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Use of periphyton assemblage data as an index of biotic integrity

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Abstract. Periphyton assemblage data collected from 233 stream site-visits (49 in 1993, 56 in 1994, and 128 in 1995) throughout the Mid-Appalachian region were used to develop a periphyton index of biotic integrity (PIBI) based on 1) algal genera richness; 2) the relative abundances of diatoms, Cyanobacteria, dominant diatom genus, acidophilic diatoms, eutraphentic diatoms, and motile diatoms; 3) chlorophyll and biomass (ash-free dry mass) standing crops; and 4) alkaline phosphatase activity. Thirty-seven diatom genera and 38 non-diatom genera were collected. The relative richness and relative abundance (RA) of these genera were used to calculate the RA metrics of the PIBI. PIBI scores ranged from 48.0 to 85.1 among the 233 site-visits with an overall regional mean ($\pm$1 SE) of 66.1 $\pm$ 0.5. The 10 metrics and the PIBI were correlated with 27 chemical, 12 physical habitat, and 3 landscape variables. Overall, PIBI was inversely correlated with stream depth, stream water color, and Fe. Component metrics were significantly correlated with several chemical (Al, acid neutralizing capacity, Cl, Fe, Mn, N, Na, P, pH, Si, SO_4, total suspended solids), physical habitat (channel embeddedness, riparian disturbances, stream depth, stream width, substrate composition), and landscape (% of the watershed in forest, agriculture, and urban land uses) variables. Canonical correlation analysis revealed significant correlations between the 10 PIBI metrics and 4 significant environmental gradients related to general human disturbances (stream acidity, stream substrate composition, and stream and riparian habitat). Analysis of variance revealed significant differences in PIBI scores for lowland vs highland streams, and among stream orders. Annual differences were explained by differences in the proportions of sampling sites in lowland streams in each year. The univariate distribution of PIBI scores was used to set threshold PIBI values for the assessment of ecological condition in Mid-Appalachian streams.

Key words: periphyton, IBI, EMAP, regional scale, stream condition, multimetrics.

The use of biological communities as indicators of water quality is evolving as our understanding of the interactions between water quality and the integrity of biological communities improves (Karr et al. 1986, Hughes et al. 1991, Dixit et al. 1992). Stream periphyton assemblages, although not widely used, are ideally suited for water-quality assessment (Patrick 1973, Stevenson and Lowe 1986, Rott 1991, Round 1991, van Dam et al. 1994, Stevenson and Pan 1999). Analysis of periphyton assemblages may focus on taxonomic or non-taxonomic features. Taxonomic descriptors (e.g., diversity indices, taxa richness, indicator species) are commonly used, and are described by several researchers (e.g., Patrick 1973, Palmer 1977, Rodgers et al. 1979, Weitzel 1979). Non-taxonomic measures (e.g., biomass and chlorophyll per unit area) can also be useful for detecting effects not indicated by taxonomic analysis. For example, toxic pollutants may cause sublethal (i.e., reproductive) effects that would not immediately be detected by taxonomic descriptors such as taxa richness, but might be indicated by changed chlorophyll content, or enzyme activity. A summary of non-taxonomic measurements is presented in Weitzel (1979).

There have been 3 basic approaches to using

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periphyton to assess stream water quality. The 1st, and oldest, approach is based on the indicator species concept, whereby known tolerances of species of algae are used to determine water quality. The oldest of these approaches is the Saprobien system (Kolkwitz and Marsson 1908), which has undergone many revisions but is still widely used in municipal water and wastewater monitoring (Lange-Bertalot 1979, Friedrich et al. 1992). The Saprobien system has proven to be less useful in monitoring stream ecosystem degradation where there is no clear link to nutrient enrichment or where assemblage richness is low (Patrick 1973, Guzkowska and Gasse 1990).

The 2nd approach is based on the assumption that pristine environments support a greater diversity of organisms than do degraded environments, and that measurement of community structure reveals health of a system. Indices of community structure (diversity, evenness, richness, similarity) have been used extensively in monitoring the impacts of point-source pollution on streams (e.g., Freidrich et al. 1992). However, several studies of community structure indices have questioned their reliability. For example, Boyle et al. (1990) reported that the most commonly used indices varied considerably in response to deletions of species from an assemblage. Patrick (1977a) demonstrated that assemblages with similar diversity scores could represent streams with markedly different chemical conditions. Similarly, Descy (1979) pointed out that diversity might be high in stressed communities with low numbers of individuals but many species. Conversely, some streams exhibit low diversity even in the absence of pollution.

Biotic indices represent the 3rd approach to using biological assemblages to assess water quality and stream ecosystem integrity (Fausch et al. 1984, Karr et al. 1986, Kerans and Karr 1994). Biotic indices have been developed to avoid the problems associated with both the indicator species and community structure approaches to water-quality assessment, and are based on correlation of assemblage data with current and past physico-chemical parameters. Multivariate analyses of environmental data and their correspondence with assemblages of organisms are a related approach for characterizing ecological conditions in lakes and streams (e.g., Fritz et al. 1993, Dixit and Smol 1994, Pan et al. 1996, Reynoldson et al. 1997). Comprehensive multimetric indices have been developed and extensively used for stream fish and macroinvertebrates (e.g., Karr et al. 1986, Kerans and Karr 1994), although similar indices based on periphyton have received much less attention (Bahls 1993, Mills et al. 1993).

Indices of biotic integrity should be designed to use measures of species richness, trophic structure, and organismal abundance. The overall index that results from the sum of these metrics should be responsive both to specific sources of stress and to general, cumulative perturbations (Karr 1993). Development of a multimetric index of stream ecosystem integrity usually requires the establishment of reference conditions against which a stream may be evaluated. This criterion may be difficult to achieve for regional-scale surveys based on random sampling. Data from probability surveys may still be subjected to testing of each metric to ensure that its response to perturbations is directional and predictable. Once a suite of metrics has been evaluated, a composite index may be evaluated (Karr 1993, Gerritsen 1995, Reynoldson et al. 1997).

Our objective was to develop a periphyton index of biotic integrity (PIBI) modeled after those developed for fish (Karr et al. 1986) and macroinvertebrates (Kerans and Karr 1994). We focused our examinations of the periphyton community on 10 measures that included taxa richness, relative abundance of indicator taxa, and trophic condition, and their associations with 42 stream chemistry, physical habitat, and landscape attributes.

Methods

Study area and survey design

The Mid-Appalachian region extends northeast from northern North Carolina to the Catskill Mountains of New York, and west from the eastern seaboard to the Western Allegheny Plateau of eastern Ohio (Fig. 1). The area includes the Blue Ridge, Central Appalachian Plateau, Central Appalachian Ridge and Valley, Northern Appalachian Plateau and Uplands, Western Allegheny Plateau, Piedmont, and Coastal Plain ecoregions (Omernik 1987). The Piedmont and Coastal Plain ecoregions were combined for our research. We also aggregated streams located in the Piedmont/Coastal Plain, Central Appalachian Valley, and Western Allegheny Plateau

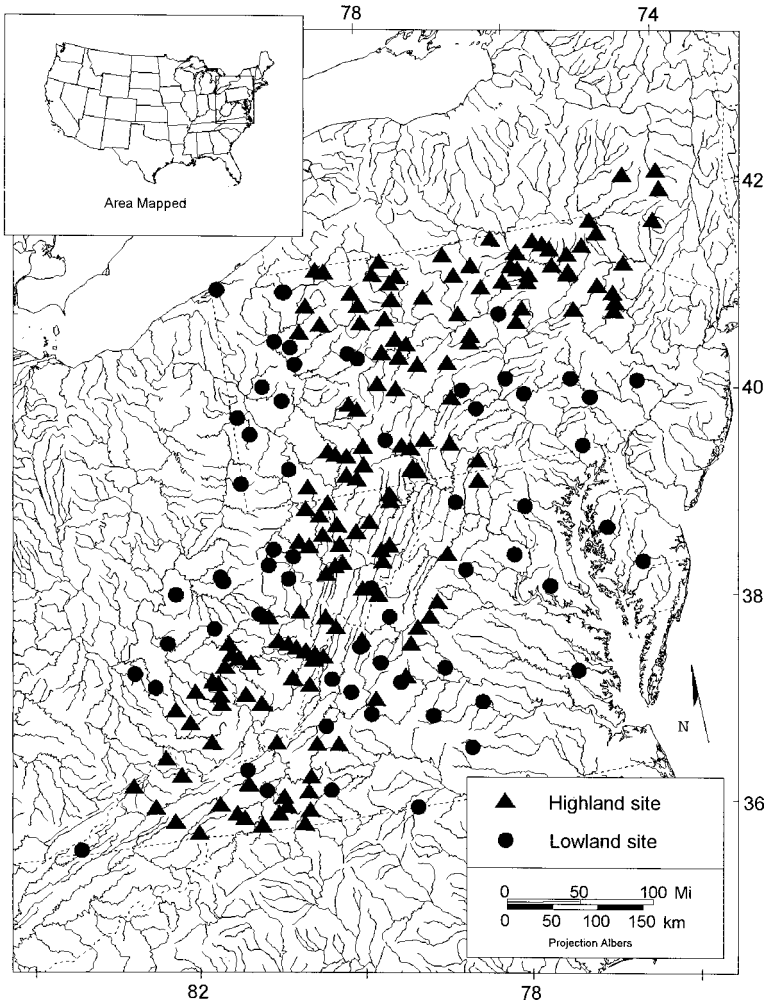

FIG. 1. Mid-Appalachian region with locations of 1993 to 1995 Environmental Monitoring and Assessment Program (EMAP) probability sites. Solid lines are major river systems. Dashed lines are state boundaries. Numbers along the north-south border are latitude (°) and those along the east-west border are longitude (°).

ecoregions into a lowland class, and the remainder were classified as highland streams.

Data used in our analyses were compiled from the US Environmental Protection Agency's (USEPA) Environmental Monitoring and Assessment Program (EMAP) surveys conducted from 1993 to 1996 (Fig. 1). Sampling sites were selected using a randomization method with a spatially systematic component (Herlihy et al., in press). The stream network on the digitized version of the 1:100,000-scale US Geological Survey topographic maps was used as the sample frame. The survey was restricted to wadeable streams defined as 1st-, 2nd-, and 3rd-order (Strahler 1957) on the 1:100,000-scale map. Sample probabilities were set so that collections would be taken from roughly equal numbers of 1st-, 2nd-, and 3rd-order streams. Streams were sampled from late April to early July each year, a period that was a compromise of the preferences among project chemists and fish, macroinvertebrate, and periphyton ecologists. Periphyton was sampled in 49 streams in 1993, 56 in 1994, and 128 in 1995. To test for changes in the biotic community related to time of year, or year of sampling, 3 sites in 1993, 4 sites in 1994, and 11 sites in 1995 were sampled both near the beginning (April–May) and end (June–July) of the

sampling period. The 1993 and 1994 revisited sites were also sampled in subsequent years to answer questions about between-year variability. Data were collected along a length of stream equal to 40 times the mean wetted width (minimum of 150 m and maximum of 500 m) centered around each randomly chosen sampling point. In total, data were collected from 199 different streams (233 site visits) representative of 164,800 km of wadeable streams in the Mid-Appalachian region.

Chemistry

A 4-L cubitainer and four 60-mL syringes of stream water were collected in flowing water near the middle of the stream at each sampling site. The syringes were sealed with a Luer-lock valve to prevent gas exchange. All samples were placed on ice and sent by overnight courier to the University of Maine's analytical laboratory. The syringe samples were analyzed for pH, dissolved inorganic carbon (DIC), and monomeric Al, and the cubitainer sample was split into aliquots and preserved within 48 to 72 h of collection (Table 1). Detailed information on the analytical procedures used for each of the aliquots can be found in USEPA (1987). In brief, base cations, Fe, and Mn were determined by atomic absorption, anions (SO_4, NO_3, Cl) by ion chromatography, dissolved organic (DOC) and inorganic (DIC) carbon by a carbon analyzer, and total N and P by persulfate oxidation and colorimetry (Table 1).

Physical habitat and riparian disturbance

Physical habitat data were collected from longitudinal profiles and from 11 cross-sectional transects evenly spaced along the stream reaches sampled. Maximum (thalweg) depth was measured at 100 (150 for streams <2.5 m wide) evenly spaced points along the stream reach. The proportion of the stream in riffles or pools was recorded while measuring the thalweg. Transect data included channel dimensions (width, depth, bank angles), visual estimates of average substrate size, channel gradient, bearing (for calculating sinuosity), riparian vegetation cover and structure, and the occurrence and proximity of riparian human disturbances (e.g., roads, buildings, agriculture) (Table 1). Physical habitat data were used as either means for the entire reach or means weighted for their proximity to the stream (Kaufmann et al. 1999). Physical habitat and riparian disturbance data were collected only in 1993 and 1994.

Landscape characterization

Land-cover data (Table 1) were derived from leaves-on and leaves-off Landsat satellite thematic mapper scenes acquired from 1991 to 1993, which were projected to Lambert Azimuthal coordinates (Vogelmann et al. 1998). The 30-m^2 picture elements (pixels) of the Landsat scenes were clustered into 100 spectrally distinct classes using an unsupervised clustering algorithm (Kelley and White 1993). Aerial photographs and supplemental data were used to interpret the classes and assign them to 1 of 15 land-cover categories. Geographic information system overlay techniques were then used to determine the % of these categories within each watershed sampled in 1993 and 1994.

Periphyton collection and identification

Periphyton were collected from randomly selected positions (left, center, or right channel) within riffles at each of the interior 9 transects along the stream reach. At each transect, periphyton were collected from a 12-cm^2 area of the stream bed within a 3.9-cm-diameter piece of PVC pipe. Periphyton were loosened from the surface of cobble-size (6-cm diameter) or larger substrates with a toothbrush and rinsed with stream water into a collection bottle. Smaller substrates were sampled by delineating the stream bed with the PVC pipe, agitating the substrate, and suctioning the overlying water. The composite periphyton sample for each reach was subsampled for subsequent taxonomic identification, chlorophyll content, biomass, and alkaline phosphatase activity (methods below). Total and subsample volumes were noted.

We focused on generic-level identification to develop an index that could be used by those who are not experts in algal taxonomy. We aggregated species-level taxonomy and environmental preferences to produce the genus-level environmental preferences of the diatom assemblage. Several researchers have proposed the genus as the appropriate taxonomic level for water-quality assessment (Coste et al. 1991, Prygiel and Coste 1993, Kelly et al. 1995). Although sev-

TABLE 1. Description and measured ranges of chemical, physical habitat, and landscape variables from streams included in this study.

Variable code	Description	Units	Range
Chemical variables (n = 233)			
ALKCALC	Calculated alkalinity	μeq/L	−32–5373
ALTD	Total dissolved Al	μg/L	0–1730
ANC	Acid neutralizing capacity	μeq/L	−48–5520
ANSUM	Sum of anions	μeq/L	78–21,419
CA	Ca	μeq/L	33–12,016
CATSUM	Sum of cations	μeq/L	89–21,478
CL	Cl	μeq/L	8–1163
CO3	CO_3	μeq/L	0–96
COLOR	Color	PCU (true color units)	0–93
COND	Conductivity	μS/cm	12–1860
FE	Fe	μg/L	0–910
HCO3	HCO_3	μeq/L	1–5333
IONSTR	Ionic strength	mM	0–80
K	K	μeq/L	7–145
MG	Mg	μeq/L	28–7107
MN	Mn	μg/L	0–7190
NA	Na	μeq/L	7–2175
NH4	NH_4	μeq/L	0–62
NO3	NO_3	μeq/L	0–722
NTL	Total N	μg/L	63–16,260
ORGION	Estimated organic anions	μeq/L	5–120
PH	pH		4.48–8.37
PTL	Total P	μg/L	1–369
SIO2	SiO_2	mg/L	1–27
SO4	SO_4	μeq/L	21–19,900
SOBC	Sum of base cations	μeq/L	87–21,416
TSS	Total suspended solids	mg/L	0–206
TURB	Turbidity	NTU (nephelometric turbidity units)	0.3–92
Physical habitat variables (n = 103)			
PCT_FN	% of the streambed as fine-grained (≤0.06-mm diameter) sediments	%	0–71
PCT_SA	% of the streambed as sand (0.06–2.0-mm diameter)	%	0–87
PCT_SAFN	% of the streambed as sand + fine-grained sediments (≤2.0-mm diameter)	%	0–98
PCT_SFGF	% of the streambed sediments ≤ small gravel (16-mm diameter)	%	4–100
RP100	Mean (n = 100) residual stream depth	cm	0.1–29
SINU	Channel sinuosity	m/m	1–2
XCDENMID	Mean canopy density, mid-channel	%	0–100
XEMBED	Mean channel embeddedness	%	4–100
XSLOPE	Mean stream reach channel slope	%	0.5–40
XWIDTH	Mean stream channel wetted width	m	0.3–16
W1_HAG	Sum of all agricultural activity adjacent to stream, proximal weighted presence, scale 0 to 5		0–1.8
W1_HALL	Sum of all human disturbances adjacent to stream, proximal weighted presence, scale 0 to 5		0–4.8
WD_RAT	Mean stream width/depth ratio	m/m	8–50

TABLE 1. Continued.

Variable code	Description	Units	Range
Landscape variables (n = 101)			
TMAGTOT	Watershed land use in agriculture, thematic mapper data	%	0–88
TMFORTOT	Watershed land use of forests, thematic mapper data	%	12–100
TMURBTOT	Watershed in urban and suburban uses, thematic mapper data	%	0–19

eral limitations have been described for genus-level indices (Round 1991, Stevenson and Pan 1999), Kelly et al. (1995) found that this level of taxonomy yielded similar results to the species level when used in benthic diatom indices.

Subsamples for identification of periphyton taxa were preserved on site with formalin (5% v/v). Each subsample was mixed in a high-speed blender and at least 300 algal cells with protoplasm were counted in a Palmer counting cell at 400× magnification. For filamentous algae, a 10-μm length was counted as a single cell. This procedure allows for unbiased characterization of algal assemblages dominated by filamentous algae. Non-diatom algae were also identified to genus.

Diatom frustules were cleaned with concentrated H_2SO_4 and $K_2Cr_2O_7$ to facilitate accurate identification (Patrick and Reimer 1966). After rinsing with distilled water, the cleaned diatom frustules were mounted in NAPHRAX mounting medium. A minimum of 500 diatom valves in each sample was counted at 1000× magnification. The primary references for diatom taxonomy were Patrick and Reimer (1966, 1975) and Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b).

The % of protoplasm-containing diatoms and non-diatom algae was determined by dividing the number counted by the total Palmer cell count. Diatom % was determined by dividing the diatom count by the sum of diatom and non-diatom algal counts. Similarly, % of Cyanobacteria was estimated as the number of Cyanobacteria cells divided by total algal count. Environmental preferences of the diatom genera were taken from published research (Lowe 1974, Christie and Smol 1993, van Dam et al. 1994), and were used to determine which genera were included in the determination of the relative abundance (RA) of acidophilic and eutraphentic diatoms (i.e., diatoms with an affinity for nutrient-enriched environments) (Table 2). Motile diatoms included raphid pennate genera (Round et al. 1990) (Table 2). The % acidophilic, eutraphentic, and motile diatoms was calculated by dividing the sum of these indicator taxa by the total diatom count.

Subsamples for chlorophyll *a* and ash-free dry mass (AFDM) were filtered onto pre-leached, pre-ashed, pre-weighed glass fiber filters (1.0 μm average pore size). Filters were wrapped in foil, frozen, and analyzed within 30 d of collection. Periphyton AFDM was determined by measurement of mass loss resulting from incineration (525°C, 30 min) of the sample collected on glass fiber filters. Chlorophyll *a* was removed from each filter by leaching in cold (4°C), 90% (v/v) acetone for 18 h. Light absorption by the leachate was measured in a spectrophotometer at 750 and 664 nm and, again, after acidification with 1 N HCl, at 750 and 665 nm. Chlorophyll *a* concentration was normalized for subsample area and AFDM (APHA 1995).

Alkaline phosphatase activity (APA) was analyzed spectrophotometrically as p-nitrophenol (p-NP) resulting from the cleavage of phosphate from p-nitrophenylphosphate (p-NPP) (Sayler et al. 1979). Samples were prepared by mixing 2 mL of filtered (1.0 μm) residue removed from the rocks with 4 mL of Tris buffer (pH 8.5) or citrate buffer (pH 4.5) and adding 1 mL of p-NPP. Because we were interested in actual rather than potential levels of APA, samples were incubated in the dark for 1 h at ambient stream temperatures, without prior sonification. Activity was stopped by the addition of 1 mL 20% K_2HPO_4. p-NP standards were used to convert absorbance of sample p-NP to units (ng) per unit periphyton AFDM.

TABLE 2. Diatom and non-diatom algal genera present in streams of the Mid-Appalachian region. Letters in parentheses indicate acidophilic (a), eutraphentic (e), and motile (m) diatom taxa, and Cyanobacteria (c).

Diatom genera	Non-diatom genera
Achnanthes	*Actinastrum*
Actinella	*Anabaena* (c)
Amphora (e)	*Ankistrodesmus*
Anomoeneis (m)	*Arthrodesmus*
Asterionella (e)	*Batrachospermum*
Aulocoseira	*Calothrix* (c)
Bacillaria	*Chaetophora*
Caloneis (m)	*Characium*
Cocconeis (e)	*Chroococcus* (c)
Cymbella	*Cladophora*
Cymatopleura (e)	*Closterium*
Cyclotella	*Cosmarium*
Diatoma (e)	*Cryptomonas*
Diploneis (m)	*Euglena*
Entomoneis (m)	*Geminella*
Eunotia (a)	*Gloecystis* (c)
Fragillaria (e)	*Hypnodium*
Frustulia (a, m)	*Lyngbya* (c)
Gomphonema (e)	*Mallomonas*
Gomphoneis	*Merismopedia* (c)
Gyrosigma (e, m)	*Microspora*
Hantzschia	*Microthamnion*
Melosira (e)	*Mougeotia*
Meridion (e)	*Oedogonium*
Navicula (m)	*Oocystis*
Neidium	*Ophiocytium*
Nitzschia (e, m)	*Oscillatoria* (c)
Pinnularia (a, m)	*Pediastrum*
Rhoicosphenia (e)	Phacus
Rhopalodia	*Phormidium* (c)
Stenopterobia	*Rhodocorton*
Stauroneis (m)	*Scenedesmus*
Stephanodiscus (e)	*Selenastrum*
Surirella (m)	*Spyrogyra*
Synedra (e)	*Staurastrum*
Tabellaria (a)	*Stigeoclonium*
Thalassiosira (e)	*Tribonema*
	Ulothrix

We expected both positive and negative responses for chlorophyll *a*, biomass, and APA to anthropogenic stressors, so median regional values for these variables were used as the reference values for these metrics. Metric scores were proportionally decreased as chlorophyll, biomass, and APA deviated from median values.

Description of metrics

The 10 PIBI metrics fell into 3 categories: 1) taxonomic richness, 2) stressor characterization, and 3) trophic condition (Table 3). All metrics were scored from 0 to 10, with decreasing metric scores corresponding to increasing anthropogenic disturbance.

Relative taxa richness.—Diatom species richness usually decreases as a result of stream contamination by organic enrichment (Amblard et al. 1990, Whitton et al. 1991), metals (Pratt et al. 1987, Crossey and La Point 1988, Scanferlato and Cairns 1990, Sudhakar et al. 1991, Whitton et al. 1991), and pesticides (Kosinski 1984), although some researchers have reported increases in richness under moderate stress (Archibald 1972, Patrick 1973, van Dam 1982, Stevenson 1984). We expected taxa richness in Mid-Appalachian streams to be inversely related to environmental stressors.

Diatom metric.—Organic enrichment or highly toxic conditions cause shifts in algal communities from domination by diatoms to domination by non-diatom taxa, especially green algae and the Cyanobacteria (Palmer 1969, Patrick 1977b, Bott and Rogenmuser 1978, Steinman et al. 1991). Therefore, we expected the % of diatoms to decline with increasing environmental stressors.

Cyanobacteria metric.—Unlike the % of diatoms, we expected the % of Cyanobacteria to increase with increases in environmental disturbances, especially as a result of nutrient and organic enrichment and exposure to toxic substances (Palmer 1969, Patrick 1977b, Bott and Rogenmuser 1978, Steinman et al. 1991, Leland 1995).

Dominant diatom metric.—The relative abundance of any genus of diatom can influence the evenness of diatom taxonomic representation within an assemblage. Species better adapted to unfavorable conditions (e.g., nutrient enrichment or toxic stress) will have an advantage resulting in an uneven distribution of individuals among taxa (Archibald 1972, Patrick 1973, Weber and McFarland 1981, Sudhakar et al. 1991, McCormick and Cairns 1997, Stevenson and Pan 1999). We expected the % dominance by a single taxon to increase with increasing environmental stress.

Acidophilic diatoms metric.—The % of acidophilic diatoms in an assemblage has been exten-

TABLE 3. Metrics included in the periphyton index of biotic integrity (PIBI). AFDM = ash free dry mass, APA = alkaline phosphatase activity, Chl = chlorophyll.

Metric	Calculation	Range	Score[a]
Relative taxa richness	No. algal genera / Expected no. algal genera[b]	0–1	0–10
Diatom metric	No. diatom cells / Total no. algal cells	0–1	0–10
Cyanobacteria metric	1—(No. Cyanobacteria cells / Total no. algal cells)	0–1	0–10
Dominant diatom metric	1—(No. dominant diatoms / Total no. diatoms)	0–1	0–10
Acidophilic diatoms metric	1—(No. acidophilic diatoms / Total no. diatoms)	0–1	0–10
Eutraphentic diatoms metric	1—(No. eutraphentic diatoms / Total no. diatoms)	0–1	0–10
Motile diatoms metric	1—(No. motile diatoms / Total no. diatoms)	0–1	0–10
Chlorophyll metric[c]	6.67 / (Absolute value of 6.67 ± Chl [mg / m^2])	0–1	0–10
Biomass metric[c]	0.006 / (Absolute value of 0.006 ± AFDM [g / m^2])	0–1	0–10
Phosphatase activity metric[c]	18.2 / (Absolute value of 18.2 ± APA [nmol g^{-1} h^{-1}])	0–1	0–10
Range of potential PIBI scores:			**0–100**

[a] Score range is calculated by multiplying raw range by 10, so the 10-metric PIBI total equals 0 to 100
[b] Expected number of genera is the observed maximum genera richness for each year
[c] Chl, AFDM, and APA are 2-tailed metrics, which have low scores when both lower and higher than median standing crops. Median values for each metric are given in the numerator of each formula

sively used in the reconstruction of lake acidification (Charles 1985, Birks et al. 1990, Dixit et al. 1992), but less often in analyses of streams (Pan et al. 1996). As pH decreases, we expect the diatom community to shift to dominance by those taxa that can tolerate pH <5.5.

Eutraphentic diatoms metric.—Eutraphentic diatoms have been widely used in the identification and assessment of sites impacted by nutrients (Palmer 1969, Lange-Berlatot 1979, Hall and Smol 1992, Christie and Smol 1993, Pan et al. 1996). We expected an increase in the % of eutraphentic diatoms with increasing nutrient and organic matter enrichment of Mid-Appalachian streams.

Motile diatoms metric.—Most anthropogenic activities within a watershed generate silt in receiving streams, even if no chemical signatures of such disturbance are present. The % of motile diatoms (Table 2) has been used as an index of siltation in Montana streams (Bahls 1993), and is cited as a potential metric by other investigators (Mills et al. 1993, Stevenson and Pan 1999). We expected the % of motile diatoms to increase with increasing siltation in streams.

Chlorophyll metric.—Chlorophyll *a* concentration has been widely used to assess nutrient enrichment of streams, even in regional-scale studies (Leland 1995, Pan et al. 1999). We used the median value of 6.67 mg chlorophyll *a* / m^2 for Mid-Appalachian streams as the reference value for the chlorophyll metric. We expected the chlorophyll metric, which decreases for both positive and negative deviations from the regional median chlorophyll *a* concentration, to decrease with increasing nutrient, organic matter, and contaminant loadings to the stream.

Biomass metric.—The relationship between standing crop and water quality is not easily interpreted. Leland (1995) reported increased periphyton biomass in response to nutrient loading and agricultural land use, whereas others have reported reductions in stream periphyton biomass in response to chemical perturbations (Sigmon et al. 1977, Clark et al. 1979, Boston et al. 1991). A median value of 0.006 g AFDM / m^2 was used as the reference value for the biomass metric. We expected the biomass metric, which decreases for both positive and negative deviations from the regional median AFDM concentrations, to decrease with increases in nutrients, organic matter, and toxicants.

Phosphatase activity metric.—Periphyton APA has proven useful as an indicator of phosphorus limitation in freshwater ecosystems (Healey and Hendzel 1979, Mulholland and Rosemond 1992), and of microbial community inhibition by toxicants (Sayler et al. 1979, 1982, Tyler 1976, Tu 1981, Baker and Morita 1982, Burton and Lanza 1987). A regional median phosphatase value of 18.2 ng g^{-1} AFDM h^{-1} was used as the reference value for the phosphatase metric. We expected the phosphatase metric, which decreases for both positive and negative deviations from the regional median APA values, to decrease with

nutrient enrichment and contaminant loading in Mid-Appalachian streams.

Evaluation of metrics

Individual metrics and the composite index were evaluated using Spearman correlation of dependent variables (metrics, index) against independent variables (physico-chemical and landscape variables). The environmental variable dataset was restricted to the 21 chemical, habitat, and landscape variables having significant correlations with the PIBI metrics (see below). The number of variables used in the canonical analyses was restricted to maintain a variable to sample ratio of <0.10 (Gittins 1985). Canonical correlation analysis was used to investigate the multivariate relationships among PIBI metrics and environmental variables. Significant canonical correlations ($p < 0.05$) were estimated as $r = \sqrt{(n - 2)/(l - r^2)}$ (Rohlf and Sokal 1969).

Individual metrics were tested for multicollinearity, the degree of co-dependence among independent variables, to ensure that the PIBI was not biased by one or a few metrics. Condition index, the square root of the largest eigenvalue to each individual eigenvalue, was used as the measure of multicollinearity. Condition indices >30 indicate potential multicollinearity (Montgomery and Peck 1982).

Single classification analysis of variance (ANOVA), with Scheffe's multiple comparison of means, was used to compare PIBI scores among landforms, drainage basins, ecoregions, and stream orders. All statistical analyses were performed using SAS software for personal computers (SAS for Windows, Release 7.0, SAS Institute, Cary, North Carolina, USA).

Results

Environmental variables

All of the chemical, physical habitat, and landscape variables were significantly correlated with at least 1 PIBI metric, but only those 3 variables with the highest R_s for each metric were selected for inclusion in canonical correlation analysis (Table 4). Canonical correlation analysis between the PIBI metrics and environmental variables resulted in 4 significant canonical axes (Table 5). The 1st canonical axis (W_1) represented an overall disturbance gradient, with significant correlations for chemical (ANC, Cl, Na, pH, SO_4), physical habitat (PCT_SAFN, PCT_SFGF, W1_HAG, W1_HALL), and land-use (TMAGTOT, TMFORTOT, TMURBTOT) variables. This axis described a gradient from forested streams to streams affected by agricultural activities. W_2 represented a stream acidification gradient, with significant correlations with dissolved Al and color on the positive end and pH on the negative end. W_3 represented a combination of chemical and habitat variables related to human activities, and was significantly correlated with Cl, Fe, pH, and % of the streambed in small gravel, sand, and fine sediments. This axis described a gradient ranging from narrow, forested streams to wider streams with less riparian cover that were also affected by Cl, Fe, and sediments. W_4 represented stream habitat features related to increasing pool habitat and riparian-zone agriculture, and described a gradient from shallow, narrow streams to wider, deeper streams having elevated ANC. Overall, these 4 axes explained 48%, 13%, 11%, and 10%, respectively, of the metric score variance for Mid-Appalachian streams.

PIBI metrics

Relative taxa richness.—Thirty-seven genera of diatoms and 38 genera of non-diatom algae were collected from streams of the Mid-Appalachian region (Table 2). Diatom richness in these streams ranged from 1 to 17 genera, with an average of 8 genera per stream. Non-diatom taxa richness ranged from 0 to 6 genera, with a mean of 2 genera per stream. Overall periphyton taxa richness ranged from 3 to 21 genera, with a regional mean of 10 genera per stream. The maximum observed richness was 19 genera in 1993, 18 genera in 1994, and 21 genera in 1995. These values were used as the standard against which all other streams within a year were evaluated for relative taxa richness. Contrary to our expectations, taxa richness was positively correlated with Cl, Fe, and Mn concentrations (Table 4), and with W_3 (Table 6).

Diatom metric.—The diatom metric was negatively correlated with mean stream depth and width, and positively correlated with % of the stream substrates ≤16 mm in diameter (Table 4). The diatom metric was not significantly correlated with any of the canonical axes, suggesting that % diatoms in the algal assemblage may

TABLE 4. Three strongest Spearman correlation coefficients (R_s) and significance ($p < x$) for the relationship between the periphyton index of biotic integrity (PIBI) and its component metrics, and chemical, physical habitat, and landscape variables in streams of the Mid-Appalachian region. Top entry: variable (abbreviations as in Table 1); middle entry: R_s; bottom entry: $p < x$.

Index / metrics	Variables		
Richness	CL	FE	MN
	0.26	0.25	0.25
	0.0001	0.0001	0.0001
Diatom	XDEPTH	XWIDTH	PCT_SFGF
	−0.24	−0.23	0.21
	0.0106	0.0171	0.0282
Cyanobacteria	SIO2	W1_HAG	W1_HALL
	−0.25	−0.21	−0.20
	0.0001	0.0294	0.0404
Dominant diatom	PCT_SFGF	NA	ANC
	0.44	0.42	0.42
	0.0001	0.0001	0.0001
Acidophilic diatoms	PH	ALKCALC	ANC
	0.61	0.60	0.60
	0.0001	0.0001	0.0001
Eutraphentic diatoms	RP100	COLOR	IONSTR
	−0.36	−0.30	−0.29
	0.0001	0.0001	0.0001
Motile diatoms	NA	COND	CATSUM
	−0.87	−0.67	−0.67
	0.0001	0.0001	0.0001
Chlorophyll	FE	COLOR	TMURBTOT
	0.21	0.15	0.14
	0.0005	0.0169	0.1395
Biomass	TMURBTOT	PCT_SAFN	TSS
	0.26	0.20	0.19
	0.0071	0.0391	0.0025
Phosphatase	W1_HAG	RP100	W1_HALL
	0.22	−0.19	−0.19
	0.0194	0.0494	0.0493
PIBI	RP 100	COLOR	FE
	−0.26	0.24	0.19
	0.0070	0.0001	0.0023

not be a meaningful measure of stream water quality (Table 6).

Cyanobacteria metric.—Cyanobacteria abundance in the algal assemblages of Mid-Appalachian streams averaged <1%, and was inversely correlated with SiO_2 and riparian zone disturbances (Table 4). As with the diatom metric, the Cyanobacteria metric was not strongly related to any of the canonical axes (Table 6), suggesting that it was not linked to water-quality constituents measured in our study streams.

Dominant diatom metric.—Dominance by 1 genus in the diatom assemblage was related to the % of the streambed composed of substrates ≤16 mm in diameter, Na, and ANC of the stream water (Table 4). The dominant diatom metric was positively correlated with W_1 and W_3 (Table 6, Fig. 2A).

Acidophilic diatoms metric.—The acidophilic diatoms metric was correlated with stream pH and stream buffering capacity (Table 4). This metric was negatively correlated with the stream acidification canonical axis (W_2) and positively correlated with the overall disturbance canonical axis (W_1) (Table 6, Fig. 2B).

Eutraphentic diatoms metric.—The eutraphentic diatoms metric was negatively correlated with residual stream depth, color, and ionic strength (Table 4), and was positively correlated with W_4 (Table 6, Fig. 2C).

TABLE 5. Correlations (r_k) between environmental variables and their canonical axes (W_x) Significant ($p < 0.05$) correlations are in **bold.** Variable abbreviations as in Table 1.

Environmental variables	Canonical variables			
	W_1	W_2	W_3	W_4
ALTD	−0.23	**0.67**	0.13	−0.10
ANC	**0.50**	−0.05	0.02	**0.32**
CL	**0.67**	0.22	**0.47**	−0.09
COLOR	−0.24	**0.46**	0.27	−0.09
FE	−0.21	0.15	**0.44**	−0.26
NA	**0.71**	0.29	0.07	−0.07
NTL	0.22	0.12	0.04	0.03
PH	**0.67**	**−0.34**	**−0.35**	0.19
SIO2	0.22	−0.16	−0.07	−0.03
SO4	**0.36**	0.21	−0.07	0.19
TSS	0.19	−0.20	0.23	0.26
PCT_SAFN	**0.39**	−0.13	**0.40**	0.10
PCT_SFGF	**0.48**	−0.13	**0.38**	0.23
XEMBED	0.22	0.12	0.16	0.20
RP100	0.19	−0.01	0.05	**−0.66**
XWIDTH	−0.12	−0.09	−0.28	**−0.45**
W1_HAG	**0.31**	−0.06	0.13	0.24
W1_HALL	**0.46**	0.22	−0.09	−0.05
TMAGTOT	**0.52**	−0.02	0.22	0.07
TMFORTOT	**−0.55**	−0.01	−0.24	−0.07
TMURBTOT	**0.50**	0.09	0.21	0.05

Motile diatoms metric.—The motile diatoms metric was negatively correlated with Na, conductivity, and the sum of all cations (Table 4), and was negatively correlated with W_1 (Table 6, Fig. 2D).

Chlorophyll metric.—Chlorophyll *a* standing crop in Mid-Appalachian streams ranged from 0 to 73.35 mg/m^2, with a regional mean of 11.72 ± 0.84 mg/m^2 of streambed. These values spanned the range of chlorophyll concentrations used by the Oklahoma Conservation Commission to categorize water quality from unimpaired to severely impaired (OCC 1993). The chlorophyll metric was correlated with Fe, color, and % of the watershed in urban and surburban land uses (Table 4), and was not significantly related to any of the canonical axes (Table 6).

Biomass metric.—Periphyton biomass in Mid-Appalachian streams ranged from 0 to 0.17 g AFDM/m^2, with a regional mean of 0.01 ± 0.001 g AFDM/m^2. The biomass metric was correlated with the % of the watershed in urban and suburban land uses, % of the streambed composed of sand and fine sediment, and total suspended solids in the stream water (Table 4). This metric was not significantly correlated with any of the canonical axes (Table 6).

Phosphatase activity metric.—APA in our study streams averaged 54.9 ± 8.4 ng g^{-1} AFDM h^{-1}, with a range of 0 to 1320 ng g^{-1} AFDM h^{-1}. The

TABLE 6. Correlations between periphyton index of biotic integrity (PIBI) metrics and the canonical axes (W_x), eigenvalues, explained variance, and significance of the canonical correlation (r_k) for the canonical axes. Significant ($p < 0.05$) correlations are in **bold.**

PIBI metrics	Canonical variables			
	W_1	W_2	W_3	W_4
Taxa richness	−0.10	−0.14	**0.55**	−0.11
Diatom	0.17	−0.05	−0.19	0.14
Cyanobacteria	−0.08	0.08	−0.05	0.05
Dominant diatom	**0.41**	0.02	**0.40**	−0.05
Acidophilic diatoms	**0.40**	**−0.48**	−0.27	−0.04
Eutraphentic diatoms	−0.11	0.01	−0.09	**0.60**
Motile diatoms	**−0.83**	−0.09	−0.18	0.06
Chlorophyll	0.01	0.08	0.08	−0.02
Biomass	0.09	0.06	0.23	0.10
Phosphatase	0.15	**0.37**	−0.05	**0.32**
PIBI	−0.06	0.03	0.11	**0.35**
Eigenvalue	3.77	1.03	0.89	0.78
Total variance explained (%)	47.6	12.9	11.2	9.8
Cumulative variance explained (%)	47.6	60.6	71.8	81.6
Significance ($p < r_k$)	0.0001	0.0001	0.0006	0.0254

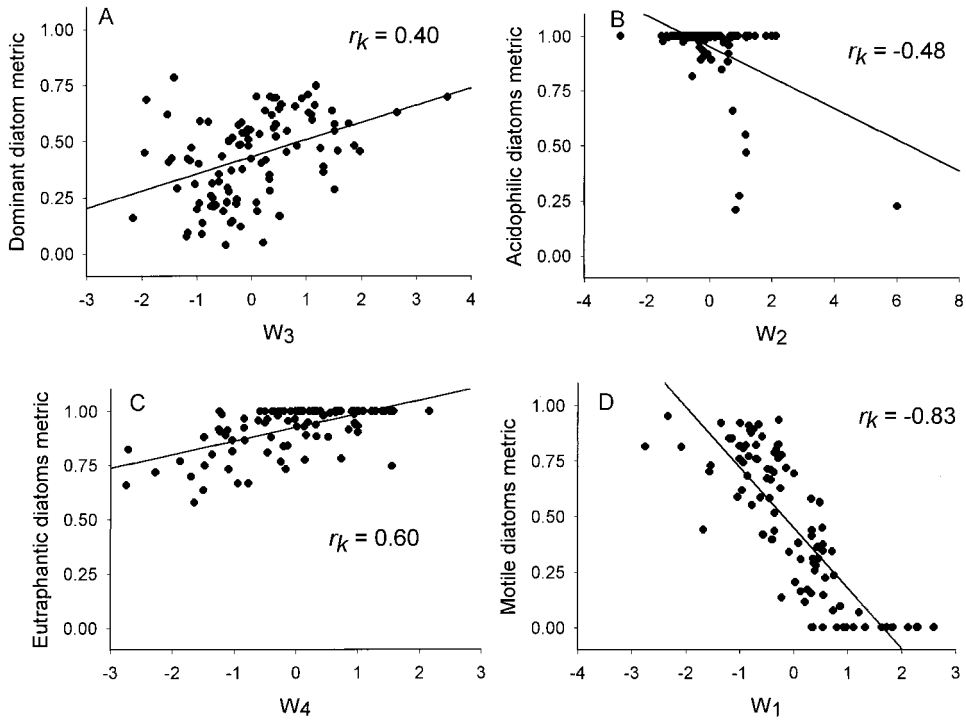

FIG. 2. Correlation of selected periphyton index of biotic integrity (PIBI) metrics (A–D) with selected canonical axes (W_x). r_k = canonical correlation coefficient.

phosphatase metric was positively correlated with the amount of agriculture in the riparian zone, and negatively correlated with human disturbances adjacent to the stream and residual stream depth (Table 4). This metric was positively correlated with W_2 and W_4 (Table 6).

PIBI composite

The PIBI was negatively correlated with residual stream depth and positively correlated with stream color and Fe (Table 4). The PIBI was positively correlated with W_4 (Table 6), suggesting that the PIBI is responding more to measures of stream size than to physico-chemical stressors. It could be argued that as stream size increases, anthropogenic effects also increase, and that an inverse relationship between the PIBI and stream size is a measure of stream condition.

All of the metrics used in calculating the PIBI, except the acidophilic and motile diatoms metrics, were significantly correlated with the PIBI; however, only the diatom and Cyanobacteria metrics were sufficiently strongly correlated ($r > 0.50$) to suggest redundancy (Table 7). Some metrics were highly significantly correlated to others (e.g., diatoms and Cyanobacteria), but none was sufficiently related (condition index >30) to suggest multicollinearity and undue bias of the final PIBI score (Table 7).

A comparison of PIBI scores among landforms, drainage basins, ecoregions, and stream orders (Table 8) using ANOVA produced no significant differences among drainage basins ($F_{2,231} = 1.42$, $p < 0.227$) or ecoregions ($F_{4,229} = 3.43$, $p < 0.095$), but highland streams had significantly higher PIBI scores than lowland streams ($F_{2,231} = 34.27$, $p < 0.0001$), and 2nd-order streams had higher scores than 1st-order streams ($F_{2,231} = 5.35$, $p < 0.005$). There were also significant differences among years (1995 > 1993 > 1994, $F_{2,231} = 68.9$, $p < 0.0001$), but these were explained by differing proportions of highland and lowland streams in each study year. In 1993, 21% of the streams sampled were lowland, compared to 36% in 1994 and 13% in 1995.

PIBI scores were compared for sites revisited within the same year and revisited in subsequent years to check index stability. PIBI scores on visits to a site within a year were signifi-

TABLE 7. Spearman correlation coefficients for the relationship between periphyton index of biotic integrity (PIBI) and its component metrics. Significant correlations ($p < 0.05$) are in **bold**. Condition index >30 indicates potential multicollinearity problems.

Condition index	Richness 1.00	Diatom 1.78	Cyano-bacteria 1.89	Dominant 2.54	Acido-philic 2.78	Eutra-phentic 2.90	Motile 3.16	Chloro-phyll 4.21	Bio-mass 4.53	Phos-phatase 4.94
Diatom	0.09									
Cyanobacteria	0.09	**0.93**								
Dominant	**0.35**	0.11	0.11							
Acidophilic	**−0.27**	**−0.26**	**−0.28**	0.08						
Eutraphentic	**−0.32**	**−0.32**	−0.29	−0.42	−0.07					
Motile	**−1.19**	0.01	0.01	**−0.56**	**−0.25**	**0.25**				
Chlorophyll	**0.16**	**0.05**	**0.03**	−0.06	0.01	−0.05	−0.01			
Biomass	0.02	−0.03	−0.03	0.09	0.05	−0.02	−0.11	0.03		
Phosphatase	0.05	0.05	0.01	0.13	0.03	0.06	−0.05	0.08	0.12	
PIBI	**0.24**	**0.60**	**0.54**	**0.23**	0.12	**−0.21**	0.11	**0.42**	**0.39**	**0.43**

cantly related to the score on the previous visit $r = 0.78$, $p < 0.01$, $n = 18$), but the same was not true between years $r = 0.24$, $p < 0.24$, $n = 7$) (Fig. 3).

Discussion

The diverse nature of streams and their stressors requires an index of integrity that is broadly based, multimetric, and responsive at both the individual and assemblage levels (Karr 1981, 1991). Such an index should be composed of individual metrics that have clear relationships between biological responses and specific stressors, and these metrics should be integrated into a composite index. The resulting index, a simplification of complex ecological information, should provide a quick assessment of the overall condition of a stream, which is easily understood by non-technical resource managers.

Multimetric indices such as the IBI for fish (Karr 1981) or the BIBI for macroinvertebrates (Kerans and Karr 1994) have been criticized because they reduce data into a single number, and because their statistical behavior and distribution are not well known (Suter 1993). Gerritsen (1995) argued that this data simplification is the goal of a multimetric index, and is the feature that allows them to be used by resource managers who may not be experts in stream ecology.

Periphyton indices of biotic integrity have not been extensively used in monitoring stream water quality. Only Kentucky, Montana, and Oklahoma have routinely used algal data in their assessments (Bahls et al. 1992, Bahls 1993, Mills et al. 1993, OCC 1993). In a survey of Montana streams, Bahls et al. (1992) found that periphyton was effective in distinguishing montane streams from plains streams, and reference streams within these broad ecoregions from streams impacted by human activities. Leland (1995) reported similar results from his study of the Yakima River, Washington. The OCC (1993) also found that, within an ecoregion, stream impairment could be distinguished using diatom-based metrics.

We used the univariate statistics and the cumulative distribution of PIBI scores for all streams within the Mid-Appalachian region as guidelines for setting assessment values relative to the 0 to 100 scale for the PIBI. PIBI scores in the upper 25th percentile (PIBI $\geq$ 72) should indicate acceptable stream conditions, and those in the lower 25th percentile (PIBI $\leq$ 60) should indicate degraded stream conditions.

We expected PIBI scores for some of the Mid-Appalachian streams to be near the upper end of the range. That no stream scored >85 may indicate inherent problems with the PIBI or its individual metrics, but it may also reflect the long history of effects from forest clearing, agriculture, mining, urbanization, and acid deposition. This view is supported by lower PIBI scores for the lowland streams, areas that have experienced greater influences of human development than other Mid-Appalachian areas (Table 8). Similarly, we expected some streams to score in the bottom ½ of the PIBI range. That these scores were not lower may reflect the re-

TABLE 8. Periphyton index of biotic integrity (PIBI) scores for streams of the Mid-Appalachian region and its drainage basins, ecoregions, and stream orders.

			PIBI score	
	n	Mean ± SE	Minimum	Maximum
Region				
Mid-Appalachian	233	66.1 ± 0.5	48.0	85.1
Major landform				
Highlands	160	67.1 ± 0.5	48.1	85.1
Lowlands	73	59.0 ± 0.1	48.0	73.7
Drainage				
Chesapeake	98	61.0 ± 1.3	48.6	73.7
Ohio	114	58.9 ± 1.1	48.0	72.1
Other	21	57.4 ± 1.8	48.7	67.2
Ecoregion				
Blue Ridge	25	66.7 ± 1.6	49.1	84.0
Central Appalachian Plateau[a]	31	62.0 ± 1.3	48.1	74.2
Acid-sensitive Plateau[b]	98	67.1 ± 0.7	52.8	85.1
Central Appalachian ridges	39	66.6 ± 1.1	48.6	80.4
Central Appalachian valleys	23	64.4 ± 1.4	48.0	78.7
Piedmont/Coastal Plain	17	66.7 ± 2.1	48.7	80.2
Stream order				
1st	111	64.3 ± 0.8	48.1	85.1
2nd	65	67.9 ± 0.8	31.3	82.6
3rd	57	66.1 ± 0.9	48.7	79.7

[a] Central Appalachian Plateau streams not considered to be acid-sensitive
[b] Acid-sensitive streams of the Central Appalachian Plateau

silient nature of streams and, especially, algal assemblages. We also expected PIBI scores to be relatively stable through time, and within a single year they were, but environmental variation, coupled with short generation times for algae, appeared to limit their use over longer temporal scales.

Several factors may confound PIBI responses to environmental variables. The 1-time sampling of these sites may have yielded discontinuities between stream environmental variables and biology. These discontinuities may have resulted in PIBI responses lagging behind chemical or habitat stressors, leading to poor correlation with these variables, and good PIBI scores in spite of bad chemical or habitat conditions. Similarly, stressors may have affected a stream and subsequently abated, resulting in good chemical or habitat conditions but poor PIBI scores. Finally, imprecision in the various metrics and the PIBI, and variance related to the spatial heterogeneity of the region, may have lessened the strength of correlations with chemistry, habitat, or landscape variables. Even with these caveats in mind, we believe that the PIBI scores were an accurate reflection of current and recent ecological conditions of these streams, and that the PIBI is a useful tool for the ecological assessment of streams.

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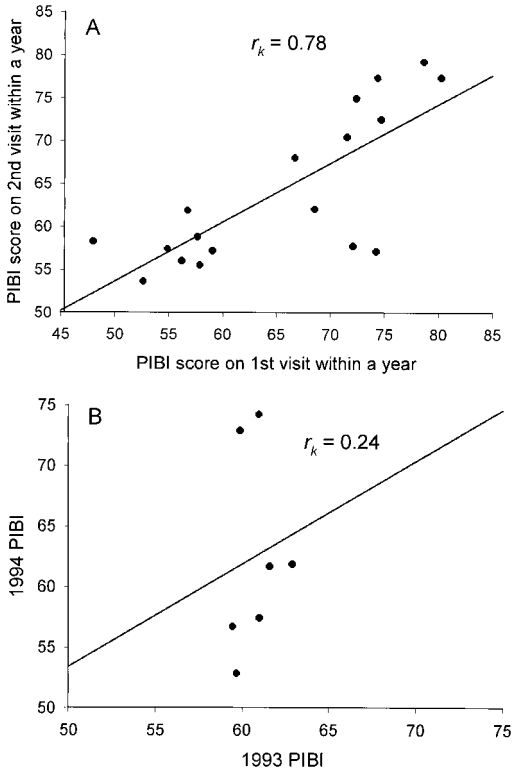

FIG. 3. Comparison of periphyton index of biotic integrity (PIBI) scores for Mid-Appalachian streams revisited within a year (A) and between years (B).

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Literature Cited

AMBLARD, C., P. COUTURE, AND G. BOURDIER. 1990. Effect of a pulp and paper mill effluent on the structure and metabolism of periphytic algae in experimental streams. Aquatic Toxicology 18:137–162.

APHA (AMERICAN PUBLIC HEALTH ASSOCIATION). 1995. Chlorophyll. Pages 10.17–10.24 *in* Standard methods for the examination of water and wastewater. 19th edition. American Public Health Association, Washington, DC.

ARCHIBALD, R. E. M. 1972. Diversity in some South African diatom associations and its relation to water quality. Water Research 6:1229–1238.

BAHLS, L. L. 1993. Periphyton bioassessment methods for Montana streams. Water Quality Bureau, Department of Health and Environmental Sciences, Helena, Montana. (Available from: Water Quality Bureau, Department of Health and Environmental Sciences, State of Montana, P. O. Box 200901, Helena, Montana 59620 USA.)

BAHLS, L., R. BUKANTIS, AND S. TRALLES. 1992. Benchmark biology of Montana streams. Water Quality Bureau, Department of Health and Environmental Sciences, Helena, Montana. (Available from: Water Quality Bureau, Department of Health and Environmental Sciences, State of Montana, P. O. Box 200901, Helena, Montana 59620 USA.)

BAKER, J. H., AND R. Y. MORITA. 1982. A note on the effects of crude oil on microbial activities in stream sediments. Environmental Pollution Series A, Ecological and Biological 31:149–157.

BIRKS, H. J. B., J. M. LINE, S. JUGGINS, A. C. STEVENSON, AND C. J. F. TER BRAAK. 1990. Diatoms and pH reconstruction. Philosophical Transactions of the Royal Society of London B, Biological Sciences 327:263–278.

BOSTON, H. L., W. R. HILL, AND A. J. STEWART. 1991. Evaluating direct toxicity and food-chain effects in aquatic systems using natural periphyton communities. Pages 126–145 *in* J. W. Gorsuch, W. R. Lower, W. Wang, and M. A. Lewis (editors). Plants for toxicity assessments. Volume 2. ASTM STP 1115. American Society for Testing and Materials, Philadelphia, Pennsylvania.

BOTT, T. L., AND K. ROGENMUSER. 1978. Effects of no. 2 fuel oil, Nigerian crude oil, and used crankcase oil on attached algal communities: acute and chronic toxicity of water-soluble constituents. Applied and Environmental Microbiology 36:673–682.

BOYLE, T. P., G. M. SMILLIE, J. C. ANDERSON, AND D. R. BEESON. 1990. A sensitivity of nine diversity and seven similarity indices. Journal of the Water Pollution Control Federation 62:749–762.

BURTON, G. A., AND G. R. LANZA. 1987. Aquatic microbial activity and macrofaunal profile of an Oklahoma stream. Water Research 21:1173–1182.

CHARLES, D. F. 1985. Relationship between surface sediment diatom assemblages and lake water characteristics in Adirondack lakes. Ecology 66: 994–1011.

CHRISTIE, C. E., AND J. P. SMOL. 1993. Diatom assemblages as indicators of lake trophic status in southeastern Ontario lakes. Journal of Phycology 29:575–586.

CLARK, J. R., K. L. DICKSON, AND J. CAIRNS. 1979. Estimating aufwuchs biomass. Pages 116–141 *in* R. L. Weitzel (editor). Methods and measurement of periphyton communities: a review. ASTM STP 690. American Society for Testing and Materials, Philadelphia, Pennsylvania.

COSTE, M., C. BOCA, AND A. DAUTA. 1991. Use of algae for monitoring rivers in France. Pages 75–88 *in* B. A. Whitton, E. Rott, and G. Friedrich (editors). Use of algae for monitoring rivers. Institut für Botanik, Universität Innsbruck, Innsbruck, Austria.

CROSSEY, M. J., AND T. W. LAPOINTE. 1988. A comparison of periphyton structural and functional responses to heavy metals. Hydrobiologia 162:109–121.

DESCY, J. P. 1979. A new approach to water quality estimation using diatoms. Nova Hedwigia 64: 305–323.

DIXIT, S. S., AND J. P. SMOL. 1994. Diatoms as indicators in the Environmental Monitoring and Assessment Program-Surface Waters (EMAP-SW). Environmental Monitoring and Assessment 31:275–306.

DIXIT, S. S., J. P. SMOL, J. C. KINGSTON, AND D. F. CHARLES. 1992. Diatoms: powerful indicators of environmental change. Environmental Science and Technology 26:23–33.

FAUSCH, K. D., J. R. KARR, AND P. R. YANT. 1984. Regional application of an index of biotic integrity based on stream fish communities. Transactions of the American Fisheries Society 113:39–55.

FRIEDRICH, G., D. CHAPMAN, AND A. BEIM. 1992. The use of biological materials. Pages 171–238 *in* D. Chapman (editor). Water quality assessments. Chapman and Hall, London, UK.

FRITZ, S. C., J. C. KINGSTON, AND D. R. ENGSTROM. 1993. Quantitative trophic reconstruction from sedimentary diatom assemblages: a cautionary tale. Freshwater Biology 30:1–23.

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

GITTINS, R. 1985. Canonical analysis: a review with applications in ecology. Springer-Verlag, New York.

GUZKOWSKA, M. A. J., AND F. GASSE. 1990. Diatoms as indicators of water quality in some English urban lakes. Freshwater Biology 23:233–250.

HALL, R. I., AND J. P. SMOL. 1992. A weighted-average regression and calibration model for inferring total phosphorus concentration from diatoms in British Columbia (Canada) lakes. Freshwater Biology 27:417–434.

HEALEY, F. P., AND L. L. HENDZEL. 1979. Fluorometric measurements of alkaline phosphatase activity in algae. Freshwater Biology 9:429–439.

HERLIHY, A. T., D. P. LARSEN, S. G. PAULSEN, N. S. URQUHART, AND B. J. ROSENBAUM. Designing a spatially balanced, randomized site selection process for regional stream surveys: the EMAP Mid-Atlantic pilot study. Environmental Monitoring and Assessment (in press).

HUGHES, R. M., T. R. WHITTIER, C. M. ROHM, AND D. P. LARSEN. 1991. A regional framework for establishing recovery criteria. Environmental Management 14:673–683.

KARR, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21–27.

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

KARR, J. R. 1993. Defining and assessing ecological integrity beyond water quality. Environmental Toxicology and Chemistry 12:1521–1531.

KARR, J. R., K. D. FAUSCH, P. L. ANGERMEIER, P. R. YANT, AND I. J. SCHLOSSER. 1986. Assessing biological integrity in running waters: a method and its rationale. Special Publication 5. Illinois Natural History Survey, Champaign, Illinois.

KAUFMANN, P. R., P. LEVINE, E. G. ROBISON, C. SEELIGER, AND D. V. PECK. 1999. Quantifying physical habitat in wadeable streams. EPA 620/R-99/003. US Environmental Protection Agency, Corvallis, Oregon.

KELLY, M. G., C. J. PENNY, AND B. A. WHITTON. 1995. Comparative performance of benthic diatom indices used to assess river water quality. Hydrobiologia 302:179–188.

KELLY, P. M., AND J. M. WHITE. 1993. Preprocessing remotely sensed data for efficient analysis and classification. Pages 24–30 *in* U. M. Fayyad and R. Uthurusamy (editors). Applications of artificial intelligence: knowledge-based systems in aerospace and industry. Proceedings of SPIE-International Society for Optical Engineering, Volume 1963. International Society for Optical Engineering, Bellingham, Washington.

KERANS, B. L., AND J. R. KARR. 1994. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee valley. Ecological Applications 4:768–785.

KOLKWITZ, R., AND M. MARSSON. 1908. Ökologie der phlanzlichen saprobien. Berichte der Deutschen Botanischen Gesellschaft 26:505–519.

KOSINSKI, R. J. 1984. The effect of terrestrial herbicides on the community structure of stream periphyton. Environmental Pollution Series A, Ecological and Biological 36:165–189.

KRAMMER, K., AND H. LANGE-BERTALOT. 1986. Bacillariophyceae, Teil 1. Naviculaceae. *In* A. Pascher, H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer (editors). Süsswasserflora von Mitteleuropa. VEB Gustav Fisher Verlag, Stuttgart, Germany.

KRAMMER, K., AND H. LANGE-BERTALOT. 1988. Bacillariophyceae, Teil 2. Epithemiaceae, Bacillariaceae, Surirellaceae. *In* A. Pascher, H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer (editors). Süsswasserflora von Mitteleuropa. VEB Gustav Fisher Verlag, Stuttgart, Germany.

KRAMMER, K., AND H. LANGE-BERTALOT. 1991a. Bacillariophyceae, Teil 3. Centrales, Fragilariaceae, Eunotiaceae, Achnanthaceae. *In* A. Pascher, H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer (editors). Süsswasserflora von Mitteleuropa. VEB Gustav Fisher Verlag, Stuttgart, Germany.

KRAMMER, K., AND H. LANGE-BERTALOT. 1991b. Bacillariophyceae, Teil 4. Achnanthaceae, Kritische Erganzungen zu Navicula (Lineolatae) und Gom-

phonema. *In* A. Pascher, H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer (editors). Süsswasserflora von Mitteleuropa. VEB Gustav Fisher Verlag, Stuttgart, Germany.

LANGE-BERTALOT, H. 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedwigia 64:285–305.

LELAND, H. V. 1995. Distribution of phytobenthos in the Yakima River basin, Washington, in relation to geology, land use, and other environmental factors. Canadian Journal of Fisheries and Aquatic Sciences 52:1108–1129.

LOWE, R. L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. EPA-670/4–74–005. US Environmental Protection Agency, Cincinnati, Ohio.

MCCORMICK, P. V., AND J. CAIRNS. 1997. Algal indicators of aquatic ecosystem condition and change. Pages 177–207 *in* W. Wang, J. W. Gorsuch, and J. S. Hughes (editors). Plants for environmental studies. CRC Lewis Publishers, Boca Raton, Florida.

MILLS, M. R., G. BECK, J. BRUMLEY, S. M. CALL, J. GRUBBS, R. HOUP, L. METZMEIER, AND K. SMATHERS. 1993. Methods for assessing biological integrity of surface waters. Division of Water, Kentucky Department for Environmental Protection, Frankfort, Kentucky. (Available from: Division of Water, Kentucky Department for Environmental Protection, Frankfort, Kentucky 40601 USA.)

MONTGOMERY, D. C., AND E. A. PECK. 1982. Introduction to linear regression analysis. John Wiley and Sons, New York.

MULHOLLAND, P. J., AND A. D. ROSEMOND. 1992. Periphyton response to longitudinal nutrient depletion in a woodland stream: evidence of upstream-downstream linkage. Journal of the North American Benthological Society 11:405–419.

OCC (OKLAHOMA CONSERVATION COMMISSION). 1993. Development of rapid bioassessment protocols for Oklahoma utilizing characteristics of the diatom community. Oklahoma Conservation Commission, Oklahoma City, Oklahoma. (Available from: Oklahoma Conservation Commission, State of Oklahoma, Oklahoma City, Oklahoma 73105 USA.)

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

PALMER, C. M. 1969. A composite rating of algae tolerating organic pollution. Journal of Phycology 5: 78–82.

PALMER, C. M. 1977. Algae and water pollution. EPA-600/9-77-036. US Environmental Protection Agency, Cincinnati, Ohio.

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

PAN, Y., R. J. STEVENSON, B. H. HILL, P. R. KAUFMANN, AND A. T. HERLIHY. 1999. Spatial patterns and ecological determinants of benthic algal assemblages in Mid-Atlantic streams, USA. Journal of Phycology 35:460–468.

PATRICK, R. 1973. Use of algae, especially diatoms, in assessment of water quality. Pages 76–95 *in* J. Cairns and K. L. Dickson (editors). Biological methods for the assessment of water quality. ASTM STP 528. American Society for Testing and Materials, Philadelphia, Pennsylvania.

PATRICK, R. 1977a. Ecology of freshwater diatoms and diatom communities. Pages 284–332 *in* D. Werner (editor). The biology of diatoms. Botanical Monographs, Volume 13. Blackwell Scientific Publishers, Oxford, UK.

PATRICK, R. 1977b. Effects of trace metals in the aquatic ecosystem. American Scientist 66:185–191.

PATRICK, R., AND C. W. REIMER. 1966. The diatoms of the United States. Volume 1. Monographs of the Academy of Natural Sciences of Philadelphia No. 13.

PATRICK, R., AND C. W. REIMER. 1975. The diatoms of the United States. Volume 2, Part 1. Monographs of the Academy of Natural Sciences of Philadelphia No. 13.

PRATT, J. R., B. R. NIEDERLEHNER, N. BOWERS, AND J. CAIRNS. 1987. Prediction of permissible concentrations of copper from microcosm toxicity tests. Toxicity Assessment 11:451–457.

PRYGIEL, J., AND M. COSTE. 1993. The assessment of water quality in the Artois-Picardie water basin (France) by the use of diatom indices. Hydrobiologia 269/270:343–349.

REYNOLDSON, T. B., R. H. NORRIS, V. H. RESH, K. E. DAY, AND D. M. ROSENBERG. 1997. The reference condition: a comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. Journal of the North American Benthological Society 16:833–852.

RODGERS, J. H., K. L. DICKSON, AND J. CAIRNS. 1979. A review and analysis of some methods used to measure functional aspects of periphyton. Pages 142–167 *in* R. L. Weitzel (editor). Methods and measurements of periphyton communities: a review. ASTM STP 690. American Society for Testing and Materials, Philadelphia, Pennsylvania.

ROLHLF, F. J., AND R. R. SOKAL. 1969. Statistical tables. W. H. Freeman and Company, San Francisco, California.

ROTT, E. 1991. Methodological aspects and perspectives in the use of periphyton for monitoring and protecting rivers. Pages 9–16 *in* B. A. Whitton, E. Rott, and G. Friedrich (editors). Use of algae for

monitoring rivers. Institut für Botanik, Universität Innsbruck, Innsbruck, Austria.

ROUND, F. E. 1991. Diatoms in river-monitoring studies. Journal of Applied Phycology 3:129–145.

ROUND, F. E., R. M. CRAWFORD, AND D. G. MANN. 1990. The diatoms. Cambridge University Press, Cambridge, UK.

SAYLER, G. S., M. PUZISS, AND M. SILVER. 1979. Alkaline phosphatase assay for freshwater sediments: application to perturbed sediment systems. Applied and Environmental Microbiology 38:922–927.

SAYLER G. S., T. W. SHERRILL, R. E. PERKINS, L. M. MALLORY, M. P. SHIARIS, AND D. PEDERSEN. 1982. Impact of coal-coking effluent on sediment microbial communities: a multivariate approach. Applied and Environmental Microbiology 44: 1118–1129.

SCANFERLATO, V. S., AND J. CAIRNS. 1990. Effect of sediment-associated copper on ecosystem structure and function of aquatic mesocosms. Aquatic Toxicology 18:23–34.

SIGMON C. F., H. J. KANIA, AND R. J. BEYERS. 1977. Reductions in biomass and diversity resulting from exposure to mercury in artificial streams. Journal of the Fisheries Research Board of Canada 34:493–500.

STEINMAN, A. D., P. J. MULHOLLAND, AND D. B. KIRSCHTEL. 1991. Interactive effects of nutrient reduction and herbivory on biomass, taxonomic structure and P uptake in lotic periphyton communities. Canadian Journal of Fisheries and Aquatic Sciences 48:1951–1959.

STEVENSON, R. J. 1984. Epilithic and epipelic diatoms in the Sandusky River, with emphasis on species diversity and water pollution. Hydrobiologia 114: 161–175.

STEVENSON, R. J., AND R. L. LOWE. 1986. Sampling and interpretation of algal patterns for water quality assessments. Pages 118–149 *in* B. G. Isom (editor). Rationale for sampling and interpretation of ecological data in the assessment of freshwater ecosystems. ASTM STP 894. American Society for Testing and Materials, Philadelphia, Pennsylvania.

STEVENSON, R. J., AND Y. PAN. 1999. Assessing ecological conditions in rivers and streams with diatoms. Pages 11–40 *in* E. F. Stoermer and J. P. Smol (editors). The diatoms: applications to the environmental and earth sciences. Cambridge University Press, Cambridge, UK.

STRAHLER, A. N. 1957. Quantitative analysis of watershed geomorphology. Transactions of the American Geophysical Union 38:913–920.

SUDHAKAR G., B. JYOTHI, AND V. VENKATESWARLU. 1991. Metal pollution and its impact on algae in flowing waters in India. Archives of Environmental Contamination and Toxicology 21:556–566.

SUTER, G. W. 1993. A critique of ecosystem health concepts and indices. Environmental Toxicology and Chemistry 12:1533–1539.

TU, C. M. 1981. Effects of pesticides on activities of enzymes and microorganisms in clay soil. Journal of Environmental Science and Health Part B, Pesticides, Food Contaminants, and Agricultural Wastes 16:179–191.

TYLER, G. 1976. Heavy metal pollution, phosphatase activity, and mineralization of organic phosphorus in forest soils. Soil Biology and Biochemistry 8:327–332.

USEPA (UNITED STATES ENVIRONMENTAL PROTECTION AGENCY). 1987. Handbook of methods for acid deposition studies: laboratory analyses for surface water chemistry. EPA 600/4-87/026. Office of Research and Development, US Environmental Protection Agency, Washington, DC.

VAN DAM, H. 1982. On the use of measures of structure and diversity in applied diatom ecology. Nova Hedwigia 73:97–115.

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

VOGELMANN, J. E., T. SOHL, AND S. M. HOWARD. 1998. Regional characterization of land cover using multiple sources of data. Photogrammetric Engineering and Remote Sensing 64:45–57.

WEBER, C. I., AND B. H. MCFARLAND. 1981. Effects of copper on the periphyton of a small calcareous stream. Pages 101–131 *in* J. M. Bates and C. I. Weber (editors). Ecological assessments of effluent impacts on communities of indigenous aquatic organisms. ASTM STP 730. American Society for Testing and Materials, Philadelphia, Pennsylvania.

WEITZEL, R. L. 1979. Periphyton measurements and applications. Pages 3–33 *in* R. L. Weitzel (editor). Methods and measurements of periphyton communities: a review. ASTM STP 690. American Society for Testing and Materials, Philadelphia, Pennsylvania.

WHITTON, B. A., E. ROTT, AND G. FRIEDRICH (EDITORS). 1991. Use of algae for monitoring rivers. Institut für Botanik, Universität Innsbruck, Innsbruck, Austria.

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