

Contributions of habitat sampling and alkalinity to diatom diversity and distributional patterns in streams: implications for conservation

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Abstract Diatoms, microscopic unicellular algae, have been widely sampled from streams around the world because of their use in biological assessments, but as a result they are often only collected from epilithic habitats in riffles, which may lead to a substantial underreporting of their diversity and distributional patterns. The goals of this study were to identify how sampling methods and water chemistry affect the characterization of local and regional diversity and distributions. Species richness, Shannon diversity, and evenness were significantly greater in multiple habitat (MH) than epilithic habitat (EH) samples (paired *t*-test; $P < 0.01$). At the local scale, MH sampling yielded 76% of the total species collected (42% unique), EH sampling captured 58% (24% unique), and 34% were shared. From MH samples, 3, 28, and 60 regionally rare species were collected from >75, >50, and >25% of sites, respectively, whereas 1, 14, and 34 were collected from EH samples, respectively. Regional abundance more strongly predicted site frequency than mean local abundance. Smaller drainage basins tended to have fewer species, likely because of species-area relationships, but local factors (% EH and alkalinity) influenced the taxonomic complexity of assemblages. Diversity was greatest at intermediate % EH, but low alkalinity reduced diversity, which is potentially important for ecosystems affected by anthropogenic acidification, as in our study. Multiple habitats need to be sampled for better documenting diatom diversity and distributions, which could improve conservation efforts at local and regional scales along with the characterization of ecological patterns and processes.

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Abbreviations

AMD	Acid mine drainage
D	Dominant taxon
E	Evenness
EH	Epilithic habitats
H	Shannon diversity
MH	Multiple habitats
S	Species richness

Introduction

Documenting species diversity and distributions is both a practical and philosophical issue. Species diversity can be important to ecosystem processes, integrity, and management of natural resources (Tilman et al. 1997; Cottingham et al. 2001; France and Duffy 2006), although its significance can vary among ecosystems and functional diversity may be as crucial, if not more so (Hooper and Vitousek 1997; Schwartz et al. 2000; Hooper et al. 2005). Efforts to characterize species diversity and distributions are becoming increasingly important and urgent as many ecological systems face escalating pressures from human induced environmental change (Tilman 1999; Hooper et al. 2005). The diversity and function of lotic ecosystems are especially sensitive to changes in climate and land use because they rely on hydrologic patterns affected by precipitation, hydro-modifications, and land use patterns in the upstream watershed (Allan 2004; Palmer et al. 2004). In addition, non-glacial freshwater comprises less than 1% of water worldwide, with less than 1% of that being streams and rivers (Wetzel 2001), thus the global distribution of many freshwater organisms is restricted to a relatively small percentage of the global land surface. Nevertheless, organisms living in streams contribute disproportionate ecosystem services, such as nutrient and biogeochemical cycling, energy flow through food webs, and processing various types of pollution, which have important terrestrial and downstream linkages (Vitousek et al. 1997; Finlay 2001; Baron et al. 2002).

Diatoms, unicellular algae with global distributions in nearly all aquatic environments (Round et al. 1990), are important structural and functional components in stream ecosystems because they are the predominant members of biofilms and primary producers (Lowe and Pan 1996; Battin et al. 2003). They have been used widely as aquatic bioindicators for decades, and their rich diversity and wide distribution make them an ideal group of organisms to study constraints on diversity and distributional patterns. Diatoms are particularly strong responders to water chemistry, such as nutrients (Winter and Duthie 2000a; Potapova and Charles 2007; Leira et al. 2009), conductivity or ionic composition (Potapova and Charles 2003), and alkalinity (Soininen 2007). However, characterizing patterns of diatom diversity has been difficult because they often show inconsistent responses to environmental gradients and pollution (Jüttner et al. 1996; Stevenson and Pan 1999). Many diatom species also display strong physical habitat specificities (Winter and Duthie 2000b; Soininen and Eloranta 2004; Townsend and Gell 2005), and diversity can differ considerably among substrata (Cattaneo et al. 1997;

Potapova and Charles 2005). Distinct substrata and habitats have different constraints on the development and structure of diatom communities (Snoeijs 1991; Burkholder 1996; Besse-Lototskaya et al. 2006; Passy and Legendre 2006), so it should be expected that documenting diversity and distributional patterns would depend on the type of sampling at the local scale (i.e., within a stream reach).

Diatoms have been widely sampled from streams around the world because of their use in biological assessments, but as a result they are often only collected from epilithic habitats in riffles (Kelly et al. 1998; Stevenson and Bahls 1999; AFNOR 2000), which may lead to a substantial underreporting of their diversity and distributional patterns. A large portion of diversity could be missed when conducting single habitat sampling because only one component of the species pool is collected (Jüttner et al. 1996; Potapova and Charles 2005), but this has rarely been directly studied. This potential underestimation would likely have considerable implications for characterizing species distributional patterns, ecological patterns and processes, monitoring species gains and losses, and conservation efforts. The objectives of this research were (1) to identify how substrata sampling and chemistry affect diatom diversity in streams using regression techniques, (2) to document any diversity potentially missed by single habitat samples (i.e., epilithic habitats in riffles) using simple descriptive statistics, species accumulation curves, bootstrapping, and paired *t*-tests for significant differences, and (3) to evaluate the subsequent implications for conserving biodiversity and characterizing species distributional patterns by investigating site frequency distributions and abundance-frequency relationships.

Methods

Study sites and sampling

Diatoms were collected from 61 streams throughout the Western Allegheny Plateau of southeast Ohio, USA (30,828 km²) during base flow conditions, August through September, 2005–2006. This region is predominantly forested with dissected topography, principally sandstone, shale, limestone and coal geology, and has a history of extensive coal mining, logging, and some agricultural activities (Omernik 1987; further details in Smucker and Vis 2009). At each site, two samples were collected, one from the epilithic habitat (EH) within a riffle (e.g., rocks and cobble), and a separate multi-habitat (MH) composite sample collected proportionately from available habitats (e.g., riffles and pools) and substrata (e.g., rock, sand, silt, etc.) in a 50 m reach encompassing the riffle sampled. Plankton and macrophytes were not sampled because this study only included wadeable streams in which the abundance of diatoms in the plankton is almost non-existent and macrophytes were absent in nearly all streams in our study region and comprised much less than 5% of the available habitat when present. Epilithic diatoms were removed by firmly holding a 7.1 cm² rubber O-ring on the upper surface of the rock while scraping with a firm-bristled toothbrush. Diatoms growing on sediments were sampled by gently placing the O-ring on the substratum while suctioning the upper surface with a pipette (Stevenson and Bahls 1999). For single substratum samples, epilithic habitats (EH) were sampled (10 O-ring scrapings) equidistantly along a zigzag path from the downstream end to the upstream end of the riffle. Multi-habitat (MH) samples were proportionately comprised of 10 O-ring scrapings of epilithic habitats or suctionings of sediments after visually estimating habitats and substrata within the 50 m reach. Each protocol sampled 71 cm² of substratum.

Samples were kept on ice until returning to the lab where 10-ml subsamples were preserved in 2.5% CaCO₃-buffered glutaraldehyde after homogenization.

Diatom samples were cleaned using 30% H₂O₂ and 50% HNO₃. After repeatedly rinsing samples with distilled water to increase pH to 7, samples were settled onto cover slips in a chamber used to randomly disperse diatom valves (Battarbee 1973), and mounted on slides with NAPHRAX™ (Brunel Microscopes Ltd., Hazelbrook, Wiltshire, UK). A total of 500 valves were counted and identified per sample in a light microscope at 1000× (Olympus BX40™, Center Valley, Pennsylvania, USA). The keys used for species identification were primarily by Kramer and Lange-Bertalot (1986), 1988, 1991a, b), but primary literature was also used and taxonomy was updated accordingly to account for recently erected genera, described species, and transferred species, which allow for the current recognition of genus and species status. Just as one example of many, genera, such as *Luticola*, *Geissleria*, *Placoneis*, *Hippodonta*, and others have been split from the genus *Navicula*.

At each site, pH, conductivity, and temperature were measured in situ using handheld probes (Waterproof ECTestr® and pHTestr 30®, Oakton, Vernon Hills, Illinois, USA). Stream water was filtered (0.45 µm pores) and stored on ice until returning to the lab for subsequent chemical analysis. Protocols accepted by the United States Environmental Protection Agency were used for measuring PO₄-P, NO₃-N, SO₄²⁻, alkalinity, and acidity with powder pills from Hach Company (Anonymous 1997), and Cl⁻ with an ion selective electrode. The cations, Ca²⁺, Mg²⁺, K⁺, and Na⁺, were measured using an inductively coupled plasma atomic emission spectrometer (Varian Vista, Palo Alta, California, USA).

Table 1 The 15 species with greatest regional relative abundance for each sampling method and the number of sampling sites, in which encountered (maximum possible = 58)

Multi-habitat samples (MH)	Relative abundance	No. of sites	Epilithic habitat samples (EH)	Relative abundance	No. of sites
<i>Achnanthydium minutissimum</i>	12.37	58	<i>Achnanthydium minutissimum</i>	17.87	57
<i>Nitzschia palea</i>	7.53	58	<i>Nitzschia dissipata</i>	6.74	55
<i>Achnanthydium deflexum</i>	5.02	46	<i>Achnanthydium deflexum</i>	6.61	33
<i>Melosira varians</i>	4.53	49	<i>Nitzschia palea</i>	5.17	57
<i>Nitzschia dissipata</i>	3.56	56	<i>Melosira varians</i>	4.48	47
<i>Brachysira vitrea</i>	2.77	20	<i>Cymbella affinis</i>	4.07	46
<i>Cymbella affinis</i>	2.51	41	<i>Brachysira vitrea</i>	3.33	19
<i>Navicula rostellata</i> *	2.08	40	<i>Nitzschia frustulum</i>	2.97	45
<i>Navicula gregaria</i> *	1.98	52	<i>Rhoicosphenia abbreviata</i> *	2.06	33
<i>Eolimna minima</i> *	1.78	38	<i>Cocconeis placentula</i> *	1.86	38
<i>Bacillaria paradoxa</i> *	1.75	29	<i>Amphora pediculus</i>	1.74	40
<i>Amphora pediculus</i>	1.54	44	<i>Synedra ulna</i> *	1.52	42
<i>Nitzschia amphibia</i> *	1.54	34	<i>Gomphonema parvulum</i> *	1.49	41
<i>Nitzschia frustulum</i>	1.49	38	<i>Navicula schroeterii</i> *	1.45	38
<i>Nitzschia linearis</i> *	1.40	41	<i>Epithemia sorex</i> *	1.29	11

Asterisk denotes a species not among the top 15 of the other sampling method

Table 2 Under-dispersed species, those below the 5% confidence interval (Fig. 8) with observed site frequencies (Freq_{obs}), predicted site frequencies (Freq_{pred}), regional relative abundances, and maximum local relative abundances

Under-dispersed species				
Multi-habitat samples (MH)	Freq _{obs}	Freq _{pred}	Regional relative abundance	Max local relative abundance
<i>Stephanodiscus hantzschii</i>	1	18.52	<0.01	17.99
<i>Surirella subsalsa</i>	1	18.65	<0.01	18.22
<i>Fragilaria capucina</i> v. <i>perminuta</i> *	4	15.06	0.23	9.16
<i>Brachysira microcephala</i>	4	12.79	1.31	5.29
<i>Pleurosigma salinarum</i>	5	16.24	0.01	14.20
<i>Nitzschia reversa</i> *	6	33.56	0.01	52.40
<i>Aulacoseira granulata</i> *	6	21.84	1.42	19.54
<i>Navicula submuralis</i>	8	25.28	0.03	11.88
<i>Nitzschia supralitorea</i>	8	18.14	0.01	6.79
<i>Epithemia sorex</i> *	13	24.86	0.30	11.87
<i>Eunotia exigua</i>	13	23.61	0.28	7.33
<i>Nitzschia sinuata</i> v. <i>tabellaria</i>	16	28.08	0.01	23.76
<i>Diploneis puella</i>	17	28.56	0.42	23.67
<i>Brachysira vitrea</i> *	20	45.32	1.25	43.11
<i>Fragilaria capucina</i> v. <i>distans</i> *	27	37.78	0.23	11.52
<i>Bacillaria paradoxa</i> *	29	40.62	1.39	42.06
Under-dispersed species				
Epilithic habitat samples (EH)	Freq _{obs}	Freq _{pred}	Regional relative abundance	Max local relative abundance
<i>Fragilaria capucina</i> v. <i>distans</i> *	1	16.41	0.16	20.92
<i>Pleurosira laevis</i>	2	16.02	<0.01	18.11
<i>Fragilaria capucina</i> v. <i>perminuta</i> *	3	11.79	0.14	10.17
<i>Nitzschia filiformis</i> v. <i>conferta</i>	3	16.02	0.01	14.46
<i>Aulacoseira granulata</i> *	4	20.09	0.73	26.80
<i>Nitzschia reversa</i> *	4	18.39	0.01	23.46
<i>Navicula cari</i>	8	18.90	0.05	8.25
<i>Rhopalodia gibba</i>	9	18.31	<0.01	7.34
<i>Eolimna minima</i>	10	24.63	0.22	22.49
<i>Nitzschia fonticola</i>	10	23.07	0.01	22.39
<i>Epithemia sorex</i> *	11	31.83	0.21	23.86
<i>Bacillaria paradoxa</i> *	19	28.44	0.70	36.53
<i>Brachysira vitrea</i> *	19	42.93	0.64	49.60
<i>Achnanthyidium deflexum</i>	33	49.06	6.57	68.59

Asterisk denotes species identified as under-dispersed in both multi-habitat (MH) and epilithic habitat (EH) samples

Table 3 Over-dispersed species, those above the 95% confidence interval of population means (Fig. 8) with observed site frequencies (Freq_{obs}), predicted site frequencies (Freq_{pred}), regional relative abundances, and maximum local relative abundances

Over-dispersed species				
Multi-habitat samples (MH)	Freq _{obs}	Freq _{pred}	Regional relative abundance	Max local relative abundance
<i>Gyrosigma scalproides</i>	21.00	10.61	0.11	1.14
<i>Navicula tripunctata</i>	25.00	15.66	0.03	2.77
<i>Amphipleura pellucida</i> *	25.00	15.95	2.80	1.77
<i>Cymbella tumida</i> *	32.00	21.74	0.54	3.78
<i>Surirella tenera</i>	32.00	22.45	<0.01	5.50
<i>Nitzschia heufleuriana</i>	34.00	21.53	0.01	3.23
<i>Navicula vandamii</i>	35.00	23.79	0.03	3.78
<i>Nitzschia apiculata</i>	36.00	24.42	<0.01	2.88
<i>Amphora montana</i> *	36.00	25.28	1.80	3.36
<i>Surirella brebissonii</i> *	36.00	25.93	<0.01	2.75
<i>Gomphonema parvulum</i>	42.00	32.92	0.13	14.09
<i>Cyclotella meneghiniana</i> *	44.00	33.20	0.85	10.47
<i>Reimeria sinuata</i> *	44.00	33.73	0.01	6.25
<i>Synedra ulna</i>	46.00	30.64	<0.01	4.09
<i>Navicula cryptocephala</i> *	51.00	37.07	0.06	5.20
<i>Navicula gregaria</i>	52.00	41.95	0.05	7.00
Over-dispersed species				
Epilithic habitat samples (EH)	Freq _{obs}	Freq _{pred}	Regional relative abundance	Max local relative abundance
<i>Navicula radiosa</i>	25.00	15.82	0.03	4.76
<i>Amphipleura pellucida</i> *	26.00	13.01	1.03	2.59
<i>Nitzschia clausii</i>	26.00	14.80	0.01	2.60
<i>Nitzschia constricta</i>	28.00	17.96	0.01	3.43
<i>Amphora montana</i> *	28.00	17.43	0.99	5.14
<i>Planothidium lanceolatum</i>	29.00	18.22	<0.01	5.20
<i>Navicula capitatoradiata</i>	34.00	23.01	0.06	10.67
<i>Encyonema minutum</i>	35.00	20.61	0.24	2.95
<i>Surirella brebissonii</i> *	37.00	22.03	0.00	3.92
<i>Cymbella tumida</i> *	38.00	26.88	0.34	5.91
<i>Cyclotella meneghiniana</i> *	39.00	28.56	0.43	11.86
<i>Nitzschia linearis</i>	40.00	26.19	0.01	7.59
<i>Reimeria sinuata</i> *	41.00	29.59	<0.01	6.90
<i>Navicula cryptocephala</i> *	46.00	30.36	0.04	3.78
<i>Nitzschia palea</i>	57.00	47.09	0.01	28.32

Asterisk denotes a species identified as over-dispersed in both multi-habitat (MH) and epilithic habitat (EH) samples

Statistical analyses

Diatom species richness (S), Shannon diversity (H'), evenness (E), and percent dominant taxon (D) were calculated for each sample. Regressions were conducted with the % epilithic substratum at each site to explore relationships with diatom diversity measures. Percent epilithic substratum was used as a simplified measure of substrata composition at each site because substrata at sites varied only in proportion of sediment to rock habitat, and diatom communities are similar among soft substrata (e.g., sand, mud, silt, etc.) in streams (Potapova and Charles 2005). After preliminary analyses, the taxonomic measures indicated nonlinear relationships with the % epilithic substratum, and subsequent nonlinear peak regressions were conducted in SigmaPlot v. 8.0 (SYSTAT Software Inc., Chicago, Illinois, USA). Patterns in the residuals from nonlinear regressions were examined in forward stepwise multiple regressions with water chemistry variables and drainage area to explore non-habitat related variation in S , H' , E , and D using Number Cruncher Statistical Systems (NCSS 2004, Kaysville, UT, USA).

Only 58 sites were used for comparing diversity and distribution patterns from EH and MH samples because three sites had no epilithic habitat in riffles. Shannon diversity, species accumulation curves, and bootstrapped species richness accumulation generated from EH and MH samples were calculated and compared using EstimateS v. 8.0 with 250 randomizations (Colwell 2005). Bootstrapped species richness is a resampling statistical method, which provides estimates of the potential richness and species accumulation curves from each protocol (Smith and Van Belle 1984; Colwell 2005). Pearson correlations were conducted to examine the relationship of S , H' , E , and D between EH and MH. Paired t -tests were conducted to test for significant differences in diversity measured between EH and MH samples. Pearson correlations were used to examine relationships of shared species and species unique to each sampling method with % epilithic substratum and water chemistry variables. Rank abundance curves of species were plotted to compare species distributional patterns produced by EH and MH sampling protocols. Occupancy-frequency distributions were compared between the two methods to explore relationships of regional abundance with distributions and shared species. The term “regional distribution,” as used in this study implies site frequency or the number of sites occupied by species (i.e., widely distributed species are species found at many sites), and the term “local scale” means at a sampling site.

Results

Environmental relationships with diatom diversity from multiple habitats

Percent epilithic habitat present in the study streams ranged from 0 to 90% (mean = 55%, 25th, 75th percentile = 40, 70%). Diatom S , H' , E , and D had unimodal relationships with % epilithic substratum present at sites, as indicated by non-linear peak regressions [$R^2 = 0.13, 0.24, 0.25, \text{ and } 0.25$, respectively (Fig. 1)]. The more homogeneous the physical habitat was at a site, the more diatom communities were dominated by a single taxon while being less species rich, diverse, and even. Greatest S , H' , and E , and subsequently least D , existed at sites with intermediate % epilithic substratum (30–70%). Diatom S was positively correlated with drainage area ($r = 0.31, P = 0.02$), but H' , E , and D were not ($P > 0.05$). H' , E , and D were correlated with alkalinity ($r = 0.41, 0.47, \text{ and } -0.42$, respectively, $P < 0.01$ for each).

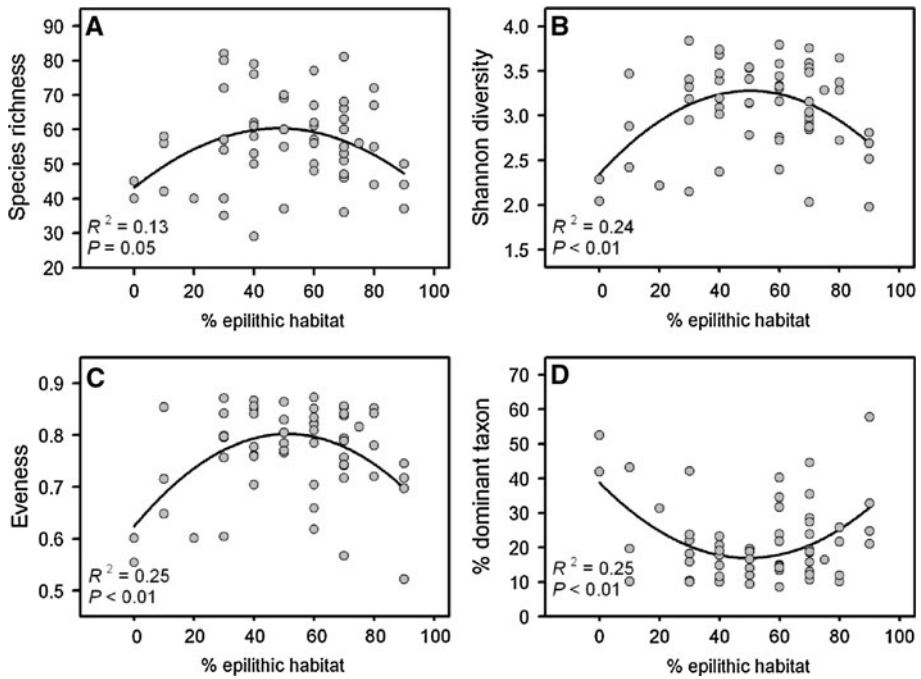


Fig. 1 Peak regressions showing relationships between % epilithic substratum and **a** species richness, **b** Shannon diversity, **c** evenness, and **d** relative abundance of the dominant taxon from multiple habitat samples

Forward stepwise multiple regressions of residuals from nonlinear regressions and environmental variables were subsequently conducted. Of the 14 variables used in stepwise multiple regressions, only drainage area was selected as a correlate with *S* and alkalinity for *H'*, *E*, and *D*. Residuals of *S* were increasingly positive with sites having larger drainage areas ($R^2 = 0.10$, $P = 0.02$), indicating that *S* was potentially overestimated in smaller drainage basins, underestimated in larger basins, and more accurately estimated in mid-sized basins by the % EH present at a site (Fig. 2a). Residuals of *H'*, *E*, and *D* were associated with alkalinity in the stepwise multiple regressions (Fig. 2b–d). *H'* and *E* residuals were increasingly positive with greater alkalinity ($R^2 = 0.16$, $P < 0.01$ and $R^2 = 0.21$, $P < 0.01$, respectively), indicating that *H'* and *E* were potentially overestimated at low alkalinities [i.e., more negative residuals in the EH regressions (Fig. 1)], underestimated at high alkalinities (i.e., more positive residuals in the EH regressions), and more accurately estimated at intermediate alkalinities by the % EH present at a site (Fig. 2b, c). *D* showed the opposite pattern, with greater dominance than predicted by % EH at lower alkalinities (Fig. 2d). Percent epilithic substratum was an important predictor of *H'*, *E*, and *D* at intermediate alkalinity, but extremes in alkalinity can override or reduce the effect of intermediate amounts of EH on diatom diversity measures.

Single habitat versus multi-habitat diversity

Regionally, a total of 416 diatom species were identified, with 300 of these species collected in epilithic habitat samples, and 327 in multi-habitat samples. Mean total *S* (i.e., all

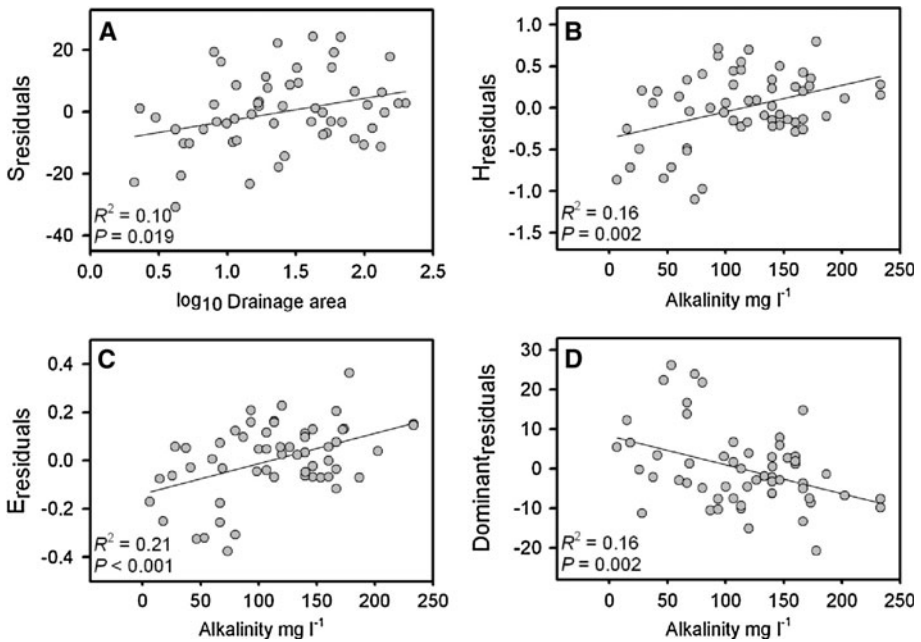


Fig. 2 Regressions showing relationships between **a** residual species richness [S] and drainage area, and **b** residual Shannon diversity [H'], **c** evenness [E], and **d** relative abundance of the dominant diatom taxon with alkalinity. Residuals are from peak-regressions of diversity measures of multiple habitat samples with % epilithic substratum

species collected by the two methods combined) at a site was 73.8 ± 2.1 (1 SE). Multi-habitat mean species richness was 56.1 ± 1.7 (1 SE), and epilithic habitat mean species richness was 42.5 ± 1.4 (1 SE). At the local scale, $76.2 \pm 0.9\%$ (1 SE) of species collected by the two methods were present in MH [$42.1 \pm 1.2\%$ (1 SE) unique to MH], $57.9 \pm 1.2\%$ (1 SE) were in EH [$23.8 \pm 0.9\%$ (1 SE) unique to EH], and $34.1 \pm 1.0\%$ (1 SE) shared (Fig. 3a). At the regional scale, 79% of species collected by the two methods were present in MH (28% unique), 72% in EH (21% unique), and 51% shared (Fig. 3b). The % shared species between MH and EH at a site was weakly correlated with % EH ($r = 0.28$, $P = 0.04$) and alkalinity ($r = 0.35$, $P = 0.01$). The % species unique to MH was negatively correlated with % EH ($r = -0.35$, $P = 0.01$).

Diatoms with high total relative abundances at the regional scale were often collected in both EH and MH samples (Fig. 4). The 15 most regionally abundant species in EH and MH samples are listed in Table 1, with nine species being common in both EH and MH samples. The six abundant species unique to MH were all highly motile biraphid species (Hill et al. 2000), whereas five of the six abundant species unique to EH were not characterized as highly motile, with the exception of *Navicula schroeterii*, and had modes of attachment or adnate growth forms (Table 1).

S, H', E, and D correlations between EH and MH were significant ($r = 0.65, 0.61, 0.58$, and 0.46 , respectively, all $P < 0.01$) (Fig 5a–d). Species richness, H', and E were significantly greater in MH samples than EH samples (paired *t*-test, $P < 0.01$), and D was significantly greater in EH samples than MH samples (paired *t*-test, $P < 0.01$). The mean

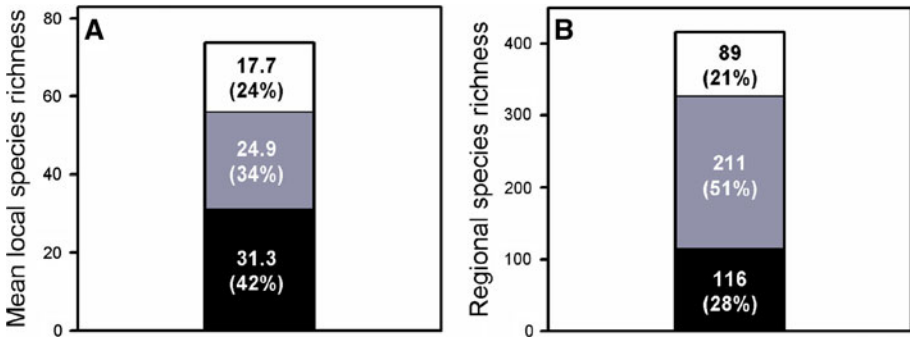


Fig. 3 **a** Mean local species richness attributed exclusively to multi-habitat samples (*black*), epilithic habitat samples (*white*), and species shared between the two protocols (*gray*) at each site. **b** Regional species richness attributed exclusively to multi-habitat samples (*black*), epilithic samples (*white*), and species shared between the two protocols (*gray*)

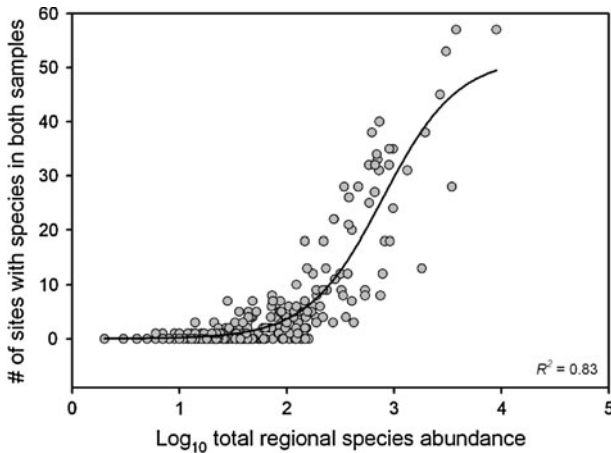


Fig. 4 Sigmoidal regression of the number of sites where each species was present in epilithic and multi-habitat samples plotted against the \log_{10} -transformed total regional abundance of each species ($P < 0.01$)

species accumulation curve for MH samples was steeper than that for EH samples, but the lower 95% confidence interval of MH and the upper 95% confidence interval of EH overlapped (Fig. 6a). Bootstrapped species richness was significantly greater in MH [366 ± 1.9 (1 SD)] than EH [343 ± 1.8 (1 SD)], and the bootstrapped species accumulation curve was steeper for MH samples than EH samples (Fig. 6a). After six sampled sites, or approximately 3,000 counted individuals, one standard deviations of bootstrapped species richness means no longer overlapped between EH and MH, giving strong statistical support for greater S , H' , and E in MH samples (Fig. 6a). The Shannon diversity accumulation curve was steeper for MH samples than EH, and regional H' was 4.25 ± 0.01 (1 SD) in MH samples and 3.90 ± 0.01 (1 SD) in EH samples (Fig. 6b). After four sampled sites, or approximately 2,000 counted individuals, one standard deviations of Shannon diversity means no longer overlapped between EH and MH (Fig. 6b).

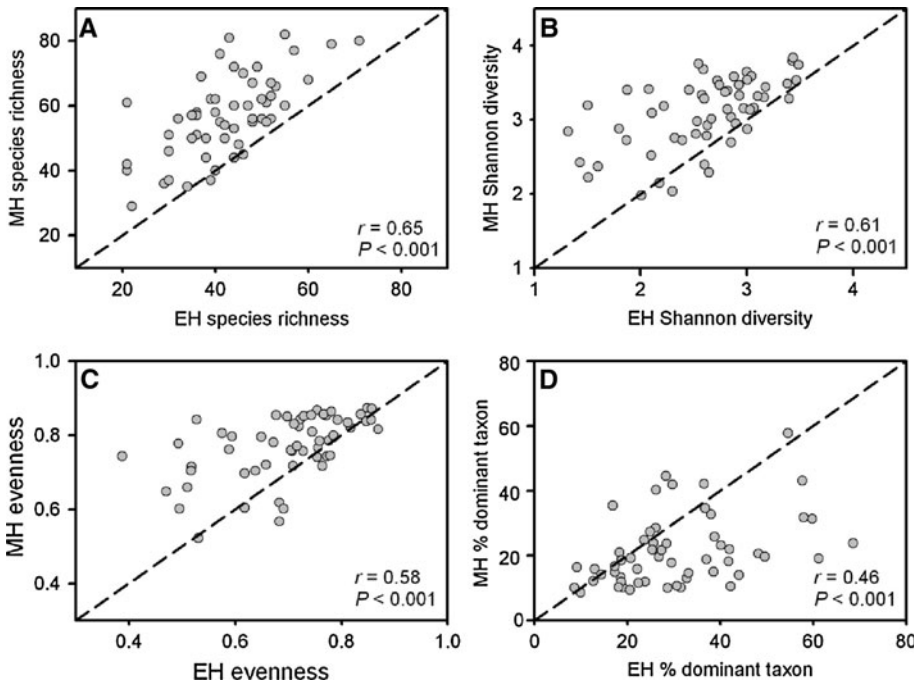


Fig. 5 Scatter plots showing Pearson correlations (r) of **a** species richness, **b** Shannon diversity, **c** evenness, and **d** % dominant taxon between epilithic habitat (EH) samples and multi-habitat (MH) samples. Dashed line is a 1:1 ratio

Species distributions

The majority of species from both sampling methods were infrequently found at a large number of sites (MH median = 4 sites, 75th, 25th % = 13, 1, EH median = 3 sites, 75th, 25th% = 10, 1). The site frequency distribution of MH species had a shallower curve than that for EH species, which indicated more frequent regional occurrences of species in MH samples than species in EH samples (Fig. 7). Of the 300 species collected from EH samples, 7, 26, and 48 species were present at >75, >50, and >25% of sites, respectively, whereas 11, 40, and 70 of the 327 species collected from MH samples were present at >75, >50, and >25% of sites, respectively (Fig. 7).

Rare species (regional relative abundance <1.5%, determined from the vertex of species rank abundance curves) had greater regional site occurrences in MH samples than EH samples. From MH samples, 3, 28, and 60 rare species were collected from >75, >50, and >25% of sites, respectively, whereas only 1, 14, and 34 rare species were collected from EH samples at >75, >50, and >25% of sites, respectively. In MH samples, 4.3% (14/327) of diatom species considered non-rare comprised 50% of all individuals counted, and in EH samples, 4.3% (13/300) of diatom species comprised 60% of all individuals counted. The rare diatom species seldom had wide distributions (Fig. 7). Only *Navicula cryptocephala* was collected in >75% of sites with <1.5% regional abundance in both EH and MH. *Synedra ulna* and *Cyclotella meneghiniana* also fit this condition in MH samples. *Brachysira vitrea* was the only species with >1.5% regional relative abundance but collected in less than 50% of sites, which further indicated the widespread distribution of

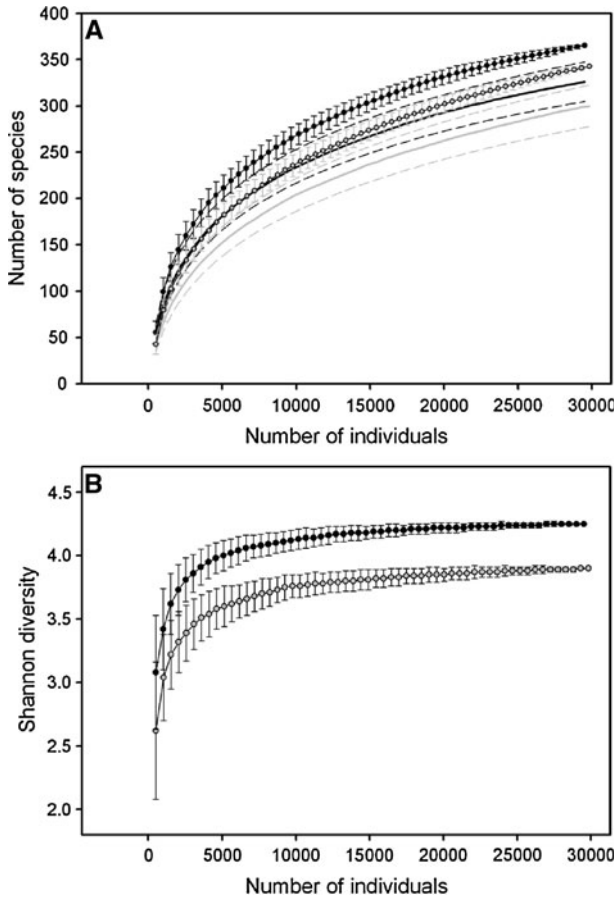


Fig. 6 Black circles or lines = multi-habitat (MH) dataset, gray circles or lines = epilithic habitat (EH) dataset. **a** Species accumulation curves (solid lines) of MH and EH samples with 95% confidence intervals (dashed lines), and bootstrapped species richness (dotted lines, error bars = ± 1 SD). **b** Relationship of Shannon diversity with increased number of sampled individuals ± 1 SD

regionally abundant species (e.g., 13/14 MH and 12/13 EH non-rare species were found at >50% of sites).

The MH and EH sampling methods produced similar sigmoidal occupancy-frequency distributions with regionally abundant species being most widely distributed and species with low regional abundances being more narrowly distributed (Fig. 8). Mean local species abundances, calculated using only sites where each species was present, were correlated with regional site distributions for both EH and MH samples ($r = 0.37, 0.47$ and $P < 0.01$, respectively), but total regional abundance was more strongly correlated with site distributions ($r = 0.73, 0.89$ and $P < 0.01$, respectively). Species with observed site frequencies outside the 5 and 95% confidence intervals were considered extremely under- or over-dispersed, respectively. Under-dispersed species in EH and MH samples were not as widely distributed as predicted by regional abundance, tended to have high local relative abundances, but were regionally rare, with the exception of *Achnanthydium deflexum* from epilithic habitats (Table 2). Over-dispersed species had wider regional distributions than

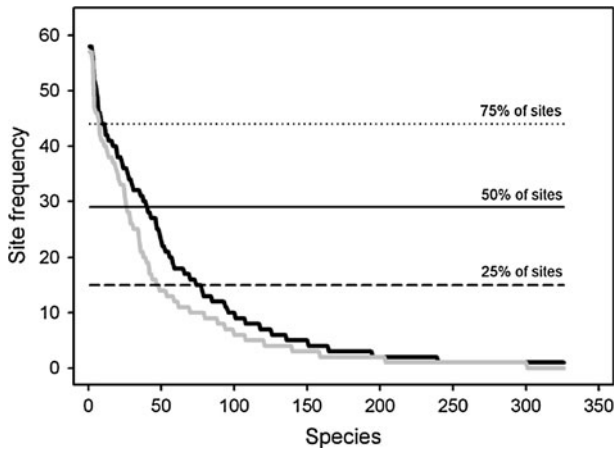


Fig. 7 Site frequency distribution of species sampled from multiple habitats (*black line*) and epilithic habitats (*gray line*)

predicted, had low local relative abundances, and low regional relative abundances, with the exception of *Amphipleura pellucida* and *Amphora montana* in multi-habitat samples (Table 3).

Discussion

Impacts to diatom diversity

Habitat sampling and alkalinity were important to the diversity in MH samples. More homogeneous substrata had lower diversity and greater dominance by a single taxon. This importance of physical substrata composition is similar to what has been documented for other aquatic (Gorman and Karr 1978; Townsend et al. 1997; Palmer et al. 2000) and terrestrial organisms (MacArthur and MacArthur 1961; Rosenzweig and Winakur 1969; Otte 1976). We feel that percent EH is meaningful for describing patterns associated with MH sampling, but we also acknowledge that the percent EH is a very simplified measure of habitat and is a measure that inextricably interacts with other biotic and abiotic variables. For example, increased heterogeneity of nutrient concentrations within a stream reach increases algal diversity (Pringle 1990), and habitat type is associated with nutrient availability (e.g., greater accumulation of nutrients in depositional habitats than in erosional, and different diffusion rates associated with boundary layers) (Cattaneo et al. 1997; Dodds 2003). Diversity of macroinvertebrates and feeding guilds in heterogeneous stream habitats (Death and Winterbourn 1995; Downes et al. 1998), current velocity and heterogeneity (Stevenson 1983), and flood disturbances interacting with algal biomass (Cardinale et al. 2006) and habitat variety (Soininen and Eloranta 2004) also affect algal diversity in streams. In addition, channelization, agriculture, and urbanization impact the habitat of stream ecosystems by reducing habitat complexity, which would be expected to decrease diversity (USEPA (United States Environmental Protection Agency) 2000a; Paul and Meyer 2001; Walsh et al. 2005). Despite the many variables that interact with habitats, percent EH, which can be affected by human impacts, was a useful proxy that was related

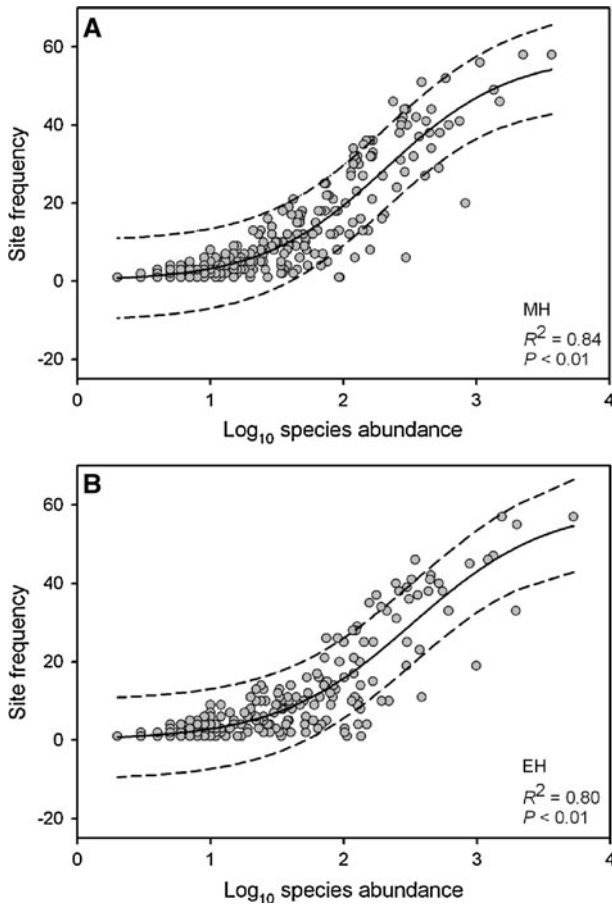


Fig. 8 Sigmodal regression of site frequencies plotted against the \log_{10} -transformed regional abundance of each species in **a** multi-habitat (MH) samples and **b** epilithic habitat (EH) samples. *Solid line* is the regression line and *dashed lines* are the 5% and 95% confidence intervals of population means

to diatom diversity. Beyond effects on diversity, sediment loads attributed to agriculture can cause a subsequent increase in the relative abundance and number of motile and high nutrient diatom species (Bahls et al. 1992; Fore and Grafe 2002; Smucker and Vis 2009), which is a relationship that could potentially be missed when only collecting EH samples (Smucker and Vis 2010).

Low and high alkalinity reduced or negated the effect of intermediate amounts of EH on diatom diversity, and increased diversity was directly and significantly correlated with greater alkalinity. Stream alkalinity is likely a predominant filter controlling assemblage structure at sites because diatoms are very sensitive to pH and acidification (Charles et al. 1991; Battarbee et al. 1999; DeNicola 2000). Alkalinity is important to diatom diversity in regions with anthropogenic acidification (Battarbee et al. 1999), but might not be as crucial in naturally low alkalinity systems (Passy 2001). Acid mine drainage (AMD) pollution, with acidic pH and high concentrations of metals, from abandoned coal waste especially threatens the diversity of lotic diatom communities (Verb and Vis 2000). AMD is a

common environmental stressor that severely impacts aquatic biota in >7000 stream kilometers throughout southeast Ohio and Appalachia (OEPA (Ohio Environmental Protection Agency) 2000; USEPA (United States Environmental Protection Agency) 2000b). In the current study, AMD is suspected of reducing alkalinity at some sites and being associated with reduced diversity and evenness of diatom communities, which is a pattern that has also been observed in streams impacted by acid precipitation (Passy 2001). Extremely high alkalinities and pH could likely reduce diatom diversity (Pither and Aarssen 2005), but a monotonic relationship between increasing diversity and greater alkalinity was observed, likely because the maximum pH in the current study was 8.3 and no highly alkaline streams were present. Beyond the effects of habitat and alkalinity, intermediate levels of water quality impairment, particularly by nutrients, can also be associated with the greatest amounts of diatom diversity (Jüttner et al. 1996; Stevenson and Pan 1999).

Drainage area was only important for the number of species at a site and not for Shannon diversity, evenness, or dominance by a taxon. This finding could indicate that dispersal throughout the watershed limits the number of species at a site, and local factors, such as substrata composition and alkalinity, constrain taxonomic complexity of diatom assemblages (e.g., Shannon diversity, evenness, and dominance by a taxon). Sites with larger drainage areas upstream likely have greater species pools than sites located closer to headwater sources, which coincides with the frequently observed trend that larger areas are capable of supporting a greater number of species than smaller areas (Broenmark et al. 1984; Rosenzweig 1995). While streams of all sizes need protection from environmental impacts, this finding suggests that smaller headwater streams are of even more conservation value because of their greater sensitivity to loss of species than sites farther downstream. In addition, promoting conservation of diversity in headwater streams could help maintain ecosystem structure and services that would benefit downstream community dynamics (Allan 2004). Further research on the role of algal species richness and species loss in ecosystem processes could improve conservation strategies.

Diversity missed by sampling only one habitat

Not surprisingly, local species richness, Shannon diversity, and evenness were significantly less in EH than MH samples, and EH samples potentially missed 42% of the species at a site, whereas MH samples potentially missed only 24%. This finding complicates the documentation of species distributions throughout the region and has subsequent implications for characterizing ecological patterns. The most regionally abundant diatom species were typically the most widely distributed and tended to be collected from both protocols, indicating potentially wide tolerances to environmental conditions and non-specificity to substratum type, which is a pattern that has been observed in many other species and ecosystems (Brown 1984). Species found in both samples at a site could be a result of some MH samples containing epilithic diatoms collected from a riffle where EH samples were also collected, which was supported by a weak, but significant, correlation of shared species with the percent EH sampled in MH samples.

Steep Shannon diversity accumulation curves indicated a large number of rare species in both EH and MH samples, which was also evident in that only ~4.3% of the species accounted for 50 and 60% of the individuals counted in MH and EH samples, respectively. The distribution of rare species was underestimated by EH samples as indicated by a much steeper rank abundance curve when compared to MH samples (Fig. 7), and the number of rare species found in MH samples at >25, >50, and >75% of sites was almost double that

for EH samples. Species accumulation curves followed similar trajectories, but the curve for MH samples was much steeper, further indicating greater richness and evenness than in EH samples. Bootstrapping showed much greater estimation of regional richness and diversity in samples collected from MH than from EH. These findings could have substantial implications for documenting species distributions and for conservation efforts because of the large underestimation of species diversity in EH samples.

Distributional patterns and conservation implications

Local and regional extinction rates are influenced by both the distribution and abundance of species (Hanski 1982). The most abundant and widely dispersed species are less likely to become locally or regionally extinct because of their greater tolerance of environmental variability, many propagules for dispersal, and wide distributions. In the current study, high mean local abundance of species did not necessarily signify greater geographic distributions, although this is a common finding with other organisms (Hanski 1982; Brown 1984; Hartley 1998) and in diatom datasets from larger spatial scales (Passy 2008). Regional abundance was much more strongly associated with the site frequency of species. Many species were capable of dominating the local community, but did not have wide distributions (i.e., under-dispersed species) (Table 2, Fig. 8). Many species with wide regional distributions also tended to have high local abundance means as previously reported in other studies, but some had wide geographic distributions and never reached high local abundances (i.e., over-dispersed species) (Table 3, Fig. 8). Under-dispersed species are at greatest risk for regional extinction because they were collected at a few sites and likely thrived under less common regional conditions. Over-dispersed species are likely at greater risk for local extinction because of their inability to be abundant at any of our sites, but their wide distributions make them less likely to become regionally extinct.

The notion that some species are under-dispersed does not necessarily mean they represent unique habitats of high conservation value, because they could represent highly impacted streams or upstream conditions, such as reservoirs, that are less common in the study region. Examples include *Eunotia exigua*, *Surirella roba*, and *Brachysira vitrea*, which are species typical of low alkalinity and AMD impacted conditions in the region (Verb and Vis 2000), *Pleurosigma salinarum*, *Nitzschia reversa*, *Pleurosira laevis*, which are species typical of anthropogenic impacted waters with extremely high conductivities (van Dam et al. 1994; Leland et al. 2001), and *Stephanodiscus hantzschii* and *Aulacoseira granulata* which are common planktonic species (Heinsalu et al. 2008) that likely washed down from upstream reservoirs and settled more readily in depositional habitats. Species such as *Rhopalodia gibba*, *Epithemia sorex*, and *Nitzschia sinuata* v. *tabellaria*, may indicate habitats of greater conservation value because they are typical of very low nitrogen conditions (Potapova and Charles 2007). Collectively considering habitat and water chemistry along with the diversity, abundance, and types of diatom species present (i.e., autecologies) is important for improving management efforts designed to effectively restore and conserve streams and biodiversity.

Recently, diatom assessments generated from epilithic habitat and multi-habitat sampling methods have shown similar conclusions or slightly stronger relationships of multi-habitat samples with environmental and landuse variables (Winter and Duthie 2000b; Smucker and Vis 2010). In addition, algal communities on hard and soft substrata may indicate similar environmental gradients, although species composition can differ (Cattaneo et al. 1997; Potapova and Charles 2005). With mounting evidence that diatoms from MH samples can be as effective as EH samples in biomonitoring studies, MH

sampling for diatoms would greatly benefit the future documentation and characterization of diatom diversity and distribution patterns, along with species losses and gains. Reducing anthropogenic contributions of nutrients and sediments to streams, conscientious land use practices, and conserving habitats are obviously imperative to sustaining stream ecosystems, but MH sampling could help guide future efforts and monitoring trends in lost or gained diversity and richness in the face of escalating human pressures on streams.

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