PERIPHYTON ASSEMBLAGES AS BIOINDICATORS OF MINE-DRAINAGE IN UNGLACIATED WESTERN ALLEGHENY PLATEAU LOTIC SYSTEMS

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Abstract. In order to determine the influence of geologic patterns and coal mining on benthic algal assemblages, 56 stream sites throughout the unglaciated Western Allegany Plateau were investigated. These sites were categorized based upon catchment mining/reclamation history. At each site, select environmental parameters such as pH, temperature, dissolved oxygen, specific conductance, metallic salts concentration, turbidity, maximum wetted width, and average thalweg depth were measured. Periphyton from riffle areas and macroscopic algal taxa from a 20 m segment were collected. Relative importance values were developed and calculated for both the periphyton and macroalgal communities. Canonical correspondence analyses of the periphyton and macroalgal data set each showed five major groups of stream reaches that were defined by specific algal taxa and environmental characteristics. Two of the groups were dominated by variables associated with acid mine drainage (AMD) and had taxa known from very acidic waters. One group was entirely composed of sites receiving treated waters from active coal mines. Another group was dominated by sites classified as alkaline mine drainage (AkMD) and the last group was primarily reference sites with a few reclaimed reaches. The AMD impacted groups had a significantly lower species richness and diversity than the other three groups. Species-based models for inferring the level of critical environmental parameters related to mining showed the periphyton-based inference model for pH was highly predictable and may be quite useful for evaluation of coal mine remediation. Other promising periphyton- and macroalgal-based models, yielded poor r^2 and root mean square error (RMSE) after cross-validation. Comparison of the relative importance values with more traditional assessments of community structure showed similar results with the diatoms and chlorophytes dominating the periphyton and macroalgae, respectively.

Keywords: acid mine drainage, alkaline mine drainage, benthic algae, bioindicator, diatoms lotic system, macroalgae, periphyton, streams, Western Allegheny Plateau

1. Introduction

Geology, physiographic history, and land use are among the most important factors influencing benthic community structure and distribution in surface waters, especially lotic systems (Biggs, 1990). The influence of these characters is wide ranging, as the weathering of local rock and soil formations leads to the input of many important (sometimes detrimental) chemical and nutrient components

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into the water (Biggs, 1990; Leland, 1995; Leland and Porter, 2000). The importance of geology and land use on aquatic systems (particularly algal assemblages) has been assessed in several studies (i.e. Biggs, 1990; Leland, 1995; Kuta and Richards, 1996; Leland and Porter, 2000). An area of the United States where land use and geologic formations have had profound and often synergistic effects on aquatic ecosystems is the unglaciated Western Allegheny Plateau (UWAP) section of the Appalachian Plateau Physiographic Province (Fenneman, 1938).

In the state of Ohio, the UWAP is within the northern portion of the Appalachian Coal Basin. Since about 1800, coal mining has been an important yet a relatively unregulated industry, and legislation regarding the reclamation of abandoned coal mines was not implemented until the 1970s. Given the amount of coal mined in Ohio (~760 million tonnes since 1816) and the number of abandoned, reclaimed, and active coal mines in the UWAP, drainage and runoff originating from these mine complexes can have extreme effects on aquatic systems (Sutton, 1970; Bell and Ungar, 1981; Eberle and Razem, 1985; Starnes, 1985).

Runoff from coal mines and associated by-products, such as pyrite (FeS_2), is termed acid mine drainage (AMD). AMD is formed when pyritic minerals are exposed to atmospheric oxygen, becoming oxidized and resulting in sulfuric acid (low pH levels), dissolved metal ions, and elevated sulfate levels (Skousen *et al.*, 1994). The United States Environmental Protection Agency has determined that AMD is the largest contributor to poor water quality in the Appalachian Region with over 16,900 km of impacted streams reported (Dugan, 1975; Office of Surface Mining, 1995). These Appalachian region systems receive approximately 3 million tonnes of acid annually and represent more than $6 billion in environmental damage (Chiras, 1988; U.S. Department of the Interior 2000).

The impact of AMD on aquatic ecosystems is largely controlled by the acid neutralizing capacity of the receiving waters. Soft waters (poorly buffered; southern UWAP) tend to be impacted more than well-buffered systems (northern UWAP). Mine drainage from the northern vicinity of the UWAP typically is circumneutral to alkaline with elevated sulfate levels (H_2S, $CaSO_4$, $MgSO_4$) and increased sedimentation rates with lower concentrations of dissolved metals, especially aluminum and iron (Banks *et al.*, 1997; Gray, 1998). However, at these sites the levels of manganese may be comparable to acidic AMD sites. In this study, we describe these sites as receiving alkaline mine drainage (Table I).

Mine drainage (particularly AMD) has damaging effects on aquatic ecosystems. In lotic systems, increases in acidity lead to decreases in algal species richness and diversity (i.e. Mulholland *et al.*, 1986; Planas, 1996; Verb and Vis, 2000). The decrease in species richness is often linked to a variety of factors including lethal levels of metallic salts and pH, with an influx of metal precipitates that may destroy suitable substrate (Leatherman and Mitsch, 1978; Keating *et al.*, 1996). In contrast to declines in species diversity, increases in algal biomass are often positively correlated with decreases in pH (Muller, 1980; Arnold *et al.*, 1981; Stokes, 1981;

TABLE I

Initial stream categories, abbreviations, and site descriptions for the 56 stream sites sampled in the unglaciated Western Allegheny Plateau

Catchment classification	Description
Acid mine drainage (AMD)	Streams still receiving AMD from abandoned coal mines.
Abandoned mine lands reclamation program (AML)	Streams receiving drainage from coal mines reclaimed with funding from the AML program.
Active mines (AM)	Streams receiving treated water from an operating coal mine.
Alkaline mine drainage (AkMD)	Streams receiving AkMD from abandoned coal mines.
Mixed systems (MS)	Streams receiving drainage from coal mines reclaimed (some under various reclamation periods) and abandoned coal mines.
Ohio revised code 1513 (ORC)	Streams draining coal mines reclaimed between 1972–1982 under ORC 1513.
Reclaimed prior to 1972 (P72)	Streams draining coal mines reclaimed prior to the year 1972.
Reference streams (RS)	Streams unimpacted by mining sources.
Surface mining control and reclamation act (SMCRA)	Streams draining coal mines reclaimed between 1982–present under SMCRA.

Parent *et al*., 1986; Stokes *et al*., 1989; Elwood and Mulholland, 1989; Turner *et al*., 1991; Verb *et al*., 2001; Verb and Vis, 2001). The exact relationship between acidity and biomass increase is not known, though it may be linked to decreases in macroinvetebrate grazing pressure (e.g., Parent *et al*., 1986), decreases in algal competition and alterations in nutrient cycling (e.g., Reice, 1981; Stokes, 1986), and increases in light availability due to riparian modifications stemming from mining activity.

A variety of organisms are employed as biological indicators in aquatic investigations. Algal and diatom assemblages in particular are rapidly being implemented in the assessment of stream systems (i.e., Lowe and Pan, 1996; Stevenson and Pan, 1999). Additionally, in lotic systems impacted by acidity (i.e., acid deposition) the diatoms have developed the reputation as "premier biological indicators of surface water acidity" and have shown promise in the evaluation of reclamation success of surface mines (Battarbee *et al*., 1999; Verb and Vis, 2000).

The main goals of this investigation were to: (1) determine the broad scale influence of geological patterns and coal mining on benthic algal assemblages throughout the UWAP, (2) compare various methods for assessing algal community structure, (3) develop weighted average (WA) regression and calibration models for inferring the levels of critical environmental parameters related to mining, and (4) describe algal assemblages which may appear useful in the bioassessment of lotic systems impacted by mine drainage.

1.1. Study region and sites

The consolidated surface sedimentary rocks of the UWAP are primarily sandstones, siltstones, and shales with interlaced thin beds of more economically desirable rocks such as coal and limestone (Stout, 1944; Riley, 1960). Presently, 17 major drainage basins exist in the UWAP (range 236–20,852 km^2) for a total catchment area of approximately 36,019 km^2 which all discharge into the Ohio River (Ohio Department of Natural Resources, Division of Water, 1985). The topography of this region is typically described as one of hills, narrow ridgetops, and dissected stream valleys.

The study area is an uneven patchwork of temperate mixed broadleaf forests (predominantly mixed oak), cropland, pasture, and mining (Sedam and Francy, 1993; Peacefull, 1996). The climate of this area tends to be fairly constant with a mean annual temperature of 10.3 °C and 99 cm average precipitation annually.

Using state records, mining permits, and USGS 7.5′ (1:24,000) topographic maps, a total of 120 (2nd–4th order) 20 m stream segments were preselected for sampling within the UWAP during May–June 1999. Of these sites, only 56 were found to be suitable for sampling due to drought like conditions in Ohio during the spring/summer of 1999 (i.e. dry stream bed, no flowing water) or a lack of access (Figure 1). Stream sites were placed into one of eight categories based upon catchment mining/reclamation history within the watershed (Table I). Additional details regarding the reclamation periods (P72, ORC, and SMCRA) are contained in Verb and Vis (2000).

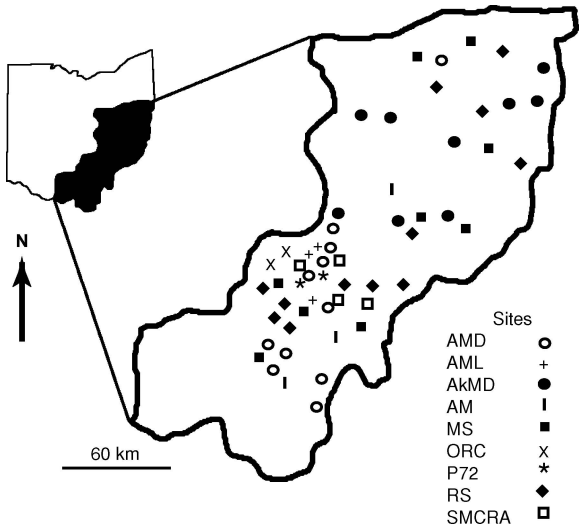

Figure 1. Map showing the portion of Ohio that is in the unglaciated Western Allegheny Plateau and the 56 sampling locations. Site categories are according to Table I.

2. Methods

2.1. Field methods

At each sampling site, a 20 m stream segment was measured for maximum wetted width and cross-sectional area at three randomly determined locations. The average thalweg depth was calculated every 2 m along the 20 m transect (11 total measurements). Current velocity was calculated from three measurements using a General Oceanics Flow Meter Model 2030, or in low flow conditions, by timing a fishing bobber (three times) as it moved over a distance of one meter. A Corning M90 portable meter was used to measure specific conductance, total dissolved solids, pH, temperature, and dissolved oxygen within the stream segment. Dissolved oxygen was cross validated in the field using a modified Winkler method (Hanna Instruments 4810). The slope of a 250 m section of the streambed was calculated using a Suunto Clinometer and a graded staff (Gordon *et al.*, 1992) and riparian canopy cover was estimated. Stream water was collected at each site and placed on ice for transportation to the laboratory. Visual assessment and estimations of canopy cover were taken from the mid-point within the 20 m stream reach (Canfield and Hoyer, 1988).

At each site, a riffle area was chosen and five rocks were randomly selected from a transect. A 5.0 cm^2 area on each rock was scraped for periphyton using a rigid O-ring and stiff toothbrush. Scraped material from the five rocks was rinsed with stream water, collected as a composite sample, and preserved with 2.5% $CaCO_3$-buffered glutaraldehyde.

Macroalgae were defined as benthic and having a discrete structure recognizable with the naked eye (Dodd, 1991; Stock and Ward, 1991; Sheath and Cole, 1992). Representative samples of each macroalgal taxon were collected from the 20 m stream reach and fixed in 2.5% $CaCO_3$-buffered glutaraldehyde to prevent morphological distortion. Percent cover of macroalgae was estimated due to the high spatial heterogeneity associated with macroalgal distribution (Mueller-Dombois and Ellenberg, 1974; Holmes and Whitton, 1981; Sheath *et al.*, 1986). The percent cover estimate was converted into a modified Braun-Blanquet cover scale to account for variability in estimation (Sheath and Burkholder, 1985). Finally, the frequency of macroalgal taxa was determined by surveying the presence/absence of macroalgal entities in 20, 400 cm^2 quadrats of the benthos, located along four, 20 m transects (5 m intervals).

2.2. Laboratory methods

Stream water turbidity was determined using a HACH 2100P^{TM} turbidity meter. For water samples having pH greater than 6.5, total alkalinity was determined using titration (Hanna Instruments 4811^{TM}). Total (phenolpthalein) acidity (Hanna Instruments 4820^{TM}) was calculated for water samples having a pH less than

5.0 (APHA 1994). For water samples having a pH between 5.0 and 6.5, both total alkalinity and acidity were determined. Water samples were filtered using Whatman GF/F 0.6 μm filters for nitrate (NO_3-N), orthophosphate (PO_4), sulfate (SO_4), calcium (Ca), and magnesium (Mg), and 0.45 μm nonglass filters for silica (SiO_2) (Wetzel and Likens, 2000). Water samples for metal analyses were preserved with nitric acid until analyzed for aluminum (Al), manganese (Mn), total iron (Fe), and zinc (Zn), the dominant metals associated with coal seams of this region (Sedam and Francy, 1993). For metal analyses, samples were pretreated using mild digestion and total metals were measured (APHA, 1994). All chemical parameters were analyzed using a Hach DR/890^{TM} colorimeter with standard protocols and powder pills from Hach Company (Anonymous, 1997).

For each periphyton sample, a small, homogenized quantity was placed in a Palmer-Maloney counting chamber to (1) enumerate 300 soft algal (non-diatom) natural units, (2) determine the ratios of living/dead algal cells, and (3) survey the basic shape morphologies, abundances, and condition of diatoms. A 10 mL periphyton subsample was extracted and cleaned using 30% H_2O_2 and concentrated HNO_3 (Stoermer *et al*., 1995). The cleaned and rinsed diatom samples were suspended in glass-distilled water and placed into evaporation chambers (Battarbee, 1973). The dried samples were prepared on slides using NAPRAX^{TM} and 600–1000 valves were enumerated along 18 mm transects at 1000X using a BX40^{TM} Olympus microscope. Soft algae and diatoms were identified to the species level when possible, primarily using Prescott (1962), Patrick and Reimer (1966, 1975), Taft and Taft (1971), Whitford and Schumacher (1984), Krammer and Lange-Bertalot (1986, 1988, 1991a,b), and Dillard (1989a,b, 1990, 1991a,b, 1993, 1999) and nomenclature was corrected using contemporary literature. For each sample, measurements of relative abundance for natural units, algal cell density, biovolume, numerical species richness, and Shannon-Weiner (H') index of diversity and evenness (J') were calculated (Magurran, 1988). A natural unit was defined as an individual unit of algae viewed under the microscope, whether it was a single cell, colony or filament. For example, a filament of *Spirogyra* was recorded as a single natural unit and it was noted that the filament was composed of 30 cells (to determine algal cell densities). Calculations for algal cell biovolumes were made using BIOVOL v.2.1 (Kirschtel, 1992) and Hillebrand *et al*. (1999).

Macroalgae were examined using an Olympus SZH-ILLD^{TM} stereoscope and discrete entities from the field notes were identified. Each entity was examined using an Olympus BX40^{TM} microscope and identified to species when possible, primarily using Prescott (1962), Taft and Taft (1971), Whitford and Schumacher (1984), and Dillard (1990, 1991a,b, 1993, 1999). Estimations of mean macroalgal thallus biovolume were conducted using the following procedure: 10 mature thalli were selected from the voucher sample, total number of cells per thalli were enumerated, cell measurements were made on 20% of the cells present in the thalli and biovolumes were calculated using formulae from Hillebrand *et al*. (1999), and the mean cell biovolume of each taxon was multiplied by the mean number of cells

per thalli. Corrections were made for taxa that varied in cell size between their main and subsequent axes (e.g., *Batrachospermum*, *Draparnaldia*) and coenocytic organisms (e.g., *Vaucheria*, *Dichotomosiphon*).

To calculate the representative biovolume of each macroalgal species at each site, we modified a technique utilized in forest ecology to calculate the relative basal area of woody plant species. In forest systems the canopy cover of trees is correlated with trunk cross-sectional area (basal area) (Barbour *et al*., 1987). Basal area (BA) is calculated by taking the mean BA of a species and multiplying this value by the species density (Smith and Smith, 1999). We altered this methodology by taking the mean biovolume of each taxon and multiplying it by its frequency (as determined from 20, 400 cm^2 quadrats) at a given sampling location:

$$\text{Macroalgal thallus biovolume} = \text{Frequency of a species} \times \text{Mean thallus biovolume.}$$

2.3. Data analysis

Relative importance values (RIV) were calculated for both the periphyton and macroalgal communities. The algal groups were treated separately because of differences in the collecting techniques and the thought that the macroalgal community may represent a less labor intensive means of examining the stream system. These importance values are based upon the concept introduced by Curtis and McIntosh (1951), which allow for an evaluation of communities that may be highly heterogeneous in their structure. These importance values provide an estimation of the assemblage structure and the dominance a given taxon may have on the given community (Curtis and McIntosh, 1951; Smith and Smith, 1999). RIV's are the sum of various relative measurements (i.e., relative abundance) for each species involved. The sum of relative measurements is divided by the total number of taxon measurements. In this study the RIV was expressed as a percentage from 0 to 100. This measurement was employed because the various algal community composition assessments (relative abundance, cell density, biovolumes) often have different advantages and disadvantages associated with them (Stevenson, 1996). We wanted to investigate if there would be any advantage to collapsing selected community measurements into a single value (RIV). RIV's for the periphyton and macroalgal communities were calculated as follows:

$$\text{Periphyton taxa RIV} = \left(\frac{(\text{Relative abundance of naturalunits}) + (\text{Relative cell density}) + (\text{Relative biovolume})}{3} \right)$$

$$\text{Macroalgae taxa RIV} = \left(\frac{\begin{array}{l}(\text{Relative cover}) + (\text{Relative thallus biovolume}) \\ + (\text{Relative frequency})\end{array}}{3} \right)$$

Soft algae and diatoms were included in multivariate techniques if they were present in a minimum of three streams with an RIV greater than or equal to at least 0.5 in at least one sample. Due to the spatial and temporal heterogeneity of macroalgae, an organism was included in analysis if it had an RIV greater than or equal to 0.5 and it was found in at least two stream segments.

Physical and chemical parameters were inspected for normality. Log_{10} and square root transformations were applied to those that exhibited skewed distributions. Ordination techniques (PCA, DCA) were performed using CANOCO version 4.0 (ter Braak and Šmilauer, 1998), cluster analyses, non-metric dimensional scaling (NMDS), and TWINSPAN were conducted using PC-ORD 4.0 (McCune and Mefford, 1999), and box plots, discriminant analyses, correlation matrices, and MANOVAs were performed with NCSS 2000 (Hintze, 2000). Detrended correspondence analysis (DCA) was used to determine the variation in the algal data sets. Based on the gradient lengths along the first axis (>three standard deviations) it was determined that canonical correspondence analysis (CCA, unimodal response model) would be the proper ordination technique to employ (ter Braak and Prentice, 1988). Patterns from the environmental and floristic data sets observed using other exploratory techniques (i.e., PCA, DCA, NMDS, UPGMA, discriminant analyses) were compared with the results of CCA. The 25 physical and chemical parameters were inspected for high correlation coefficients ($r > 0.85$) and high variance inflation factors $\geq$10 (Pan *et al*., 1996; ter Braak and Šmilauer, 1998). The significance of the first four CCA axes was tested using Monte Carlo permutations tests (1000 random permutation, $\alpha = 0.05$).

Periphyton and macroalgal based regression and calibration models were constructed to investigate the relationship between critical environmental parameters and the algal community. Weighted-average (WA) regression was constructed on the RIV and of selected periphyton and macroalgal taxa to determine their optimum and tolerances to specific environmental gradients using WACALIB v.3.3 and CALIBRATE v.0.8 (Juggins and ter Braak, 1992; Line *et al*., 1994). Error levels associated with the inference models were estimated using jackknifing and bootstrap cycles. Additional models were conducted using the different measurements of the algal community structure (i.e., relative abundance, percent cover) for comparison with RIV.

Two unbalanced multivariate analyses of variance (MANOVA) were conducted using SAS (SAS, 1996). Stream groups from multivariate analyses were the fixed effect with measurements of the algal assemblages (density, biovolume, macroalgal percent cover, species diversity, evenness, and richness) and selected environmental parameters as response variables. Bonferroni (Dunn) multiple comparison

tests were employed to investigate significant differences among the category types.

3. Results

3.1. Taxa survey and algal diversity

A total of 594 infrageneric algal taxa were recorded from these stream sites. Diatoms were the most abundant and diverse algal group (359 taxa), followed by chlorophytes (121), Cyanobacteria (42), euglenophytes (31), xanthophytes (14), chrysophytes (12), rhodophytes (6), dinophytes (6), and cryptophytes (3). The diatom *Achnanthidium minutissimum* was the most widespread taxon in this survey, being present at 53 stream sites (95%). Other prevalent diatoms included *Navicula cryptotenella* (73% of streams), *Eunotia exigua* (68%), *Encyonema minuta* (66%), *Gomphonema parvulum* (66%), *Fragilaria capucina* (64%), and *Synedra ulna* (64%) (appendix). Frequently encountered microscopic soft algae included *Pseudoanabaena* spp. (59%), *Trachelomonas* spp. (54%) (though no one species >7%), *Chlorella vulgaris* (45%), and *Audouinella hermannii* (36%). *Mougeotia* spp. (43%) and *Cladophora glomerata* (36%) had the highest frequency levels among the macroalgal taxa (appendix).

The original data set of algae encountered in these stream systems was restricted to 200 periphyton taxa (diatoms and soft-bodied organisms) and 65 macroalgae species for use in computational investigations. Periphyton and macroalgae taxa were retained for use in multivariate analyses if they had a RIV >0.5 from at least two (macroalgae) or three (periphyton) stream sites.

3.2. CCA results

Of the original 25 variables measured from the stream sites, 9 were excluded due to problems associated with autocorrelation and variance inflation. For example Al, REDOX, and SiO_2 were removed since they were highly correlated with total acidity ($r > 0.86$). Additionally, TDS, along with cations such as Ca and Mg, were also removed because they were correlated ($r > 0.88$) with specific conductance. The first four axes of the periphyton CCA were statistically significant (Monte Carlo permutation, $P = 0.001$) and explained 18.4% of the species variance (Table II). The correlation between the species and environmental variables was highest along the first axis ($r = -0.93$ and 0.80) and dropped in the following axes ($r = 0.33$–0.64; Table II). Similarly, the macroalgal data set showed the first four CCA axes also to be statistically significant ($P = 0.009$). The loadings on the first CCA axis were higher ($r = -0.85$, -0.73 and 0.69) and subsequently lower for axes 2–4 ($r = -0.34$–0.37; Table II). In both algal data sets there was an influence of mine drainage along the first two axes. The first axis was strongly correlated with pH for

TABLE II
Summary of CCA results for the first four axes of the periphyton and macroalgae RIV data sets

Periphyton (axis)	λ	*S*	SER	Environmental parameter (*r*)
I	0.449	8.9	22.5	pH (−0.93), acidity (0.80)
II	0.193	3.9	9.7	Specific conductance (0.64), SO_4 (0.41)
III	0.156	3.1	7.8	Specific conductance (0.53)
IV	0.127	2.5	6.4	Canopy (0.33)
	TVE	18.4	46.4	
Macroalgae (axis)				
I	0.684	9.3	20.6	pH (−0.85), Total alkalinity (−0.73), Acidity (0.69)
II	0.367	4.9	11.0	SO_4 (0.36), Specific conductance (0.35)
III	0.328	4.5	9.9	Mean current velocity (0.37), Dissolved oxygen (−0.34)
IV	0.254	3.4	7.7	NO_3 (−0.36)
	TVE	22.1	49.2	

Notes: λ is the eigenvalue, *S* the percent variance explained by the corresponding axis, SER the percent species-environment relation, TVE the total variance explained, and *r* the correlation coefficient between axis and influential environmental parameters. All axes were statistically significant ($P < 0.05$) as determined by Monte Carlo permutation tests (1000 bootstrap replicates).

both periphyton ($r = -0.93$) and macroalgal assemblages ($r = -0.85$) (Table II). In both data sets the second axis also depicted a relationship with two secondary AMD variables, SO_4 and specific conductance ($r = 0.64$ and 0.36).

3.3. Stream group characteristics

Patterns of site groups from the various multivariate techniques (CCA, DCA, PCA, NMDS, cluster analysis) were generally in agreement. Therefore, only the CCA biplots are shown. The environmental and biological measurements (periphyton and macroalgal data sets) typically showed five major groups of stream reaches (Figures 2 and 3), which were defined by certain shared attributes. These groups not only displayed specific assemblages of periphyton and macroalgae, but also particular physical and chemical variables. In both data sets, Group I is composed primarily of stream sites receiving AMD from unreclaimed coal mines and a small number of reclaimed sites (Figures 2 and 3). The second biplot group was composed entirely of samples from streams receiving treated waters from active surface coal mines (AM) (Figures 2 and 3; Group II). Sites initially classified as AkMD composed one-half of Group III (Figures 2 and 3) and 94% of the samples were from locales found in the northern region of the UWAP (Figure 1). Group IV was an agglomeration of reference sites and also contained some reclaimed stream reaches (Figures 2 and 3). The final CCA group, Group V, was a conglomeration of different reclamation types (P72, ORC, AML, MS) moderately impacted by AMD (Figures 2 and 3).

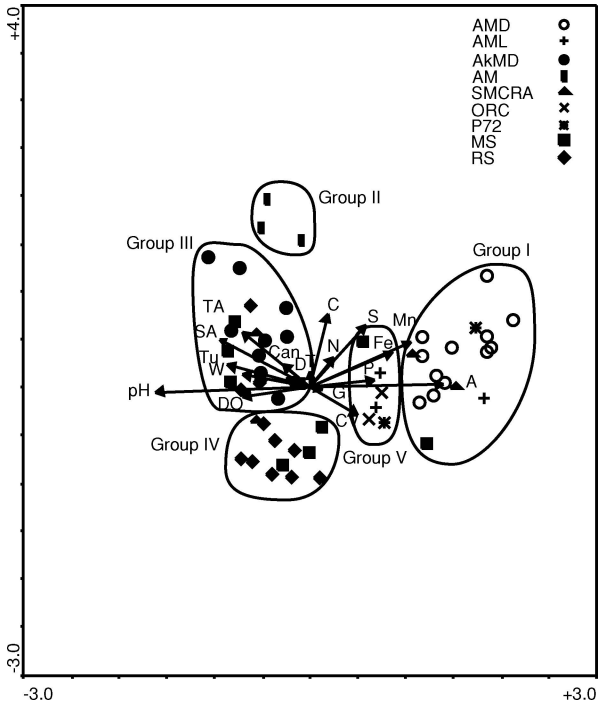

Figure 2. Periphyton-based CCA biplots of unglaciated Western Allegheny Plateau stream sites with environmental variables represented by arrows. A: total acidity, C: specific conductance, Can: canopy cover, CV: current velocity, D: thalweg depth, DO: dissolved oxygen, G: streambed slope, N: nitrate, P: orthophosphate, SA: stream cross-sectional area, TA: total alkalinity, Tu: turbidity, W: maximum wetted width. Symbol categories according to Table I.

3.4. Environmental variables

Many physical and chemical parameters displayed great divergences among the various stream segments in this investigation (Table III). Extreme examples of these deviations can be found in environmental variables (i.e., Al, alkalinity, acidity, conductance, Fe, Mn, pH, SO_4) that are commonly used as indicators of mine drainage pollution (Wetzel and Hoffman, 1989; Sedam and Francy, 1993). Group I sites had the lowest water pH levels (MANOVA and Bonferroni Dunn multiple comparison test: $P < 0.001$) and the highest concentrations of acidity ($P < 0.001$), Al ($P < 0.001$), Fe ($P < 0.001$), and Mn ($P < 0.001$). The stream water at Group II (AM) sites had circumneutral pH levels, the highest specific conductance levels (MANOVA, $P < 0.001$), and elevated SO_4 and total alkalinity levels. The SO_4 levels were comparable (Bonferroni-Dunn) to those found at AMD (Group I) sites and some AkMD and Group V sites, but without the levels of Fe and Al often associated with these sites (Table III). Group III stream sites were characterized by circumneutral to alkaline water pH (6.8–8.5) with high levels of

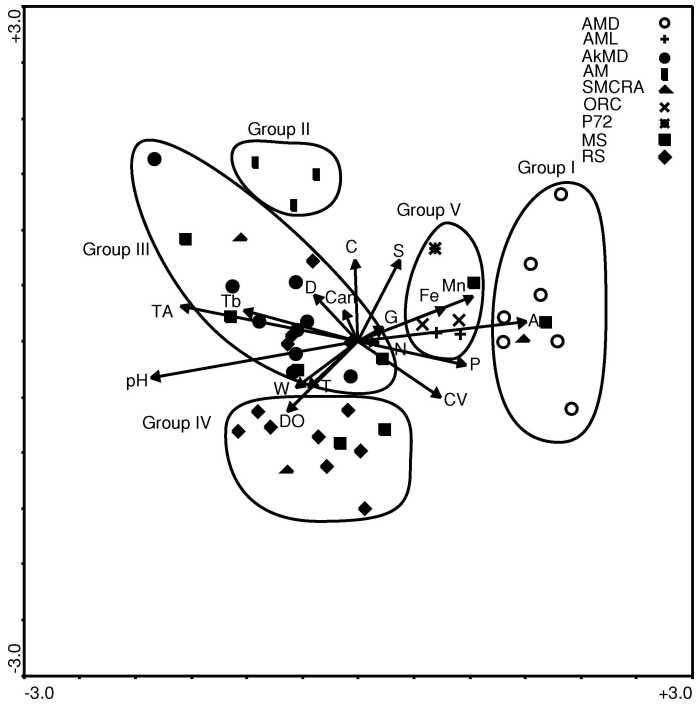

Figure 3. Macroalgae-based CCA biplots of unglaciated Western Allegheny Plateau stream sites with environmental variables represented by arrows. A: total acidity, C: specific conductance, Can: canopy cover, CV: current velocity, D: thalweg depth, DO: dissolved oxygen, G: streambed slope, N: nitrate, P: orthophosphate, SA: stream cross-sectional area, TA: total alkalinity, Tu: turbidity, W: maximum wetted width. Symbol categories according to Table I.

specific conductance, SO_4, and total alkalinity. The metals Fe and Mn were found at an intermediate concentration range, higher than that found at AM (Group II) and Group IV sites, but lower than concentrations present at AMD (Group I) and Group IV systems ($P < 0.05$) (Table III). The Group IV water chemistry was relatively circumneutral in pH, with the lowest levels of specific conductance and metals (Table III). Furthermore, these sites showed lower levels of total alkalinity than those in Groups II and III ($P < 0.001$). Group V streams had moderately acidic conditions (pH 4.5–5.5), which were significantly different ($P < 0.001$) from all other systems. In addition, the waters had low total alkalinity levels, moderate specific conductance, and elevated concentrations of SO_4, Fe, and Mn (Table III).

3.5. Algal communities

The five most influential members (based on percent RIV's) of the periphyton community at AMD sites (Group I) were *Eunotia* spp. (specifically *E. exigua*), *Frustulia rhomboides*, *Pinnularia subcapitata*, *Euglena mutabilis*, and *Nitzschia*

TABLE III

Summary of descriptive statistics for selected physical, chemical, and habitat variables (median value with ranges below) for 56 streams sampled in the unglaciated Western Allegheny Plateau

	Multivariate group				
Environmental parameters	I ($n = 17$)	II ($n = 3$)	III ($n = 18$)	IV ($n = 12$)	V ($n = 6$)
Al (mg/L)	7.68 (1.11–47.44)	0.23 (0.08–0.68)	0.26 (0.00–0.55)	0.17 (0.00–0.30)	0.62 (0.10–1.91)
Specific conductance (μS/cm)	1112 (639–2700)	4555 (4025–6470)	748 (432–1999)	302 (215–495)	592 (428–1847)
Current velocity (cm/s)	10 (3–25)	9 (3–16)	5 (2–28)	6 (1–20)	11 (5.3–23)
Dissolved oxygen (mg/L)	6.2 (0.0–9.8)	6.7 (4.9–7.6)	7.8 (5.3–10.2)	8.3 (4.4–9.8)	6.7 (4.3–8.4)
Fe (mg/L)	4.40 (1.33–18.45)	0.26 (0.25–1.33)	0.74 (0.03–3.32)	0.40 (0.02–0.83)	1.61 (0.69–6.43)
Maximum wetted width (m)	2.8 (0.9–6.6)	3.4 (2.8–3.8)	4.9 (2.8–17.1)	4.8 (1.1–8.3)	3.8 (3.0–10.5)
Mn (mg/L)	6.77 (1.32–30.24)	2.10 (0.82–4.33)	3.62 (0.00–18.21)	0.67 (0.00–1.33)	3.87 (1.54–19.39)
NO_3 (mg/L)	0.07 (0.01–1.40)	0.03 (0.01–0.20)	0.09 (0.01–4.00)	0.02 (0.00–0.60)	0.10 (0.20–1.50)
Open canopy (%)	75 (5–100)	30 (25–95)	73 (5–100)	27 (8–88)	81 (10–95)
pH	3.2 (2.4–4.3)	7.4 (7.3–7.7)	7.5 (6.7–7.9)	7.1 (6.4–7.7)	5.0 (4.5–5.5)
PO_4 (mg/L)	0.14 (0.03–0.70)	0.13 (0.09–0.14)	0.11 (0.02–0.36)	0.07 (0.02–0.69)	0.12 (0.06–0.24)
SiO_2 (mg/L)	21.0 (14.3–45.8)	7.6 (3.6–13.7)	6.7 (2.8–12.3)	8.1 (5.3–11.7)	11.4 (9.8–14.9)
SO_4 (mg/L)	780 (620–1800)	1480 (1370–1520)	385 (34–1520)	69 (24–270)	403 (240–1280)
Stream cross-sectional area (m^2)	22.81 (5.50–143.64)	39.85 (9.98–129.10)	75.39 (14.67–306.45)	34.24 (28.2–101.34)	59.50 (18.71–106.50)
Temperature (°C)	23 (13–30)	25 (22–28)	23 (16–29)	20 (15–26)	20 (15–28)
Thalweg depth (cm)	16 (5–37)	25 (12–34)	18 (8–57)	15 (4–24)	20 (10–26)
Total alkalinity (mg/L)	0 0	153 (42–168)	138 (124–260)	52 (15–123)	15 (0–42)
Total acidity (mg/L)	300 (150–1000)	0 0	0 0	0 0	5 (3–48)
Turbidity (NTU)	0.59 (0.14–68.50)	1.66 (0.71–3.21)	3.94 (0.50–24.40)	2.99 (0.77–7.32)	3.73 (1.14–8.80)

Note: Stream categories according to ordinations groups described in the text. NTU is the nephelometric turbidity units.

capitellata (appendix). The macroalgal community was composed primarily of filamentous chlorophytes, especially *Klebsormidium rivulare*, *Microspora tumidula*, *Microspora pachyderma*, and *Mougeotia* spp., along with *Euglena mutabilis* mats (appendix). At eight heavily impacted AMD sites no macroalgae were detected.

Prevalent taxa comprising the benthic microalgal communities at Group II sites included the diatoms *Achnanthidium minutissimum*, *Enotmoneis paludosa*, *Diatoma tenue*, *D. moniliformis*, and the rhodophyte *Audouinella hermannii* (appendix). Group II macroalgal communities were dominated by the filamentous chlorophytes *Cladophora glomerata*, *Chara* spp., and *Spirogyra* spp. Furthermore, conspicuous mats of *Vaucheria polysperma* and *Oscillatoria nigra* were present at the AM sites (appendix).

The taxon of most importance in the periphyton communities at Group III systems was *Achnanthidium minutissimum*. Additional dominant constituents included *Navicula lanceolata*, *Cymbella affinis*, *Nitzschia dissipata*, and *Navicula cryptotenella* (appendix). Expansive beds of *Cladophora glomerata* dominated macroalgal communities, but other widespread taxa were *Batrachospermum helminthosum*, *Vaucheria* spp. (*V. geminata* and *V. polysperma*), *Mougeotia* spp., and *Microspora amoena* (appendix).

The algal communities in Group IV showed less of a divergence in RIV's than the previous three ordination groups. Periphyton communities were characterized by *Achnanthidium minutissimum*, *Pseudoanabaena* spp., *Cymbella affinis*, *Brachysira vitrea*, and *Synedra ulna* (appendix). *Batrachospermum gelatinosum*, along with four filamentous chlorophytes, *Cladophora glomerata*, *Spirogyra* spp., *Draparnaldia plumosa*, and *Microspora stagnorum*, were shown to be the main components of the macroalgal community (appendix).

Periphyton communities associated with Group V sites were characterized by *Brachysira vitrea*, *Achnanthidium minutissimum*, *Plectonema* sp., *Phormidium inundatum*, and *Eunotia exigua* (appendix). The macroalgal community was dominated by *Vaucheria polysperma*, *Mougeotia* spp., and *Klebsormidium rivulare*, with additional contributions from *Microspora tumidula* and *Phormidium retzii* (appendix).

3.6. Comparisons algal community measurements

Groups II, III, and IV from the multivariate analyses had levels of species richness and diversity which were significantly ($P < 0.05$) greater than the measurements from Groups I and V (Figure 4A, B). Periphyton assemblages present in AMD impacted systems (Group I and V) displayed significantly depressed ($P < 0.05$) levels of algal cell density and biovolume (Figure 4C and D). Shannon's index of Evenness (not shown) did not differ significantly ($P < 0.05$) among groups. Significant elevations ($P < 0.05$) in levels of macroalgal cover (not shown) were also found in sites relatively unimpacted by AMD (Groups II, III, and IV).

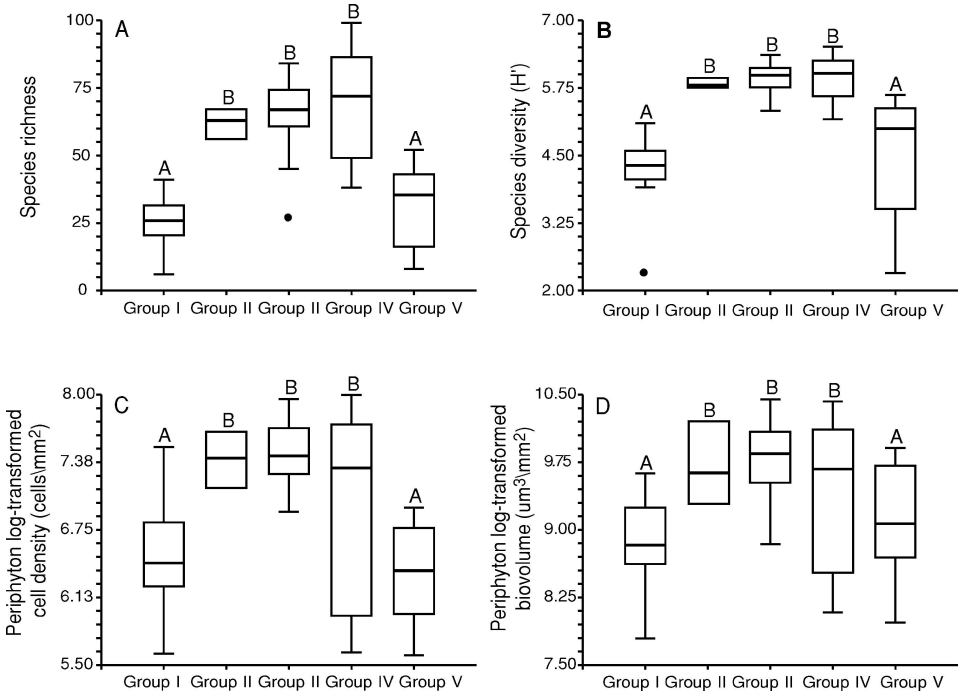

Figure 4. Periphyton community statistics. Box represents the 25th and 75th percentiles, whiskers represent the 5th and 95th percentiles, and dots represent outliers outside the 95th percentile. Line in box represents median values for multivariate group. Boxes with the same letters are not significantly different ($P < 0.05$) as determined by an unbalanced MANOVA and Bonferroni-Dunn multiple comparison test.

3.7. Species based inference models

Weighted-average regression and calibration were used to develop periphyton and macroalgal-based inference models to quantify relationships between algal RIV's and environmental variables related to mine drainage. These variables were first tested using constrained CCA's to determine if they had significant influence on the species distributions. Periphyton-based models were highly correlated with pH and exhibited high levels of predictability (Table IV and Figure 5), resulting in strong correlations between the algal-inferred and observed values. Initial values for sulfate and specific conductance were promising, but cross-validation procedures yielded poor r^2 and RMSE (Table IV and Figure 6). Many of the WA inference models based on the macroalgal data set performed poorly (Table IV and Figure 5). Models of pH yielded strong correlations with the taxa ($r = 0.80$), but the high RMSE (0.82) raised concern about the models' validity. WA SO_4 models displayed moderate correlations ($r^2 = 0.54$, RMSE = 0.35) with the taxa, but cross validation procedures further reduced the performance of these models ($r^2 = 0.05$,

TABLE IV

Predictive levels of periphyton and macroalgae-based simple weighted averaging (WA) calibration models for unglaciated Western Allegheny Plateau streams

Parameters	WA ($n = 56$)		WA(tol) ($n = 56$)	
Periphyton	r^2	RMSE	r^2	RMSE
pH	0.89 (0.81)	0.49 (0.58)	0.95 (0.68)	0.40 (0.52)
SO_4	0.63 (0.35)	0.40 (0.49)	0.70 (0.39)	0.26 (0.47)
Conductivity	0.68 (0.32)	0.22 (0.30)	0.65 (0.25)	0.24 (0.32)
Acidity	0.78 (0.63)	0.27 (0.32)	0.83 (0.22)	0.48 (0.38)
Macroalgae	($n = 45$)		($n = 45$)	
pH	0.80 (0.71)	0.82 (0.93)	0.87 (0.62)	0.73 (0.87)
SO_4	0.54 (0.05)	0.35 (0.55)	0.59 (0.01)	0.33 (0.62)
Conductivity	0.41 (0.02)	0.39 (0.58)	0.45 (<0.01)	0.36 (0.66)
Acidity	0.64 (0.50)	0.32 (0.36)	0.66 (0.31)	0.49 (0.36)

Note: The r^2 is the coefficient of determination of regression between periphyton-inferred and measured environmental variables. RMSE is the root mean squared error. The r^2 and RMSE in parentheses were derived from jackknifing. WA(tol) is the weighted averaging with tolerance down-weighted option.

RMSE = 0.55) (Table IV and Figure 6). Similar results were obtained with models based on specific conductance and the metals Fe and Mn. One WA model that did perform well was related to the environmental variable acidity (Table IV).

Many species in this study exhibited strong relationships with selected environmental parameters. Estimated species optima and tolerances for pH, specific conductance, SO_4, and mean RIVs (for all 56 sites) are listed for taxa with an RIV > 0.5 at three or more stream segments (appendix).

3.8. Evaluation of relative importance values (RIV)

We compared the performance of the periphyton RIV's with output from more traditional assessments of community structure (relative abundance of natural units, relative biovolume, and relative cell density) using CCA and WA (pH). The cumulative species variance explained by the first four CCA axes ranged from 18.3 to 23.9% (Table V). In all four analyses the first axis was highly correlated with pH while the second CCA axis was influenced by secondary mine drainage parameters (i.e., specific conductance, SO_4, Mn; Table V). Periphyton WA pH inference models also produced similar results with apparent r^2 ranging from 0.82–0.89 (Table V).

Few protocols are available for the assessment and evaluation of macroalgal communities. Hence, the performance of macroalgae RIV was compared with species percent cover. The amount of variation explained by the two data sets was comparable (Table V). The percent cover based WA inference models displayed the same

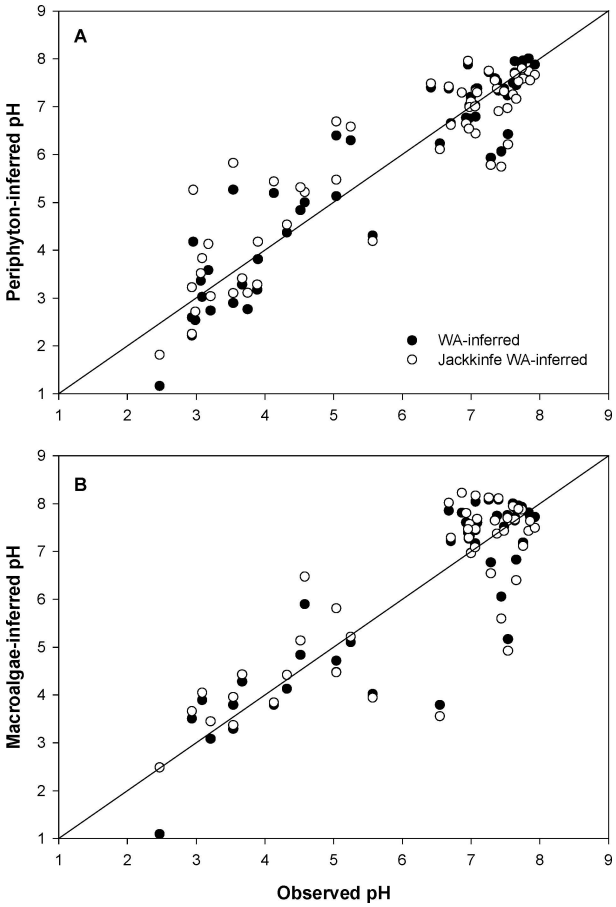

Figure 5. Relationship between calculated pH and macroalgae (A) and periphyton-inferred (B) stream pH using weighted-averaging regression and calibration models, with and without jackknifing. The line was drawn at a 1:1 ratio.

shortcomings as the RIV data set with high species correlation and concerning, high levels of variance (Table V).

The influence and profusion of the different algal divisions as displayed by the various community measurements was further evaluated. There were no major differences in the results obtained from the four periphyton measurements; diatoms were the dominant community constituents in all measurements. The contributions of the chlorophytes, Cyanobacteria, euglenophytes, and rhodophytes also varied slightly between community measures (Figure 7). Both macroalgal RIV's and relative cover showed that lotic UWAP assemblages were dominated by the Chlorophyta (Figure 8). The relative cover showed a nearly equal distribution of the xanthophytes, rhodophytes, and Cyanobacteria (Figure 8). However, the macroalgal RIV placed a greater importance on the Xanthophyta and Rhodophyta (Figure 8).

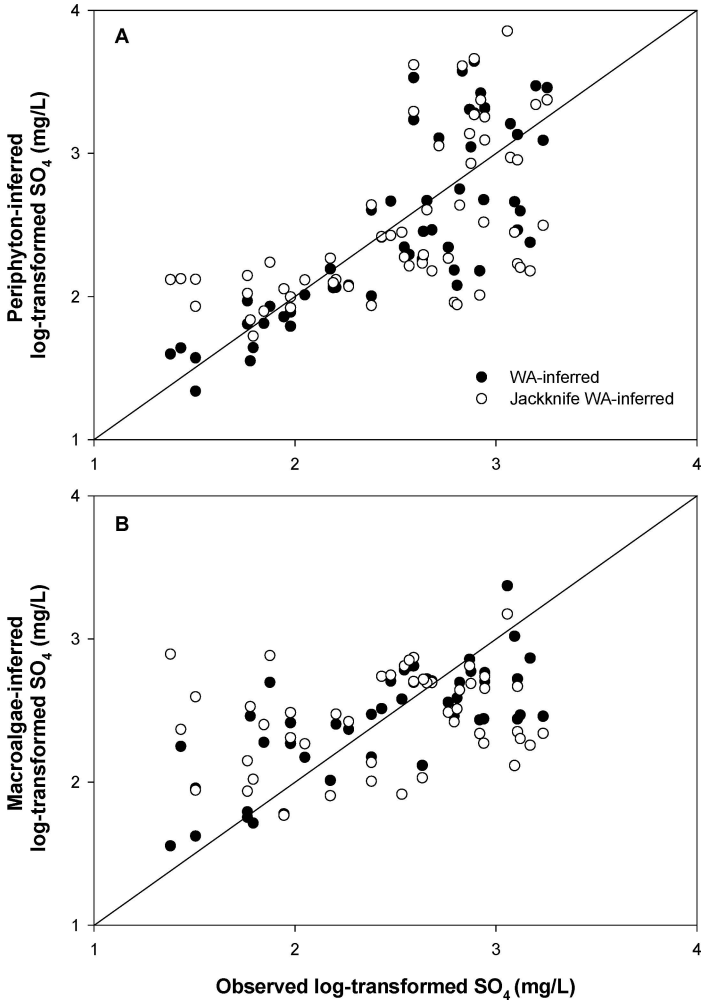

Figure 6. Relationship between calculated SO_4 and macroalgae (A) and periphyton-inferred (B) stream $\log_{10}$-transformed SO_4 using weighted-averaging regression and calibration models, with and without jackknifing. The line was drawn at a 1:1 ratio.

4. Discussion

4.1. Relationships between geology, mining, and algal patterns

In this study, many low order stream systems within the UWAP were influenced by geologic patterns, coal mining, and mine drainage. Major distinctions in site and algal assemblage distributions can be correlated with inputs of AMD and the ability of natural waters to neutralize this pollutant. This investigation led to the appearance of two influential environmental gradients, a dominant pH derived gradient from

TABLE V

The performance of various community composition assessment metrics in canonical correspondence analysis (CCA) and weighted-averaging (WA) calibration models

Measurement	CCA TVE[a](%)	Axis 1 (r)	Axis 2 (r)	pH WA r^2	pH WA RMSE
RIV (periphyton)	18.3	pH (−0.93)	Conductivity (0.64)	0.89	0.49
RA (periphyton)	23.9	pH (−0.97)	Alkalinity (0.50)	0.88	0.60
RBIOV (periphyton)	21.9	pH (−0.96)	SO_4, Mn (0.42)	0.85	0.71
RCD (periphyton)	21.9	pH (−0.97)	SO_4 (−0.34)	0.82	0.73
RIV (macroalgae)	22.1	pH (−0.85)	SO_4 (0.36)	0.80	0.82
RC (macroalgae)	24.9	pH (−0.90)	SO_4 (−0.62)	0.72	0.79

Note: RIV is the relative importance values, RA the relative abundance of natural units, RBIOV the relative biovolume, RCD the relative cell density, and RC the relative percent cover.
[a]Sum of first four statistically significant CCA axes (Monte Carlo permutation test, $P < 0.05$).

AMD and a secondary spectrum relating to high electrolyte levels resulting from the input of sulfate brines and AMD-related metallic ions. Our results show that there appears to be a strong relationship between pH and the distribution of periphyton and macroalgae. This relationship was not unexpected since our study sites spanned a wide spectrum of pH values (2.4–7.9). It is not unusual for these environmental parameters to be influential in algal community composition (i.e., Pan *et al*., 1996; Planas, 1996). Furthermore, the importance of pH and electrolyte gradients in algal distributions have been noted in other regional studies of Appalachian lotic systems (Pan *et al*., 1996; Verb and Vis, 2000, 2001).

In much of the southern UWAP, AMD and subsequent reductions in pH had detrimental impacts on benthic algal assemblages. Groups I and V display significantly lower levels of species richness and production. There is ample evidence in the literature supporting reductions in species richness in acid impacted aquatic systems due to increased hydrogen ion activity and elevations in the concentrations of metallic ions such as Al (e.g., Yan and Stokes, 1978; Mulholland *et al*., 1986; Stokes *et al*., 1989; Verb and Vis, 2000). However, most studies seem to support the concept that increases in acidification lead to increases in algal biomass (e.g., Muller, 1980; Arnold *et al*., 1981; Stokes, 1981; Parent *et al*., 1986; Stokes *et al*., 1989; Turner *et al*., 1991; Winterbourn *et al*., 1992; Planas, 1996; Verb *et al*., 2001). In this study, sites that were moderately to heavily impacted by AMD showed significantly lower levels of periphyton biovolume and macroalgal cover. Even within the AMD impacted systems there were wide ranges of cell densities, biovolumes, and percent covers (Figure 4C and D). The paradox of some AMD impacted systems showing high levels of biomass accrual while others display rather depauperate production was also noticed in two previous studies which incorporated diatom and macroalgae communities (Verb and Vis, 2000, 2001). At some sites these studies suggested an inverse production relationship

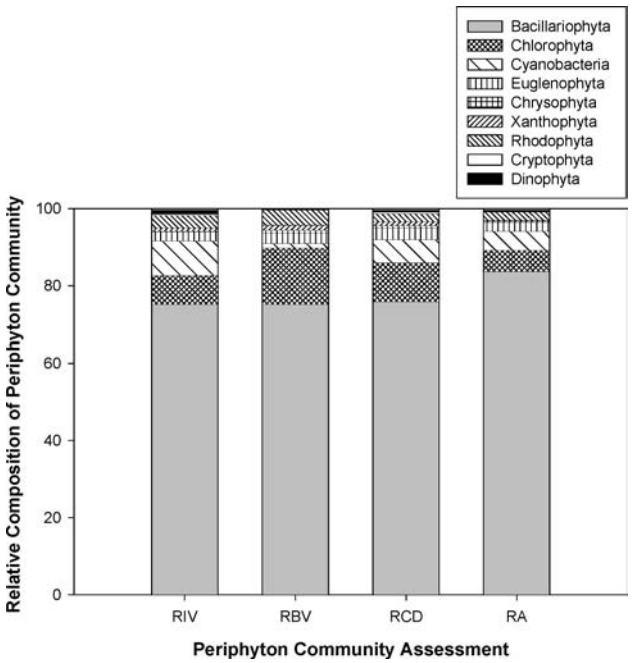

Figure 7. Stacked bar charts examining the distribution of major algal divisions when employing various metrics of periphyton community composition. RIV: relative importance value, RBV: relative biovolume, RCD: relative cell density, RA: relative abundance of natural units.

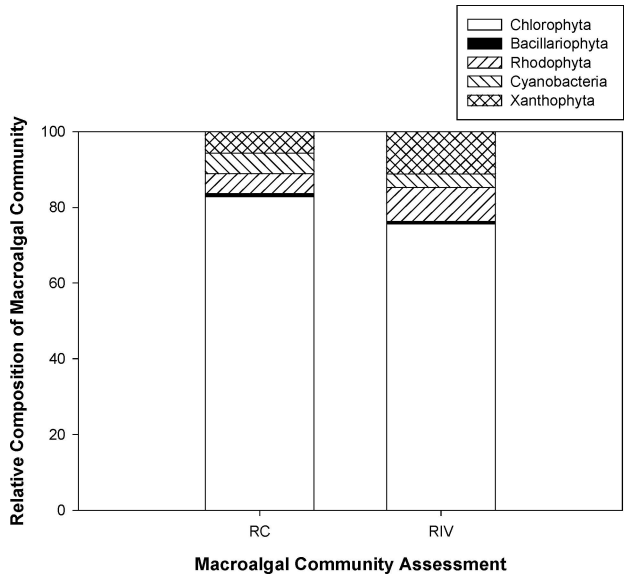

Figure 8. Stacked bar charts showing the distribution of major algal divisions using two measures of macroalgal community composition. RIV: relative importance value, RC: relative percent cover.

between the diatoms and macroalgae. For example, sites having high diatom densities displayed low levels of macroalgal cover and vice versa. However, in this study there was a weak negative correlation between periphyton (typically dominated by diatoms) biomass and macroalgal cover, but it was not supported statistically ($r_s = -0.37$; $P = 0.22$). Furthermore, all of these studies were unable to link any of the measured environmental variables to the various measurements of algal production.

In systems moderately impacted by AMD (Group V), the periphyton community was dominated by two diatoms, *Brachysira vitrea* and *Achnanthidium minutissimum*. In a 12-month study of reclaimed systems within the Hocking River Drainage Basin (southeastern Ohio) these two taxa were dominant at two unusual reclaimed sites. These two sites were unique because the stream water oscillated between acidic and circumneutral pH throughout the year and they had very low levels of overall diatom densities (Verb and Vis, 2000). These two sites were revisited and sampled again for this study and were within Group V (Figures 2 and 3). The ordinational location of these sites, coupled with the importance and dominance of *Brachysira vitrea* and *Achnanthidium minutissimum* in Group V, the overall low periphyton cell densities, and little to no buffering capacity, allows us to theorize that many or all of these sites may also be oscillating in pH (Table III, Figures 2–4; appendix). Further research utilizing temporal monitoring of these streams will be needed to confirm or deny our suspicions.

The effects of increased hydrogen ion activity from AMD were neutralized by local watershed geology (i.e., calcareous shales, limestone, marly clays) at all but one of the sites in the northern UWAP. The singular northern AMD site was sampled very close to the abandoned mine and exposed pyrite piles. This could, in part, explain the lack of acid neutralization and the switch from AMD to AkMD that we typically find in the northern UWAP. The resulting AkMD seemed to have little impact on diversity and production levels of the algal communities when compared to reference streams. While these systems appear to show no ill effects from the AkMD, the major impact from mining seems to stem from high sediment loads (from both sediment and iron floc) and habitat degradation from embeddedness (qualitative habitat evaluation, data not included). This observation could perhaps explain the highest numbers of motile algae, especially raphid diatoms (i.e., *Navicula* and *Nitzschia*), in the Group III periphyton samples (appendix). With increased sedimentation we postulated that there might be a corresponding increase in turbidity or available phosphorus or decreases in species diversity, however our analyses detected no significant differences.

4.2. Initial site classifications and implications of restoration

Site categories (Table I) which consistently grouped together in multivariate analyses included AkMD, AM, AMD, and AML (found in Group I and V). Other reclamation periods and reference systems showed a great deal of overlap and

dispersion in ordinal space. From this study, and others about the region, it appears that coal mines reclaimed prior to 1982 are still inundated with periodic or continual AMD (Verb and Vis, 2000, 2001). Mixed systems (MS) also showed wide variation, but surprisingly only two of these sites appear to be impacted consistently by AMD. The four SMCRA samples displayed perhaps the most confounding patterns of distribution. Verb and Vis (2000) reported that of all the reclamation periods, SMCRA sites most closely resembled reference streams despite a shorter time frame since abandonment (though this prior study did not include several mining categories such as AkMD, AML, and AM). The reasoning was that this regulation (SMCRA) requires stricter mine remediation and water treatment (active and passive) prior to bond release (Ohio Department of Natural Resources, Division of Mines and Reclamation, 2001). However, it should be noted that the Verb and Vis (2000) study and this investigation examined a different number of SMCRA sites (2 versus 4) and a different number of study sites (10 versus 56). In this current study, two of the SMCRA sites were found associated with Groups III or IV. However, the other two SMCRA sites were acidic in water chemistry and floristic composition. Exact reasoning for these acidic occurrences was not determined, but perhaps stemmed from failed reclamations or the intrusion of contaminated groundwater (given the drought conditions of 1999). Reclamation projects seem to be very difficult to categorize (via abiotic and biotic means) based on the legislation under which they were remediated. The primary cause of this phenomenon may be collaboration from a variety of factors ranging from different mining, reclamation, and remediation techniques to variation in local geological strata associated with the mines.

4.3. Algal communities as indicators of mine drainage and reclamation

The results indicated that the epilithic periphyton community could be reliably related to pH levels and other highly correlated variables (i.e., acidity). The performance of the periphyton pH inference models was comparable to other lotic and lentic studies. For example, Pan *et al*. (1996) showed diatom communities produced apparent r^2 values of 0.79–0.81 from 49 streams in the Mid-Atlantic Highlands. In addition, there have been several pH calibration models developed for acidic lakes that have apparent r^2 values (0.78–0.91) similar to levels derived from our data sets (Birks *et al*., 1990; Dixit *et al*., 1991).

Predictive periphyton models of secondary AMD compounds, such as SO_4, specific conductance, and Mn, performed poorly. The weak performance of these models was even more apparent after cross-validation. One possible explanation for the poor performance of these models is the pH of these stream segments. For instance, AkMD, AMD, and AM (Group I–III, V) streams all have inflated concentrations of SO_4 and levels of specific conductance. However, there are differences in the floristic composition of these streams based on the more dominant

pH spectrum (AMD, acidic; AkMD, AM, circumneutral). Hence the results may be skewed due to the presence of these two vastly different algal communities, both of which may indicate elevations in specific conductance and SO_4, but are influenced and separated by the different pH conditions.

WA models based on the macroalgal community performed inadequately. While there were strong correlations between certain taxa and the environmental variables, we found the high levels of variance (RMSE) to be troublesome. The performance of the data set when cross-validation procedures were applied provided further concerns regarding performance and reliability. The substandard predictability of the macroalgal community may stem from the spatial and temporal heterogeneity of these organisms (Sheath *et al*., 1986). Since approaches in sampling macroalgal communities are highly variable, it is difficult to make comparisons with other studies. Furthermore, to our knowledge, there have not been any attempts to ascertain the performance of macroalgal data sets in the creation of WA regression calibration models. Part of the analytical difficulty may stem from the patchiness of these organisms, a characteristic that may make it difficult for some analyses that search for linear or unimodal responses to detect consistent patterns and distributions. To properly investigate the distribution patterns of macroalgae, it may be necessary to apply analyses which are capable of taking spatial and temporal components into account (i.e., geostatistics).

4.4. Evaluation of algal RIV's

In the benthic algal literature there are many techniques employed to assess community composition (Stevenson, 1996). While there exist positive and negative aspects to all of these protocols we had hoped that the use of importance values would assist in eliminating or minimizing the drawbacks associated with the various community composition assessment metrics. However, there was no clear-cut advantage in the employment of RIV's versus traditional means of algal community assessment in multivariate analyses and WA regression (Table V). RIV explained less of the species variation in both the periphyton and macroalgae data sets. The RIV's from both data sets did perform slightly better in the WA inference models. It may be argued that the differences between the RIV and other measurements are not great enough to warrant the time that is required (i.e., biovolume calculations). However, the use of importance values may be a valuable tool for minimizing inherent problems, incorporating strengths associated with the different community measurements, and dealing with the heterogeneous composition of algal assemblages. Furthermore, while we proposed and utilized a three component RIV for both data sets, the composition of the RIV's is quite flexible and may be modified to employ different types of relative community assessments. For instance, the values of relative abundance of natural units and relative cell densities may yield fairly similar results if the community is dominated by unicellular organisms (e.g., diatoms). In systems dominated by single-celled taxa, the use of the two

aforementioned measurements may be considered repetitive and thus only one of the two should be used in conjunction with biovolumes. Additionally, the RIV's seem to have performed well in distinguishing taxa, which may serve as dominant and index species for the various algal communities associated with our multivariate mine drainage groups. While the RIV's served well in determining algal community structure it remains to be seen if RIV's are truly a viable option in such investigations.

In conclusion, the lotic benthic algal communities reflected two major environmental gradients present within this region of the UWAP. Both gradients, pH and electrolyte, were primarily induced from different varieties of anthropogenic mine drainage discharging into natural waterways. Periphyton-derived models generally demonstrated a stronger, quantifiable relationship with the environmental parameters than macroalgae. There are unique assemblages of influential algal taxa which seem to define the various multivariate groups. Employment of RIV's to assess algal community structure and response to the environmental gradients yielded mixed results. Given the somewhat limited success of the periphyton data set, it is conceivable that these assemblages may be employed in the mitigation and risk assessment of moderate and heavily impacted AMD watersheds and the evaluation of coal mine remediation and reclamation efforts. If macroalgal communities are to be employed in bioassessment activities they require further investigation, perhaps employing different analytical techniques than have traditionally been applied or focusing on individual taxa that seem important.

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Appendix

Apparent optimum and tolerance of pH and SO_4 of algal taxa in this study with mean relative importance values (RIV) in ordinations, weighted-averaging regression, and calibration inference models. N is the frequency of occurrence throughout the 56 sites from the unglaciated Western Allegheny Plateau.

Taxon Periphyton	N	pH		SO_4 (mg l^{-1})		RIV for groups[1]	Mean RIV[2]
		Optimum	Tolerance	Optimum	Tolerance		
				Bacillariophyta			
Achnanthes spp.	12	7.44	0.32	203	210		0.25
A. deflexa C. W. Reimer	3	7.07	0.09	32	368		0.15
A. pseudoswazi Carter	15	6.05	1.39	245	320		0.16
A. suchlandtii Hust.	4	7.41	0.36	102	188		0.24
Achnanthidium minutissimum (Kütz) Czarn.	53	6.66	1.28	447	457	I (1.66); II (23.67); III (21.41); IV (13.98); V (22.04)	16.10
Adlafia minuscula (Grunow) Lange-Bert.	16	7.32	0.74	311	385		0.16
Amphipleura pellucida (Kütz.) Kütz.	13	7.11	0.80	483	608	IV (1.04)	0.41
Amphora inariensis Krammer	20	7.45	0.61	269	294	III (3.40)	1.14
Amphora montana Krasske	9	7.01	0.42	288	496		0.08
Amphora ovalis (Kütz.) Kütz.	4	7.69	0.43	574	525		0.03
Aulacoseira ambigua (Grunow) Simonsen	3	7.04	0.21	240	78	III (1.01)	0.32
Aulacoseira granulata (Ehrenb.) Simonsen	3	7.14	0.40	240	38	III (1.00)	0.32
Bacillaria paxillifer (O. F. Müll.) Hendey	5	6.59	1.60	378	459		0.12
Brachysira vitrea (Grunow) Ross *in* Hartley	29	6.13	1.50	391	299	III (1.37); IV (4.09); V (25.89)	5.27
Caloneis spp.	19	7.56	0.65	721	574	III (1.91)	0.70
C. bacillum (Grunow) Cleve	4	6.88	1.09	268	502		0.04
Cocconeis pediculus Ehrenb.	19	7.28	0.53	373	388		0.23
C. placentula Ehrenb.	19	7.29	1.02	261	327		0.22
Craticula cuspidata (Kütz.) D. G. Mann	7	3.93	1.66	667	335	I (1.06)	0.36
C. halophila (Grunow) D. G. Mann	26	7.01	1.06	284	300		0.30
Ctenophora pulchella (Ralfs *ex* Kütz.) Round & D. M. Williams	20	6.92	1.10	243	421	II (1.16); III (1.93)	0.88
Cyclotella meneghiniana Kütz.	20	7.21	0.63	996	643		0.26
Cyclotella striata (Kütz.) Grunow	8	7.49	0.35	1029	697	II (1.73)	0.08

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Taxon Periphyton	N	pH		SO_4 (mg l^{-1})		RIV for groups[1]	Mean RIV[2]
		Optimum	Tolerance	Optimum	Tolerance		
Cymatopleura solea (Bréb.) W. Sm.	12	6.06	1.89	280	321	IV (1.01)	0.48
Cymbella affinis Kütz.	34	7.13	1.03	157	204	III (6.14); IV (4.53)	3.20
Cymbella cymbiformis C. Agardh	5	7.07	0.10	61	156	IV (1.07)	0.26
Cymbella delicatula Kütz.	7	6.87	1.34	89	256	IV (1.68)	0.39
Cymbella lanceolata (C. Agardh) C. Agardh	3	7.53	0.12	170	111		0.06
Cymbella mexicana (Ehrenb.) Cleve	3	6.55	1.00	75	368		0.13
Cymbella microcephala Grunow	5	6.89	0.39	149	421		0.04
Cymbella naviculiformis Auersw. *ex* Heib.	8	5.86	1.56	234	159		0.07
Cymbella tumida (Bréb.) VanHeurck	15	6.19	2.09	339	472	IV (1.04)	0.36
Denticula kuetzingii Grunow	7	7.21	0.37	153	298		0.03
Diatoma moniliformis Kütz.	5	7.70	0.61	1307	57	II (7.31)	0.04
D. tenue C. Agardh	8	7.62	0.55	1440	317	II (9.34)	0.75
D. vulgare Bory	13	7.22	0.70	130	130		0.42
Encyonema lange-bertalotii Krammer	21	6.51	1.28	169	227		0.23
E. minutum (Hilse *ex* Rabenh.) D. G. Mann	29	6.65	1.40	387	471	II (1.10); IV (1.49)	0.66
E. muelleri (Hust.) D. G. Mann	5	5.64	2.13	179	171		0.04
E. prostratum (Berk.) Kütz.	9	7.46	0.34	195	142		0.08
E. silesiacum (Bleisch *ex* Rabenh.) D. G. Mann	36	6.49	1.58	477	581	III (1.46); IV (1.38); V (1.04)	1.08
Entomoneis paludosa (W. Sm.) C. W. Reimer	3	7.38	0.30	1773	198	II (12.18)	0.70
Eolimna minima (Grunow) Lange-Bert.	3	6.87	1.00	62	368		0.00
Eunotia arcus Ehrenb.	4	6.01	2.89	388	282		0.24
E. curvata (Kütz.) Lagerst.	7	5.79	1.75	442	532		0.13
E. exigua (Bréb.) Rabenh.	37	3.46	0.86	858	458	I (48.05); V (12.61)	16.01

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Taxon Periphyton	N	pH		SO_4 (mg l^{-1})		RIV for groups[1]	Mean RIV[2]
		Optimum	Tolerance	Optimum	Tolerance		
E. implicata Nörpel, Lange-Bert. & Alles	3	7.44	0.23	411	190		0.03
E. incisa W. Sm.	9	3.97	0.79	766	539	I (1.05); V (1.20)	0.43
E. parallela Ehrenb.	3	2.94	1.00	740	368		0.18
E. pectinalis (O. F. Müll.) Rabenh.	5	4.25	0.84	383	154		0.16
E. steineckii Petersen	22	3.54	0.95	755	342	I (2.58)	0.85
E. tenella (Grunow) Hust.	11	4.13	1.08	574	442	I (1.00); V (1.89)	0.47
Fragilaria spp.	5	7.12	0.41	143	274		0.09
F. capucina Desm.	36	6.30	1.21	555	514	II (1.56); III (2.77); IV (2.84); V (10.13)	2.54
Fragilariforma virescens (Ralfs) D. M. & Williams Round	3	4.50	0.18	541	209		0.10
Frustulia rhomboides (Ehrenb.) DeToni	17	3.80	1.32	738	460	I (7.85); V (1.79)	1.69
F. vulgaris (Thwaites) DeToni	19	6.50	1.08	199	214		0.20
Geissleria decussis (Østrup) Lange-Bert. & Metzeltin	7	7.03	0.31	100	198		0.03
Gomphoneis olivacea (Hornemann) Bréb.	19	7.30	0.78	372	470		0.42
Gomphonema sp. 1	13	7.24	0.66	362	328		0.15
Gomphonema sp. 2	3	7.76	1.90	608	130		0.03
Gomphonema sp. 3	12	6.83	0.95	144	197		0.17
G. acuminatum Ehrenb.	7	7.12	0.24	119	130		0.12
G. gracile Ehrenb. emend. VanHeurck	7	7.14	0.25	220	338		0.04
G. micropus Kütz.	4	5.90	1.87	155	152		0.04
G. minutum C. Agardh	20	6.68	1.26	208	199		0.18
G. parvulum (Kütz.) Kütz.	37	6.32	1.33	284	360	IV (1.22)	0.58
G. sarcophagus W. Greg.	17	6.48	1.08	276	410		0.38
G. truncatum Ehrenb.	5	7.18	0.27	78	61		0.03
Gyrosigma acuminatum (Kütz.) Rabenh.	5	7.09	0.58	469	517		0.08
G. reimeri Sterrenb.	6	7.71	0.22	741	598		0.05
Hantzschia amphioxys (Ehrenb.) Grunow	3	5.32	1.21	213	176		0.03

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Taxon Periphyton	N	pH		SO_4 (mg l^{-1})		RIV for groups[1]	Mean RIV[2]
		Optimum	Tolerance	Optimum	Tolerance		
Hippodonta capitata (Ehrenb.) Lange-Bert., Metzeltin & Witkowski	4	7.37	0.42	448	559		0.01
Luticola mutica (Kütz.) D. G. Mann	9	6.47	1.20	140	166		0.11
Melosira varians C. Agardh	8	7.19	0.34	606	559		0.12
Meridion circulare (Grev.) C. Agardh	21	6.41	1.34	140	172	IV (1.21)	0.37
Navicula capitatoradiata Germain	12	7.62	0.28	254	181		0.33
N. cincta (Ehrenb.) Ralfs	4	7.51	0.21	463	530		0.02
N. cryptocephala Kütz.	26	7.08	0.48	186	317		0.29
N. cryptotenella Lange-Bert.	41	6.71	1.59	540	516	III (3.42)	1.74
N. gregaria (Donkin)	31	6.88	1.01	336	369	III (1.39)	0.67
N. lanceolata (C. Agardh) Kütz.	25	7.25	0.71	387	446	III (6.28); V (1.35)	2.36
N. menisculus Schum.	10	7.37	0.45	254	341		0.09
N. radiosa Kütz.	16	7.14	0.89	444	462		0.20
N. rhynchocephala Kütz.	5	6.15	1.20	242	335		0.09
N. tripunctata (Müll.) Bory	22	7.31	0.80	453	417	III (1.39)	0.54
N. trivialis Lange-Bert.	16	6.44	1.43	421	413		0.16
N. veneta Kütz.	3	7.44	0.35	153	83		0.01
N. viridula (Kütz.) Ehrenb.	20	7.11	0.52	382	521		0.20
Nitzschia acicularis (Kütz.) W. Sm.	18	7.46	0.26	608	602	II (1.39)	0.44
N. agnita Hust.	18	7.46	0.26	608	602		0.14
N. amphibia Grunow	11	7.45	0.39	492	614		0.08
N. angusteforminata Lange-Bert.	10	7.26	0.84	289	370		0.02
N. calida Grunow	3	7.76	0.09	699	604		0.05
N. capitellata Hust.	28	4.67	1.85	923	641	I (3.19); V (1.10)	1.11
N. communis Rabenh.	3	6.62	1.17	693	582		0.03
N. constricta (Kütz.) Ralfs	28	7.24	0.14	370	436	III (1.00)	0.10
N. dissipata (Kütz.) Grunow	18	7.41	0.38	455	545	II (1.35); III (3.61); IV (2.37)	1.85
N. dubia W. Sm.	35	7.24	0.87	357	446	III (1.09)	0.21
N. graciliformis Lange-Bert. & Simonsen	12	7.20	0.39	634	587		0.13
N. gracilis Hantzsch	7	6.95	0.38	252	184	IV (1.02)	0.31
N. inconspicua Grunow	18	6.91	1.10	612	594	II (1.03); III (2.90)	1.13

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Taxon Periphyton	N	pH		SO_4 (mg l^{-1})		RIV for groups[1]	Mean RIV[2]
		Optimum	Tolerance	Optimum	Tolerance		
N. linearis (C. Agardh) W. Sm.	22	7.47	0.50	313	395	II (1.27); III (1.09); IV 1.71	0.93
N. littoralis Grunow	32	6.72	1.33	368	446		0.07
N. microcephala Grunow	6	7.41	0.31	1417	916	II (2.55)	0.15
N. nana Grunow	9	5.83	1.77	460	349		0.12
N. ovalis Arn.	3	5.00	0.66	519	222	V (1.00)	0.20
N. palea (Kütz.) W. Sm.	35	7.12	0.83	452	479	III (1.17); IV (1.00)	0.70
N. perminuta (Grunow) M. Perag.	10	7.50	0.38	503	594	III (1.01)	0.13
N. recta Hantzsch	5	7.09	0.37	285	315		0.05
N. sigma (Kütz.) W. Sm.	4	7.26	0.35	408	566		0.03
N. vermicularis (Kütz.) Hantzsch	8	7.01	0.51	282	191		0.07
Pinnularia abaujensis (Pant.) Ross	3	4.16	0.41	584	155		0.16
P. braunii (Grunow) Cleve	10	4.28	1.46	580	438	I (1.02)	0.13
P. microstauron (Ehrenb.) W. Sm.	21	4.64	1.79	487	399	I (2.37); II (2.68);	3.35
P. obscura Krasske	12	4.82	1.26	318	266	V (4.97)	0.09
P. subcapitata W. Greg.	24	3.82	1.14	668	451	I (5.88); V (1.77)	1.19
Planothidium lanceolatum (Bréb.) Round & Bukht.	26	7.14	0.79	235	296	III (2.67)	0.54
Reimeria sinuata (W. Greg.) Kociolek & Stoermer	20	7.30	0.45	109	105	III(1.40)	0.59
Rhoicosphenia curvata (Kütz.) Grunow	27	7.28	0.93	707	588	IV (3.87)	1.16
Sellaphora pupula (Küz.) Mereschk.	4	7.22	0.91	95	124		0.02
Surirella sp. 1	3	7.44	0.70	270	368		0.01
Surirella sp. 2	4	7.28	0.53	673	679		0.04
S. amphioxys W. Sm.	11	5.23	1.56	246	273	V (11.07)	1.27
S. angusta Kütz.	20	6.86	0.88	290	350		0.13
S. brebissonii Krammer & Lange-Bert.	3	5.25	1.00	436	368		0.00
S. linearis W. Sm.	4	7.36	0.20	414	405	III (2.34)	0.02
S. minuta Bréb.	17	6.60	0.85	193	364		0.28
S. ovalis Bréb.	31	7.03	0.99	332	434	II (1.40); III (1.53); IV(1.59)	0.97
S. tenera W. Greg.	7	7.58	0.36	551	594		0.15

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Taxon Periphyton	N	pH		SO_4 (mg l^{-1})		RIV for groups[1]	Mean RIV[2]
		Optimum	Tolerance	Optimum	Tolerance		
Synedra delicatissima W. Sm.	7	6.15	1.91	215	151	II (1.43); IV (1.81)	0.60
Synedra tenera W. Sm.	13	6.74	1.15	168	225		0.22
S. ulna (Nitzsch) Ehrenb.	16	6.72	0.96	909	577	I (1.74); II (3.12); III (2.51); IV (4.21); V (1.65)	2.48
Tabellaria fenestrata (Lyngb.) Kütz.	36	6.07	1.82	484	583		0.11
T. flocculosa (Roth) Kütz.	8	5.62	1.27	265	187		0.13
			Chlorophyta				
Ankistrodesmus convolutus Corda	4	6.92	0.70	69	110		0.02
A. falcatus (Corda) Ralfs	3	5.81	2.16	434	616		0.12
Chaetophora elegans (Roth) C. Agardh	1	7.09	1.00	32	368	IV (1.74)	0.37
Chlamydomonas sp.	25	5.04	1.73	528	394		0.16
C. globosa Snow	11	7.15	0.67	247	476		0.07
Chlorella sp. 1	14	3.52	0.65	731	458	I (1.70); V (1.69)	0.67
Chlorella spp.	8	4.96	1.99	595	441		0.07
C. ellipsoidea Gerneck	11	6.70	0.99	133	154		0.11
C. vulgaris Beyerinck	25	7.11	0.80	404	432	IV (1.46)	0.34
Cladophora glomerata (L.) Kütz.	3	7.62	0.47	336	329	III (3.09)	0.99
Closterium acutum Bréb.	5	4.81	1.79	511	401		0.03
C. moniliferum Ehrenb.	9	6.94	0.48	261	416		0.14
Cosmarium spp.	6	6.53	1.94	597	632		0.27
C. contractum Kirch.	19	6.07	1.79	291	359	IV (1.56)	0.49
C. tetrapedia (Kirch.) West & West	1	6.96	1.00	27	368	III (1.20)	0.39
Crucigeniella rectangularis (Nägeli) Komárek	1	7.07	1.00	32	368		0.21
Gleocystis gigas (Kütz.) Lagerh.	6	3.75	1.41	651	314		0.15
Klebsormidium rivulare (Kütz.) Morison & Sheath	7	3.93	0.44	851	356	I (2.29)	0.70
Micrasteria radiata Hass.	3	6.66	0.75	86	78	III (1.17); IV (2.81)	0.98
Microspora amoena (Kütz.) Rabenh.	7	4.03	1.66	620	245		0.23

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Taxon		pH		SO_4 (mg l^{-1})		RIV for	Mean
Periphyton	N	Optimum	Tolerance	Optimum	Tolerance	groups[1]	RIV[2]
M. stagnorum (Kütz.) Lagerh.	8	3.44	0.96	670	279		0.23
M. tumidula Hazen	3	3.82	0.66	584	212		0.20
Microthamnion kuetzingianum Naegeli	13	5.04	2.13	502	407		0.09
Mougeotia spp.	10	4.67	1.64	614	489	I (1.60); III (1.24); IV (1.04)	1.18
Mougeotiopsis cf *calospora* Palla	2	3.56	0.35	949	212	I (1.31)	0.40
Oedogonium spp.	6	6.79	0.93	293	179		0.08
Scenedesmus bijuga (Turp.) Lagerh.	3	6.97	0.97	49	162		0.04
Sphaerocystis spp.	3	7.40	0.71	1520	368	II (2.21)	0.12
Spirogyra spp.	5	7.53	0.41	68	46	III (2.47)	0.12
Stigeoclonium spp.	3	7.03	0.21	196	640	IV (1.04)	0.10
Ulothrix spp.	11	5.35	1.62	629	497		0.23
U. subtilissima Rabenh.	7	6.54	0.70	146	406		0.11
			Chrysophyta				
Mallomonas spp.	4	7.38	0.53	24	368		0.05
M. cf *acaroides* Perty	6	6.86	0.44	172	245		0.03
M. pseudocoronata Prescott	3	4.30	1.11	794	453	V (1.18)	0.27
			Cyanobacteria				
Hapalosiphon aureus West & West	9	3.65	1.09	637	353	I (1.86)	0.59
Oscillatoria sp. 1	3	6.52	1.50	179	92		0.02
Oscillatoria spp.	4	7.37	0.25	209	551		0.03
O. anguina (Bory) Gomont	12	6.30	1.66	272	281	IV (2.87); V (6.57)	1.50
Jaaginema angustissimum (West & West) ex Anagn. et Komárek	9	7.08	0.37	468	502	II (1.63)	0.89
O. planktothris Gomont	2	7.65	0.74	60	1	III (2.42)	0.24
Phormidium inundatum Kütz.	9	5.46	1.05	987	597	III (1.01); V (11.07)	1.56
Plectonema sp.	23	5.58	1.63	542	454	I (1.01); III (2.22); IV (2.83); V (13.82)	2.53
Pseudoanabaena spp.	19	5.88	1.48	471	515	II (6.08); III (2.48); IV (7.13); V (1.88)	3.39

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Taxon Periphyton	N	pH		SO_4 (mg l^{-1})		RIV for groups[1]	Mean RIV[2]
		Optimum	Tolerance	Optimum	Tolerance		
Scytonema coactile Montagne	3	7.61	0.58	185	368		0.15
			Dinophyta				
Glenodinium spp.	3	7.01	1.11	137	193		0.08
G. cf *palustre* (Lemmerm.) Schiller	4	4.38	1.80	626	255		0.11
Gymnodinium spp.	5	5.28	2.05	1041	863		0.05
Peridinium spp.	5	4.89	2.01	433	299		0.03
P. cinctum (O. F. Müll.) Ehrenb.	8	4.31	1.97	492	375		0.06
			Euglenophyta				
Euglena sp. 1	3	5.16	1.74	233	182	I (2.41); V (1.09)	0.10
Euglena sp. 2	17	5.54	1.83	462	406	V (1.08)	0.37
E. minuta Prescott	8	2.82	1.44	1046	519		1.12
E. mutabilis Schmitz	8	3.45	0.50	925	363	I (3.56); V (1.03)	0.73
Phacus spp.	4	6.53	0.83	240	519		0.07
P. longicauda (Ehrenb.) Dujardin	3	7.69	0.05	612	662		0.20
Trachelomonas spp.	14	5.16	2.07	539	420		0.09
T. rotunda Swirenko	4	7.44	0.35	246	304		0.23
			Rhodophyta				
Audouinella hermanii (Roth) Duby	15	4.77	1.85	638	682	II (6.46); III (3.30); IV (3.16)	2.10
Batrachospermum spp.	3	6.76	0.20	60	368	III (2.47)	0.79
Chantransia "pygmaea"[3]	3	7.07	0.46	83	53	IV (2.90)	0.57
B. gelatinosum (L.) DeCanolle	3	7.46	0.70	88	368	IV (2.90)	0.62
			Xanthophyta				
Ophiocytitum parvulum (Perty) A. Braun	3	6.91	0.14	57	21		0.09
Tribonema affine West	3	5.59	2.03	77	97		0.12
T. bombycinum var. *tenue* Hazen	2	6.12	2.13	119	223	I (1.52)	0.03
T. bombycinum (C. Agardh) Derbés & Solier	4	5.51	2.00	513	589		0.15
Vaucheria spp.	3	6.01	0.20	323	289	III (1.50)	0.48
			Macroalgae				
			Bacillariophyta				
Melosira varians C. Agardh	7	7.39	0.32	335	377		0.59

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Taxon Periphyton	N	pH Optimum	pH Tolerance	SO_4 (mg l^{-1}) Optimum	SO_4 (mg l^{-1}) Tolerance	RIV for groups[1]	Mean RIV[2]
			Chlorophyta				
Chaetophora elegans (Roth) C. Agardh	4	7.27	0.37	392	810	II (2.93); III (4.44); IV (1.94)	0.77
C. incrassata (Huds.) Hazen	3	7.02	0.20	35	42	IV (4.84)	0.12
Chara spp.	3	7.02	0.21	1071	120	II (13.23)	0.39
Cladophora glomerata (L.) Kütz.	20	7.47	0.35	525	549	II (46.31); III (55.32); IV (21.51)	27.40
Dichotomosiphon tuberosus (A. Braun) Ernst	3	7.10	0.28	161	120		0.36
Draparnaldia plumosa (Vauch.) C. Agardh	5	7.20	0.30	52	38	IV (8.02)	0.18
Klebsormidium rivulare (Kütz.) Morison & Sheath	14	4.69	1.39	527	342	I (41.76); III (1.02); V (32.4)	13.72
Microspora amoena (Kütz.) Raben.	3	6.98	0.12	535	358	III (3.23)	
M. pachyderma (Wille) Lagerh.	7	4.28	2.07	482	292	I (10.54); III (1.26); V (1.66)	2.75
M. stagnorum (Kütz.) Lagerh.	3	7.36	0.51	299	215	IV (7.36)	1.92
M. tumidula Hazen	8	3.75	0.97	601	272	I (23.89); V (12.56)	6.06
Mougeotia spp.	24	5.48	1.63	550	403	I (9.53); II (2.18); III (4.57); IV (3.51); V (22.93)	7.47
Oedogonium spp.	10	7.48	0.21	387	258	II (2.58); III (1.74)	0.90
Protoderma viride Kütz.	3	7.10	0.51	996	806		0.38
Spirogyra spp.	14	7.17	0.40	154	147	II (7.83); III (1.24); IV (8.15)	3.57
Stigeoclonium subsecundum Kütz.	3	6.99	0.25	280	200	III (1.15)	0.56
Tetraspora gelatinosa (Vauch.) Desvaux	3	7.42	0.36	142	116	III (3.18); IV (1.83)	1.65

(*Continued*)

(*Continued*)

Taxon Periphyton	N	pH		SO_4 (mg l^{-1})		RIV for groups[1]	Mean RIV[2]
		Optimum	Tolerance	Optimum	Tolerance		
Ulothrix subtilissima Rabenh.	5	6.68	1.57	584	344	II (1.66)	0.40
Ulothrix tenerrima Kütz.	3	7.03	0.28	1040	812		0.36
Zygnema spp.	4	6.16	1.61	309	388	I (1.15); IV (1.95)	0.68
Cyanobacteria							
Geitlereinema splendidum (Grev.) *ex* Anagn. *et* Komárek	3	5.62	1.43	365	69	II (3.24); V (4.27)	0.66
Oscillatoria anguina (Bory) Gomont	2	7.35	0.38	715	774		0.10
Phormidium amoenum (Kütz.) *ex* Anagn. *et* Komárek	2	7.26	0.28	398	860		0.14
P. nigrum (Vauch.) *ex* Anagn. *et* Komárek	3	7.35	0.18	370	178	II (3.28)	0.29
P. retzii (ag.) Gomont	2	5.38	1.85	220	107	V (6.29)	1.08
Pseudoanabaena spp.	4	7.17	0.37	53	40	IV (1.4)	0.38
Euglenophyta							
Euglena mutabilis Schmitz	3	2.58	1.22	1116	258	I (11.89)	2.28
Rhodophyta							
Batrachospermum gelatinosum (L.) DeCandolle	5	7.38	0.39	134	163	IV (18.51)	4.80
Batrachospermum helmentosum (Bory) St.Vincent	6	7.16	0.54	595	362	III (11.79); IV (1.98)	2.99
Xanthophyta							
Vaucheria spp.	7	7.13	0.36	538	360	II (2.42); III (4.64); IV (3.82)	2.29
V. geminata (Vauch.) DeCandolle	3	7.15	0.46	1072	623	III (4.31); IV (1.26)	1.95
V. polysperma Hassall	12	5.63	1.33	392	121	II (11.3); III (3); V (25.65)	5.69

[1]RIVs for influential taxa which had mean RIV > 1.00 in a given multivariate group. Group I–V correspond with the descriptions in the text.

[2]Mean RIV for taxon across all 56 stream sites.

[3]Alternate life history stage of taxa in the Batrachospermales, but may not be the alternate stage for the gametophyte present.

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