	Habitat shall be characterized as *unimpaired*[e]. Discharges shall not cause adverse impacts to aquatic life. Receiving water shall be of sufficient quality to support all aquatic species *indigenous*[f] to the receiving water *without detrimental changes*[g] in the *resident biological community*[h].
C	Acceptable water quality. Maintains the interim goals of the United States Clean Water Act (i.e., fishable/swimmable). Discharge of well treated effluent permitted. Impoundments allowed.	Habitat for fish and other aquatic life. Discharges may cause some changes to aquatic life, provided that the receiving waters shall be of sufficient quality to support all species of fish indigenous to the receiving water and maintain the *structure*[i] and *function*[j] of the resident biological community.
Impoundment	Riverine impoundments not classified as *Great Ponds*[k] and managed for hydropower generation	Support all species of fish indigenous to those waters and maintain the structure and function of the resident biological community.

[a] The narrative aquatic life criterion is the same for Class AA and Class A

[b] *Natural* means living in or as if in, a state of nature not measurably affected by human activity (State of Maine 2004b)

[c] *Aquatic life* means any plants or animals that live at least part of their life cycle in fresh water (State of Maine 2004b)

[d] *As naturally occurs* means conditions with essentially the same physical, chemical, and biological characteristics as found in situations with similar habitats, free of measurable effects of human activity (State of Maine 2004b)

[e] *Unimpaired* means without a diminished capacity to support aquatic life (State of Maine 2004b)

[f] *Indigenous* means supported in a reach of water or known to have been supported according to historical records compiled by State and Federal agencies or published in scientific literature (State of Maine 2004b)

[g] *Without detrimental changes* means no significant loss of species or excessive dominance by any species or group of species attributable to human activity (State of Maine 2004b)

[h] *Resident biological community* means aquatic life expected to exist in a habitat, which is free from the influence of the discharge of any pollutant. This shall be established by accepted biomonitoring techniques (State of Maine 2004b)

[i] *Community structure* means the organization of a biological community based on numbers of individuals within different taxonomic groups and the proportion each taxonomic group represents of the total community (State of Maine 2004b)

[j] *Community function* means mechanisms of uptake storage and transfer of life-sustaining materials available to a biological community which determine the efficiency of use and the amount of export of the materials from the community (State of Maine 2004b)

[k] *Great Ponds* means any inland body of water which in a natural state has a surface area in excess of 10 acres (40,000 m^2) and any inland body of water artificially formed or increased which has a surface area in excess of 30 acres (120,000 m^2) except for the purposes of this article, where the artificially formed or increased inland body of water is completely surrounded by land held by a single owner (State of Maine 2004a)

APPENDIX 2. Biological Condition Gradient (BCG) framework for Maine stream algae. Numeric ranges (e.g., >5%) in this final version replaced qualitative descriptions in the original version. TP = total P, Cond = specific conductance (µS/cm), Dev = % developed watershed (% no longer forest or wetland).

	BCG Tier					
	Tier 1	Tier 2	Tier 3	Tier 4	Tier 5	Tier 6
Description	Natural condition	Minimal changes in community structure	Evident changes in community structure and minimal changes in ecosystem function	Moderate changes in community structure and minimal changes in ecosystem function	Major changes in community structure and moderate changes in ecosystem function	Severe changes in community structure and major changes in ecosystem function
Corresponding Maine water-quality classification	AA/A	AA/A	B	C	NA	NA
BCG attributes						
II Highly sensitive taxa III Sensitive taxa	Usually >20% of species and >10% of biovolume; most metrics within the range of natural condition	Usually >20% of species and >10% of biovolume; most metrics within the range of natural condition	Usually >15% of species and >5% of biovolume; most metrics within or near the range of natural condition	Richness and biovolume usually well below the range of natural conditions; several sensitive taxa metrics are low	Absent or richness and biovolume very low; occasionally can have a sensitive filamentous alga with high biovolume; most or all sensitive taxa metrics low	Absent
IV Opportunistic taxa of intermediate tolerance	Usually 30–70% of richness and 0–90% of biovolume	Usually 30–70% of richness and 0–90% of biovolume	Usually 50–85% of richness and 25–99% of biovolume	Usually 50–80% of richness and 40–99% of biovolume	Usually 30–60% of richness and 0–70% of biovolume	Richness <30%
V Tolerant taxa	Usually <18.5% of taxon richness, 2% of cell density, and 4% of biovolume; most tolerant metrics within range of natural condition	Usually <18.5% of taxon richness, 2% of cell density, and 4% of biovolume; most tolerant metrics are within range of natural condition	Usually <25% of taxon richness and 10% of biovolume; some tolerant-taxa metrics may be elevated	Usually <40% of taxon richness and 50% of biovolume; several tolerant-taxa metrics are elevated	Usually >40% of taxon richness and 50% of biovolume; ratio of the number of tolerant taxa to sensitive taxa is high; most tolerant-taxa metrics are elevated	Usually comprise most of the assemblage; often at very low or very high densities; most tolerant taxa metrics are highly elevated
VI Nonnative taxa	None observed			Very little is known about which species of algae are native or nonnative to Maine. The greatest known risk is from the diatom *Didymosphenia geminata*, which has been observed in Vermont and New Hampshire. It can form extensive mats that smother the stream bottom. The greatest risk is to high-quality streams and rivers because it has the potential to colonize many oligotrophic-mesotrophic streams and rivers and overgrow native algae.		

APPENDIX 2. Continued.

	BCG Tier					
	Tier 1	Tier 2	Tier 3	Tier 4	Tier 5	Tier 6
VII Diatom community inference models	Predict very low TP, Cond, Dev	Inferred TP, Cond, and Dev usually <18, 100, and 20, respectively; 1 may be elevated	≥1 inference models is elevated above the range of natural conditions	Variable; inference models may be somewhat elevated; ≥1 may be much higher than expected	Predict high TP, SPC, Dev	Predict high TP, SPC, Dev
VIII Ecosystem function	Typically well oxygenated because of cold water, riffles, or low to moderate algal growth	Typically well oxygenated because of cold water, riffles, or low to moderate algal growth	Increased algal growth may increase food supply for algivores and begin to alter habitat for benthic organisms	Algal growth may alter O_2 regime; presence of filamentous forms may alter habitat; increased cyanobacteria may raise concern of cyanotoxins	Excessive algal growth may alter habitat or cause aesthetic problems; O_2 may greatly increase during the day and decrease at night; abundant cyanobacteria may raise concern of cyanotoxins	Algal community may no longer be dominant and may be replaced by saprobic community (“sewage fungus”)

Appendix 3. The algal discriminant model replicated Biologist Classifications and used 9 metrics to classify samples into 4 groups based on Maine's water-quality classifications of Class AA/A, Class B, Class C, and nonattainment (NA). The 9 metrics are relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA), relative abundance of erect diatoms (EREC_RA), richness of diatoms that require high O_2 concentrations (HIGH_R), relative richness of intermediate taxa (INT_RR), relative biovolume of intermediate taxa (INT_RB), intermediate-tolerant richness ratio (INTTOL), relative biovolume of sensitive taxa (SEN_RB), relative richness of sensitive taxa (SEN_RR), and sensitive-tolerant richness ratio (SENTOL). Table A1 lists the metric coefficients of the algal discriminant model classification functions.

Table A1. Metric coefficients of the algal discriminant model classification functions.

Metric	Transformation	AA/A	B	C	NA
Constant		−402.743	−345.655	−271.173	−212.396
BCRS_RA	$\sqrt[4]{}$	103.154	101.749	99.952	112.145
EREC_RA	$\sqrt[4]{}$	−22.778	−20.192	−21.129	−14.504
HIGH_R	$\sqrt{}$	−0.355	0.008	0.269	−2.056
INT_RB	$2/\pi$ arcsin	64.054	63.318	53.664	30.441
INT_RR	$2/\pi$ arcsin	52.328	73.567	47.320	25.235
INTTOL	$\sqrt[4]{}$	540.168	488.664	444.500	408.181
SEN_RB	$\sqrt[4]{}$	87.324	86.118	74.211	45.088
SEN_RR	$2/\pi$ arcsin $\sqrt{}$	1749.161	1580.800	1394.386	1244.261
SENTOL	$\sqrt[4]{}$	631.965	−576.899	−519.906	−468.459

Applying the algal discriminant model to new samples

For each sample (x), the discriminant score for each group (Z_g) is computed with the following equation and the metric values (M) and coefficients for each group (C_g) from Table A1:

$$Z_g(x) = \text{constant} + C_{1g}M_1 + C_{2g}M_2 + \ldots C_{ng}M_n$$

For example, the discriminant score equation of the AA/A group for sample x would be as follows:

$$Z_{\mathrm{AA/A}}(x) = -402.743 + 103.154\ \mathrm{BCRS\_RA} - 22.778\ \mathrm{EREC\_RA} + \ldots - 631.965\ \mathrm{SENTOL}$$

The probability of a sample belonging to a water-quality group [$P_g(x)$] is computed with the following equation:

$$P_g(x) = \frac{e^{Z_g(x)}}{e^{Z_{AA/A}(x)} + e^{Z_B(x)} + e^{Z_C(x)} + e^{Z_{NA}(x)}}$$

For example, the probability of a sample (x) belonging to the AA/A group would be computed as follows:

$$P_{\mathrm{AA/A}}(x) = \frac{e^{Z_{AA/A}(x)}}{e^{Z_{AA/A}(x)} + e^{Z_B(x)} + e^{Z_C(x)} + e^{Z_{NA}(x)}}$$