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Ecoregions and benthic diatom assemblages in Mid-Atlantic Highlands streams, USA

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Abstract. Ecoregional differences in geology and hydrology may affect physical and chemical conditions in streams and, consequently, the species composition of algal assemblages. Stresses resulting from human disturbance, however, may constrain species membership in algal assemblages and reduce regional diversity. We expected that ecoregional differences in diatom assemblages, if they were present, would be more evident in relatively undisturbed sites than in randomly selected sites. Benthic diatom and water chemistry samples were collected from streams in 7 ecoregions of the Mid-Atlantic Highlands to evaluate correspondence between ecoregional classification and diatom assemblages. Ecoregional differences were assessed using 196 randomly selected stream sites (probability sites) and 60 sites with less disturbance by humans (reference sites). Multivariate analyses showed that significant ecoregional differences in diatom assemblages were observed only in probability sites and not in reference sites. Water chemistry was significantly different among ecoregions, both for probability sites and for reference sites. Significant differences in diatom assemblages and water chemistry were, however, evident only among ecoregions grouped by topography (i.e., montane, high plateau, and low plateau/valley). Ecoregional differences between montane regions or low plateau/valley regions were subtle. Stream sites grouped by catchments were also significantly different in water chemistry but not in diatom assemblages, both for probability sites and for reference sites. Our data suggest that diatom assemblages respond to land use, especially agricultural activities, and thus may correspond to the ecoregional classification when land use differs significantly among these ecoregions (e.g., montane vs valley ecoregions). Diatom assemblages that lack a region-specific feature may be ideal as unbiased indicators of stream water quality.

Key words: benthic diatom, catchment, ecoregion, index, Mid-Atlantic Highlands, multivariate analysis, probability sites, reference sites.

A major goal in predictive ecology is to identify a few assembly rules for biological assemblages (Keddy 1992). Ecologists have searched for these predictors for decades (MacArthur 1972, Southwood 1977, Grime 1979, Orians 1980). An emerging view is that physical environments function like a filter and thus provide a template for biological assemblages (Southwood 1977, Keddy 1992, Townsend and Hildrew 1994, Poff 1997). A better understanding of biological assemblages in relation to their environments can enhance our predictive abilities in ecological studies and resource management.

Omernik's (1987, 1995) ecoregion classification is an attempt to divide a spatially complex landscape into regions with relatively homogeneous ecosystems, based on climate, soil, geology, and potential vegetation. The classification may provide a framework for water-resource management (Omernik 1987). Bioassessment of stream conditions has been widely recognized as a valuable tool in water-resource management (Karr 1991, Karr and Chu 1999). Benthic diatoms, for example, can respond to and integrate environmental changes through time (see review by Stevenson and Pan 1999). However, the accuracy and precision with which biological indicators or indices can be used are largely determined by how well we can distinguish the variation in biota caused by natural fluctuations from the variation resulting from human-related disturbance. Ecoregional classification may distinguish natural variability in biota between ecoregions, so that robust and sensitive biocriteria can be developed for water-resource management (Hughes and Larsen 1988).

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TABLE 1. Characterization of the ecoregions in the Mid-Atlantic Highland region (modified from Woods et al. 1996). 60 = Northern Appalachian Plateau and Uplands, 62 = North Central Appalachians, 66 = Blue Ridge Mountains, 67 = Central Appalachian Ridges and Valleys (divided into Ridge and Valley), 69 = Central Appalachians, 70 = Western Allegheny Plateau.

Ecoregion	Land-surface form	Land use	Soil parent material	Vegetation
60	Low plateau	Cropland, pasture, and woodland	Shales and siltstones	Appalachian oak forests
62	Elevated plateau	Forests	Sandstone, shale, and till	Northern hardwoods
66	Mountainous ridges	Forests	Metavolcanic and igneous rocks	Appalachian oak/northern hardwoods
67 Ridge	Mountainous ridges	Forests	Sandstone and shale	Appalachian oak and hickory/pine forests
67 Valley	Valley	Cropland, woodland	Limestone and shale	Appalachian oak and hickory/pine forests
69	High plateau	Forests, cropland	Sandstone, shale, conglomerate, and coal	Appalachian oak, northern hardwood, and mixed mesophytic forests
70	Unglaciated low plateau	Urban-industrial fields, pasture, forests, and mines	Sandstone, shale, siltstone, limestone, and coal	Appalachian oak/mixed mesophytic forests

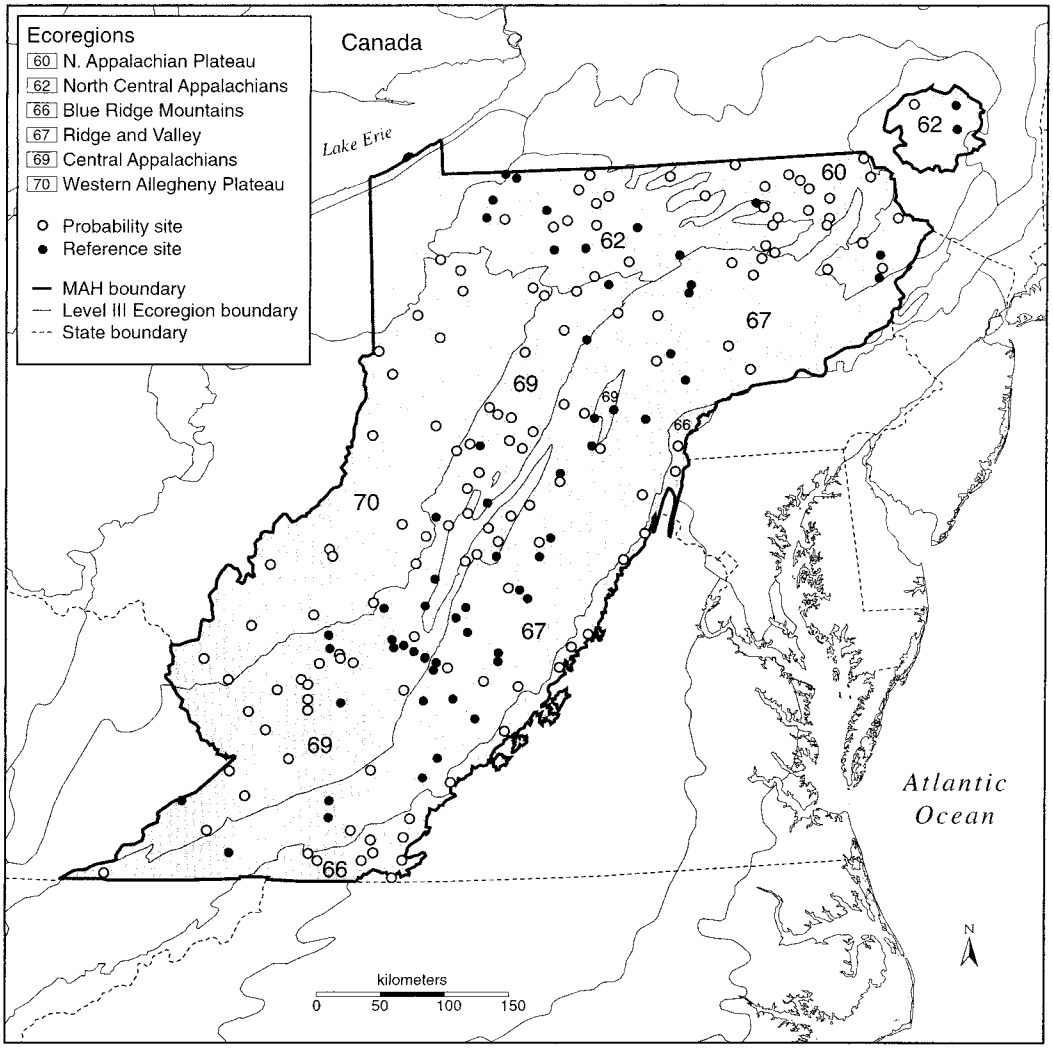

FIGURE 1. The Mid-Atlantic Highlands (MAH) region showing sampling locations.

The main objective of this study was to examine correspondence between the Omernik ecoregion classification and benthic diatom assemblages in Mid-Atlantic Highlands (MAH) streams. First, we expected that diatom assemblages would be more similar within ecoregions than among ecoregions if ecoregional properties (e.g., climate, geology, vegetation, and soil) have significant impacts on diatom ecology in streams. Second, we expected that ecoregional differences in diatom species composition would be more evident among relatively undisturbed sites than among randomly selected sites. Human disturbance is likely to reduce spatial heterogeneity, constrain the species represented at a site, and mask ecoregional differences. Also, we looked for correspondence between benthic diatom assemblages and catchments to assess the effectiveness of an alternative stream classification system.

Study Area

The MAH region is one of the most diverse physical and ecological regions in the US (Jones et al. 1998). Topographic variation creates a mo-

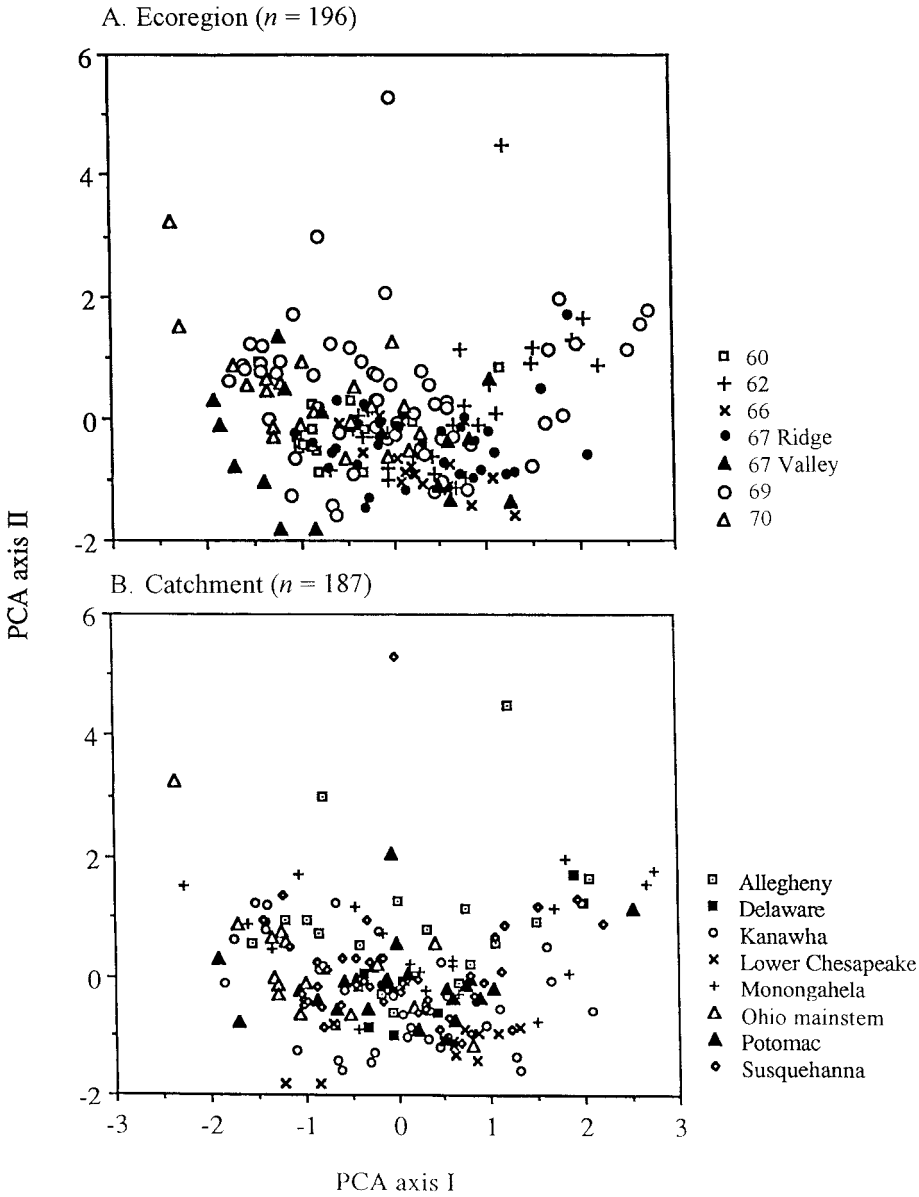

FIGURE 2. Principal component analysis (PCA) ordination diagrams of the probability sites based on environmental variables (only the first 2 PCA axes were plotted). Ecoregions (A) and catchments (B) are indicated by different symbols. 60 = Northern Appalachian Plateau and Uplands, 62 = North Central Appalachians, 66 = Blue Ridge Mountains, 67 = Central Appalachian Ridges and Valleys (divided into Ridge and Valley), 69 = Central Appalachians, 70 = Western Allegheny Plateau.

saic of strikingly different landscapes. Land-surface form varies from valley to plateau and mountainous ridges. Land cover/land use includes dense forests, industrial fields, urban areas, mining fields, and agricultural fields (Woods et al. 1996, Jones et al. 1998). The region has been delineated into several ecoregions (Omernik's 1987 Level III ecoregion classifica-

TABLE 2. Land use/land cover (%) for Omernik (1987) ecoregions (Level III) in the Mid-Atlantic Highlands region based on the thematic mapper (TM) version 2 data. Ecoregion codes as in Table 1.

Ecoregion	Urban	Agriculture	Forest	Water	Wetlands	Mines/quarries	Other
60	0.84	32.11	64.52	1.58	0.76	0.02	0.17
62	0.73	4.89	91.59	0.88	1.10	0.22	0.59
66	0.38	14.55	84.25	0.39	0.13	0.03	0.27
67	2.64	30.49	64.85	0.94	0.38	0.36	0.34
Ridge	1.03	13.31	84.03	0.59	0.21	0.42	0.41
Valley	3.74	47.23	47.01	1.15	0.50	0.13	0.24
69	0.87	10.30	85.89	0.58	0.31	1.72	0.33
70	3.81	22.02	72.06	0.94	0.13	0.73	0.31

tion), which include the Northern Appalachian Plateau and Uplands, the North Central Appalachians, the Blue Ridge Mountains, the Central Appalachian Ridges and Valleys, the Central Appalachians, and the Western Allegheny Plateau. For our study, the Central Appalachian Ridges and Valleys ecoregion was further divided into 2 separate ecoregions (Ridge, Valley) because of its contrasting topography. Major characteristics of the 7 ecoregions are summarized in Table 1. Detailed descriptions of the region and its ecoregions can be found in Woods et al. (1996) and Jones et al. (1998).

Methods

Data sets

Correspondence between the ecoregion or catchment classification and benthic diatom assemblages was evaluated using 2 data sets: probability-based sites and reference sites.

Probability based data set.—A total of 196 sites within 7 ecoregions was used for ecoregional analyses (Fig.1). Stream sites were selected using a systematic randomized design. The stream population in the region was estimated from USGS 1:100,000 scale topographic maps. Sample probabilities were set so that roughly equal numbers of 1st-, 2nd-, and 3rd-order streams would appear in the sample. These wadeable stream sites were sampled for periphyton and water chemistry from late April to early July in 1993, 1994, and 1995. The sampled sites were distributed among 11 major catchments: Allegheny, Delaware, Hudson, Kanawha, Lower Chesapeake, Monongahela, Ohio mainstem, Potomac, Roanoke, Susquehanna, and Tennessee. Because of small sample sizes, sites in Hudson (2), Roanoke (4), and Tennessee (3) catchments were not included in the final catchment analysis.

Reference data set.—Two sets of criteria were used to select relatively undisturbed sites from 196 randomly selected sites. Previous studies indicated that acidification and eutrophication were major environmental problems in streams of the region (Herlihy et al. 1990, 1998, Pan et al. 1996, 1999). Sites were eliminated if acid neutralizing capacity (ANC) was <0 μeq/L or if $SO_4^=$ concentration was >300 μeq/L (Herlihy et al. 1990) and if total P concentration ([TP]) was >25 μg/L and total N concentration ([TN]) was >700 μg/L (Dodds et al. 1998).

Most selected reference sites were in 4 ecoregions (Ridge, Valley, North Central Appalachians, and Central Appalachians) and 6 catchments (Allegheny, Kanawha, Lower Chesapeake, Monongahela, Potomac, and Susquehanna). A total of 60 and 53 sites were used for reference-based ecoregional and catchment analyses, respectively.

Data collection

Land use.—Land cover/land use for each ecoregion in the MAH region was estimated from Landsat satellite images (Thematic Mapper version 2 data). The resolution of the land cover/land use data was 30 m (Jones et al. 1998).

Water chemistry.—A cubitainer (4 L) and 4 syringes (each 60 mL) of stream water were collected at a flowing-water area near the middle of the stream. The syringes were sealed with a Luer-lock valve to prevent gas exchange. Within 48 to 72 h of collection, water from the syringe samples was analyzed for closed headspace measurements of pH, and the cubitainer sample

TABLE 3. Principal component analysis (PCA) loadings of environmental variables in the Mid-Atlantic Highlands streams. ANC = acid neutralizing capacity, DIC = dissolved inorganic carbon, DOC = dissolved organic C, TSS = total suspended solids, TN = total N, TP = total P.

	PCA axis (reference sites)			PCA axis (probability sites)		
Variables	I	II	III	I	II	III
pH	0.90	−0.32	0.09	−0.84	−0.50	0.01
ANC	0.92	−0.32	0.08	−0.91	−0.33	0.05
Conductivity	0.96	0.12	0.01	−0.90	0.35	−0.12
Ca	0.95	−0.17	−0.06	−0.92	0.23	−0.04
Mg	0.82	0.11	0.10	−0.85	0.36	−0.17
K	0.44	0.21	0.01	−0.80	0.24	−0.07
Na	0.60	0.74	0.12	−0.78	0.25	−0.19
Cl	0.49	0.81	0.08	−0.62	0.33	−0.08
SO_4	0.49	0.44	−0.18	−0.61	0.59	−0.24
Al	−0.39	0.31	−0.41	0.42	0.63	0.21
DIC	0.90	−0.34	0.07	−0.90	−0.18	0.06
DOC	0.03	0.08	−0.40	−0.20	0.11	0.52
SiO_2	0.08	0.06	0.03	−0.32	0.01	0.02
Turbidity	0.19	−0.15	−0.67	−0.38	0.15	0.72
TSS	0.17	−0.30	−0.73	−0.30	0.12	0.81
TP	0.32	−0.02	−0.60	−0.42	0.01	0.75
TN	0.37	0.14	−0.75	−0.50	0.32	0.36
NH_4	0.34	0.30	−0.10	−0.28	0.30	0.22
NO_3	0.32	0.23	−0.65	−0.43	0.29	0.07
Temperature	0.12	−0.07	−0.12	−0.28	−0.01	0.14
Elevation	−0.26	−0.40	0.05	0.44	−0.09	0.03
% of variance	0.46	0.18	0.11	0.51	0.14	0.09

TABLE 4. Mahalanobis distance measures (D^2) among the ecoregion centroids of principal component analysis scores on environmental variables (lower left triangle) and nonmetric multidimension scaling scores on diatom composition (upper right triangle). Bold numbers indicate that distances were significant at $p < 0.05$. Ecoregion codes as in Table 1.

A. Probability sites Ecoregions	60	62	66	67 Ridge	67 Valley	69	70
60	**	0.16	0.09	0.36	0.63	**0.61**	0.62
62	**2.56**	**	0.08	0.22	**0.61**	**0.35**	**0.84**
66	**1.64**	**1.74**	**	0.09	0.31	0.24	0.41
67 Ridge	**1.47**	**0.51**	0.44	**	0.11	0.04	0.30
67 Valley	0.26	**2.03**	**0.98**	**0.81**	**	0.12	0.09
69	**1.31**	**0.51**	**2.27**	**0.92**	**1.32**	**	**0.40**
70	0.42	**3.37**	**3.54**	**0.81**	**1.14**	**1.41**	**

B. Reference sites Ecoregions	62	67 Ridge	67 Valley	69
62	**	0.21	0.30	0.07
67 Ridge	0.89	**	0.73	0.20
67 Valley	**1.86**	0.60	**	0.16
69	**1.54**	0.51	**1.65**	**

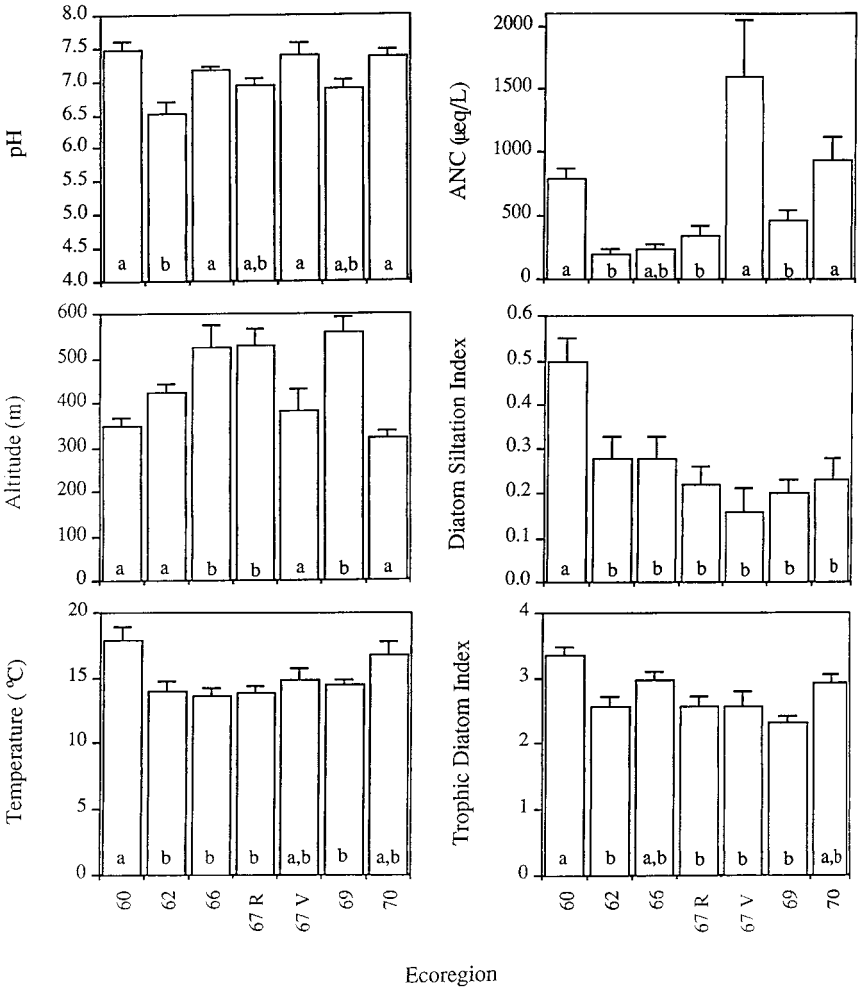

FIGURE 3. Selected environmental variables and diatom indices (mean ±1 SE) with significant (all p = 0.0001) ecoregional differences (probability sites only; different letters in the figure show significant differences at $p < 0.05$). Ecoregion codes as in Fig. 2. R = Ridge, V = Valley. ANC = acid neutralizing capacity, DOC = dissolved organic C, TP = total P, TSS = total suspended solids.

was split into aliquots and preserved (Pan et al. 1996). Base cations were determined by atomic absorption, anions by ion chromatography, dissolved organic C concentration ([DOC]) by a C analyzer, and [TN] and [TP] by persulfate oxidation. Detailed information on the analytical procedures can be found in USEPA (1987).

Periphyton.—Periphyton samples were collected from a study reach (40× the mean wetted channel width) at each site. The study reaches, ranging from 150 to 500 m long, were each divided into 11 cross-section transects with equal length intervals. A periphyton sample was collected in riffle habitats at each of the 11 transects and combined into a composite sample. Periphyton was scraped off coarse substrata from a defined area of stream bed (12 cm^2) with a toothbrush and rinsed with stream water. For fine substrata, periphyton was sucked into a 60-mL syringe. Composite periphyton samples were then preserved with 37% formalin. A subsample of the preserved periphyton suspension was acid-cleaned and mounted in HYRAX® to enumerate diatom species (Patrick and Reimer 1966). A minimum of 500 diatom valves was counted at 1000× magnification. Patrick and

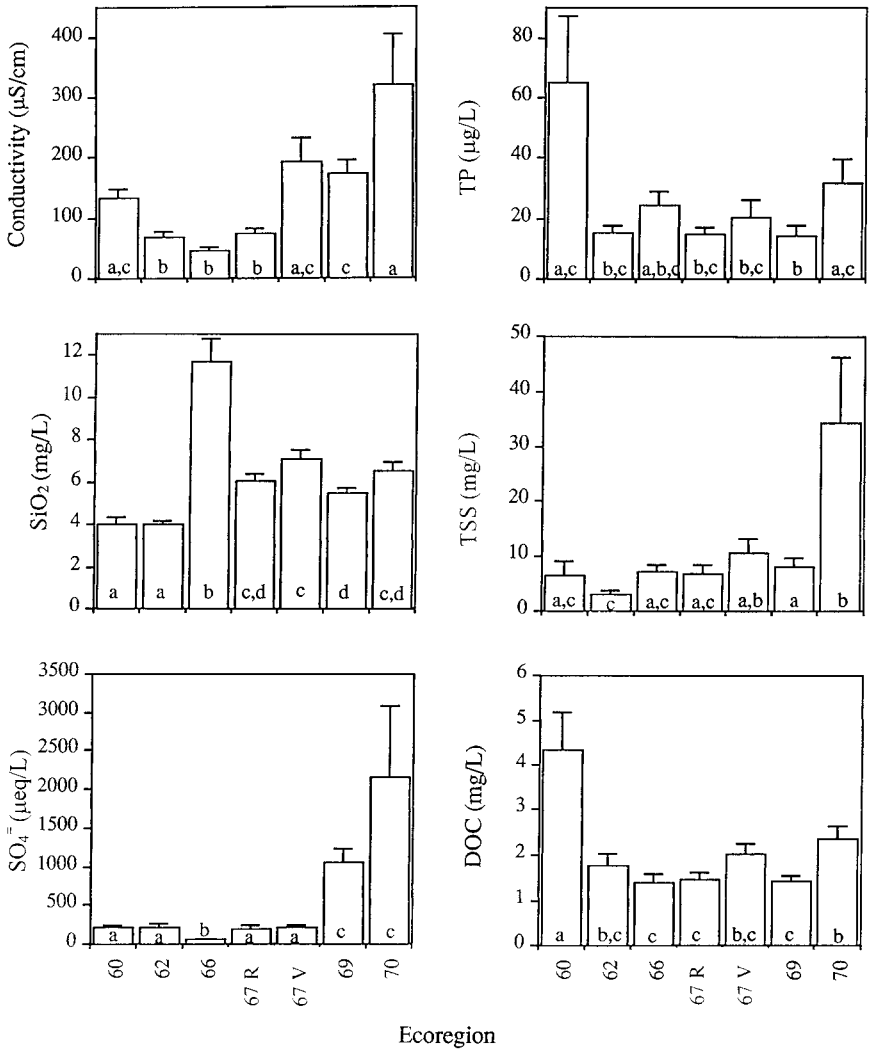

FIGURE 3. Continued.

Reimer (1966, 1975) and Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b) were used as primary references for diatom taxonomy.

Data analysis

Periphyton assemblages are dominated by diatoms in the MAH streams (Pan et al. 1999). Because of their high abundance and well-known taxonomy, only diatoms were used for analyses in this study.

Principal components analysis (PCA) was used to identify and summarize major patterns or trends of water chemistry in the region. All environmental variables except pH were log-transformed before analysis. Nonmetric multidimensional scaling (NMDS), a multivariate ordination technique (Kenkel and Orloci 1986), was used to identify diatom distribution patterns with relation to the ecoregion or catchment classification. If the ecoregion or catchment classification could group streams with similar biota, we expected that sites within an ecoregion or catchment should be more similar than sites in different ecoregions or catchments, based on biotic assemblages. Therefore, distinctive clusters of stream sites should be evident, grouped by ecoregions or catchment. Pair-wise ecological

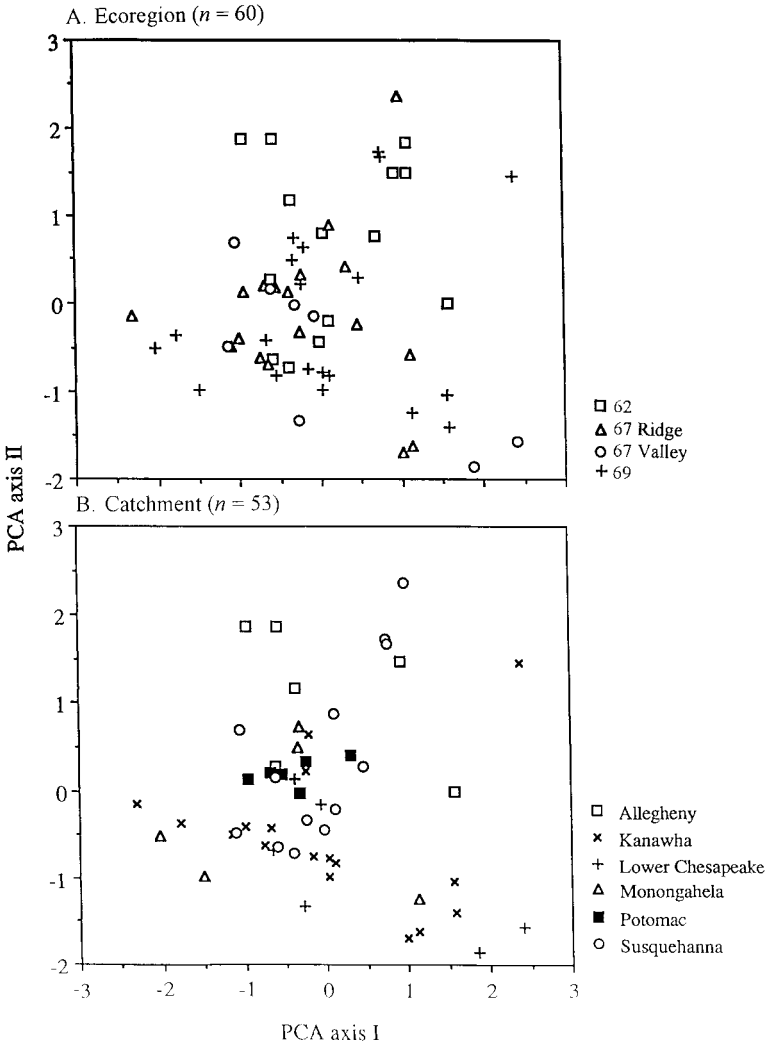

FIGURE 4. Principal component analysis (PCA) ordination diagrams of the reference sites based on environmental variables (only the first 2 PCA axes were plotted). Reference sites in ecoregions (A) and catchments (B) are indicated by different symbols. Ecoregion codes as in Fig. 2.

distances (normalized Euclidean distance) were calculated for all sites based on diatom species composition (relative abundance). Euclidean distances between sites were then projected to 2-dimensional plots with a minimum distortion by using the NMDS method. The NMDS was performed using SYSTAT (L. Wilkinson. 1992. SYSTAT: statistics, version 5.2, SYSTAT, Inc., Evanston, Illinois). A flexible unweighted pair-group method using arithmetic averages (UPGMA) clustering method was also used to classify stream sites based on diatom data (relative abundance or presence/absence). The UPGMA was performed using the Bray–Curtis association measure with a β value of -0.1, using PATN (L. Belbin. 1993. PATN, pattern analysis package, Division of Wildlife and Ecology, CSIRO, Canberra, Australia).

The PCA (3 axes) and NMDS (2 axes) sample scores were used as composite variables of water chemistry and diatom assemblages, respectively, in canonical discriminant analyses (CDA) to assess ecoregion or catchment (Jackson and Harvey 1989). Overall ecoregion or catchment

TABLE 5. Correspondence between ecoregion- and diatom-based stream classification. The numbers are sampled stream sites. Stream sites were classified based on diatom relative abundance and presence/absence (binary) data using the UPGMA (unweighted pair-group method using arithmetic averages) clustering method. Ecoregion codes as in Table 1.

A. Probability sites

	Diatom-based stream clusters													
	Abundance data							Binary data						
Ecoregions	I	II	III	IV	V	VI	VII	I	II	III	IV	V	VI	VII
60	0	0	0	8	6	1	1	1	13	2	0	0	0	0
62	1	7	3	3	8	0	9	4	10	6	1	4	3	3
66	2	2	3	2	1	10	0	2	2	10	2	3	1	0
67 Ridge	7	2	4	5	6	6	4	7	8	5	2	2	9	1
67 Valley	1	2	9	2	0	0	2	1	0	0	4	7	4	0
69	12	7	10	3	7	6	12	18	12	9	10	3	4	1
70	3	5	4	2	8	0	0	2	1	2	7	8	2	0

B. Reference sites

	Diatom-based stream clusters						
	Abundance data				Binary data		
Ecoregions	I	II	III	IV	I	II	III
62	0	3	9	2	1	11	2
67 Ridge	7	3	7	1	7	4	7
67 Valley	3	3	0	2	2	1	5
69	5	4	7	4	9	8	3

differences were assessed using multivariate analysis of variance (MANOVA). Mahalanobis distances, which are squared distance measures between centroids of ecoregions or catchment in the space defined by CDA, were used to evaluate group-specific differences among ecoregions or catchments using SAS (1985. SAS user's guide: statistics, SAS Institute, Cary, North Carolina; Jackson and Harvey 1989). Univariate statistical analyses (ANOVA and Tukey test) were also used to assess ecoregion or catchment differences in terms of individual environmental variables (Zar 1984).

The Siltation Index, a summation of the relative abundance of all motile diatoms, was calculated following Bahls (1993). The Trophic Diatom Index was calculated following Kelly and Whitton (1995).

Results

Ecoregional classification and benthic diatom assemblages

Dominant types of land cover/land use varied along a topographic gradient (Table 2). Montane and elevated plateau ecoregions (Blue Ridge Mountains, Ridge, North Central Appalachians, and Central Appalachians) were covered by a high proportion of forests (mean = 86.4%). Agricultural activities were relatively intense in valley and low plateau ecoregions (Valley, Northern Appalachian Plateau and Uplands, and Western Allegheny Plateau, mean = 33.8%).

Probability sites.—The PCA showed 3 major water chemistry gradients among probability stream sites (Fig. 2). Three PCA axes accounted for 74% of variance in the data set (Table 3). Axis I may represent a geochemical gradient. The axis was highly correlated with ionic strength (e.g., ANC, conductivity, and pH) (Table 3). Axis II may indicate effects of acidification on stream water chemistry. This axis was correlated positively with total dissolved Al and $SO_4^=$ concentrations and negatively with pH. Axis III was correlated with total suspended solids (TSS), [TP], and [DOC], variables commonly associated with agricultural activities.

Measured environmental characteristics at probability sites were significantly different among ecoregions (MANOVA, Wilks's λ $F_{18,529}$ =

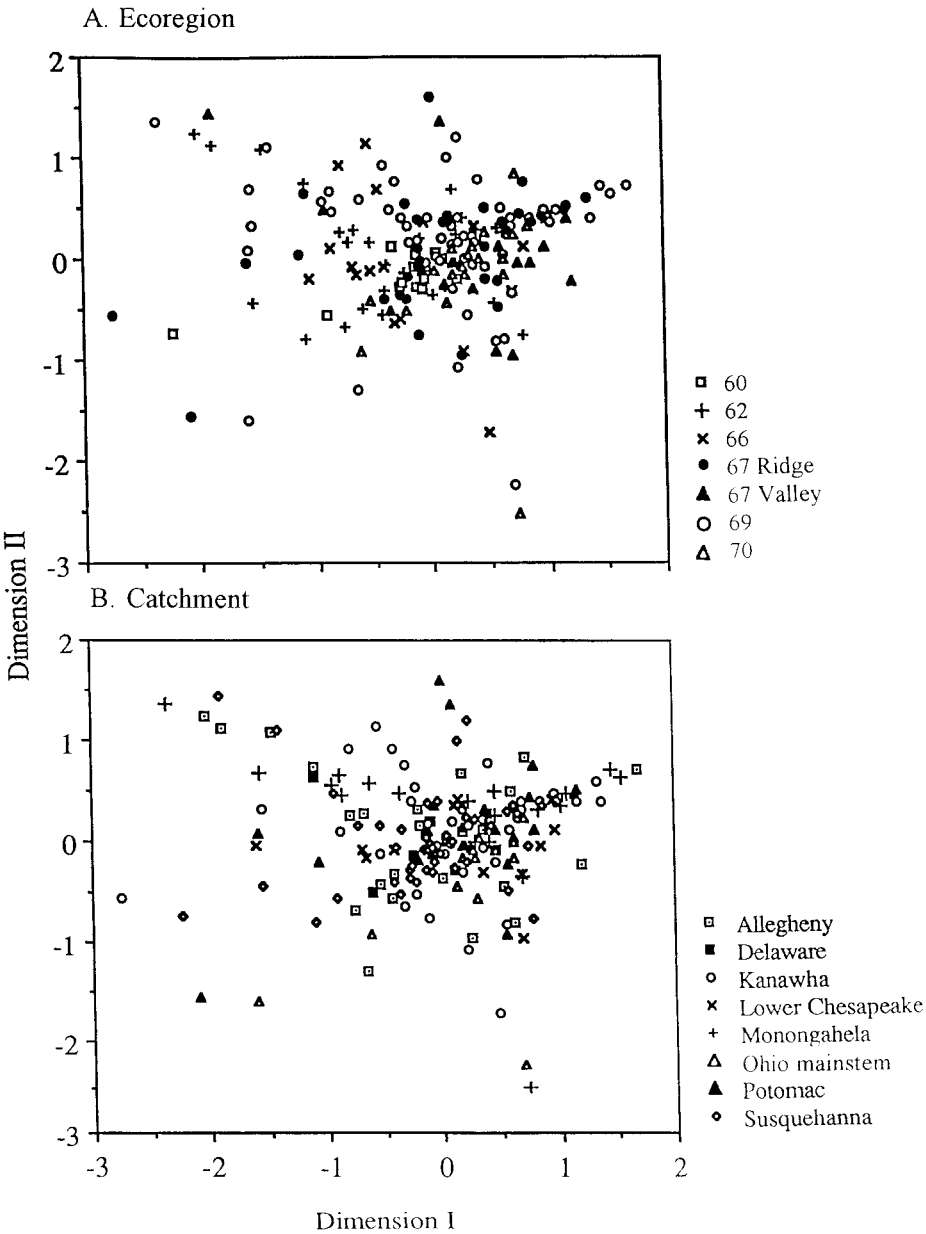

FIGURE 5. Nonmetric multidimensional scaling ordination diagrams of the probability sites based on diatom assemblages (only the first 2 ordination axes were plotted). Ecoregions (A) and catchments (B) are indicated by different symbols. Ecoregion codes and numbers of sites as in Fig. 2.

6.03, $p = 0.0001$). Mahalanobis distances of the 3 PCA axis scores on all environmental variables divided the 7 ecoregions into 4 groups: 1) low plateau and valley ecoregions (Northern Appalachian Plateau and Uplands [60], Western Allegheny Plateau [70], and Valley [67]), 2) mountainous ridge ecoregions (Ridge [67] and Blue Ridge Mountains [66]), 3) northern high plateau (North Central Appalachians [62]), and 4) north-central high plateau (Central Appalachians [69]) (Table 4). These groups were delineated based on significant differences among

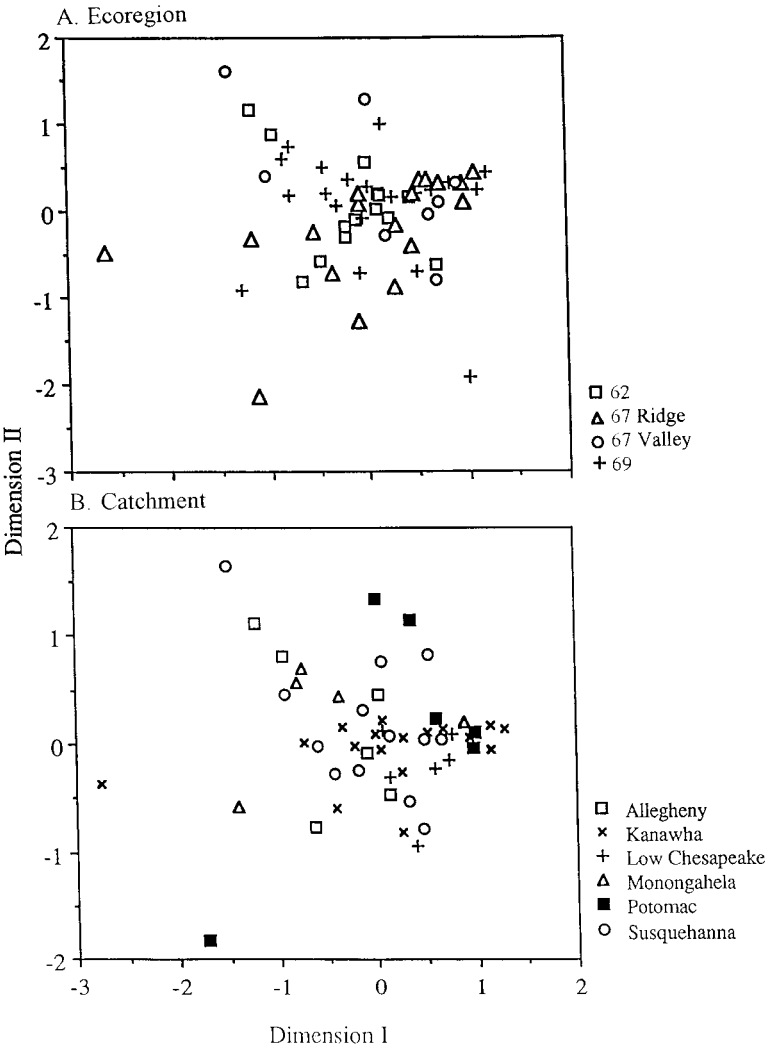

FIGURE 6. Nonmetric multidimensional scaling ordination diagrams of the reference sites based on diatom assemblages (only the first 2 ordination axes were plotted). Ecoregions (A) and catchments (B) are indicated by different symbols. Ecoregion codes as in Fig. 2 and numbers of sites as in Fig. 4.

ecological distance measures. For example, the Mahalanobis distances were not significantly different among the 3 ecoregions within group 1. Multiple comparison tests showed that some ecoregions were significantly different in altitude, temperature, ionic strength (e.g., conductivity), and nutrients (e.g., [TP]) (Fig. 3). For example, the West Allegheny Plateau Ecoregion (70) had significantly higher conductivity than the North Central Appalachians (62), Blue Ridge Mountains (66), Ridge (67), and Central Appalachians (69). However, overlaps in these environmental variables among some ecoregions were evident (Fig. 3).

Species composition of diatom assemblages at probability sites was significantly different among some ecoregions (MANOVA, Wilks's λ $F_{12,376} = 1.95$, $p = 0.03$). The centroid of the North Central Appalachians (62) was significantly different from that of the Central Appalachians (69), Western Allegheny Plateau (70), and Valley (67) ecoregions ($p < 0.05$) (Table 4). The centroid of the Central Appalachians was also significantly different from that of the

Northern Appalachian (60) and Western Allegheny Plateau (70) ecoregions ($p < 0.05$). Two diatom indices were significantly different among probability sites grouped by ecoregions (Fig. 3). A multiple comparison test showed that the Siltation Index was significantly higher in the Northern Appalachian Plateau and Uplands (60) than in the rest of the ecoregions ($p < 0.05$). Probability sites in ecoregion 60 also had a significantly higher Trophic Diatom Index value than sites in the North Central Appalachians (62), Central Appalachians (69), and Ridge and Valley (67) ecoregions ($p < 0.05$) (Fig. 3).

Reference sites.—The PCA also showed 3 major water chemistry gradients among reference stream sites (Fig. 4). Three PCA axes accounted for 75% of variance in the data set (Table 3). The environmental gradients represented by the first 3 PCA axes were similar to those in the probability sites (Table 3). Ecoregions also differed significantly in environmental characteristics at reference sites (MANOVA, Wilks's λ $F_{9,132} = 2.47$, $p = 0.01$). Ecoregional centroids were significantly different between the Valley (67) and high plateau ecoregions (62 and 69) (Table 4). Differences among ecoregions in measured environmental variables were not statistically significant, except for SiO_2. Concentrations of SiO_2 in the Ridge (67) and Valley (67) ecoregions were significantly higher than in the North Central Appalachians (62) and Central Appalachians (69) ecoregions ($p < 0.05$).

Species composition of diatom assemblages at reference sites was not significantly different among 4 ecoregions (MANOVA, Wilks's λ $F_{6,110} = 0.84$, $p = 0.54$). There were no statistically significant differences at reference sites among ecoregions for the Trophic Diatom Index ($p = 0.42$) and Siltation Index ($p = 0.34$).

Although diatom assemblages differed significantly among some ecoregions, overall correspondence between ecoregion- and diatom-based stream classification was poor, regardless of data set (probability or reference sites) or data type (relative abundance or presence/absence) (Figs 5 and 6, Table 5). Common diatom species distributed in >3 sites in at least 1 ecoregion are listed in the appendix.

Catchment classification and benthic diatom assemblages

Probability sites.—Stream sites grouped by catchments were significantly different in environmental characteristics (MANOVA, Wilks's λ $F_{21,509} = 3.47$, $p = 0.0001$). The centroid of the Lower Chesapeake catchment was significantly different from those of the rest of the catchments ($p < 0.05$) (Table 6). The centroid of the Allegheny catchment also differed significantly from those of the rest of the catchments, except for the Delaware and Monongahela catchments ($p < 0.05$) (Table 6). Multiple comparison tests showed that sites in some catchments were significantly different in altitude, temperature, ionic strength (e.g., conductivity), and nutrients (e.g., [TN]) (Fig. 7). For example, the Ohio mainstem catchment had significantly higher pH and ANC than those of the Allegheny and Monongahela catchments (Fig. 7). Overlaps were also evident in these environmental variables among some catchments.

The MANOVA of the NMDS scores showed no significant difference among probability catchment sites in diatom species composition (Wilks's λ $F_{14,356} = 1.65$, $p = 0.06$). However, the Trophic Diatom Index differed significantly among probability catchment sites ($p = 0.003$) (Fig. 7), and was significantly lower in the Monongahela catchment than in the Ohio mainstem and Susquehanna catchments.

Reference sites.—The MANOVA of reference sites showed a significant difference among catchments in measured environmental variables (Wilks's λ $F_{15,125} = 2.65$, $p = 0.002$), but no significant difference in diatom species composition (Wilks's λ $F_{6,110} = 0.84$, $p = 0.54$). There were also no statistically significant differences among reference catchment sites for the Trophic Diatom Index ($p = 0.40$) and Siltation Index ($p = 0.61$).

Discussion

Ecoregional classification and benthic diatom assemblages

Biological assemblages are often the result of a complex interplay among several ecological processes at different temporal and spatial scales (O'Neill et al. 1986, Menge and Olson 1990). As a result, membership of the biological assemblages may be determined by the relative importance of the multiple factors (e.g., regional vs local) (Ricklefs 1987, Jackson and Harvey 1989). Correspondence between the ecoregional classification and the biota should depend on the extent to which environmental variables differ between ecore-

TABLE 6. Mahalanobis distance measures (D^2) among the catchment centroids of principal component analysis scores on environmental variables (lower left triangle) and nonmetric multidimensional scaling scores on diatom composition (upper right triangle). Bold numbers indicate that distances were significant at $p < 0.05$.

A. Probability sites

Catchment	Allegheny	Delaware	Kanawha	Lower Chesapeake	Monongahela	Ohio mainstem	Potomac	Susquehanna
Allegheny	**	0.07	0.27	0.37	0.34	**1.21**	0.30	0.07
Delaware	0.87	**	0.12	0.13	0.30	0.71	0.18	0.00
Kanawha	**1.67**	0.19	**	0.05	0.10	**0.71**	0.01	0.16
Lower Chesapeake	**4.87**	**1.71**	**1.06**	**	0.28	0.39	0.10	0.16
Monongahela	0.41	0.75	**1.09**	**3.72**	**	**1.32**	0.04	0.35
Ohio mainstem	**1.51**	1.01	**1.10**	**3.94**	**1.72**	**	**0.88**	**0.71**
Potomac	**0.87**	0.00	0.16	**1.73**	0.72	**0.92**	**	0.22
Susquehanna	**0.91**	0.21	0.20	**2.09**	0.43	**0.80**	0.16	**

B. Reference sites

Catchment	Allegheny	Kanawha	Lower Chesapeake	Monongahela	Potomac	Susquehanna
Allegheny	**	0.75	1.92	0.03	0.69	0.22
Kanawha	**4.15**	**	0.27	1.00	0.14	0.24
Lower Chesapeake	**6.49**	1.43	**	2.33	0.58	1.01
Monongahela	**5.29**	1.43	**5.69**	**	0.85	0.32
Potomac	1.46	0.94	**3.27**	1.69	**	0.13
Susquehanna	0.92	**1.25**	**3.64**	2.06	0.17	**

gions, and on the strength of linkages between the biota and ecoregional variables.

Ecoregions, a nonhierarchically based classification system, may poorly correspond to components of the biota that are strongly regulated by local factors. Algae, macroinvertebrate, and fish assemblages respond to environmental changes at different temporal and spatial scales because of their differing life histories, physiologies, and mobilities. Thus, changes in each assemblage may reflect environmental conditions of hierarchically organized stream habitats and associated human influences (e.g., microhabitat, channel unit, reach, and catchment) (Frissel et al. 1986). Whittier et al. (1988) compared ecoregional differences in periphyton, macroinvertebrates, and fish in 48 Oregon streams, and found that correspondence between ecoregions and the biota was clearest in fish and very subtle in periphyton. Their results are consistent with other studies showing scale-specific determinants for the biota. For instance, ~50% of variability in fish assemblages measured as the Index of Biological Integrity (Karr 1991) was explained by land use in catchments (Roth et al. 1996). Macroinvertebrate species traits and species compositions exhibited much stronger relationships to local environmental conditions (e.g., at reach scale) than to catchment variables (Carter et al. 1996, Richards et al. 1997). Pan et al. (1999) showed that spatial patterns of diatom assemblages in the MAH were best discriminated by both land cover/land use in catchments and by site-specific factors such as riparian conditions. Benthic diatoms in the MAH streams may respond much more strongly to local conditions than to regional factors such as climate, geology, soil, and vegetation.

Ecoregions, benthic diatom assemblages, and probability sites

Diatom assemblages may correspond to the ecoregional classification when overall land use differs significantly among these ecoregions. Correspondence between the biota and ecoregions is often clearest among ecoregions along topographic gradients (e.g., montane vs valley ecoregions) (Larsen et al. 1986, Whittier et al. 1988, Harding et al. 1997, Maret et al. 1997). Cluster analysis of the 89 MAH stream sites showed that eutrophic stream sites were more

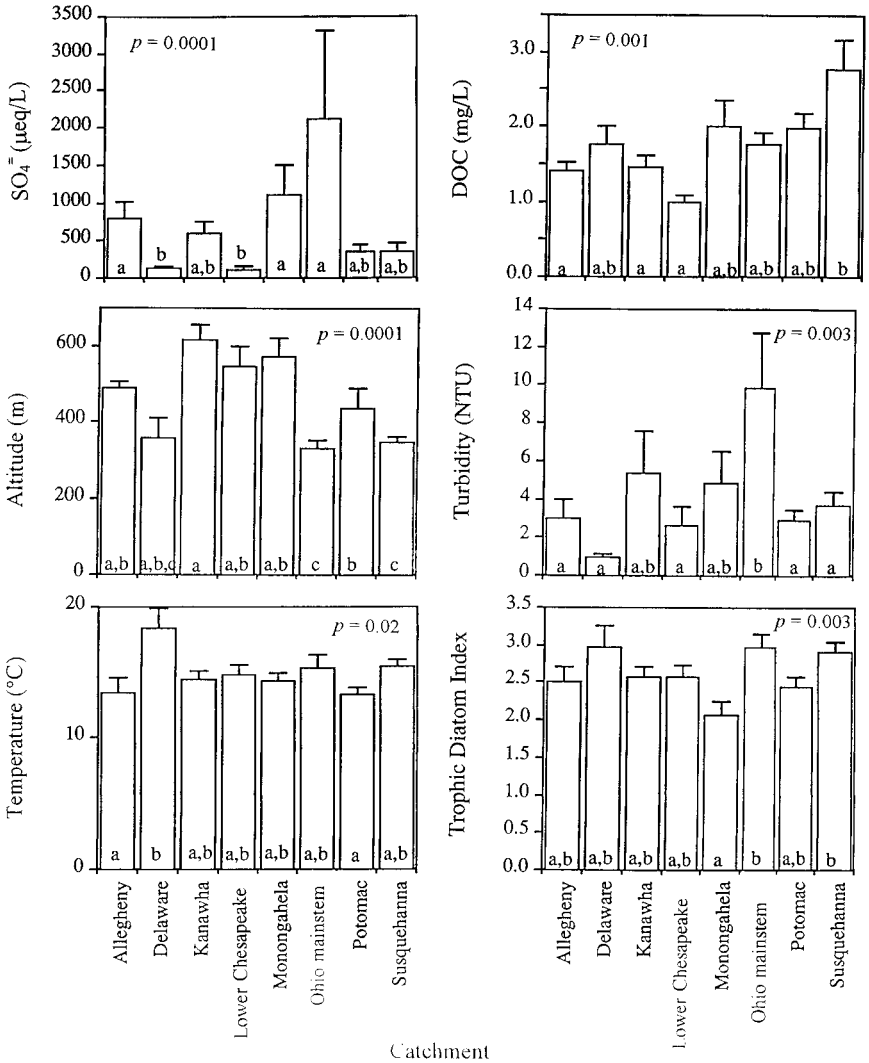

FIGURE 7. Selected environmental variables and diatom indices (mean ±1 SE) with significant (p-values shown in each panel) differences among catchments (probability sites only; different letters in the figure show significant differences at $p < 0.05$). ANC = acid neutralizing capacity, DOC = dissolved organic C, TN = total N.

frequently present in valley, low plateau, and coastal plain ecoregions with dense human settlement and intensive land use (Pan et al. 1999). Relatively few eutrophic sites were present in other ecoregions. Significant ecoregional differences in the Trophic Diatom Index and Siltation Index, diatom-based indices that indicate trophic changes (Bahls 1993, Kelly and Whitton 1995), suggested ecoregional differences in land-use activities. Our data showed that ecoregional differences in diatom assemblages became subtle after we removed the sites that were severely affected by eutrophication and acidification, and assessed only the reference sites. Correspondence between ecoregions and diatoms along the topographic gradient may largely result from variations in the frequency of occurrence of disturbed sites in ecoregions with different topography.

Ecoregions, benthic diatom assemblages, and reference sites

The lack of correspondence between ecoregions and diatoms in reference sites may be

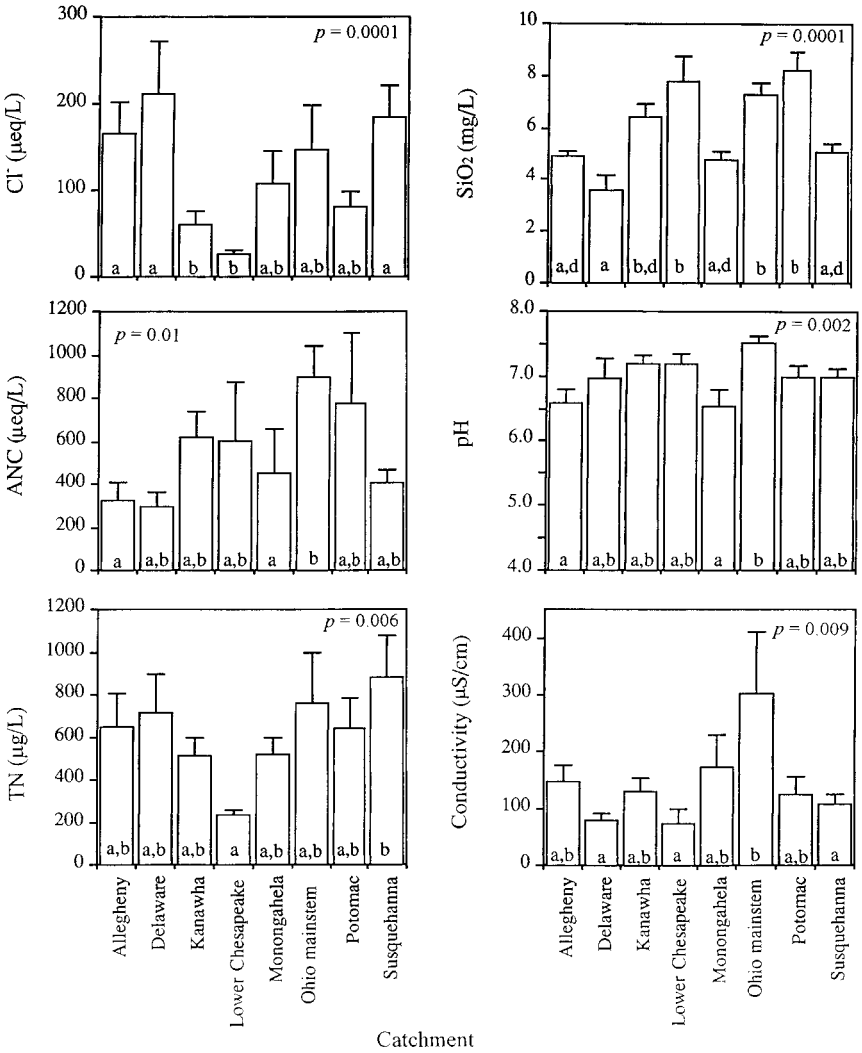

FIGURE 7. Continued.

partly related to how reference sites are defined and selected (Hughes 1995, Omernik 1995). Our reference sites were defined by the same sets of criteria for all ecoregions. Other workers' criteria for selecting reference sites may be ecoregion-specific (i.e., they select the least-impacted sites in each ecoregion) (Whittier et al. 1988). The least-impacted sites in a highly disturbed ecoregion such as valleys may be highly impacted compared to the least-impacted sites for a relatively undisturbed montane ecoregion. Ecoregion-specific criteria for reference-site selection may increase the likelihood of detecting ecoregional differences, but this approach may be problematic because of circularity in the process of environmental assessment (Omernik 1995).

Ecoregions were delineated on a composite of several qualitative variables (Omernik 1995). The relative importance of each variable may vary from one ecoregion to another. If sampled stream sites are selected from the most typical portion of the ecoregion that shares all 4 land characteristics (e.g., climate, soil, geology, and potential vegetation, Whittier et al. 1988), there may be more likelihood of detecting ecoregional differences. In our study, the stream sites were selected randomly from the region. A random sampling design may include many sites that

are located in transitional zones among ecoregions. Overlaps among ecoregions were evident in both PCA and NMDS plots. These sites may mask ecoregional differences if boundaries between ecoregions are not always sharp (Woods et al. 1996).

Dense riparian vegetation in small streams in the MAH region and temporal variability in diatom assemblages may also contribute to the lack of ecoregional differences in diatom assemblages. Of the 89 sites randomly selected from the MAH region, 46% were 1st-order streams and the rest were 2nd- and 3rd-order streams in approximately equal proportions (Pan et al. 1999). The mean wetted stream width was 5.1 m. Stream channels were usually well-shaded, averaging 83.4% bank-side and 76.1% mid-channel riparian canopy. Shading in small streams may constrain the number of diatom species. Diatom assemblages in the MAH streams are dominated by a few species such as *Achnanthes minutissima, Cymbella minuta,* and *Synedra rumpens.* Most of them are cosmopolitan and are early successional species (Lowe 1974). Diatom samples were collected over 3 mo in each year for 3 y. Temporal variation, although not assessed in this study, can potentially be a confounding factor.

River catchment classification and benthic diatom assemblages

Correspondence between river catchments and benthic diatom assemblages was worse than between ecoregions and diatom assemblages. Stream sites grouped by catchments were significantly different in altitude, temperature, and water chemistry. However, diatom assemblages were not significantly different among any catchments for either probability or reference sites. Catchments, delineated by topographic divides, are hydrological units. Such a spatial framework may not necessarily correspond to spatial patterns of biota and their ecological determinants (Omernik and Griffith 1991).

In summary, benthic diatoms may respond to local environmental conditions more strongly than to ecoregional variables. Therefore, the nonhierarchically based ecoregion classification may not correspond well to benthic diatom assemblages in the MAH streams. The similarity among ecoregions in diatom species composition at reference sites makes it possible to use diatoms as indicators of stream conditions and ecological health. A single set of diatom indicators of stream conditions should be applicable over relatively broad regions, be sensitive to environmental changes, and be able to distinguish between natural environmental changes and those induced by human activities. Our results suggest that this indicator system should be precise without establishing different reference expectations and without varying metrics among regions. In larger streams, or in other regions with different hydrogeomorphology or greatly different climate, the same diatom metric system may not be as readily transferable as it is within the MAH. It will be necessary to assess differences in sensitivity and precision of algal indicators of environmental conditions and biotic integrity among stream types and among greatly different regions before accepting diatoms as a reliable and universal tool for the environmental assessment of streams.

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APPENDIX. Common benthic diatom species in the Mid-Atlantic Highlands streams. Taxa listed occurred at >3 sites in each ecoregion. Sites from which a taxon was absent were excluded in the calculation of mean relative abundance. 60 = Northern Appalachian Plateau and Uplands, 62 = North Central Appalachians, 66 = Blue Ridge Mountains, 67 = Central Appalachian Ridges and Valleys (divided into Ridge [R] and Valley [V]), 69 = Central Appalachians, 70 = Western Allegheny Plateau.

	Occurrence							Mean relative abundance (%)						
Ecoregion:	60	62	66	69	67 R	67 V	70							
Taxa / No. of sites:	16	31	20	57	34	16	22	60	62	66	69	67 R	67 V	70
Achnanthes biasolettiana (Kütz.) Grun.	12	16	2	13	9		1	5.4	7.1	5.5	12.4	9.0		3.3
A. bioreti Germain	6	14	5	3	6		2	1.8	0.9	0.4	0.3	1.8		1.0
A. chlidanos Hohn & Hellerm.	2	10	5	10	8		3	1.2	3.8	0.9	0.9	1.1		0.4
A. clevei Grun.	3	4	3	2	5	2	2	0.3	1.8	0.3	0.4	1.0	0.4	0.4
A. daodensis Lange-Bertalot		1	6						7.2	23.7				
A. deflexa Reim.	1	6	3	15	7	12	13	16.3	16.8	0.5	23.4	18.6	18.2	12.0
A. exigua Grun.	1			4	4			2.2			5.1	0.4		
A. flexella (Kütz.) Brun	5	3		9	6		1	0.5	0.4		0.9	1.0		1.7
A. lanceolata (Bréb.) Grun.	15	21	14	28	24	6	16	9.8	4.5	7.9	1.9	6.2	6.4	4.7
A. lapidosa Krasske		4	7	9	6				0.9	1.8	0.6	1.6		
A. marginulata Grun.		7	2	7	3	1			3.4	0.5	0.9	1.7	0.2	
A. minutissima Kütz.	15	26	19	56	32	15	22	9.9	15.6	14.3	26.3	25.7	28.7	25.8
A. pusilla (Grun.) DeT.	2	5	11	26	15	1	2	14.5	9.4	30.8	9.1	9.2	1.4	2.2
Amphipleura pellucida (Kütz.) Kütz.	1			6			2	0.8	0.4		1.3			2.3
Amphora inariensis Kram.	8	6	1	5	5		1	1.4	0.6	1.2	1.4	0.8		0.2
A. pediculus (Kütz.) Grunow	1	5		2	1	3	5	2.7	0.9		0.7	0.4	2.8	1.4
A. perpusilla (Grun.) Grun.	1	2		6	2			0.2	1.2		0.6	2.5		
Anomoeoneis brachysira (Bréb.) Grunow		3		9	4	1	1				1.9	4.9	13.9	2.4
A. vitrea (Grun.) Ross		2	1	19	11	3	6		0.9	0.4	5.8	1.9	1.3	6.2
Caloneis bacillum (Grun.) Cl.	5	7	2	8	5	1	6	0.3	0.5	1.6	0.5	0.5	0.7	1.1
Cymbella affinis Kütz.	3	7	2	17	9	5	2	0.1	0.5	0.1	1.0	0.3	3.4	0.7
C. cistula (Ehr.) Kirchn.	1	4	10	10	4	1	6	0.3	0.2	3.2	0.5	0.8	0.1	0.7
C. delicatula Kütz.		1	1	28	8	3	5		<0.1	<0.1	1.0	0.4	1.0	0.6
C. mesiana Choln.	3	1		11	3	1	1	<0.1	<0.1		0.1	0.1	0.1	<0.1
C. microcephala Grun.		1		7	3	4	4			<0.1	0.1	0.1	0.3	0.2
C. minuta Hilse ex Rabh.	9	21	7	26	14	12	17	5.4	4.9	1.0	1.3	0.7	2.1	1.5
C. silesiaca Bleisch	14	17	12	30	15	0	5	4.8	1.6	2.7	1.8	1.6	0.0	0.2

APPENDIX. Continued.

Taxa	Ecoregion: / No. of sites:	Occurrence							Mean relative abundance (%)						
		60	62	66	69	67 R	67 V	70							
		16	31	20	57	34	16	22	60	62	66	69	67 R	67 V	70
C. placentula Ehr.		12	12	10	17	11	6	8	4.1	2.8	0.9	1.6	0.8	2.7	0.3
C. pediculus Ehr.		3			4	1	1		0.3			0.8	2.4	0.2	
Cyclotella meneghiniana Kütz.		12	4		3	3	2	3	1.8	0.5		0.6	4.1	0.9	1.1
Diatoma mesodon (Ehr.) Kütz.		1	11	3	9	8	2	1	8.0	3.6	0.3	0.5	13.9	0.6	0.4
D. moniliformis Kütz.		1			5			1	0.4			5.3			5.6
D. tenue Ag.			1		4	2		1		0.2		1.6	5.1		0.2
D. vulgare Bory		5		1	10	5	4	4	0.3		1.7	1.1	1.5	1.4	1.4
Eunotia bilunaris (Ehr.) Mills		2	8	2	9	5	1	2	1.8	0.8	0.6	3.0	5.0	2.6	2.0
E. exigua (Bréb. ex Kütz.) Rabh.		5	15	3	25	15	4	6	1.4	25.1	1.1	19.1	5.1	28.3	1.5
E. flexuosa Bréb. ex Kütz.		2	4		6	1			0.2	5.6		0.9	0.2		
E. implicata Norp. & L.B. & Alles		1	9	5	10	13		3	0.2	2.6	1.2	2.1	2.8		1.9
E. incisa W. Sm. ex Greg			1	2	11	6				0.8	0.9	8.4	29.0		
E. intermedia (Krasske ex Hust.) Nörpel La.-Bert.		2	12	5	11	9	1	1	2.6	3.7	5.5	1.8	0.7	2.2	0.2
E. paludosa Grun.			3	1	1	2	4			1.0	0.2	2.9	6.3	9.8	
E. pectinalis (O.F. Müll.) Rabh.		1	4	7	8	3	1	1	0.4	1.3	1.0	0.9	1.4	0.8	1.0
E rhomboidea Hust.				1	6	1					3.8	9.4	0.3		
Fragilaria bidens Heiberg		4	2	1	4	1		3	0.6	0.2	1.5	0.8	1.6		5.4
F. capucina var. *gracilis* (Østr.) Hust.		6	10	7	11	13		1	2.2	3.1	2.2	5.4	3.2		1.1
F. pinnata Ehr.		5	5		2	3	2		0.8	3.4		0.2	0.4	2.2	
F. vaucheriae (Kütz.) Peters.		14	20	14	34	18	4	13	1.7	1.6	4.0	2.7	1.8	1.0	1.9
F. virescens Ralfs		3	9	2	11	5	1	4	24.3	1.0	4.0	0.5	1.2	3.4	3.0
Frustulia rhomboides (Ehr.) DeT.		1	10	4	14	5	3	5	0.2	1.3	0.3	2.4	0.9	1.1	0.5
F. vulgaris (Thwaites) DeT.		3	3	6	6	1			0.5	1.0	0.3	0.2	0.2		
Gomphonema angustum Agardh		9	18	19	42	28	11	20	3.3	6.1	4.7	4.5	6.5	2.1	6.7
G. gracile Ehr. emend. V.H.		3			7	3		2	0.2			0.2	0.9		0.4
G. minutum (Agardh) Agardh		11		4	9	3		1	2.5		2.9	0.7	0.6		0.4
Gomphonema olivaceum var. *minutissimum* Hust.		8	6	3	12	7			1.4	2.1	0.9	1.3	1.5		
G. olivaceum (Lyngb.) Kütz.			1	1	5	1		5		2.0	1.5	3.1	3.2		1.0

APPENDIX. Continued.

Taxa	Ecoregion:	Occurrence							Mean relative abundance (%)						
		60	62	66	69	67 R	67 V	70							
	No. of sites:	16	31	20	57	34	16	22	60	62	66	69	67 R	67 V	70
G. parvulum (Kütz.) Kütz.		11	21	17	37	23	9	18	1.6	2.7	3.3	2.3	3.2	2.7	2.7
G. pumilum (Grun.) Reic. & L-B.		3	1	12	20	8	8	6	1.2	0.4	12.3	1.2	5.8	2.9	6.8
G. ventricosum Greg.				1	4						0.4	2.0			
Gomphoneis herculeana (Ehr.) Cl.		6	2	1	6	3			2.2	0.5	0.2	0.4	0.6		
Meridion circulare (Grev.) Ag.		12	20	16	25	25	6	10	1.5	2.6	3.2	1.0	3.0	1.2	1.9
Melosira varians Ag.		8	9	8	11	3	3	4	0.7	1.0	0.5	1.3	0.3	1.9	0.6
Navicula angusta Grun.			2	5	4	2				0.2	1.3	0.5	0.2		
N. capitatoradiata Germain		10	3	2	8	6			1.8	10.9	1.4	2.0	1.5		
N. capitata Ehr.			1	16		2	4	6		8.6	0.6		2.8	0.9	1.0
N. cryptocephala Kütz.		13	19		34	20	10	17	2.7	1.1		1.1	1.2	4.0	1.7
N. decussis Østr.		5	3	6	1	1		3	0.5	0.3	1.8	0.2	0.2		0.5
N. gregaria Donk.		15	21	11	27	9	7	15	3.9	5.3	2.6	1.9	8.7	3.2	3.9
N. gysingensis Foged		4	3		4	1		1	0.3	1.1		0.2	1.1		7.6
N. harderii Hust.		6		2	1	4			0.7		0.6	0.2	0.9		
N. lanceolata (Ag.) Ehr. sensu K & L-B. 1986			4		8	1	3	11		8.1		14.5	0.4	1.1	9.7
N. minima Grun.		10	13	11	18	12	8	10	1.2	1.3	1.4	1.0	1.6	5.2	2.7
N. mutica Kütz.		3	4	1	1	2	2		0.2	3.1	0.4	1.0	0.2	0.2	
N. pelliculosa Hilse		4	3	1	2	1		1	4.7	2.6	1.9	0.5	1.1		0.8
N. rhynchocephala Kütz.		7	6	5	9	4	2	3	0.5	0.3	0.8	0.7	0.7	0.6	0.7
N. seminulum Grun.		1	2		1	4			0.4	0.5		0.8	0.7		
N. tripunctata (O.F. Müll.) Bory		1		1	6	3	6	2	0.2		0.4	0.7	0.4	3.8	0.4
N. trivialis Lange-Bert.		1			3	3	4	2	1.4			1.5	6.7	1.4	0.5
N. veneta Kütz.		1	5	3	6	3		1	0.2	0.5	1.0	2.0	1.2		0.4
N. viridula var. *avenacea* (Bráb. ex Grun.) V.H.		9	14	3	13	11	2	5	1.6	4.7	2.0	2.4	3.2	2.1	2.3
Neidium alpinum Hust		1	1		4	1			0.2	0.4		0.7	0.2		
Nitzschia acicularis (Kütz.) W. Sm.		3	1		4	2	2	5	0.3	3.9		1.5	0.3	3.6	2.7
N. amphibia Grun.		4		1	2	1			1.5		0.2	0.5	0.6		
N. dissipata (Kütz.) Grun.		14	17	5	29	18	5	15	4.3	2.7	2.2	1.8	1.1	3.1	7.3
N. frustulum (Kütz.) Grun.		2	2	1	5	4			1.2	2.1	0.2	1.9	0.7		

APPENDIX. Continued.

Taxa	Ecoregion:	Occurrence							Mean relative abundance (%)						
		60	62	66	69	67 R	67 V	70							
	No. of sites:	16	31	20	57	34	16	22	60	62	66	69	67 R	67 V	70
N. gracilis Hantz. ex Rabh.		3	5	3	4	3	2	5	0.4	2.6	0.2	0.6	7.5	0.8	0.5
N. inconspicua Grun.		10	6		1	7	3	3	6.6	1.2		3.5	1.9	8.4	6.7
N. linearis (C.A. Ag. ex W. Sm.) W. Sm.		7	7	15	16	12	2	13	0.3	0.9	1.5	0.5	2.2	2.6	2.5
N. palea (Kütz.) W. Sm		15	22	11	27	13	6	16	2.9	2.1	1.6	1.9	0.8	2.1	2.3
N. perminuta (Grun.) Peragallo		9	4	5	10	9	2	5	0.7	0.3	0.5	0.5	0.4	0.9	1.0
N. recta Hantz. ex Rabh.		7	6	8	16	5	1	2	1.0	0.9	0.4	0.6	0.2	0.2	2.6
N. sociabilis Hust.		7	9	2	10	3	5	12	0.7	0.6	0.7	1.4	0.9	2.1	1.0
N. tropica Hust.		4	1	3	5	2		1	0.5	1.5	1.7	0.6	0.6		0.2
Pinnularia microstauron (Ehr.) Cl.					4	1						2.7	0.2		
Reimeria sinuata (Greg.) Kociolek & Stoermer		15	20	10	29	22	7	13	7.5	2.8	1.9	2.3	2.2	1.6	2.4
Rhoicosphenia curvata (Kütz.) Grun. ex Rabh.		12	11	6	17	12	3	9	4.0	1.1	6.2	4.7	4.5	1.1	7.4
Surirella augusta Kütz.		8	6	5	14	8	3	7	0.5	1.3	1.2	0.7	0.8	0.9	1.5
S. brebisonii K. & L-B.			1	1	3	3	3	8		0.4	0.7	0.4	2.1	0.9	3.9
S. minuta Bréb		13	14	4	17	11	1	8	0.8	2.7	0.5	1.7	0.9	2.6	6.0
S. delicatissima W. Sm.		4		1	7	1		1	1.0		0.4	0.8	0.2		8.3
Synedra rumpens Kütz.			11	6	40	18	8	11		8.4	5.9	6.6	3.9	5.3	7.6
S. ulna (Nitz.) Ehr.		8	17	17	46	24	5	11	0.9	2.0	4.4	2.7	4.1	3.7	2.2
Tabellaria fenestrata (Lyngb.) Kütz.		1	8	2	1	2			0.4	6.9	0.5	3.8	0.2		
T. flocculosa (Roth) Kütz.		1	7		12	5	3	2	0.2	8.8		1.3	0.9	0.6	0.4
T. quadriseptata Knud.			4		1	1				11.7		0.2	1.8		
T. vantricosa Kütz.		3	3		6	6			0.3	2.2		2.7	0.6		