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Developing and testing diatom indicators for wetlands in the Casco Bay watershed, Maine, USA

Yi-Kuang Wang[1,4], R. Jan Stevenson[1,*], P. Roger Sweets[2] & Jeanne DiFranco[3]
[1]*Department of Zoology, Michigan State University, East Lansing, MI, 48824, USA*
[2]*Biology Department, University of Indianapolis, Indianapolis, IN, 46227, USA*
[3]*Maine Department of Environmental Protection, 312 Canco Rd., Portland, ME, 04103, USA*
[4]*General Education Center, Nan-Hua University, Taiwan, R.O.C.*
*(*Author for correspondence: E-mail: rjstev@msu.edu)*

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Abstract

Diatom indicators of wetland condition were developed and tested by assessing human disturbance, water chemistry, and species composition of benthic, epiphytic, and planktonic diatoms from 20 wetlands sampled for 2 years. One sample from each site was randomly selected to form a development data set, while the rest were used as the test data set. Human disturbance indicated substantial differences among wetlands in hydrologic modification, impervious surface, and potential for non-point source contamination. These landscape alterations were related to increases in pH, non-nutrient ions, and nutrients and decreases in dissolved organic carbon and water color. Pre-existing diatom indicators, calculated with autecological information from lakes and aquatic habitats, correlated highly to relevant water chemistry and human disturbance scores. Weighted average models (WAM) of Cl^-, conductivity, pH, and alkalinity derived with the Maine development data set correlated to relevant water chemistry and human disturbance of the test wetlands. Diatom assemblage attributes that correlated with human disturbance were selected to combine into a multimetric index of biotic condition (IBC). IBCs and WAMs from benthic and epiphytic diatoms were usually more precisely related to relevant environmental factors than planktonic diatoms. These results showed that human disturbance alkalized wetlands, enriched them with nutrients, and diatom assemblages responded to these changes. Indicator development protocols for streams can be readily adapted for use in wetlands.

Introduction

Wetlands are important elements of landscapes and provide natural capital and many ecosystem services, such as migratory bird habitat, biodiversity hotspots, water retention, groundwater recharge, flood reduction, and carbon, sediment, and nutrient sequestration (Gale et al., 1993; Drewien et al., 1996; Mitsch & Gosselink, 2000; Rapalee et al., 2001; Bendjoudi et al., 2002; Severo et al., 2002; Leibowitz, 2003). A historic lack of understanding of wetland ecosystems has led to mass destruction of wetlands for economic development. Recent public awareness has stimulated protection of wetlands by government agencies. In 1998, however, ecological condition had only been assessed by state agencies for 9 % of the wetlands in the conterminous U.S. (U.S. EPA, 1998a). Sedimentation, filling and draining, and habitat alteration were the major threats to wetland integrity (U.S. EPA, 1998a). Facing increasing pressure from human development, effective assessment tools are needed for consistent evaluation of the condition and stressors of wetland resources and to provide information for solving problems.

The goals of ecological assessment are to evaluate biological condition (*sensu* Cairns, 1977; Karr, 1991) and other valued ecological attributes, and to assess human disturbance, contaminants, and habitat alterations that could impair valued ecological attributes (Smith et al., 1997; D'Elia et al., 2003; Stevenson et al., 2004). Then, water quality criteria and stressor–response relationships (Roux et al., 1999; Stevenson & Hauer, 2002; Yuan & Norton, 2003) can be used to synthesize all information for management purposes (Stevenson, 1998; U.S. EPA, 1998b; Jackson et al., 2000; Wang & Stevenson, 2002; Stevenson et al., 2004).

Diatom indicators can be used to evaluate both biological condition and pollutants affecting biological condition. Most diatom indicators have been developed as weighted average models (WAMs) to infer chemical or physical conditions of streams and lakes. Recently diatom indicators were developed to assess biological condition independently from inferences of pollution (Hill et al., 2000; Wang et al., 2005). Existing wetland studies have evaluated WAMs of water chemistry in Kentucky and Michigan wetlands (Pan et al., 1996; Stevenson et al., 1999). In the Everglades, changes in algal species composition and biomass have been related to environmental factors (McCormick & O'Dell, 1996; Slate & Stevenson, 2000; Pan et al., 2000; Stevenson et al., 2002a) However, no wetland studies of algae have developed multiple metrics of biological condition and tested multimetric indices of biological condition (Stevenson et al., 2002b). In addition, no wetland studies have evaluated which habitat in wetlands is best for sampling and assessing algal assemblages in wetlands.

The objectives of this study were to develop and test a set of diatom indicators of wetland conditions and to determine which habitats were best to sample. We developed and tested separate indicators of biological condition and indicators of physicochemical condition. Biological condition in this study was defined as the condition of algal assemblages in wetlands with low human disturbance. First we related changes in water chemistry of wetlands to human activities around them. We then tested metrics calculated with species autecological information from lake and stream literature by relating them to changes in human activities and related water chemistry of wetlands. WAMs were developed and tested with Maine wetlands data to characterize species environmental optima and tolerances in wetlands and to compare to metrics calculated with pre-existing information. Finally, we developed and tested metrics and multimetric indices of biological condition in wetlands with benthic, epiphytic, and planktonic diatoms.

Materials and methods

Wetland sampling and sample analysis

Twenty wetlands were sampled in 1998 and 1999 in the Casco Bay watershed of Maine (Fig. 1), with two different wetlands sampled in each year. Sites were selected along an urban gradient, with landscapes conditions around wetlands ranging from natural to suburban and urban. This watershed was glaciated and had sand, gravel, and silt deposited over granite bedrock. About two-thirds of sites were riverine wetlands, while one-third were isolated marshes. Because of the small sample size, different wetland types were not analyzed separately, which could improve precision of indicators. Eighteen wetlands were sampled during 2 years to assess annual variability in plants, invertebrates, and water chemistry, which are not reported in this paper. Based on results from studies in streams (Catherine Riseng et al., University of Michigan, unpublished data), we assumed that repeated measures of algal species composition and water chemistry in the same wetland across years were independent, because annual variability in these factors was as great among wetlands of similar disturbance regimes as within each wetland.

Human disturbance was characterized using a field assessment of the wetland landscape developed by Maine Department of Environmental Protection. Similar approaches have been adopted by state agencies, such as Washington Department of Ecology (Washington DE, 1993) and Ohio EPA (Mack, 2001). Field assessments were calibrated between people in charge of the assessments for consistency. The assessment was conducted by walking around the wetlands. Five categories of disturbance were characterized: vegetation modi-

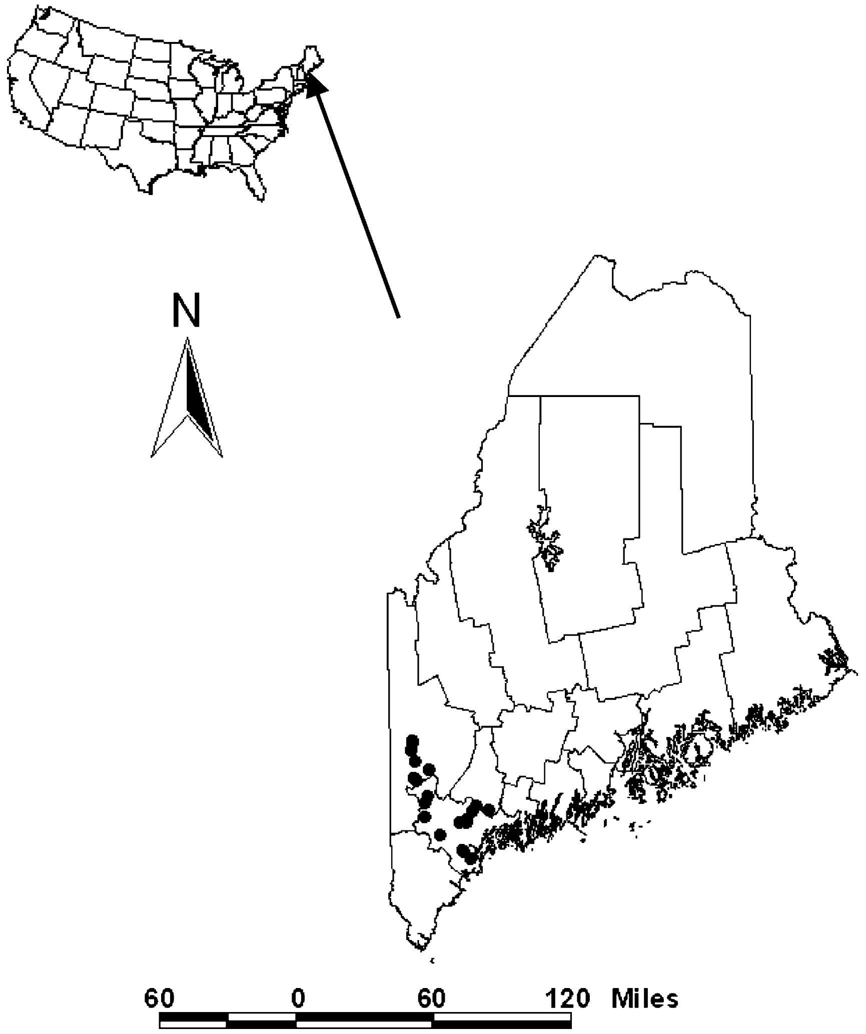

Figure 1. Locations of sampling sites in Maine and USA.

fications (VM), hydrological modifications (HM), the evidence of chemical pollutants (CP), impervious surface (IS), and potential for non-point source pollution (NPS). Each category had five questions and each question was answered with a ranking from 0 to 5 (none observed (0), minimal (1), moderate (3), to severe (5)). A maximum total score of 25 could be assigned to each category. The total human disturbance score (THDS) was the sum of scores from these five categories.

Water grab samples were collected from multiple locations in each wetland site and composted for chemical analysis. Ca^{+2}, Mg^{+2}, K^{+}, Na^{+}, Si, Cl^{-}, NO_3–N, NH_4–N, total N, SO_4, PO_4–P, total P, and dissolved organic carbon were measured in the lab with standard methods (APHA, 1998). A grab sample of 500 ml was acidified with HNO_3 and stored in a refrigerator for Ca^{+2}, Mg^{+2}, K^{+}, and Na^{+} analyses, while a 250- and 500-ml grab samples were stored on ice in the field and kept in a refrigerator for nutrients (NO_3–N, NH_4–N, total N, PO_4–P, total P) analyses in the lab respectively. All metals were analyzed by inductively coupled plasma-atomic emission spectrometry, while nutrients were analyzed by automated or semi-automated colorimetry. Conductivity, temperature (Hanna hand-held meter, model HI 9635), and dissolved oxygen (Hanna hand-held meter, model HI 9142) were measured in the field. pH and alkalinity were analyzed in the lab.

Diatoms were sampled qualitatively from three habitats (water depth <1 m): the water column, plants, and sediments. Composite algae samples were collected randomly. Phytoplankton was sampled by collecting water from several locations in the wetland and composting them in a 1-l bottle for each site. Epiphytic algae were sampled by randomly selecting plants from the wetland, cutting stems, placing underwater sections of plant stems in a Whirl-Pak bag with distilled water, rubbing plants together to remove epiphyton, and then removing the stems from the bag. Sediment algae were sampled by using a turkey baster, which is like a large pipette with a 0.5-cm opening. Diatoms were cleaned with boiling nitric acid catalyzed by potassium dichromate, neutralized with de-ionized water, and mounted on slides with Naphrax®. Diatom valves were identified and counted until a minimum of 600 valves and at least 10 valves for 10 different taxa were observed. Diatom taxonomy was based on Krammer and Lange-Bertalot (1986, 1988, 1991a, b) and Patrick and Reimer (1966, 1975).

Data analysis

First, we separated the data set into development and test data sets. One of two samples from each site was randomly selected to form a development data set so that year of sampling was not a confounding variable during testing. The rest of samples were used in a test data set. Differences in water chemistry between data sets were tested with a Mann-Whitney U test.

We tested the hypothesis that differences in water chemistry among wetlands were related to human activities around wetlands. Pearson correlation coefficients were calculated to characterize relationships between individual and combined scores of human disturbance categories with each water chemistry variable measured. Separate analyses were performed for 1998 and 1999 because water levels were substantially higher in 1999.

Diatom indices of water chemistry were calculated with existing autecological information from two major sources to evaluate transferability of metrics across regions and habitat types, to evaluate generic indicators, and to develop and test WAMs as diagnostic indices. These indices were calculated with diatom relative abundances in planktonic samples only, to limit the length of results to report in this paper. Planktonic samples were chosen for these analyses over epiphytic and benthic samples because we assumed plankton would most directly reflect measured water chemistry conditions. WAMs were calculated using species environmental optima and their relative abundances (Dixit & Smol, 1994). van Dam's indices (1994) were calculated for N, salinity, pH, trophic state, and saprobity using the categorical autecological classification of diatoms, which were derived from multiple habitat types in Netherlands and adjacent countries. Dixit's WAMs were calculated for TP, pH, and Cl^- using species optima from Dixit et al. (1999), which were derived from a large number of lakes in northeastern U.S. WAMs are capable of inferring quantitative values of water quality, while biotic indices based on categorical autecological ranks simply infer relative ranks of water quality. Due to different taxonomic systems used in different studies, species names in this study were matched with species names used in van Dam's indices and Dixit's WAMs before calculations. Generic indicators were calculated with relative abundance of genera in which most species favor acidic environments, are motile, or require high nutrients to survive (eutraphentic), according to the classification of genera in Wang et al. (2005).

Transferability of metrics across regions and habitat types was evaluated by comparing the magnitude of correlations between diatom indicators and human disturbance scores and relevant environmental factors (i.e., those for which the indicator was developed, such as Cl^-, TN, and TP concentrations and pH). Thus correlations between Dixit's WAMs or van Dam's indices and relevant environmental factors were compared to correlations between the same environmental factors and respective Maine wetland WAMs. If correlations between environmental factors and Dixit's WAMs or van Dam's indices were similar to correlations with Maine wetland WAMs, then transferability was considered high.

WAMs were calculated for environmental variables in Maine wetlands that were highly correlated to THDS by using the development data set and CALIBRATE (Juggins & ter Braak, 1992). WAMs were calculated with and without downweighting the importance of species with high tolerances (ter Braak & van Dam, 1989) and referred to as WA and WATol models, respectively. The Maine WAMs were tested in three ways. First, WAMs were tested using bootstrapped techniques with the development data set. Second, WAMs derived with the development data set were then tested with the test data set by inferring conditions at test sites with the species relative abundances and measured conditions at test sites. Third, WAM-inferred conditions were compared with human disturbance scores at test sites. WAMs were calculated for all three habitat types. Differences in precision of WAMs among habitats were compared by correlations between inferred conditions and relevant environmental factors.

Multimetric indices of biotic condition (IBCs) were developed, tested, and compared among habitats. The first step in IBC development was to select a set of diatom attributes that could respond to human disturbance. Both biotic condition and diagnostic metrics were included in IBC (Barbour et al., 1999). Six categories of attributes were used in index development: diversity, biotic indices inferring stressors, similarity to reference condition, sensitive and tolerant species, growth forms, and genus-level community structure. In the analyses, reference sites were defined as those with THDS smaller than 7, the 25th percentile of THDS among sites.

Five diversity indices were assessed. Shannon diversity (Shannon & Weaver, 1949), Hurlbert's evenness, and species and generic richness indices were expected to decrease with increasing human disturbance, while Simpson's dominance index was expected to increase with increasing human disturbance (Odum, 1985).

Similarity measures have been recognized as a powerful tool to assess human disturbance on aquatic communities (Boyle et al., 1990). Average similarity between species composition of the assemblage in a test site and assemblages in reference sites is an overall measurement of community structural change resulting from human disturbance (Sheehan, 1984). We used the Bray–Curtis dissimilarity index to evaluate similarity of assemblages between a test site and reference sites. Similarity was calculated by subtracting the dissimilarity value from 1. We expected that average similarities with reference sites would decrease with increasing human disturbance.

Ecosystems lose sensitive species and gain tolerant species under increasing stress (Schindler, 1987). Sensitive and tolerant species were distinguished in the development data set based on the presence or absence at reference or impaired sites, respectively, with indicator species analysis (Dufrene & Legendre, 1997). The number and percentage of sensitive species were expected to decrease with increasing human stressor levels, while the number and percentage of tolerant species were expected to increase with increasing stressor levels.

Diatoms are sensitive to nutrients and take up nutrients as primary producers in stream ecosystems. Therefore, we categorized diatom functional groups with their preferences to nutrient conditions. The index values usually increase with relevant water chemistry levels. van Dam's indices and generic groups were expected to increase with human disturbance.

The relative abundance of diatoms with different growth forms indicates assemblage developmental status (Peterson, 1996). Prostrate diatoms grow below the boundary layer and can avoid scouring, and are usually dominant during early successional stages or heavy grazing. Erect and stalked diatoms are capable of overgrowing prostrate diatoms and appear during mid and late succession. Unattached diatoms are planktonic and accumulate in slow moving water. Motile diatoms are capable of moving through sediments, which may indicate the level of sedimentation and bank erosion.

Shifts in assemblage composition at family and genus levels of organization are commonly used in the assessment of biological condition. Some diatom genera are also capable of indicating specific environmental conditions. For example, *Eunotia* and *Stenopterobia* prefer acidic waters; *Frustulia*, *Pinnularia*, and *Tabellaria* prefer soft waters (Round, 1990). Most *Nitzschia*, *Planothidium*, *Amphora*, *Anomoeneis*, and *Stauroneis* are high-trophic status indicators, whereas most *Brachysira*, *Cymbella*, *Eunotia*, *Frustulia*, and *Stenopterobia* are low-nutrient indicators (Kelly, 1998). Relative abundances of *Achnanthidium* and *Cym*bella are less abundant in streams with high human disturbance in a region studied by Wang et al. (2005). Therefore, relative abundances of each genus were evaluated as potential metrics of biological condition.

Metrics of the IBC were selected according to the following procedures. Diatom attributes were selected as metrics if they were among the three highest Pearson correlation coefficients with each human disturbance categories and if they were significantly correlated with THDS ($p < 0.05$). Correlations among selected metrics were evaluated to reduce redundant metrics. Each metric was scaled to a 0–10 scoring system. If the metric decreased with human disturbance, the score was scaled by dividing the 95th percentile of metric values, and then multiplied by 10. If the metric increased with disturbance rank, the score was calculated by dividing the metric value by the 95th percentile, subtracting the quotient from 1, and then multiplying that difference by 10. The total IBC score was a summation of values of selected metrics.

IBCs were developed with the development data set and tested on both data sets for three habitat specific assemblages. They were evaluated and compared with Pearson correlation analysis to assess their relationships with three indicators of human disturbance: THDSs for each site; site scores on the first principal component axis of a principal component analysis (PCA) of human disturbance scores; and site scores on the first principal component axis of a PCA of water chemistry data. PCA was used to summarize multivariate environmental gradients into a single latent variable. The first principal component axis usually explains most of the variance, hence can represent the overall gradient in conditions at the sites. Statistical analyses were performed with SPSS software.

Results

Human disturbance and physicochemical attributes

THDS ranged from 1 to 25 with a mean of 8.7 and had a distribution skewed toward low scores (Fig. 2). Variation was relatively great for hydrologic modification with scores ranging from 0 to 6, impervious surfaces ranging from 0 to 9, and non-point source pollution ranging 0 to 9. Less than half of the sites had vegetation modification scores greater than 0 (maximum = 3); only three sites had chemical pollution scores greater than 0 (maximum = 5). Because of low variation in the vegetation and chemical disturbance scores, they were not included in most analyses.

Physicochemical variables varied greatly among wetlands and correlated with the THDS (Tables 1 and 2). Indicators of ionic content of waters were usually low with medians of 3.87 mg Cl l^{-1}, 29 μS cm^{-1} of conductivity, and 6.7 pH (Table 1). Indicators of nutrient concen-

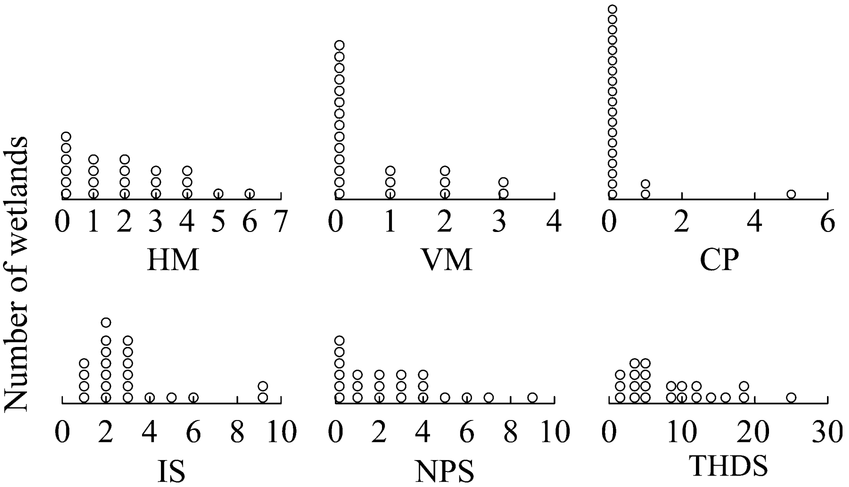

Figure 2. Distribution of scores for different categories of human activities near Maine wetlands: HM, hydrologic modification; VM, vegetation modification; CP, chemical pollution; IS, impervious surface; NPS, non-point source pollution; SumHDS, sum of human disturbance ranks for all categories.

Table 1. Summary statistics for the development and test data sets ($n = 20$)

Statistic		Cl^- (mg l^{-1})	NO_3–N (mg l^{-1})	NH_4–N (mg l^{-1})	TN (mg l^{-1})	PO_4^{-2} (μg l^{-1})	TP (μg l^{-1})	Cond (μS cm^{-1})	SiO_2–Si (mg l^{-1})	pH
Development data set										
Minimum		0.7	0.01	0.01	0.23	1	11	15	0.11	6.2
Percentiles	25th	2.33	0.01	0.02	0.56	2	22	19.8	1.68	6.4
	50th	3.87	0.03	0.05	0.74	3	42	29	3.11	6.7
	75th	10.95	0.04	0.05	0.93	5	51	74.8	4.37	6.9
Maximum		357	1.34	0.17	22	12	300	1345	5.4	8.1
Test data set										
Minimum		0.52	0.01	0.01	0.4	1	12	14	0.6	6.2
Percentiles	25th	3.04	0.01	0.02	0.49	2	24	27.6	1.72	6.6
	50th	5.34	0.03	0.04	0.71	3	55	39.1	2.67	6.8
	75th	7.87	0.03	0.05	1.06	5	71	45	4.26	7
Maximum		388	0.89	0.32	2.7	14	120	1820	5.2	7.6

Table 2. Pearson correlation coefficients characterizing relationships between physiochemical variables and human disturbance rank scores

Pchem variable	HM		IS		NPS		THDS	
	1998	1999	1998	1999	1998	1999	1998	1999
Ca^{+2}	**0.51**	**0.46**	**0.79**	**0.80**	**0.51**	**0.56**	**0.65**	**0.67**
Mg^{+2}	**0.54**	**0.49**	**0.77**	**0.79**	**0.57**	**0.58**	**0.70**	**0.68**
K^+	0.10	**0.50**	**0.68**	**0.72**	0.24	**0.54**	0.37	**0.66**
Na^+	**0.53**	0.39	**0.66**	**0.74**	**0.56**	**0.52**	**0.65**	**0.60**
Cl^-	**0.56**	0.36	**0.61**	**0.73**	**0.52**	**0.49**	**0.60**	**0.57**
Cond	**0.53**	**0.50**	**0.72**	**0.79**	**0.54**	**0.52**	**0.67**	**0.66**
Alk	**0.45**	**0.50**	**0.79**	**0.69**	**0.59**	**0.69**	**0.70**	**0.72**
SO_4^{-2}	0.31	**0.45**	0.31	**0.48**	−0.11	**0.44**	0.12	**0.54**
NH_4–N	0.21	0.12	**0.72**	0.10	0.37	0.40	**0.49**	0.36
NO_3–N	**0.07**	−0.31	0.37	−0.07	0.02	−0.29	0.08	−0.25
Total N	0.04	0.26	0.30	**0.50**	0.36	0.34	0.28	0.41
PO_4–P	0.11	−0.08	0.08	0.34	0.07	0.28	0.09	0.22
Total P	0.31	**0.48**	0.29	0.35	**0.60**	**0.56**	**0.47**	**0.54**
Si	0.28	0.32	0.23	0.28	0.29	**0.73**	0.29	**0.60**
Chl a	0.22	**0.57**	0.10	**0.58**	0.40	**0.57**	0.31	**0.66**
DOC	−0.32	−0.28	0.01	−0.13	0.17	−0.24	−0.04	−0.28
pH	0.31	0.32	**0.80**	**0.62**	0.36	**0.54**	**0.55**	**0.59**
Color	**−0.46**	**−0.48**	**−0.56**	−0.38	0.03	−0.40	−0.30	**−0.49**

Bold coefficients indicate $p < 0.05$ ($n = 20$).

trations were also low, with median NO_3, NH_4, and TN of 0.03, 0.05, and 0.74 mg N l^{-1}, a median Si of 3 mg l^{-1}, and median PO_4 and TP of 3 and 42 μg P l^{-1}, respectively. No significant differences were observed in physicochemical variables between the development and test data sets (Mann-Whitney U tests, $p > 0.05$) (Table 1). For example, non-nutrient ionic factors (Ca^{+2}, Mg^{+2}, K^+, Na^+, Cl^-, SO_4^{-2}, alkalinity, and conductivity) significantly correlated to scores of disturbance categories and to sums of disturbance scores in more than 90% of the combinations of disturbance and physicochemical variables. Variables related to nutrient enrichment (NO_3–N, NH_4–N, TN, PO_4, TP, Si, and *chl* a) were positively correlated ($p < 0.05$, $n = 20$) to human disturbance categories in less than 30% of the variable combinations (15 of 56), but results of correlation analyses were two times as likely to be significant during 1999 vs. 1998. DOC was not related to human disturbance, but pH was often positively related and color was often negatively related to human disturbance variables.

Functional groups, WAMs, and biotic indices

Indices based on pre-existing autecological information were highly correlated with both physicochemical and human disturbance variables. Diatom functional groups (van Dam's indices) were significantly correlated with most relevant disturbance and water chemistry variables (Table 3). Scores of van Dam's saprobity and nitrogen indices were correlated to most physicochemical and human disturbance variables. Scores of van Dam's indices were always correlated with Cl, pH, and IS scores. All of Dixit's WAMs were also significantly correlated with relevant variables and more than 50% of variables in Table 3.

Two of the three genus-level indices were correlated with most relevant variables (Table 3). Percentages of diatoms in eutraphentic genera did not correlate with any relevant variables. Percentages of diatoms in acidic genera negatively correlated to 6 of 8 relevant variables. Percentages of motile diatoms was significantly correlated with 7 of the 8 relevant variables.

WAMs derived from the Maine development data set were correlated with many relevant variables for assemblages in all three habitats when tested by bootstrapping within the development data set, applied to the test data set, and related to THDS (Table 4). For example, the benthic pH WAM correlated with pH in the development data set, applied to the test data set, and with THDS ($r = 0.58$, $p = 0.007$; $r = 0.58$, $p = 0.007$; $r = 0.69$, $p < 0.001$; respectively). Benthos had more significant relationships than epiphyton ($p < 0.05$ for 26 and 21 of 60, respectively). Plankton had only 16 significant correlations of the 60 possible tests. More significant correlations were observed between WAMS and THDS than between WAMS and relevant variables with the test data set (32/60 vs. 19/60, respectively). Few correlations were found between WAMS and measured environmental variables when bootstrapped with the development data set (12/60).

WAMs derived from the Maine development data set for ionic variables (Cl, conductivity,

Table 3. The correlations of planktonic functional groups and indices with relevant disturbance scores and water chemistry for the plankton samples of the test data set

	VD trophic	VD saprobity	VD N	% Eutrophentic diatom	% Acidic diatom	% Motile diatom	Dixit's TP	Dixit's pH	Dixit's Cl	VD Salinity	VD pH
Cl^-	**0.64**	**0.75**	**0.72**	−0.24	**−0.64**	**0.64**	**0.56**	**0.68**	**0.54**	**0.56**	0.11
TN	0.36	**0.76**	**0.74**	0.18	−0.38	**0.80**	**0.61**	0.37	**0.56**	0.29	−0.03
TP	**0.51**	**0.70**	**0.70**	0.08	**−0.48**	**0.67**	**0.54**	**0.54**	**0.52**	0.40	0.20
DOC	−0.13	0.04	−0.02	0.27	0.18	0.02	0.20	−0.09	0.24	0.01	0.06
pH	**0.63**	**0.60**	**0.63**	−0.04	**−0.71**	0.26	0.35	**0.77**	0.41	**0.69**	0.31
Chl a	0.16	**0.50**	**0.50**	0.14	−0.25	**0.77**	**0.49**	0.27	**0.50**	0.09	−0.21
HM	0.19	0.30	0.26	−0.30	−0.31	**0.60**	0.30	0.26	0.29	0.13	−0.11
CP	**0.50**	**0.83**	**0.80**	−0.01	**−0.47**	**0.86**	**0.68**	**0.48**	**0.61**	0.36	0.02
IS	**0.51**	**0.68**	**0.62**	−0.27	**−0.62**	**0.51**	**0.55**	**0.59**	**0.54**	**0.55**	0.00
NPS	0.38	**0.49**	**0.50**	0.11	**−0.46**	**0.57**	0.28	0.42	0.31	0.31	0.11
THDS	**0.49**	**0.66**	**0.64**	−0.07	**−0.59**	**0.70**	**0.49**	**0.55**	**0.49**	**0.44**	0.04

Bold face marks significant correlation. IS – impervious surface rank; NPS – non-point source pollution rank; ns – non-significance ($n = 20$).

Table 4. Correlation statistics for WAMs developed with and without down-weighting species for their environmental tolerance (WA and WATol, respectively)

WAM variable	Habitat	Dev data set		Test data set		THDS	
		WA	WATol	WA	WATol	WA	WATol
Cl^-	Benthos	0.01	0.36	**0.86**	**0.46**	**0.59**	**0.67**
Cond	Benthos	0.20	**0.53**	**0.90**	**0.92**	**0.63**	**0.71**
Alk	Benthos	**0.66**	**0.64**	**0.79**	**0.95**	**0.69**	**0.71**
pH	Benthos	**0.58**	0.31	**0.58**	**0.49**	**0.69**	**0.65**
SO_4	Benthos	0.00	0.02	**0.57**	−0.04	**0.45**	−0.02
Si	Benthos	0.03	0.10	0.21	0.26	0.12	0.25
NH_4	Benthos	0.25	0.01	0.03	0.03	**0.68**	**0.70**
NO_3	Benthos	0.00	0.00	−0.01	−0.03	**0.47**	0.39
TN	Benthos	0.00	0.07	−0.05	0.16	−0.13	**0.41**
TP	Benthos	0.11	0.11	0.10	0.03	0.40	0.31
Cl^-	Epiphyton	0.02	0.27	0.22	−0.05	**0.59**	0.20
Cond	Epiphyton	0.13	0.30	0.24	−0.02	**0.62**	0.36
Alk	Epiphyton	**0.77**	**0.60**	**0.62**	**0.56**	**0.71**	**0.64**
PH	Epiphyton	**0.54**	**0.43**	**0.70**	**0.57**	**0.60**	**0.53**
SO_4	Epiphyton	0.00	0.00	0.26	−0.01	**0.51**	0.08
Si	Epiphyton	0.03	0.03	0.30	0.14	**0.53**	0.26
NH_4	Epiphyton	0.22	0.00	0.26	−0.06	**0.75**	**0.56**
NO_3	Epiphyton	0.00	0.01	−0.01	−0.06	**0.52**	−0.08
TN	Epiphyton	0.00	0.01	−0.05	0.05	0.05	**0.46**
TP	Epiphyton	0.02	0.01	0.16	−0.04	**0.65**	0.13
Cl^-	Plankton	0.01	0.04	**0.49**	0.29	0.39	0.60
Cond	Plankton	0.12	0.19	**0.55**	0.10	0.40	0.42
Alk	Plankton	**0.66**	**0.46**	0.19	**0.86**	**0.44**	**0.60**
PH	Plankton	**0.55**	**0.37**	**0.44**	0.35	**0.46**	**0.57**
SO_4	Plankton	0.01	0.05	**0.42**	0.10	0.35	0.03
Si	Plankton	0.00	0.01	**0.45**	0.37	**0.72**	0.68
NH_4	Plankton	0.15	0.02	−0.03	−0.06	0.34	**0.47**
NO_3	Plankton	0.00	0.07	−0.02	−0.05	0.35	0.36
TN	Plankton	0.04	0.06	−0.04	−0.04	−0.09	−0.35
TP	Plankton	0.07	0.00	0.09	0.05	0.35	0.22

Development (Dev) data set coefficients relate measured and inferred conditions with the development data set based using bootstrapping. Test data set coefficients relate measured physicochemical conditions of the test data set and conditions inferred for test data sites using species optima from the development data set and species relative abundances from the test data set. Human dist rank coefficients relate human disturbance ranks of test sites with conditions inferred for test data sites using species optima from the development data set and species relative abundances from the test data set. Coefficients in bold indicate that $p < 0.05$ ($n = 20$ for epiphyton and benthos, $n = 19$ for plankton).

alkalinity, and pH, excluding the SO_4 WAM) were related to relevant variables in 20 out of the 24, 14 of the 24, and 12 of the 24 tests of benthos, epiphyton, and plankton, respectively (Table 4). The WAMs for SO_4 and nutrients were significantly correlated in less than 10% of cases with the development or test data sets for any assemblage, however they were usually related to THDS. Nutrient WAMs were significantly related to THDS at test sites for only 4, 6, and 2 of 10 possible relationships (WA and WATol for 5 nutrient parameters) for benthos, epiphyton, and plankton, respectively.

WAMs for Cl, pH, and nutrients using autecological information from the Maine wetlands were not always better related to relevant environmental variables than WAMs using existing autecological indices. Correlation coefficients of Cl and pH WAMs derived from Maine wetlands with relevant variables were higher when assessing the test data set and were lower when assessing the development data set with bootstrapping (Table 4). Correlation coefficients for Cl and pH WAMs were usually higher with THDS at test sites when using autecologies derived from the Maine development data set than when using existing autecologies. However, correlations for nutrient WAMs were lower in all situations when using autecological information from Maine wetlands than when using existing autecologies.

Biotic condition

Six metrics of benthic assemblages were selected for the benthic IBC according to correlations with human disturbance scores (Table 5). Selected metrics were: % motile diatom, % Synedra, no. sensitive species, average similarity with reference sites, % Neidium, and % Eunotia (Table 5). Percent *Nitzschia* and *Surirella* both had high correlations with % motile diatom ($r = 0.94$, $p < 0.001$; $r = 0.70$, $p < 0.001$), so both were not included in the IBC.

Eight metrics were selected for the epiphytic IBC according to correlations with human disturbance scores (Table 5). The 8 metrics were: % motile diatom, % Eunotia, no. sensitive species, average similarity with reference sites, no. tolerant species, van Dam's saprobity index, van Dam salinity index, and van Dam's nitrogen index (Table 5). Percent Nitzschia was not included in the IBS due to high correlations with % motile diatom ($r = 0.97$, $p < 0.001$).

Eight metrics were selected for the planktonic IBC according to correlations with human disturbance scores (Table 5). The 8 metrics were: % prostrate diatoms, % motile diatoms, no. sensitive species, van Dam's saprobity index, % *Eunotia*, % *Neidium*, % *Pinnularia*, and average similarity with reference sites. Percent sensitive species was highly correlated with no. of sensitive species, hence it was not included in the planktonic IBC.

Multimetric biotic indices from each of the three habitats significantly correlated with three evaluation criteria in the development data set (Tables 6–8). In the PCA results of water physical-chemistry variables, the 1st axis explained 57.4% variance, which was around five times the variance explained by the 2nd axis (Table 6). In the PCA results of human disturbance variables, the 1st axis explained 51% variance, which was about twice the variance of the 2nd axis (Table 7). In the development data set, the epiphytic IBC had the highest average correlation with evaluation criteria ($r = -0.87$, $p < 0.001$; $r = -0.98$, $p < 0.001$; $r = -0.81, p < 0.001$) and the benthic IBC was the second ($r = -0.84$, $p < 0.001$; $r = -0.95$, $p < 0.001$; $r = -0.79$, $p < 0.001$) (Table 8). In the

Table 5. Selected metrics for each habitat

Habitat	Disturbance categories			
	HM	CP	IS	NPS
Benthos	% *Nitzschia* (0.60)	% Synedra (0.91)	No. sensitive species (−0.84)	% *Neidium* (−0.57)
	% Motile (0.59)	% Motile (0.80)	Average similarity (−0.82)	% *Eunotia* (−0.57)
	% *Synedra* (0.56)	% *Surirella* (0.72)	% *Synedra* (0.74)	No. sensitive species
Epiphyton	% Motile (0.6)	% *Surirella* (0.74)	No. sensitive species (−0.83)	VD saprobity (0.65)
	% *Nitzschia* (0.59)	No. sensitive species (−0.73)	Average similarity (−0.76)	VD N (0.62)
	% *Eunotia* (−0.57)	VD saprobity (0.70)	No. tolerant species (0.76)	VD salinity (0.63)
Plankton	% Prostrate (0.58)	% *Nitzschia* (0.83)	No. sensitive species (−0.82)	% *Neidium* (−0.57)
	% Motile (0.55)	% Motile (0.82)	Average similarity (0.73)	% *Pinnularia* (−0.53)
	% Sensitive species (−0.55)	VD saprobity (0.80)	% *Eunotia* (−0.69)	% *Nitzschia* (0.51)

Attributes with the highest three correlation coefficients with each disturbance rank category were selected as metrics. ( ) encloses Pearson correlation coefficient ($n = 20$ for plankton and epiphyton, $n = 19$ for benthos).

Table 6. Results of PCA on water chemistry variables

	PCA axis 1	PCA axis 2
% Variance explained	57.35	12.40
Ca^{+2}	0.98	0.01
Mg^{+2}	0.98	0.05
K^{+}	0.95	0.11
Na^{+}	0.96	0.08
Si	0.52	−0.15
Cl^{-}	0.93	0.02
NH_4	0.53	−0.46
NO_3–N	0.03	−0.50
TN	0.68	0.53
PO_4	0.23	−0.27
TP	0.73	0.43
SO_4	0.70	0.25
DOC	−0.17	0.78
Cond	0.97	−0.03
Alkalinity	0.96	−0.02
PH	0.87	−0.25
Color	−0.61	0.58

Variance explained and correlations of PCA axes with water chemistry variables were shown in the table.

Table 7. Results of PCA on human disturbance variables

	PCA axis 1	PCA axis 2
% Variance explained	51	24.7
HM	0.72	−0.11
VM	0.09	0.97
CP	0.81	−0.35
IS	0.81	−0.07
NPS	0.84	0.39

Variance explained and correlations of PCA axes with human disturbance variables were shown in the table.

test data set, the benthic IBC had the highest average correlation with evaluation criteria ($r = -0.79$, $p < 0.001$; $r = -0.69$, $p < 0.001$; $r = -0.68, p < 0.001$) and the planktonic IBC was the second ($r = -0.71$, $p < 0.001$; $r = -0.73$, $p < 0.001$; $r = -0.64$, $p < 0.001$). The epiphytic IBC had the largest decrease in Pearson correlation coefficients from the development data set to the test data set. The multiple-habitat IBC had higher correlations with evaluation criteria in both development and test data sets than IBCs from the three specific habitats.

Table 8. Correlation between IBC from development data set and IBC from test data set for each habitat and evaluation criteria

Habitat	Evaluation criteria		
	DR PCA1	Water PCA1	Human DR
Benthos			
	−0.84	−0.95	−0.79
($n = 19$)	(<0.001*)	(<0.001*)	(<0.001*)
	−0.79	−0.69	−0.68
($n = 20$)	(<0.001*)	(0.001*)	(0.001*)
Epiphyte			
	−0.87	−0.98	−0.81
($n = 20$)	(<0.001*)	(<0.001*)	(<0.001*)
Test	−0.61	−0.67	−0.62
($n = 20$)	(0.005*)	(0.001*)	(0.004*)
Plankton			
	−0.71	−0.83	−0.74
($n = 20$)	(<0.001*)	(<0.001*)	(<0.001*)
Test	−0.71	−0.73	−0.64
($n = 19$)	(<0.001*)	(<0.001*)	(<0.001*)
Multihabitat			
	−0.88	−0.95	−0.85
($n = 20$)	(<0.001*)	(<0.001*)	(<0.001*)
Test	−0.79	−0.72	−0.70
($n = 20$)	(<0.001*)	(<0.001*)	(0.001*)

DR PCA1 denotes disturbance rank principal component analysis axis 1. Water PCA1 represents water chemistry principal component analysis axis 1. Human DR is total human disturbance rank score. Values in the table are Pearson correlation coefficient. Sample sizes were 20. Values in ( ) denote probability; * indicates statistical significance.

Discussion

Many attributes of diatom assemblages were related to physicochemistry and human disturbance of the Casco Bay watershed wetlands in both development and test sets of data. These attributes included deviations in taxonomic composition from reference condition and new and existing indicators of water chemistry. Correlations among human disturbance scores, measured water chemistry, and diatom indices of water chemistry showed that human activities alkalized and enriched these wetlands. Diatom assemblages changed from naturally occurring taxa that tolerate low pH and low nutrient conditions to those that require circumneutral to alkaline pH and elevated nutrients.

No studies have developed and tested simple and multimetric indices of water chemistry and biological condition in wetlands as this study for the Casco Bay watershed wetlands. Although our sample size was relatively small, we did test metrics with a relatively "naïve" data set and demonstrated the reliability of indices at spatial and temporal scales that better simulated sources of variability than the bootstrapping approach commonly used to test WAMs.

Our results show that existing metrics are transferable across regions and habitats. Ten of the 12 indices based on existing autecological information and species composition of plankton were correlated significantly with THDS. Almost all species level indicators based on Dixit's and van Dam's autecological information were significantly correlated with relevant environmental variables. In many cases, index precision was as good or better when calculated with autecological information gathered from existing literature for lakes and streams as when calculated with species autecologies derived in this study. Nineteen of the 30 metrics based on new autecological information were correlated with THDS. Less than 50% of Maine-derived WAMs were statistically significant.

Even though indices based on existing autecological data were correlated with relevant environmental factors and THDS as well or better than indices based on autecologies developed in this study, we argue that regional refinement of indicators is very important. Regional differences in population phenotypes and cryptic species (*sensu*, Sáez and Lozano, 2005) likely reduce accuracy of transferring species autecological information among regions. The small sample size of wetlands in this study probably constrained development of precise autecological information. Constraining indicator development to specific types of wetlands would probably increase precision of metrics. More precise measure of environmental factors with relatively high-temporal variability would likely increase precision of metrics. For example, the WAMs developed in this study were more precisely related for Cl and pH than to nutrient concentrations. Nutrient concentrations in shallow waters such as wetlands vary greatly at daily and diurnal temporal scales due to exogenous weather factors and endogenous metabolic regulation of uptake and release from periphyton, macrophytes, and sediments. Cl probably varies little diurnally. Both Cl and pH probably vary less than nutrients with weather related factors. Weather related temporal variability in nutrient relationships with human disturbance was indicated by higher correlations during 1999 vs. 1998, which was probably due to higher water levels in 1999. Thus, more thorough assessment of water chemistry with repeated visits of wetlands and characterizing biota and chemistry of more wetlands should improve precision and accuracy of WAMs and regionally refined indicators.

Metrics from diatom assemblages in all three habitats provided reliable assessments of ecological conditions in wetlands. WAMs and IBCs calculated with species composition of benthic and epiphytic diatoms were more precise than with planktonic diatoms. No justification for using multiple assemblages was detected, such as differing responses of assemblages to contaminants or categories of human activities. Thus benthic or epiphytic diatom assemblages should provide similar characterizations of wetland conditions, and plankton could be used if a slight increase in ease of sampling was more important than the loss in precision of assessments.

Down-weighting tolerant species in WAMs and combining multiple metrics into a single multimetric index may be valuable, respectively, for improving precision of some metrics and providing a summary statistic for characterizing wetland health. However, application of these indicator modification techniques should be chosen with appropriate justification. The lack of pattern in whether WA or WATol models were more precise for different types of chemical variables (e.g., ions or nutrients), and more precise in one habitat or another, showed that differences in indicator performance were random. Therefore, down-weighting tolerant species in WAMs may just add another source of variability and may not be worthwhile.

In this study, we emphasized the development and testing of indices that were more directly related to measures of biological condition than WAMs of stressor conditions. Metrics such as number of sensitive and tolerant species, similarity of species composition to reference condition, and

% individuals of specific genera should be included in multimetric indices of biological condition (Stevenson and Smol, 2002; Wang et al., 2005). They more independently measure biological condition than WAMS of stressor condition. Maintaining independence in measures of biological conditions and measures of pollutants and human activity is important in ecological assessment (Stevenson et al., 2004). If the goals of assessment are to evaluate valued ecological attributes and to diagnose stressors and human activities causing problems, then measures of valued attributes and stressors must be as independent as possible to avoid circularity when arguing cause-effect relationships. Many indicators of biological condition were highly correlated to human disturbance, such as those mentioned above and % *Synedra*, *Nitzschia*, *Neidium*, and *Eunotia*. The high performance of many metrics of biological condition show that this approach can be used with diatoms as with other organisms.

In conclusion, diatom indicators of biological condition and water chemistry of wetlands provide reliable methods for assessing wetland condition and diagnosing potential stressors. This study demonstrated procedures and measures involved in developing a set of tools that assess ecological condition and diagnose potential causes of impairment. Classifying the great diversity of wetland types according to their expected condition and response to stressors should improve precision of assessments. These additional steps can be taken to refine indicator as more data on biological condition, contamination, habitat alteration, and human activities are accumulated for wetlands.

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Appendix 1.

Correlation coefficients between human disturbance scores of test sites and conditions inferred for test data sites using species optima from the development data set and species relative abundances from the test data set. Coefficients in bold indicate that $p < 0.05$ ($n = 20$ for Epiphyton and Benthos, $n = 19$ for Plankton).

Habitat	IndVar	HM		VM		CP		IS		NPS	
		WA	WATol	WA	WATol	WA	WATol	WA	WATol	WA	WATol
Benthos	Cl–	**0.43**	0.38	−0.21	−0.09	**0.60**	**0.94**	**0.79**	**0.63**	0.31	**0.63**
Benthos	Cond	**0.43**	0.39	−0.20	−0.19	**0.68**	**0.85**	**0.81**	**0.89**	**0.81**	**0.89**
Benthos	Alk	**0.42**	0.33	−0.16	−0.15	**0.81**	**0.94**	**0.83**	**0.85**	**0.83**	**0.85**
Benthos	pH	0.39	0.35	−0.07	−0.11	**0.74**	**0.66**	**0.78**	**0.84**	**0.78**	**0.84**
Benthos	SO_4^{-2}–S	0.39	0.02	−0.21	−0.15	0.39	−0.02	**0.67**	0.06	**0.67**	0.06
Benthos	Si	0.01	0.10	**0.48**	**0.48**	−0.22	−0.04	−0.18	−0.07	−0.18	−0.07
Benthos	NH_4^+–N	0.39	0.35	−0.18	−0.18	**0.82**	**0.95**	**0.85**	**0.84**	**0.85**	**0.84**
Benthos	NO_3^-–N	**0.40**	0.13	−0.15	0.14	0.36	0.27	**0.67**	**0.55**	**0.67**	**0.55**
Benthos	TN	−0.04	0.29	−0.22	−0.13	−0.01	**0.45**	0.05	**0.56**	**0.05**	**0.56**
Benthos	TP	0.33	0.15	−0.21	0.17	**0.46**	0.33	**0.53**	**0.47**	**0.53**	**0.47**
Epiphyton	Cl^-	0.33	**0.43**	0.15	−0.20	**0.59**	0.17	**0.44**	−0.07	**0.44**	−0.07
Epiphyton	Cond	0.33	**0.46**	0.15	−0.06	**0.63**	0.33	**0.49**	0.09	**0.49**	0.09
Epiphyton	Alk	0.38	**0.42**	0.13	−0.08	**0.73**	**0.72**	**0.62**	**0.59**	**0.62**	**0.59**
Epiphyton	pH	0.26	0.20	0.18	0.02	**0.60**	**0.69**	**0.58**	**0.59**	**0.58**	**0.59**
Epiphyton	SO_4^{-2}–S	0.22	0.05	0.16	−0.02	**0.55**	0.13	**0.44**	0.08	**0.44**	0.08
Epiphyton	Si	**0.43**	0.20	0.37	0.38	0.21	0.04	0.19	−0.11	0.19	−0.11
Epiphyton	NH_4^+–N	0.40	0.35	0.14	−0.10	**0.75**	**0.40**	**0.63**	**0.79**	**0.63**	**0.79**
Epiphyton	NO_3^-–N	0.21	−0.39	0.24	**0.49**	**0.53**	−0.19	**0.41**	0.08	**0.41**	0.08
Epiphyton	TN	0.05	0.33	0.19	0.23	−0.05	0.31	−0.06	0.28	−0.06	0.28
Epiphyton	TP	**0.48**	0.16	0.31	−0.01	**0.42**	0.06	**0.41**	0.02	**0.41**	0.02
Plankton	Cl	0.22	0.40	−0.02	−0.18	0.32	**0.84**	**0.59**	**0.47**	**0.59**	**0.47**
Plankton	Cond	0.22	0.14	0.00	−0.19	0.32	**0.85**	**0.59**	0.38	**0.59**	0.38
Plankton	Alk	0.23	0.14	0.07	−0.11	0.34	**0.91**	**0.57**	**0.76**	**0.57**	**0.76**
Plankton	pH	0.18	0.25	0.19	−0.13	0.29	**0.61**	**0.58**	**0.83**	**0.58**	**0.83**
Plankton	SO_4^{-2}–S	0.20	−0.28	−0.05	0.04	0.28	0.25	**0.58**	**0.28**	**0.58**	0.28
Plankton	Si	**0.52**	**0.47**	0.01	0.18	**0.58**	**0.47**	**0.58**	**0.47**	**0.58**	**0.47**
Plankton	NH_4^+–N	0.13	0.28	0.12	−0.18	0.23	0.39	**0.47**	**0.79**	**0.47**	**0.79**
Plankton	NO_3^-–N	0.19	0.21	−0.04	−0.08	0.29	0.19	**0.60**	**0.65**	**0.60**	**0.65**
Plankton	TN	0.08	−0.17	0.07	0.19	0.01	−0.31	−0.17	−0.37	−0.17	−0.37
Plankton	TP	0.37	0.18	−0.06	−0.15	0.35	0.35	0.29	0.26	0.29	0.26