

Assessment of streams of the eastern United States using a periphyton index of biotic integrity

Brian H. Hill ^{a,*}, Alan T. Herlihy ^b, Philip R. Kaufmann ^c,
Susanna J. DeCelles ^d, Mark A. Vander Borgh ^{d,1}

^a US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory,
6201 Congdon Boulevard, Duluth, MN 55804, USA

^b Department of Fisheries and Wildlife, Oregon State University, c/o US Environmental Protection Agency,
200 SW 35th Street, Corvallis, OR 97333, USA

^c US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory,
200 SW 35th Street, Corvallis, OR 97333, USA

^d SoBran Inc., c/o US Environmental Protection Agency, National Exposure Research Laboratory,
26 W. Martin Luther King Drive, Cincinnati, OH 45268, USA

Abstract

Benthic algae were collected from 272 eastern United States streams and rivers and analyzed for diatom species richness and dominance, the relative abundance of acidobiontic, eutrathentic, and motile diatoms, standing crops of chlorophyll and biomass, and alkaline phosphatase activity. These data were used to calculate a periphyton index of biotic integrity (PIBI), and values of the index were compared among reference, moderately impacted, and disturbed streams. The level of disturbance was based on stream chemistry, riparian disturbance, or a combined classification. Analyses of variance showed that PIBI was significantly higher in reference streams for all classifications. The PIBI and its metrics were correlated with many of the chemistry and habitat variables, and canonical correlation analysis revealed three significant environmental gradients which extracted 84% of the variance in the PIBI and its metrics. We used the mean 75th, 25th, and 5th percentile scores from the reference sites to set thresholds for excellent, good, fair, or poor condition. Applying these criteria to the cumulative distribution of total stream length in the region, we found that 4.3% of the stream length was in excellent condition; 20.8% in good condition; 56.4% in fair condition; and 18.5% in poor condition. The sensitivity of the PIBI and its component metrics to environmental stressors supports the use of this index for monitoring ecological conditions in streams in the eastern United States and as a tool to aid in diagnosing the causes of their impairment.

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1. Introduction

Stream biological integrity reveals itself in the condition, abundance, and diversity of its biota, and biological surveys of stream communities have long been used to assess the impacts of human activities on these systems. Over the past two decades, biological

* Corresponding author. Fax: +1-218-529-5003.

E-mail address: hill.brian@epa.gov (B.H. Hill).

¹ Present address: North Carolina Department of Environment and Natural Resources, Raleigh, NC 27699, USA.

monitoring has risen to the forefront of environmental impact assessments and stream monitoring programs. During this time, the scope of biological monitoring has evolved from the collection of biological data in support of toxicity determinations of waters to the collection of biological information for water quality prediction. Data collected from these surveys are analyzed typically by either multimetric or multivariate techniques, the merits of each having been argued extensively (Norris, 1995; Gerritsen, 1995; Reynoldson et al., 1997). During this same time period the United States Environmental Protection Agency (US EPA) mandated that states establish water quality criteria based on biological information and determine the causes of impairment (US EPA, 1990, 2000a). To meet this challenge states are collecting biological data from a large number of sites scattered across many different landscapes. The usefulness of these data may depend not just on our ability to determine the extent of impairment of stream conditions, but also on our ability to diagnose the likely causes of the impairment.

In previous studies we demonstrated the use of algal assemblage data for stream monitoring (Hill et al., 2000) and the effect of diatom taxonomic resolution on the assessment of stream conditions (Hill et al., 2001). Our present study has three objectives: (1) to adapt our earlier periphyton index of biotic integrity for use with diatoms only, (2) to compare diatom assemblage attributes and an index of biotic integrity among reference and disturbed stream classes, and (3) to explore the ability of this index of biotic integrity to diagnose the likely causes of impairment in eastern USA streams.

2. Study area and survey design

Our survey included streams sampled during 1997 and 1998 by the US EPA in the Appalachian Mountain, Piedmont, and Coastal Plains regions of the eastern United States (Fig. 1). We classified streams as either reference, disturbed, or moderate on the basis of chemistry or riparian disturbance. Reference streams met all of the following criteria: acid neutralizing capacity (ANC) > 50 meq/l, $\text{Cl}^- < 100$ meq/l, $\text{SO}_4^{2-} < 400$ meq/l, total P < 25 mg/l, and total N < 700 mg/l; and disturbed streams met any one of

these criteria: $\text{ANC} < 0$ meq/l, $\text{Cl}^- > 1500$ meq/l, $\text{SO}_4^{2-} > 2500$ meq/l, total P > 200 mg/l, or total N > 5000 mg/l. Streams meeting neither of these criteria were placed in a “moderately impacted” category. These chemical criteria were derived from our data with guidance from prior regional studies and US EPA nutrient criteria guidelines (Herlihy et al., 1990, 1991, 1993, 1998; Kaufmann et al., 1991; US EPA, 2000c,d). Similarly, reference streams based on riparian disturbances met all of these criteria: proximity weighted presence of agriculture in the riparian zone <0.3, proximity weighted presence of all human disturbances in the riparian <1.1, and canopy cover >98% (Kaufmann et al., 1999), and disturbed streams met any of these criteria: proximity weighted presence of agriculture in the riparian zone >1.3, proximity weighted presence of all human disturbances in the riparian zone >3.4, or canopy cover <10%). Riparian disturbance criteria were developed from historical conditions (Denevan, 1992) in conjunction with our regional dataset. We combined the chemistry and riparian disturbance classifications into a combined reference classification, using the same logic in defining reference and disturbed classifications. Again, streams meeting neither the reference nor disturbed criteria were classified as moderately impacted.

We present data from 272 streams sampled during the US EPA’s Environmental Monitoring and Assessment Program (EMAP) surveys conducted in 1997 and 1998 (Fig. 1). Twenty-nine streams were revisited 4–8 weeks following their initial sampling yielding a total of 296 stream visits. Sampling sites were selected using a randomized, systematic design with a spatial component (Herlihy et al., 2000). The stream network on digitized 1:100,000 scale United States Geological Survey topographic maps was used as the sample frame, so our survey included all streams and rivers represented by map blue lines in the study area. Each sample site has a sample weight (expansion factor), calculated as the inverse of the probability of selecting that site; summing the sample weights of all sites in the data set yields the total stream length in the study population. Thus, by using the sample weights in data analysis, inference can be made to the entire population (196,500 km) of streams in the study area. All sampling was done during the summer (July–September) under low-flow conditions.

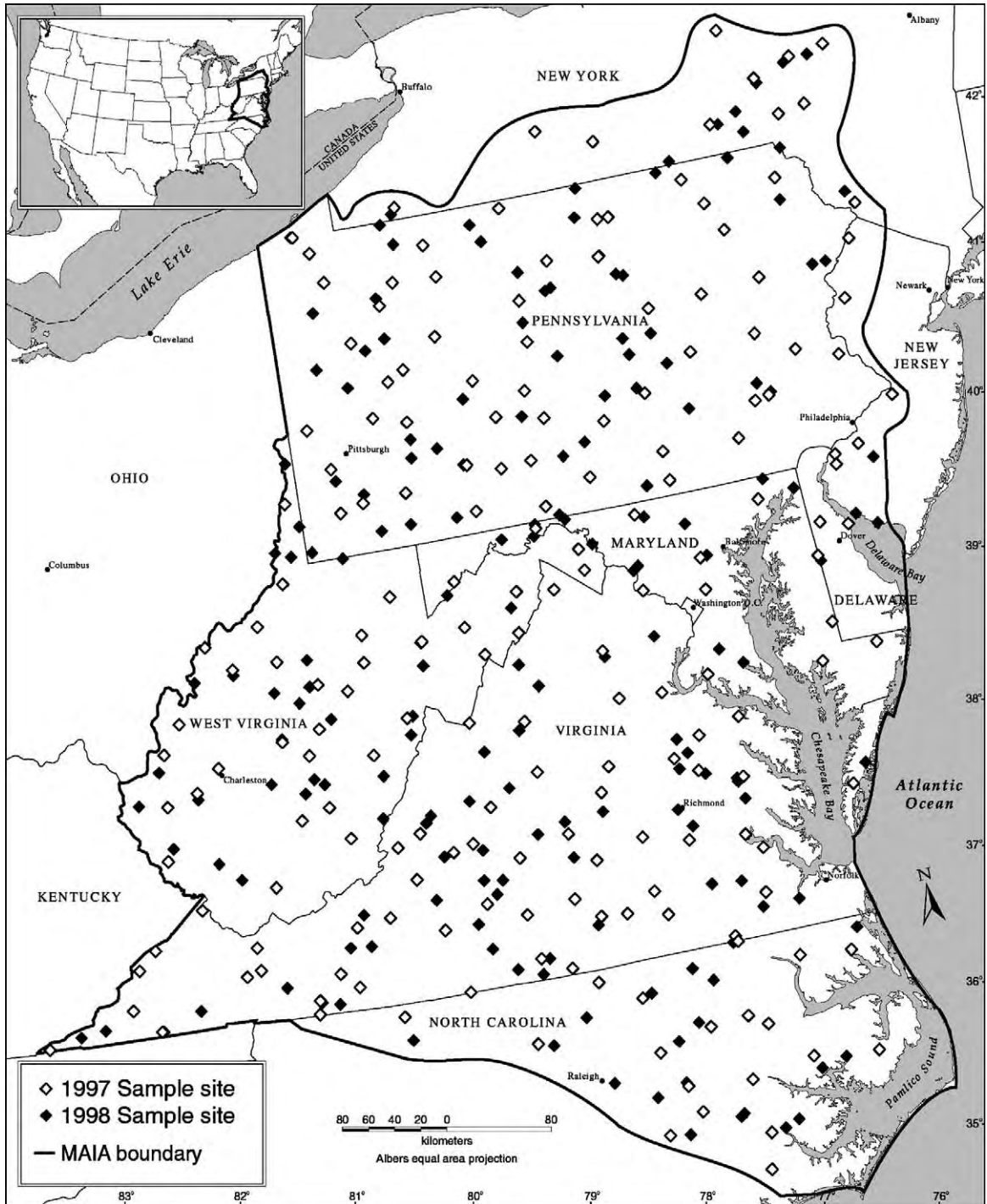


Fig. 1. Mid-Appalachian region with drainage boundaries and locations of 1997–1998 US Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) probability-based stream sampling sites.

3. Methods

3.1. Chemistry

A 4 l grab-sample and two 60 ml syringes of stream water were collected in flowing water near the middle of the stream at each sampling site (Lazorchak et al., 1999, 2000). 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 analytical laboratory. The syringe samples were analyzed for pH, and dissolved inorganic carbon (DIC), and the cubitainer sample was split into aliquots and preserved within 48–72 h of collection. Detailed information on the analytical procedures used for each of the aliquots can be found in US EPA (1987). In brief, Fe, Mn, and the base cations were determined by atomic absorption, anions (SO_4^{2-} , NO_3^- , Cl^-) by ion chromatography, dissolved organic carbon (DOC) by a carbon analyzer, and total N and P by persulfate oxidation and colorimetry.

3.2. Physical habitat structure and riparian disturbances

Physical habitat data were collected from longitudinal profiles and from 11 cross-sectional transects evenly spaced along the stream reaches sampled (Lazorchak et al., 1999, 2000). Stream reach lengths were calculated as 40 times their low-flow wetted widths, but not less than 150 m. Maximum depth was measured in the thalweg at 100 (150 for streams less than 2.5 m wide) evenly spaced points along the stream reach. Transect data included channel dimensions (width, depth, bank angles); substrate size determinations; channel gradient; bearing (for calculating sinuosity); riparian vegetation cover; size structure and composition; and the occurrence and proximity of riparian human disturbances (e.g. roads, buildings, agriculture) (Kaufmann et al., 1999). In wadeable streams, substrate was determined from a 105 particle pebble count; in non-wadeable rivers, dominant and subdominant substrates were determined visually or by probing the bottom with a pole.

3.3. Algal collection and diatom identification

Algae from wadeable streams were collected from randomly selected positions (left, center, or right

channel) within riffles and pools at each of the interior nine transects along the stream reach (Lazorchak et al., 1999). In non-wadeable streams algae were collected along either the right or left margin of the stream (depth <0.5 m) (Lazorchak et al., 2000). At sites where algae were collected from more than one habitat, only samples from the dominant habitat were included in our analyses. At each transect, algae were collected from a 12 cm² area of the stream bed within a 3.9 cm-diameter piece of PVC pipe. Algae were loosened from the surface of cobble-size (6–25 cm-diameter) substrates with a toothbrush and rinsed with stream water into a collection bottle. Substrates smaller or larger than cobble were sampled by delineating the stream bed with the PVC pipe, agitating the substrate, and aspirating the overlying water. The composite algal sample for each reach was sub-sampled for diatom identification, chlorophyll *a*, biomass, and alkaline phosphatase activity. Total and sub-sample volumes were recorded for all analyses (Lazorchak et al., 1999, 2000).

Sub-samples for diatom identification were preserved on site with formalin (5%, v/v). In the laboratory, an aliquot was removed from each algal sub-sample and at least 300 algal cells with protoplasm were counted in a Palmer counting cell at 400× magnification. Additional aliquots from each algal sub-sample were removed for diatom identification and counting (Pan et al., 1996). Diatom frustules were cleaned with concentrated H_2SO_4 and $\text{K}_2\text{Cr}_2\text{O}_7$ 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 frustules 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,b). Permanent diatom mounts are archived at the US EPA National Health and Environmental Effects Laboratory, Duluth, Minnesota.

Sub-samples for chlorophyll *a* and ash-free dry mass (AFDM) were each 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 biomass (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), buffered, 90% (v/v) acetone for 18 h under subdued lighting. Light absorption by the leachate was measured in a spectrophotometer at 750 and 664 nm and, again, after acidification with 1N HCl, at 750 and 665 nm (APHA, 1998). Chlorophyll *a* and biomass were normalized for sub-sample area.

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% K₂HPO₄. The *p*-NP standards were used to convert absorbance of sample *p*-NP per unit periphyton AFDM.

We compared (analyses of variance with Scheffe's means test) diatom assemblage data, chlorophyll, biomass, and alkaline phosphatase activity in riffles and pools, where both habitats were sampled. With the exception of biomass, which was greater in pools, there were no significant differences in these attributes between these habitats. Therefore, only data from the dominant habitat were used in analyses. We used the periphyton assemblage metrics and the PIBI described

by Hill et al. (2000), with the exception of the relative abundances of total diatoms and cyanobacteria, which were not included in our index calculations (Table 1).

3.4. Assessment of ecological condition

We used the average of the chemistry, riparian disturbance, and combined classifications cumulative distributions of PIBI scores at reference sites to set thresholds defining excellent, good, fair, and poor conditions. Our approach is a modification of the US EPA guidelines for setting nutrient criteria (US EPA, 2000b). These guidelines suggest the 25th percentile scores from reference streams to set the threshold between acceptable and unacceptable stream conditions. For datasets inclusive of all streams, the suggested thresholds are set using the 5th and 25th percentiles. We have used the 75th, 25th, and 5th percentiles from reference streams to establish four classes of stream condition, rather than two. Our approach is similar to that used by McCormick et al. (2001) in their assessment of fish assemblages in Appalachian streams. Excellent streams in our study had average PIBI scores exceeding the 75th percentile for reference streams (PIBI > 95); good streams had PIBI scores between 25th and 75th reference percentiles (PIBI 85–95); fair streams had PIBI scores between the 5th and 25th reference percentiles (PIBI 65–85); poor streams had PIBI scores less than the 5th reference percentile (PIBI < 65). We then used the stream population length-weighted frequency distribution of

Table 1

Periphyton assemblage metrics included in the diatom index of biotic integrity (PIBI)

Metric	Calculation
Species richness	No. species – 16/(33–16)
Species dominance	1 – (% dominant species – 39.5)/(73 – 39.5)
Acidobiontic diatoms ^a	1 – (% acidobiontic diatoms – 0.17)/(11.1 – 0.17)
Eutraphentic diatoms ^b	1 – (% eutraphentic diatoms – 14.2)/(78.2 – 14.2)
Motile diatoms	1 – (% motile diatoms – 2.2)/(33 – 2.2)
Chlorophyll	1 – (Chl., µg m ⁻² – 4.8)/(111 – 4.8)
Biomass	1 – (AFDM, g m ⁻² – 20.4)/(155 – 20.4)
Phosphatase	1 – (APA, ng g ⁻¹ AFDM h ⁻¹ – 713)/(11101 – 713)

All metrics are scored 0–1 then multiplied by 100/8 (12.5) to yield a PIBI score range of 0–100 (Hill et al., 2000). Upper limits on scoring ranges were set at the 50th percentile scores from reference sites; lower limits were based on the 5th (positive scoring metrics) or 95th (negative scoring metrics) percentiles from non-reference sites.

^a Optimal occurrence at pH <5.5 (van Dam et al., 1994; see Table 4). Diatoms for which pH optima were not reported were excluded from this metric calculation.

^b Optimal occurrence in waters classified as eutrophic (van Dam et al., 1994; see Table 4).

all PIBI scores to estimate the percentage of the total length of streams in the study area in each condition class (Herlihy et al., 2000).

3.5. Statistical analyses

The relationships between the PIBI, its component metrics, and environmental variables were evaluated using Spearman rank correlation (r_s) to avoid problems associated with non-normal data distribution. Environmental variables significantly correlated with PIBI and its metrics were selected for subsequent canonical correlation analysis (CCA). Canonical CA computes a series of canonical functions that do the best job possible of summarizing the relationship between a linear combination of dependent (diatom index and metrics) variables and a linear combination of independent (environmental) variables. Where two or more variables were highly correlated ($r > 0.5$) (e.g. NO_3^- , NH_4^+ and total N; pH and ANC; Cl and specific conductance), only the variable with the greatest correlation with the biotic variables was included in CCA. The significance of the canonical correlations ($P < 0.05$) was tested using a t -test of the null hypothesis that $r_k = 0$, $t = r_k / \sqrt{(1 - r_k^2)/(n - m)}$, where r_k is the canonical correlation coefficient, n the sample size and m the number of variables (Rohlf and Sokal, 1969). All statistical analyses were performed using SAS for Windows, release 8.2 (SAS, 2001).

Table 2

Five strongest Spearman rank correlation coefficients (r_s) for the relationship between PIBI and its component metrics, and chemical and physical habitat variables in eastern US streams

Variables	Index/metrics								
	Richness	Dominance	Acidobiotic	Eutrphentic	Motile	Chlorophyll	Biomass	Phosphatase	PIBI
Channel substrate	-0.440	-0.327		0.238	0.2997			-0.215	-0.187
P_{total}	0.436	0.407		-0.549	-0.453			0.239	
N_{total}	0.302	0.263		-0.421			-0.158		
ANC	0.285	0.258	0.397	-0.513	-0.385	-0.340			
Canopy			-0.261			0.421	0.157	0.260	0.257
SO_4							-0.230		-0.263
Cl				-0.430	-0.406	-0.317	-0.134		-0.279
Zn			-0.213						
Slope	-0.267	-0.226			0.310		0.198		
Width			0.199			-0.338		-0.242	
Depth								-0.189	
Riparian disturbance			0.188			-0.287			-0.222

All correlations were significant at $P < 0.05$.

4. Results

4.1. Environmental variables

The PIBI and its metrics were significantly correlated with nutrients, chemicals associated with watershed disturbances (Cl^- and SO_4^{2-}), and habitat features (channel substrate size, canopy cover, stream width, channel slope and proximity-weighted sum of human disturbances in the riparian zone) (Table 2). Canonical correlation analysis revealed three significant environmental gradients, the first (W_1), associated with stream chemistry, was positively correlated with ANC, total P, total N Cl^- and SO_4^{2-} . The second canonical axis (W_2), associated with habitat features, is positively correlated with channel substrate size and ANC, and negatively correlated with riparian canopy cover. The third canonical axis (W_3) represents a disturbance gradient and is negatively correlated with human disturbances in the riparian zone, SO_4^{2-} , and Cl^- . Overall, these three axes extracted 46, 26, and 11% of the variance in the correlations of index and metric scores to the environmental variables for eastern United States streams (Table 3).

4.2. Species richness

Thirty-one genera and 227 species of diatoms were collected from the study streams (Table 4). The species richness metric was positively correlated with

Table 3
Correlations of environmental, diatom metrics, and PIBI with the canonical correlation axes (W_1 , W_2 , W_3)

	W_1	W_2	W_3
Environmental variables			
lnANC	0.721	0.411	0.342
Canopy cover	-0.110	-0.500	0.103
Channel substrate size	-0.300	0.735	-0.054
ln Cl^-	0.625	0.151	-0.399
ln N , total	0.638	-0.102	-0.137
ln P , total	0.674	-0.328	-0.228
ln SO_4^{2-}	0.325	-0.046	-0.403
Riparian disturb.	0.176	0.325	-0.580
Stream width	-0.181	-0.357	0.064
Diatom variables			
Species richness	0.433	-0.281	0.165
Species dominance	0.378	-0.172	0.074
Acidobiontic species	0.182	0.391	0.282
Eutraphentic species	-0.617	-0.027	0.104
Motile species	-0.422	0.020	0.169
Chlorophyll	-0.239	-0.398	0.139
Biomass	-0.239	0.225	-0.072
Phosphatase	0.062	-0.296	0.075
PIBI	-0.158	-0.256	0.296
Variance extracted (%)	47.6	25.9	10.9
Significance of the canonical axis ($P < r_k$)	<0.0001	<0.0001	<0.0001

Significant correlations ($P > r_k 0.05$) are indicated in bold. Log transformed variables = $\ln(\text{variable value} + 1)$.

nutrients and Cl^- , and negatively correlated with channel substrate size and channel slope (Table 2). The richness metric was significantly correlated with the first canonical axis (Table 3, Fig. 2a).

4.3. Species dominance

The dominance metric was positively correlated with nutrients and ANC, and negatively correlated with channel substrate size and channel slope (Table 2). Dominance was significantly correlated with the first canonical axis (Table 3, Fig. 2b).

4.4. Acidobiontic diatoms

The acidobiontic diatom metric positively correlated with ANC, stream width, and riparian disturbances; and negatively correlated with riparian canopy cover and Zn^{3+} (Table 2). This metric was

Table 4
Diatom species collected from streams in the eastern United States and the number of streams, in parentheses, at which they were found

<i>Achnanthes biasolettiana</i> (248)
<i>A. bioretii</i> (12)
<i>A. clevei</i> (32) E
<i>A. daodensis</i> (15)
<i>A. deflexa</i> (10)
<i>A. exigua</i> (60)
<i>A. grana</i> (22)
<i>A. holsatica</i> (8)
<i>A. hungarica</i> (6)
<i>A. laevis</i> (9)
<i>A. lapidosa</i> (18)
<i>A. minutissima</i> (273)
<i>A. nodosa</i> (13)
<i>A. pseudoswartzii</i> (30)
<i>A. pusilla</i> (9)
<i>A. stewartii</i> (25)
<i>A. subatomoides</i> (45)
<i>A. suchlandtii</i> (43)
<i>Amphipleura pellucida</i> (50)
<i>Amphora libyca</i> (21)
<i>A. montana</i> (37) E
<i>A. ovalis</i> (8)
<i>A. pediculus</i> (136) E
<i>A. veneta</i> (30) E
<i>Anomooneis brachysira</i> (10) A
<i>A. vitrea</i> (38)
<i>Aulacoseira granulata</i> (74) E
<i>A. italica</i> (28) E
<i>Bacillaria paradoxa</i> (24) M,E
<i>Caloneis bacillum</i> (46) E
<i>C. hyalina</i> (34)
<i>Capartogramma crucicula</i> (14)
<i>Cocconeis pediculus</i> (54) E
<i>C. placentula</i> (179) E
<i>C. scutellum</i> (12)
<i>Cyclotella atomus</i> (28) E
<i>C. bodanica</i> (10)
<i>C. iris</i> (17)
<i>C. meneghiniana</i> (112) E
<i>C. stelligera</i> (40)
<i>C. stelligeroides</i> (6)
<i>Cymatopleura solea</i> (12)
<i>Cymbella affinis</i> (71) E
<i>C. amphicephala</i> (17)
<i>C. aspera</i> (5)
<i>C. caespitosa</i> (5)
<i>C. cistula</i> (24)
<i>C. cuspidata</i> (5)
<i>C. delicatula</i> (26)
<i>C. gracilis</i> (49) A
<i>C. hebridica</i> (6) A
<i>C. mesiana</i> (5)
<i>C. microcephala</i> (37) E

Table 4 (Continued)

<i>C. minuta</i> (41)
<i>C. prostrata</i> (10)
<i>C. silesiaca</i> (225)
<i>C. sinuata</i> (191)
<i>C. tumida</i> (106)
<i>C. tumidula</i> (7) E
<i>C. turgidula</i> (9)
<i>Diatoma mesodon</i> (11)
<i>D. tenuis</i> (7) E
<i>D. vulgaris</i> (73) E
<i>Diploneis elliptica</i> (8)
<i>D. oblongella</i> (17)
<i>D. parma</i> (22)
<i>Eunotia arcus</i> (20) A
<i>E. bilunaris</i> (50) A
<i>E. diodon</i> (7) A
<i>E. exigua</i> (69) A
<i>E. flexuosa</i> (9) A
<i>E. formica</i> (6) A
<i>E. implicata</i> (11) A
<i>E. incisa</i> (27) A
<i>E. meisteri</i> (5) A
<i>E. monodon</i> (9) A
<i>E. musicola</i> (15) A
<i>E. pectinalis</i> (5) A
<i>E. rhomboidea</i> (19) A
<i>Fragilaria capucina</i> (192)
<i>F. construens</i> (10) E
<i>F. crotonensis</i> (8)
<i>F. goulardii</i> (12)
<i>F. leptostauron</i> (5) E
<i>F. nanana</i> (10)
<i>F. parasitica</i> (11)
<i>F. pinnata</i> (21)
<i>F. tenera</i> (26)
<i>F. virescens</i> (20)
<i>Frustulia rhomboides</i> (106) A
<i>F. weinholdii</i> (52) A
<i>Gomphonema acuminatum</i> (8) E
<i>G. affine</i> (5)
<i>G. angustatum</i> (41)
<i>G. angustum</i> (45)
<i>G. auger</i> (19) E
<i>G. brasiliense</i> (8)
<i>G. clevei</i> (12)
<i>G. gracile</i> (42)
<i>G. insigne</i> (15)
<i>G. minutum</i> (8) E
<i>G. olivaceum</i> (50) E
<i>G. parvulum</i> (221)
<i>G. pumilum</i> (16)
<i>G. truncatum</i> (11) E
<i>Gyrosigma acuminatum</i> (25) E
<i>G. attenuatum</i> (52)
<i>G. nodiferum</i> (10)
<i>G. scalproides</i> (6) E

Table 4 (Continued)

<i>Hantzschia amphioxys</i> (5) M
<i>Melosira varians</i> (159) E
<i>Meridion circulare</i> (93)
<i>Navicula angusta</i> (41) M
<i>N. arvensis</i> (55) M
<i>N. capitata</i> (56) E,M
<i>N. capitatoradiata</i> (96) E,M
<i>N. confervacea</i> (7) M
<i>N. contenta</i> (20) M
<i>N. cryptocephala</i> (196) E,M
<i>N. cryptotenella</i> (181) M
<i>N. cuspidata</i> (5) E,M
<i>N. decussis</i> (73) M
<i>N. elginensis</i> (18) E,M
<i>N. erifuga</i> (5) E,M
<i>N. goeppertiana</i> (30) E,M
<i>N. gregaria</i> (111) E,M
<i>N. halophila</i> (68) E,M
<i>N. heimansii</i> (15) A,M
<i>N. indifferens</i> (9) A,M
<i>N. lanceolata</i> (8) E,M
<i>N. lenzii</i> (5) M
<i>N. margalithii</i> (25) M
<i>N. menisculus</i> (72) E,M
<i>N. meniscus</i> (25) M
<i>N. minima</i> (168) M
<i>N. minusculoides</i> (9) M
<i>N. mutica</i> (22) E,M
<i>N. phyllepta</i> (13) M
<i>N. pupula</i> (94) E,M
<i>N. pygmaea</i> (11) E,M
<i>N. radiosa</i> (58) E,M
<i>N. rhynchocephala</i> (57) M
<i>N. salinarum</i> (7) E,M
<i>N. schadei</i> (17) M
<i>N. schroeterii</i> (47) M
<i>N. seminulum</i> (50) M
<i>N. submeniscula</i> (12) M
<i>N. subtilissima</i> (16) M
<i>N. tenelloides</i> (78) E,M
<i>N. tenera</i> (9) M
<i>N. tripunctata</i> (63) M
<i>N. trivialis</i> (12) E,M
<i>N. veneta</i> (25) E,M
<i>N. viridula</i> (170) E,M
<i>Neidium affine</i> (6) E
<i>N. alpinum</i> (12) A
<i>N. ampliatus</i> (8) E
<i>N. dubium</i> (15) E
<i>Nitzschia acicularis</i> (33) E,M
<i>N. acidoclinata</i> (25) A,M
<i>N. agnita</i> (14) M
<i>N. amphibia</i> (125) E,M
<i>N. bremensis</i> (12) M
<i>N. capitellata</i> (6) E,M
<i>N. clausii</i> (26) M

Table 4 (Continued)

<i>N. coarctata</i> (12) E,M
<i>N. fonticola</i> (25) E,M
<i>N. frustulum</i> (39) E,M
<i>N. gracilis</i> (48) M
<i>N. hantzschiana</i> (7) M
<i>N. incognita</i> (5) E,M
<i>N. inconspicua</i> (144) E,M
<i>N. intermedia</i> (6) M
<i>N. laevis</i> (7) E,M
<i>N. lanceolata</i> (7) E,M
<i>N. levidensis</i> (42) E,M
<i>N. linearis</i> (87) E,M
<i>N. nana</i> (16) M
<i>N. palea</i> (235) E,M
<i>N. paleacea</i> (20) E,M
<i>N. perminuta</i> (18) M
<i>N. pumila</i> (8) M
<i>N. pusilla</i> (6) M
<i>N. recta</i> (44) M
<i>N. sigmoidea</i> (29) E,M
<i>N. sinuata</i> (29) M
<i>N. tropica</i> (8) M
<i>N. tubicola</i> (8) E,M
<i>Pinnularia appendiculata</i> (32) A
<i>P. borealis</i> (7)
<i>P. divergentissima</i> (22)
<i>P. gibba</i> (31)
<i>P. interrupta</i> (17)
<i>P. legumen</i> (6)
<i>P. lundii</i> (14) A
<i>P. subcapitata</i> (23) A
<i>P. viridis</i> (9)
<i>Pleurosira laevis</i> (6) E
<i>Rhoicosphenia curvata</i> (156)
<i>Stauroneis anceps</i> (19) E
<i>S. kriegeri</i> (6)
<i>S. phoenicenteron</i> (10) E
<i>S. producta</i> (10)
<i>S. smithii</i> (28)
<i>S. thermicola</i> (10)
<i>Stenopterobia delicatissima</i> (5) M
<i>S. hantzschii</i> (24)
<i>Surirella amphioxys</i> (10) M
<i>S. angusta</i> (68) E,M
<i>S. brebissonii</i> (11) E,M
<i>S. minuta</i> (110) E,M
<i>S. subsalsa</i> (10) M
<i>S. tenera</i> (45) M
<i>Synedra acus</i> (10) E
<i>S. ulna</i> (167) E
<i>Tabellaria flocculosa</i> (28) A
<i>Thalassiosira weissflogii</i> (11) E

The letters following the parentheses indicate the acidobiontic (A) and eutraphentic (E) and motile (M) taxa.

significantly correlated with the second canonical axis (Table 3, Fig. 2c).

4.5. Eutraphentic diatoms

The eutraphentic diatoms metric was positively correlated with channel substrate size, and negatively correlated with total P, ANC, Cl^- , and total N (Table 2). This metric was negatively correlated with the first canonical axis (Table 3, Fig. 2d).

4.6. Motile diatoms

The motile diatom metric was positively correlated with channel substrate size and channel slope, and negatively correlated with total P, ANC, Cl^- , and riparian disturbances (Table 2). The motile diatom metric was negatively correlated with the first canonical axis (Table 3, Fig. 2e).

4.7. Chlorophyll

The chlorophyll metric was positively correlated with riparian canopy cover; negatively correlated with ANC, stream width, and Cl^- , and riparian disturbance (Table 2), and negatively correlated with the second canonical axis (Table 3, Fig. 2f).

4.8. Biomass

The biomass metric was positively correlated with channel slope and riparian canopy cover, and negatively correlated with SO_4^{2-} , total N, and Cl^- (Table 2). The biomass metric was not significantly correlated with any canonical axis (Table 3).

4.9. Alkaline phosphatase activity

The phosphatase metric was positively correlated with canopy cover and total P, and negatively correlated with stream width and depth, and channel substrate size (Table 2). This metric was not correlated with any canonical axis (Table 3).

4.10. Periphyton index of biotic integrity (PIBI)

The PIBI was positively correlated with riparian canopy cover, and negatively correlated with Cl^- ,

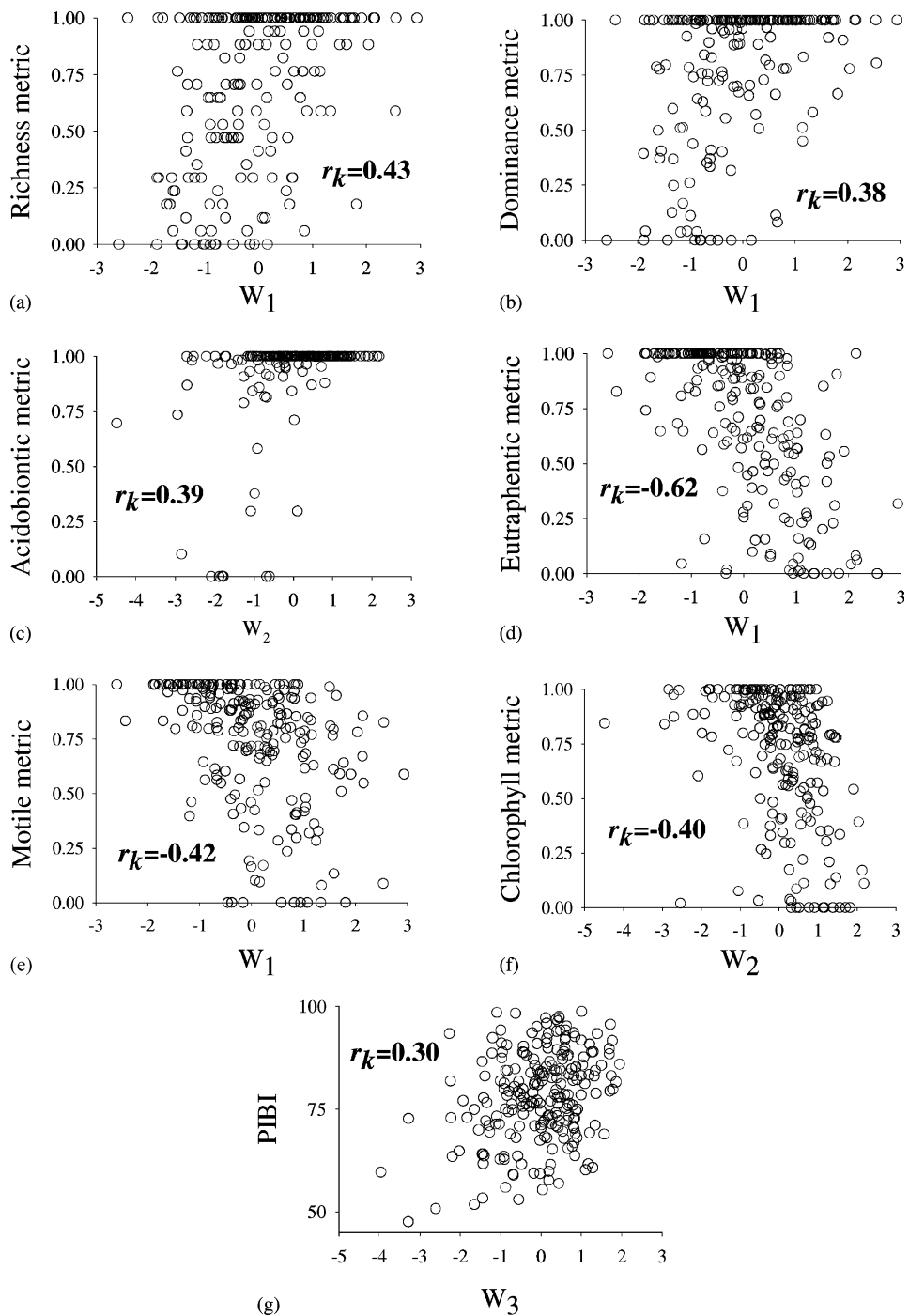


Fig. 2. Correlation PIBI metrics and index scores with the canonical axes (W_1 , W_2 , W_3): (a) richness metric; (b) dominance metric; (c) acidobiontic metric; (d) eutraphentic metric; (e) motile metric; (f) chlorophyll metric; and (g) PIBI. r_k : canonical correlation coefficient.

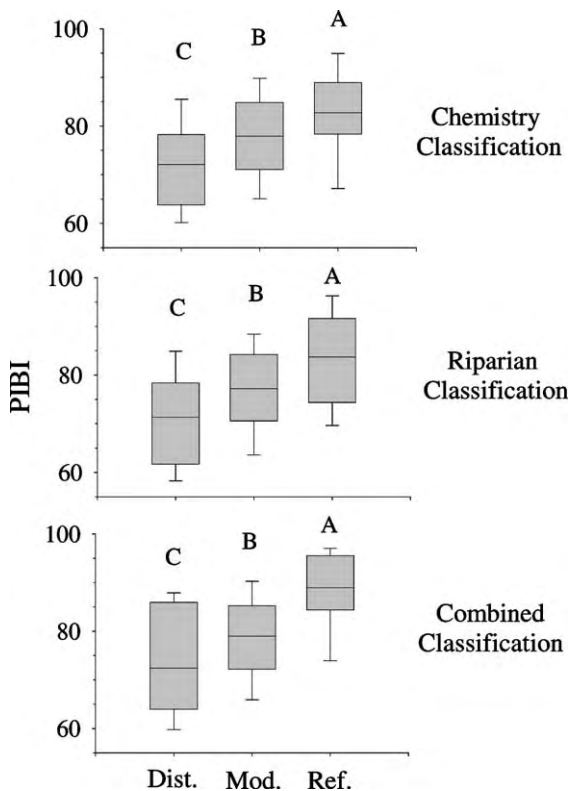


Fig. 3. Box plot of PIBI scores for disturbed (Dist.), moderately impacted (Mod.), and reference (Ref.) streams classified by stream chemistry (top), riparian disturbance (middle), and the combined reference classes (bottom). The boxes represent the 25th and 75th percentiles; the line across the box is the median; the error bars are the 10th and 90th percentiles. Letters above the boxes indicate their inclusion in Scheffe groups, an a posteriori means test of analysis of variance. Different letters are significantly different at the 0.10 level.

SO_4^{2-} , riparian disturbances, and channel substrate size (Table 2). The PIBI was positively correlated with the third canonical correlation axis (Table 3, Fig. 2g). For all stream classifications, the PIBI was significantly higher in reference stream classes, followed by moderate and disturbed streams (Fig. 3).

4.11. Assessment of ecological condition

Our survey of these 272 streams represents 196,500 km of streams in the eastern United States. Using criteria determined from the univariate distribution of PIBI scores from our reference streams and

the expansion factors associated with each stream, we classified 4.3% of this total stream length in excellent condition, 20.8% in good condition, 56.4% in fair condition, and 18.5% in poor condition (Fig. 4).

5. Discussion

Section 305(b) of the Clean Water Act (CWA) requires states, territories, tribes, and interstate commissions to assess the conditions of their waters and the extent that these waters meet water quality standards and support designated uses. The most recent report indicated that 45% of total stream length in the United States is impaired (35%) or threatened with impairment (10%) (US EPA, 2000a). States in the Mid-Atlantic region report similar findings: e.g. Delaware 63% in poor condition; Pennsylvania 34%; West Virginia 11% (US EPA, 2000a). Monitoring agencies are also required by the CWA (section 303(d)) to prioritize impaired waters as a step towards their restoration. Inherent in this process is the need to determine the causes of impairment. The most recent summary of 305(b) and 303(d) reporting lists siltation (40% of stream miles) and nutrients (30%) as leading stressors in streams and rivers, and agricultural practices (58% of stream miles) as the probable source of stressor loading (US EPA, 2000a).

Our estimates of stream condition based on diatoms, analogous to the CWA aquatic life support criteria, are less optimistic than those reported by monitoring agencies. These agencies found that 58% of the stream length monitored was in good condition, 31% was either threatened or in fair condition, and only 10% was in poor condition (US EPA, 2000a). Our estimates of streams in excellent or good condition (25%), while much lower than estimates by state monitoring agencies, are similar to those based on a fish (29%) and macroinvertebrate (25%) IBI for small Appalachian streams (US EPA, 2000e; McCormick et al., 2001). We used US EPA recommendations (US EPA, 2000b,c,d) and the univariate distribution of our reference site data to set assessment criteria. The results of which, for our study region, are that only a quarter of those sites scored well.

Streams may be simultaneously impacted by many anthropogenic stressors, especially as their watersheds are cleared for agriculture and urban development.

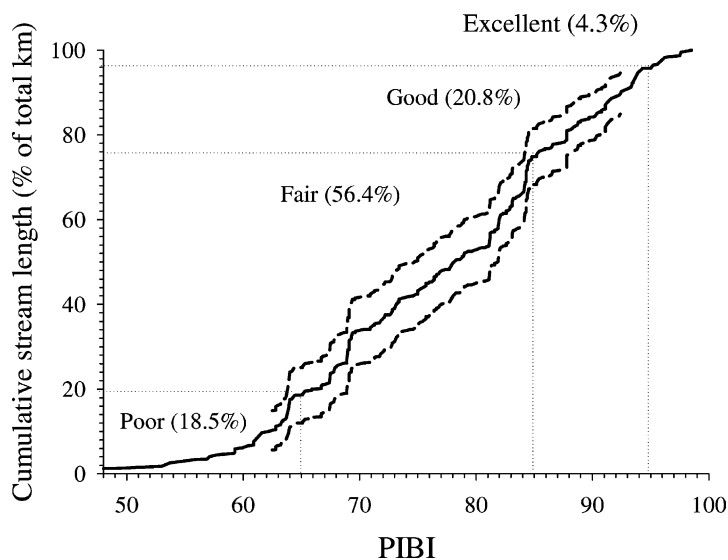


Fig. 4. Cumulative stream length of streams in excellent, good, fair, or poor condition. The solid line is the cumulative stream length (as percent of total stream length) bounded by the upper and lower 90th percentile confidence interval (dashed lines). Thresholds for assessment categories are based on the 75th, 25th, and 5th percentile scores for the distribution of PIBI scores at reference sites.

Assessments of these resources require an approach that is sensitive to environmental changes and responsive to specific stressors. The use of diatoms for monitoring streams is well established (Pan et al., 1996; Hill et al., 2000; Leland et al., 2001), but not widely used. Multimetric biotic indices and multivariate analyses represent the most recent approaches to biological monitoring of streams (Gerritsen, 1995; Norris, 1995; Reynoldson et al., 1997). The multimetric indices are based on correlations of assemblage data with environmental parameters, and multivariate analyses are based on the correspondence of environmental data with biological assemblages.

Multimetric indices have been criticized because they reduce data into a single number, and because their statistical behavior and distribution are not well known (Suter, 1993; Norris, 1995). Gerritsen (1995), however, argued that data simplification is the goal of a multimetric index, and it is this feature that allows them to be used by resource managers who may not be experts in stream ecology.

Regardless of the approach taken, the resulting index should be composed of individual biological metrics that have clear relationships to specific environmental stressors, and these metrics should lend themselves to aggregation into a composite index.

The index should provide a quick assessment of the overall condition of a stream, which is easily understood by non-technical resource managers, while the individual metrics should provide insight into the causes of impairment (Karr, 1991; Cairns, 1993).

6. Conclusions

In order for diatom assemblages to be most useful in the monitoring and management of streams in these changing environments, their attributes and indices should respond both to specific and multiple environmental stressors in a predictable manner. The diatom assemblage attributes and periphyton index used in this study were selected because of reported relationships between these attributes and indices and environmental degradation. The PIBI is not influenced significantly by stream size nor watershed area, both of which are significant factors influencing fish IBIs. We think this is one of the fundamental differences between fish IBIs and those developed for lower trophic levels, and this is demonstrated by species richness, which for diatoms does not exhibit significant increase with increasing stream size, whereas such an expectation is valid for fish. The PIBI is

relatively independent of specific assemblage composition, depending more on functional attributes of the assemblage, which, because of the cosmopolitan distribution of diatom species, allows its use across broad geographical ranges.

The PIBI and its component metrics were responsive to both individual chemical and habitat variables, and to the suites of environmental variables represented by the canonical correlation axes. The composite PIBI also demonstrated sensitivity to disturbance gradients. As a result, we believe that the PIBI and its component metrics are useful measures of ecological conditions in streams, and provide insight into the causes of impairment of these resources. We further believe that the PIBI, in conjunction with established assessment criteria, presents a realistic and defensible assessment of the ecological condition of streams of the region and a benchmark against which future studies may be compared.

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