

## A comparison of diatom assemblages generated by two sampling protocols

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**Abstract.** We investigated whether 2 stream sampling protocols collected different diatom assemblages within sampling reaches and, consequently, influenced the outcome of bioassessment. We analyzed data collected at 71 sites by both reach-scale (Environmental Monitoring and Assessment Program [EMAP]) and targeted-habitat (US Environmental Protection Agency [EPA] Science to Achieve Results [STAR]) sampling protocols as part of the US EPA EMAP survey. Overall, diatom assemblages generated by the 2 protocols were similar. Median Bray–Curtis (BC) similarity between EMAP and STAR diatom counts was 70% (range 19–91%). Taxon richness ( $r^2 = 0.7$ ), autecological metrics (e.g., siltation index:  $r^2 = 0.9$ , trophic diatom index:  $r^2 = 0.8$ ), and morphological metrics (e.g., % erect taxa:  $r^2 = 0.7$ , % prostrate taxa:  $r^2 = 0.8$ ) were comparable between the 2 protocols. Relationships between diatom assemblages (summarized as nonmetric multidimensional scaling ordination axes) and environmental variables were similar between the 2 protocols, with diatom assemblages relating more to instream water-quality variables (e.g., total P, conductivity) than to physical-habitat or watershed characteristics. Diatom assemblages generated by the 2 protocols did diverge under a specific set of environmental conditions. Sites with the lowest BC similarities between EMAP and STAR counts tended to be larger and less shaded and to have higher values of water-quality variables affected by human disturbance (e.g., conductivity, total P, % fine sediment) than sites with higher values of BC similarity between protocols. We conclude that EMAP and STAR sampling protocols, in general, collect similar diatom assemblages. However, researchers should exercise caution when combining diatom data sets collected with different sampling protocols, particularly in large streams, where the potential for sampling of multiple habitats is increased.

**Key words:** diatoms, periphyton, bioassessment, sampling method, ordination, Bray–Curtis similarity, EMAP.

Aquatic bioassessment studies often are undertaken at the local level. Regional studies exist in the US (e.g., US Environmental Protection Agency [EPA] Environmental Monitoring and Assessment Program [EMAP], US Geological Survey National Water Quality Assessment Program [NAWQA]), but a more comprehensive understanding of the biological integrity of the nation's waters could be garnered through synthesis of smaller studies into regional or country-wide assessments. Data from many sources, collected over multiple years and processed by different analysts, will have to be merged to accomplish this task. Differences among sampling protocols can influence

the outcomes of analyses (McGeoch and Gaston 2002), and one of the many issues that must be resolved before merging these data sets is the amount of variability in the data that is a consequence of these differences. The influence of sampling protocol and habitat sampled has been assessed for macroinvertebrates and fish (e.g., Parsons and Norris 1996, Cao et al. 2005), and the effect of habitat sampled on species–environment relationships has been explored for periphyton (e.g., Pan et al. 1996, Potapova and Charles 2005). However, a comparison of diatom assemblages generated by different reach-scale periphyton sampling protocols has not been undertaken to date. The purpose of our study is to determine how periphyton sampling protocol may influence bioassessments.

The 2 main quantitative periphyton sampling approaches used in stream bioassessment are the reach-scale and targeted-habitat protocols. Investigators using the reach-scale approach collect a predeter-

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mined number of samples throughout the study reach. Periphyton usually is collected and composited from a set number of equally spaced points along a transect spanning the sample reach. The reach-composite sample may contain a mixture of periphyton growing on different substrates (e.g., cobble, bedrock, sand) and in different habitats (e.g., riffle, run, pool). The rationale of the reach-scale approach is that the sample will be representative of all periphyton within the sampling reach by inclusion of periphyton from all habitats and substrates (Stevenson and Bahls 1999). However, different groups of periphyton may be present in different stream habitats, so the reach-scale approach may introduce heterogeneity into the periphyton sample that may mask differences in environmental conditions among sites. In addition, the use of transects may make it impossible to match habitat conditions between reference and impacted sites (Biggs and Kilroy 2000). In comparison, investigators using the targeted-habitat approach collect samples from a single, dominant habitat within the study reach. The rationale behind this approach is that targeted-habitat samples should reflect water-quality differences among streams more precisely than reach-composite samples because the heterogeneity of the periphyton assemblages within the stream is minimized (Stevenson and Bahls 1999).

Large-scale river/stream assessment programs undertaken in different countries and by different agencies within the same country use different sampling protocols, and this inconsistency may limit the potential for comparison and merging of data from different sources. For example, much of the European Union currently uses a targeted-habitat approach in which the same substrate is sampled for all streams within a project, and the sampling location is within the main channel and has ample light (CEN 2003). In contrast, New Zealand's State of the Environment monitoring program uses a reach-scale approach (Biggs and Kilroy 2000).

In the US, the NAWQA quantitative survey uses a targeted-habitat approach. Separate samples are collected from the richest targeted habitat and a depositional targeted habitat (Porter et al. 1993). The richest targeted habitat, usually erosional, is where maximum taxon richness is likely to be observed. This approach may miss impacts to nondominant habitats and is subjective, leaving field crews to decide where to concentrate their sampling efforts. In addition, the targeted habitat may differ among streams within a study area. Potapova and Charles (2005) found that algal biovolume and diversity differed significantly between samples from the richest targeted habitat and the depositional targeted habitat at the same site, but

that the strengths of the relationships between algal assemblages and environmental variables did not vary in a consistent manner between substrate types.

The EMAP survey uses the reach-scale approach. This protocol is based on studies of the influence of sampling effort on richness of fish taxa and has been used in several periphyton studies (Pan et al. 1996, 2000, Hill et al. 2000). The length of the sampling reach is defined as 40× the mean wetted width of the channel and the number of transects is held constant at 11, regardless of stream size. The practice of holding transect length constant at 40× the wetted width of the channel may introduce bias into the bioassessment. As reach length increases, transects become further apart and may cover several instream habitat types. In addition, as reach length increases, the stream area covered by a composite sample increases. Therefore, habitat heterogeneity will probably increase, possibly influencing taxon composition and leading to higher taxon richness. State bioassessment programs (e.g., Regional Environmental Monitoring and Assessment) are using the EMAP approach with increasing frequency.

No systematic comparisons have been made between reach-scale and targeted-habitat approaches to sampling periphyton across regions and environmental settings to date. Therefore, we compared diatom assemblages generated from reach-scale and targeted-habitat approaches in 3 US states. Our main objective was to determine whether diatom assemblages collected by the 2 sampling protocols were related to different environmental variables and therefore might yield different bioassessment results. Our specific objectives were to: 1) compare diatom assemblages collected using reach-scale and targeted-habitat approaches, 2) quantify and compare the relationships between diatom assemblages and environmental variables shown by the 2 sampling approaches, and 3) quantify the environmental conditions under which the 2 protocols generate different diatom assemblages.

## Methods

### *Study area*

Data were collected at 71 sites in 3 states (Idaho [ID]:  $n = 15$ , Nevada [NV]:  $n = 27$ , South Dakota [SD]:  $n = 29$ ) as part of the EMAP-West pilot study in 2002. Stream sites were selected using a systematic randomized sampling design (Herlihy et al. 2000) from digitized versions of 1:100,000-scale US Geological Survey (USGS) topographic maps. Periphyton, water chemistry, and physical habitat were sampled from May through August 2002.

Field sampling

*Sampling reach.*—A study reach with a total length equal to 40× the average wetted channel width (minimum: 150 m, maximum: 1240 m) was centered around each sampling site. Each study reach was divided into 10 intervals of equal length, and 11 cross-sectional transects (including transects at the start and end of each reach) were established.

*Periphyton.*—Periphyton samples were collected using 2 sampling protocols: 1) reach-scale (EMAP), and 2) targeted-habitat (US EPA Science to Achieve Results [STAR]). For the EMAP protocol, periphyton samples were collected at each of the 11 transects (Hill 1998). After a random start, samples were collected at ¼ (left), ½ (center), or ¾ (right) of the distance from the stream bank (Fig. 1A). To sample coarse substrate, 1 cobble was removed from the water at each sampling point and periphyton was scraped from a 12-cm<sup>2</sup> area with a toothbrush. The toothbrush was rinsed into a sample jar with deionized water. To sample fine substrate in depositional habitats, 1 sample of periphyton and sediments was sucked into a 60-mL syringe at each sampling point. Samples from all habitats and substrates within a reach were combined into one composite sample and preserved with 37% formalin.

For the STAR protocol, periphyton samples were collected from the dominant habitat (rock or sand/silt) in each reach (USEPA, unpublished method; Fig. 1B). When riffles were the dominant habitat, periphyton

was collected from rock substrates. Sampling points were distributed among 8 riffles throughout the study reach. At each riffle, 2 pieces of substrate were sampled from water depths between 15 and 20 cm (deepest part when depth <15 cm), for a total of 16 pieces of substrate per reach. If <8 riffles were present, additional pieces of substrate were collected from each riffle for a total of 16 pieces of substrate per reach. Periphyton was scraped from each piece of substrate as previously described. When riffles were not the dominant habitat, periphyton was collected from sand/silt substrates (as previously described) in depositional habitats from a depth of 15 to 20 cm (deepest part when depth <15 cm) until 16 samples were collected. Sampling points were evenly distributed among transects with depositional habitats. Samples from all points within a reach were combined into one composite sample and preserved with 37% formalin.

*Water chemistry and physical habitat.*—Water samples were taken near the middle of the stream in flowing water and analyzed for pH, total N (TN), total P (TP), dissolved organic and inorganic C (DOC and DIC, respectively), and major base cations and anions. Detailed information on the analytical procedures used for each of the analyses can be found in USEPA (1987) and in table 1 in Pan et al. (1996). Characterization of instream habitat included systematic spatial sampling of channel dimensions, gradient, and substrate size and type, habitat complexity and cover, riparian vegetation cover and structure, anthropogenic alterations, channel-riparian interaction, and stream discharge. Substrate characterization (size and embeddedness) was based on size classification of 105 individual particles collected at 5 equidistant locations at 21 evenly spaced transects throughout the reach. Detailed field methods can be found in Kaufmann and Robison (1998).

Laboratory analyses

*Periphyton.*—An aliquot of homogenized algal suspension was used for identification of diatoms. Diatom samples were digested in acid and mounted on microscope slides (Patrick and Reimer 1966). Slides were scanned in transects by a single analyst using an Olympus BH-2 microscope outfitted with a differential interference contrast lens at 1000× magnification until ≥600 diatom valves were enumerated and identified to the lowest taxonomic level possible (mainly species level). The primary references for diatom taxonomy were Krammer and Lange-Bertalot (1986, 1988, 1991a, b, 2000) and Patrick and Reimer (1966, 1975). Diatom assemblage diversity measures (e.g., richness, Shannon

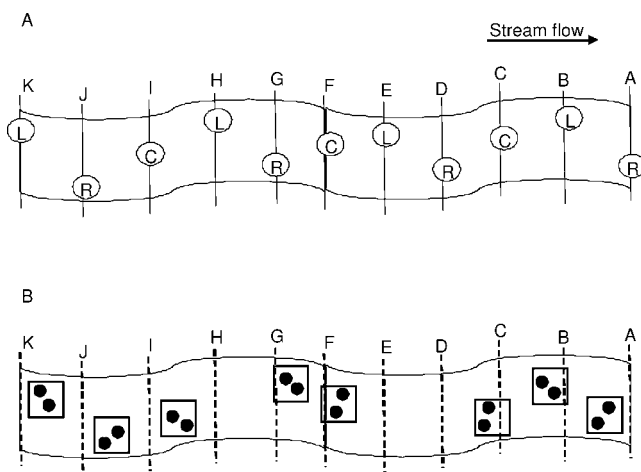


FIG. 1. Periphyton sampling design for US Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) (A) and Science to Achieve Results (STAR) (B) protocols. EMAP: L = left bank, C = stream center, and R = right bank. STAR: boxes represent targeted-riffle units and circles represent individual coarse substrates. Sand/silt substrates were sampled if riffles were not the targeted habitat.

diversity index [ $H'$ ], dominance [relative abundance of most abundant species] in a sample, and Hill's  $N_2$  diversity index) were calculated for EMAP and STAR assemblages at each site. Diatom autecological indices and morphological metrics (% silt-tolerant taxa, trophic diatom index, % erect taxa, % prostrate taxa, % stalked taxa) were calculated for EMAP and STAR assemblages at each site and were based on the species characterizations in Molloy (1992), Bahls (1993), and Kelly and Whitton (1995). The trophic diatom index is based on the relationships between aqueous P concentrations and the abundance of 86 common diatom taxa. Each species is assigned a sensitivity value between 1 and 5 based upon the concentration at which the taxon is most abundant and an indicator value based on the spread of values around this peak. The index is calculated as the weighted average of the indicator and sensitivity values. The value of the trophic diatom index can range between 1 (very low nutrient concentrations) and 5 (very high nutrient concentrations).

*Land use/cover.*—Watershed boundaries above the sampled sites were delineated on 1:250,000-scale USGS topographic maps and digitized into a geographic information system (GIS), from which watershed areas were calculated. Watershed slope was calculated as the difference in elevation between the sample site and the highest point in the watershed divided by the distance between them. The elevation of each stream site was determined from the contour lines on 1:24,000-scale USGS maps.

#### Data analysis

*Diatom assemblage.*—Diatom taxon counts were represented as the proportion of total individuals at each site (relative abundances). Bray–Curtis (BC; relative abundance data) and Jaccard's (presence/absence data) similarities were calculated between EMAP and STAR diatom counts at each site. Sites were ranked based on their BC similarity and divided into 4 groups of equal number (low-BC group:  $n = 17$ , all other groups:  $n = 18$ ). Differences in environmental variables among BC groups were tested with 1-way analysis of variance (ANOVA) and Tukey's multiple comparison tests using a Bonferroni-corrected  $\alpha$ . Stream sites were ordinated on the basis of diatom species composition (BC similarities) with nonmetric multidimensional scaling (NMDS; PRIMER, version 5; PRIMER-E, Plymouth, UK; Clarke and Warwick 1994). Separate ordinations were done for diatom data generated by the EMAP and STAR sampling protocols. Pearson correlations calculated between environmental variables and the axes of the 2 NMDS ordinations

were used to identify a subset of environmental variables that covaried with diatom species composition among sites. All environmental variables (except pH) were transformed ( $\log_{10}[x]$  or  $\arcsine\sqrt{x}$ ) prior to analysis.

## Results

### Environmental variables

Environmental variables varied widely among sites (Table 1). For example, pH ranged from 6.5 to 9.0, and TP ranged from 2 to 964  $\mu\text{g/L}$ . Stream depth ranged from 1 to 83 cm, and stream width ranged from 0.1 to 28 m. The percentage of the stream bed consisting of fine substrate ranged from 0 to 95%, and midchannel canopy cover ranged from 0 to 74%. Watershed area ranged from 1 to 15,952  $\text{km}^2$ .

### Diatom assemblages

A total of 432 diatom taxa was identified in counts generated by the 2 sampling protocols; 380 taxa were identified in the EMAP counts, and 363 taxa were identified in the STAR counts. Fifty taxa identified in the EMAP counts were not found in the STAR counts, whereas 40 taxa identified in the STAR counts were not found in the EMAP counts. Dominant species were similar between counts generated by the 2 sampling protocols (Table 2). *Achnantheidium minutissimum* (Kützing) Czarnecki had the highest median abundance in counts generated by both sampling methods.

In general, diatom assemblages were similar between the 2 samples within a reach. However, taxa present and relative abundances were very different between samples in several reaches. BC similarity between samples within reaches ranged from 19 to 91% (lower quartile = 60%, median = 70%, upper quartile = 78%). Jaccard's similarity ranged from 36 to 84% (lower quartile = 58%, median = 65%, upper quartile = 70%). Jaccard's similarity was less than BC similarity in 25 reaches, indicating that rare species influenced dissimilarities between EMAP and STAR counts in these reaches. Taxon richness was similar between counts within reaches ( $r^2 = 0.7$ ; EMAP: median = 42 taxa, range = 12–87; STAR: median = 40 taxa, range = 18–78; Table 2, Fig. 2A). The EMAP sampling protocol yielded higher taxon richness than the STAR sampling protocol in 45 of the 71 reaches.

The 2 sampling protocols produced statistically similar autecological and morphological metric values in all cases ( $r^2 = 0.7\text{--}0.9$ ,  $p < 0.05$ ; Table 2, Fig. 2B–F). Divergence in autecological and morphological metrics tended to be greatest in reaches with the lowest BC values (Fig. 2B–F). BC similarity values were only

TABLE 1. Mean ( $\pm 1$  SD) values of environmental variables for all sites sampled and for sites grouped by Bray–Curtis (BC) similarity between samples collected using US Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) and Science to Achieve Results (STAR) sampling protocols. Groups that share a superscript letter are not significantly different from one another (1-way analysis of variance and Tukey's multiple comparison tests,  $p < 0.05$ ).

Environmental variable	All sites	BC >78%	BC = 68–78%	BC = 60–68%	BC <60%
<b>Water chemistry</b>					
Conductivity ( $\mu\text{S}/\text{cm}$ )	1020 $\pm$ 1578	1365 $\pm$ 2519	471 $\pm$ 859	1176 $\pm$ 1085	1072 $\pm$ 1270
Turbidity (NTU)	15 $\pm$ 29	8.6 $\pm$ 20.2	11 $\pm$ 27	20 $\pm$ 32	21 $\pm$ 36
pH	8.0 $\pm$ 0.5	7.8 $\pm$ 0.4	8.0 $\pm$ 0.6	8.0 $\pm$ 0.4	8.1 $\pm$ 0.5
Total P ( $\mu\text{g}/\text{L}$ )	108 $\pm$ 165	97 $\pm$ 95	67 $\pm$ 95	122 $\pm$ 143	145 $\pm$ 273
Total N ( $\mu\text{g}/\text{L}$ )	868 $\pm$ 1319	715 $\pm$ 738	623 $\pm$ 775	908 $\pm$ 900	1246 $\pm$ 2299
SO <sub>4</sub> ( $\mu\text{g}/\text{L}$ )	10,287 $\pm$ 29,967	11,684 $\pm$ 23,854	2043 $\pm$ 6119	20,280 $\pm$ 52,421	6957 $\pm$ 12,723
Cl ( $\mu\text{g}/\text{L}$ )	1095 $\pm$ 4294	2830 $\pm$ 8291	2043 $\pm$ 6119	196 $\pm$ 284	638 $\pm$ 1233
SiO <sub>2</sub> ( $\mu\text{g}/\text{L}$ )	23 $\pm$ 18	25 $\pm$ 19	31 $\pm$ 20	21 $\pm$ 14	16 $\pm$ 14
<b>Physical habitat</b>					
Reach length (m)	240 $\pm$ 198	213 $\pm$ 138 <sup>ab</sup>	151 $\pm$ 3.2 <sup>a</sup>	287 $\pm$ 278 <sup>b</sup>	315 $\pm$ 227 <sup>b</sup>
Depth (cm)	28 $\pm$ 18	22 $\pm$ 19 <sup>a</sup>	23 $\pm$ 16 <sup>a</sup>	38 $\pm$ 17 <sup>b</sup>	30 $\pm$ 13 <sup>ab</sup>
Width (m)	5.1 $\pm$ 5.7	3.9 $\pm$ 4.4 <sup>ab</sup>	2.0 $\pm$ 1.3 <sup>a</sup>	7.0 $\pm$ 7.3 <sup>b</sup>	7.8 $\pm$ 6.3 <sup>b</sup>
% canopy density midchannel	36 $\pm$ 34	47 $\pm$ 38	45 $\pm$ 32	25 $\pm$ 30	27 $\pm$ 33
% coarse substrate	37 $\pm$ 31	43 $\pm$ 33	35 $\pm$ 25	35 $\pm$ 29	36 $\pm$ 36
% fine sediment	25 $\pm$ 26	20 $\pm$ 24	24 $\pm$ 27	26 $\pm$ 24	30 $\pm$ 31
% embeddedness	75 $\pm$ 18	72 $\pm$ 19	74 $\pm$ 18	75 $\pm$ 18	77 $\pm$ 19
% riffle habitat	30 $\pm$ 35	28 $\pm$ 37	43 $\pm$ 37	21 $\pm$ 32	25 $\pm$ 29
% pool habitat	12 $\pm$ 24	6 $\pm$ 7	9 $\pm$ 22	19 $\pm$ 34	12 $\pm$ 24
<b>Watershed</b>					
Area (km <sup>2</sup> )	1050 $\pm$ 2533	487 $\pm$ 1175 <sup>ab</sup>	82 $\pm$ 256 <sup>a</sup>	1500 $\pm$ 2462 <sup>b</sup>	2196 $\pm$ 4130 <sup>b</sup>
Elevation (m)	1292 $\pm$ 678	1268 $\pm$ 721 <sup>ab</sup>	1663 $\pm$ 583 <sup>a</sup>	1075 $\pm$ 700 <sup>b</sup>	1152 $\pm$ 588 <sup>ab</sup>
% forested land cover	25 $\pm$ 33	29 $\pm$ 39	19 $\pm$ 31	24 $\pm$ 33	28 $\pm$ 36
% agricultural land use	22 $\pm$ 35	26 $\pm$ 39	7 $\pm$ 17	35 $\pm$ 40	21 $\pm$ 36

weakly related to measures of diatom community diversity, but these relationships were statistically significant in all cases ( $r^2 = 0.1$ ,  $p < 0.05$ ; Fig. 3A–D). BC similarity was negatively related to taxon richness (Fig. 3A),  $H'$  (Fig. 3B), and Hill's N2 diversity index (Fig. 3D). BC similarity was weakly positively related to dominance by a single taxon in a reach (Fig. 3C).

#### Ordinations

Most reaches clustered more closely in the NMDS ordination of EMAP-generated data than in that of STAR-generated data (Fig. 4A–B). Relationships between diatom species assemblages (represented as ordination axes) and environmental variables were similar between the 2 sampling protocols (Table 3). The

TABLE 2. Median (range) relative abundances (% of total count) of the 5 most abundant diatom species and diatom metrics for samples collected using US Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) and Science to Achieve Results (STAR) sampling protocols for all sites and for sites with low values of Bray–Curtis (BC) similarity between samples collected with the 2 protocols. Coefficients of determination are between EMAP and STAR relative abundances and metrics.

Diatom variable	All sites			Low BC sites		
	EMAP	STAR	$r^2$	EMAP	STAR	$r^2$
<i>Achnanthes minutissimum</i> (Kütz.) Czarnecki	4 (0–74)	3 (0–67)	0.8	3 (0–74)	2 (0–41)	0.4
<i>Cocconeis placentula</i> Ehrenberg	1 (0–50)	2 (0–51)	0.7	1 (0–23)	2 (0–13)	0.4
<i>Fragilaria capucina</i> Desmazières	1 (0–59)	1 (0–61)	0.9	1 (0–27)	1 (0–3)	0.3
<i>Planorhynchium lanceolatum</i> (Bréb. ex Kütz.) Round and Bukhtiyarova	1 (0–44)	1 (0–46)	0.9	0 (0–15)	1 (0–14)	0.4
<i>Nitzschia frustulum</i> (Kütz.) Grunow	1 (0–19)	1 (0–13)	0.3	1 (0–13)	1 (0–5)	0.4
<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-Bertalot	1 (0–22)	1 (0–24)	0.9	1 (0–10)	1 (0–7)	0.7
Taxon richness	42 (12–87)	40 (18–78)	0.7	56 (12–87)	43 (25–68)	0.5
Trophic diatom index	3.3 (2.1–4.5)	3.3 (1.9–4.2)	0.8	3.2 (2.1–4.2)	3.0 (1.9–4.1)	0.5
Siltation index (%)	51 (6–100)	50 (5–100)	0.9	70 (16–100)	72 (34–100)	0.7

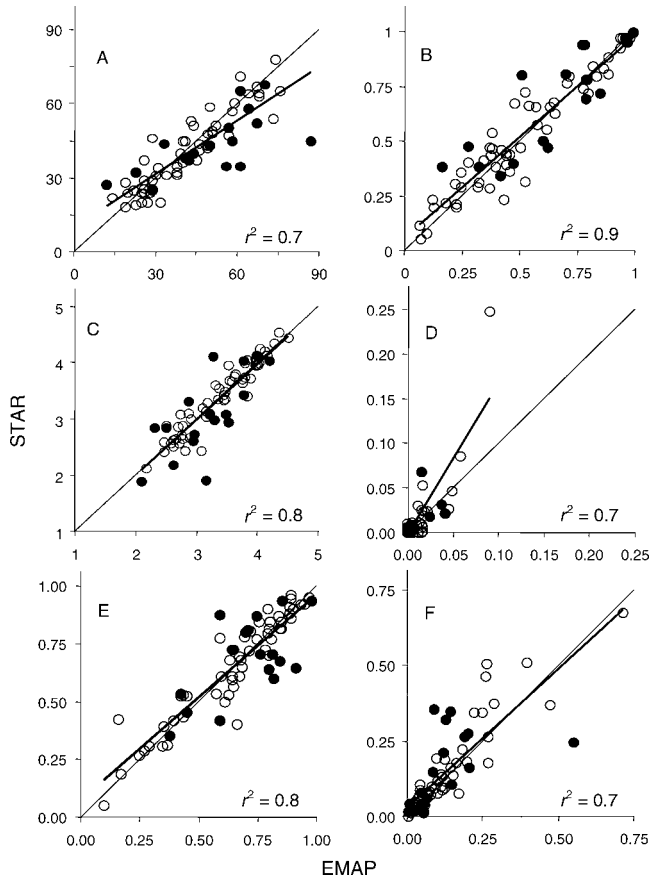


FIG. 2. Relationships between values of taxon richness (A), % silt-tolerant taxa (B), trophic diatom index (C), % erect taxa (D), % prostrate taxa (E), and % stalked taxa (F) for diatom counts generated by US Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) and Science to Achieve Results (STAR) protocols. Thin lines are 1:1 lines and heavy lines are regression lines.

1<sup>st</sup> ordination axis appeared to represent stream size and disturbance gradients for both EMAP and STAR data. Watershed area was significantly correlated with axis 1 for both EMAP ( $r = -0.76$ ) and STAR ( $r = -0.76$ ) ordinations. Instream physical-habitat (e.g., % canopy cover midchannel, reach slope, % coarse substrate, % riffle habitat) and watershed variables (e.g., elevation, watershed slope) that characteristically have higher values in smaller streams were significantly positively correlated with axis 1 (Table 3). Water-quality (e.g., conductivity, TP, TN, turbidity), physical-habitat (e.g., % fine sediment), and watershed variables that characteristically have higher values in larger or more disturbed streams were significantly negatively correlated with axis 1 (Table 3). Axis 2 was not strongly correlated with any measured environmental variable (except turbidity with EMAP axis 2; Table 3). Rela-

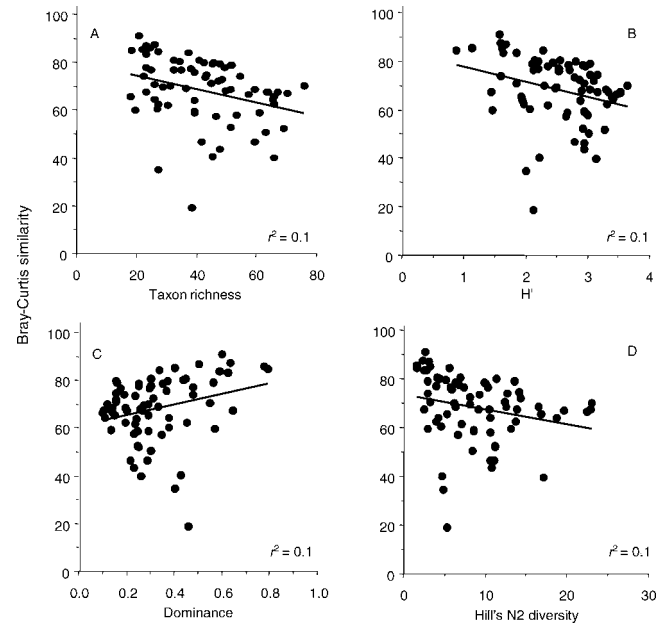


FIG. 3. Regressions of Bray-Curtis (BC) similarity between diatom assemblages collected using US Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) and Science to Achieve Results (STAR) protocols as a function of taxon richness (A), Shannon diversity index ( $H'$ ) (B), dominance (relative abundance of most abundant species) (C), and Hill's  $N_2$  diversity index (D).

tionships between ordination axes and water-quality variables tended to be stronger than relationships between ordination axes and instream physical habitat variables and watershed characteristics, regardless of sampling protocol (Table 3).

#### *Sites with low values of BC similarity*

Diatom assemblages from the subset of reaches with the lowest values of BC similarity were examined further. Reaches were regarded as having a low BC similarity if the value was in the lowest quartile of similarities ( $BC < 60\%$ ,  $n = 17$ ). For this group of reaches, median taxon richness was lower for counts generated by the STAR sampling protocol than for counts generated by the EMAP sampling protocol (Table 2). Individual values of taxon richness were lower in counts generated by the STAR protocol than in counts generated by the EMAP protocol at 13 of 17 low BC sites.

Dominant taxa were similar in counts generated by the 2 protocols (Table 2). However, in individual low-BC reaches, the dominant taxa varied considerably between the 2 counts. Differences in relative abundances of specific taxa between EMAP and STAR

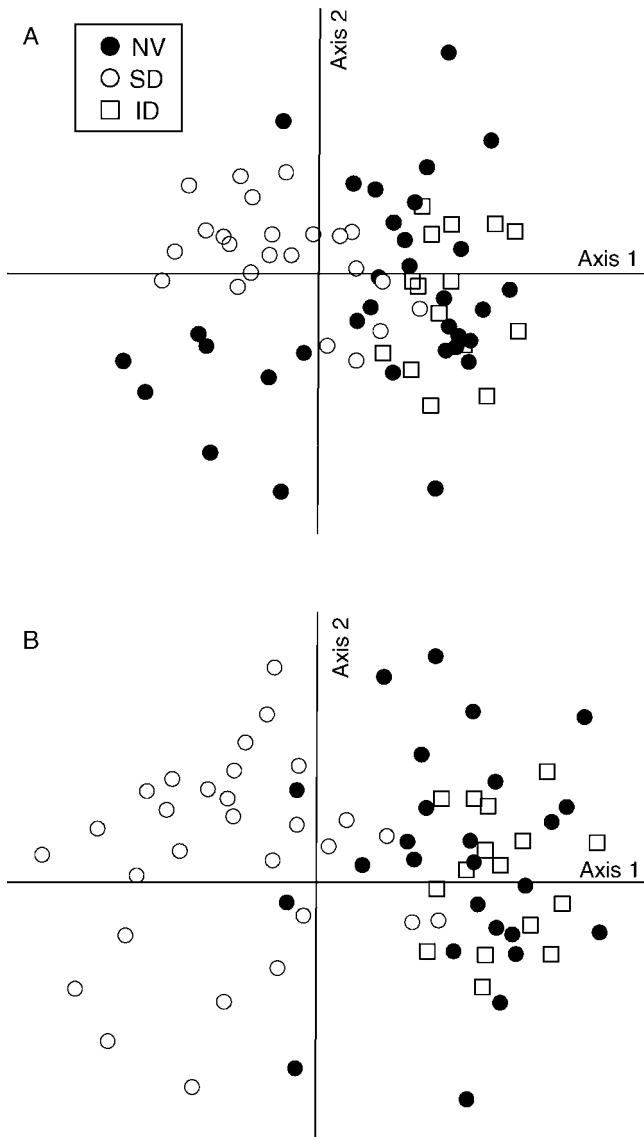


FIG. 4. Nonmetric multidimensional scaling (NMDS) ordinations of diatom assemblages collected using US Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) (A) and Science to Achieve Results (STAR) (B) protocols.

counts ranged from 0 to 68%. The mean difference in relative abundances of the 5 most common species in each reach ranged from 4 to 20% (mean = 10%). No consistent pattern in taxonomic group, morphology, or autecology could be identified for the taxa that differed between counts in a reach. However, channel morphology and size differed consistently between streams in the low-BC group and other streams (Table 1). Streams in the low-BC group tended to be larger and less shaded and to have more fine sediment, longer sampling reach lengths, and greater watershed area than other streams. Moreover, for streams in the

low-BC group, the targeted habitat (EMAP protocol) was not always similar to the dominant habitat (STAR protocol) sampled within the reach. For example, in the 7 streams with reach lengths <300 m where rock/gravel substrates were targeted, mean % coarse substrate within the reach was only 49%. For the 2 streams where sand/silt substrates were targeted, mean % fine substrate within the reach was only 56%.

**Discussion**

*Habitat heterogeneity and stream size*

Reach-scale (EMAP) and targeted-habitat (STAR) sampling approaches produced samples with similar diatom assemblage (e.g., species richness), autecological (e.g., siltation index, trophic diatom index), and morphological metrics within a reach. The median BC similarity between samples within a reach was 70%. This BC value was higher than expected because variability in diatom assemblages, diatom sampling, and laboratory analysis is relatively high (Kelly et al. 2002). For example, mean BC similarity between diatom assemblages from different habitats within the same stream sampled multiple times was 68% (Stevenson and Hashim 1989). Mean BC similarity between replicate diatom counts ( $n = 3$ ) on the same slide by the same analyst was 79% for 3 slides with taxon richness that ranged from 30 to 70 (K. Manoylov, Michigan State University, unpublished data). Kelly (2001) proposed BC similarity  $\geq 60\%$  between analysts as a numerical criterion for data-quality assessment in benthic diatom analysis. Thus, the BC similarity between counts produced by the EMAP and STAR protocols at most (76%) of the sites in our study is well within the acceptable range of bioassessment values.

The maximum attainable BC similarity among multiple samples from the same reach will be reduced by within-reach heterogeneity among diatom assemblages or by using sampling methods that are not comparable. The effects of these 2 mechanisms are often difficult to distinguish. In our study, diatom assemblage diversity measures, which can be viewed as surrogates of stream habitat diversity, were significantly related to BC similarity. This result indicates that both habitat heterogeneity and sampling method incompatibility influenced the differences between diatom assemblages in the 2 samples at some sites. The high values of BC similarity between samples within reaches in our study may be attributed to the fact that the 2 protocols usually sampled the same habitat. Reach-scale (EMAP) composite samples tended to be dominated by subsamples collected from the dominant targeted habitat (STAR). For example, in reaches where STAR samples were collected from

TABLE 3. Pearson correlation coefficients between mean values of environmental variables and nonmetric multidimensional scaling ordination axes based on diatom data generated by US Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) and Science to Achieve Results (STAR) sampling protocols. Bold font indicates statistically significant correlations ( $p < 0.05$ ).

Environment variable	EMAP		STAR	
	Axis 1	Axis 2	Axis 1	Axis 2
<b>Water quality</b>				
Conductivity ( $\mu\text{S}/\text{cm}$ )	<b>-0.79</b>	-0.15	<b>-0.83</b>	-0.18
pH	<b>-0.29</b>	-0.18	-0.23	-0.39
Turbidity (NTU)	<b>-0.69</b>	<b>0.28</b>	<b>-0.68</b>	0.08
Dissolved organic C ( $\mu\text{g}/\text{L}$ )	<b>-0.78</b>	0.12	<b>-0.78</b>	0.07
Total P ( $\mu\text{g}/\text{L}$ )	<b>-0.64</b>	0.42	<b>-0.63</b>	0.24
Total N ( $\mu\text{g}/\text{L}$ )	<b>-0.71</b>	0.05	<b>-0.73</b>	0.06
SO <sub>4</sub> ( $\mu\text{g}/\text{L}$ )	<b>-0.80</b>	-0.06	<b>-0.83</b>	-0.07
Cl ( $\mu\text{g}/\text{L}$ )	<b>-0.73</b>	-0.12	<b>-0.78</b>	-0.16
<b>Physical habitat</b>				
Depth (cm)	<b>-0.25</b>	-0.11	-0.20	-0.16
Width (m)	<b>-0.43</b>	-0.06	<b>-0.40</b>	-0.11
Reach slope (%)	<b>0.61</b>	0.08	<b>0.64</b>	-0.03
% canopy cover midchannel	<b>0.58</b>	-0.12	<b>0.58</b>	-0.03
% coarse substrate	<b>0.55</b>	-0.04	<b>0.62</b>	-0.11
% fine sediment	<b>-0.52</b>	0.07	<b>-0.58</b>	-0.05
% embeddedness	<b>-0.53</b>	-0.08	<b>-0.60</b>	0.04
% riffle habitat	<b>0.48</b>	0.00	<b>0.55</b>	-0.05
% pool habitat	<b>-0.35</b>	0.01	<b>-0.36</b>	0.08
<b>Watershed characteristics</b>				
Area (km <sup>2</sup> )	<b>-0.76</b>	-0.15	<b>-0.76</b>	-0.12
Elevation (m)	<b>0.69</b>	-0.13	<b>0.75</b>	-0.11
Watershed slope (%)	<b>0.76</b>	-0.08	<b>0.81</b>	-0.05
Distance from ocean (km)	<b>-0.68</b>	-0.05	<b>-0.71</b>	0.03
% forested land cover	<b>-0.57</b>	0.21	<b>-0.61</b>	0.20
% agricultural land use	<b>0.58</b>	-0.21	<b>0.53</b>	-0.14
% disturbed land use	<b>-0.57</b>	0.21	<b>-0.61</b>	0.21

sandy substrates, the mean percentage of the channel covered by sand/fine substrate was 75%, indicating that much of the EMAP sample was collected from sand/fine substrate.

However, diatom assemblages produced by EMAP and STAR sampling protocols tended to diverge for larger streams. BC similarities were below the median value in 83% of sites with reach lengths >300 m (Fig. 5). Both protocols call for longer sampling reaches in larger streams while holding sampling effort constant. As reach length increases, the potential that the EMAP protocol will require sampling different habitats increases. Our results suggest that, for reach lengths >300 m, the constant transect number used by the EMAP protocol may sample the stream poorly, adequately sampling neither all habitats within the reach (larger sampling area) nor a single dominant

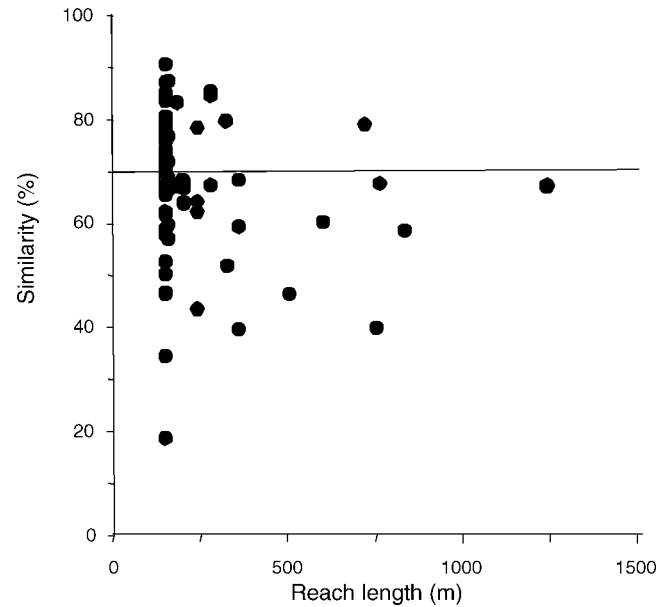


FIG. 5. Bray-Curtis (BC) similarity between diatom assemblages collected using US Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) and Science to Achieve Results (STAR) protocols as a function of reach length. Line indicates median BC similarity = 70%.

habitat (equidistant transects may miss habitats). In our study, higher-order streams tended to have a mixture of pool and riffle habitat and rock and sand substrates. Investigators using the EMAP protocol may have collected periphyton samples from several of these habitats, depending on the placement of transects. In contrast, investigators using the STAR protocol would have sampled only the dominant habitat, thereby leading to a potential discrepancy between EMAP and STAR diatom assemblages in these reaches. Moreover, habitat heterogeneity appears to have been the major factor influencing comparability between the 2 sampling protocols even for sites with reach lengths <300 m ( $n = 9$ ) because the targeted habitat represented only ~50% of the substrates present in the reach.

#### *Ability of protocols to sample assemblages adequately*

One of the basic premises of bioassessment is that the species assemblage has been characterized accurately within the study unit (e.g., sampling reach). Both sampling approaches provided similar information about the diatom assemblages, but our study did not inform us as to whether the reaches had been sampled adequately. The reach length used in the EMAP protocol (40× the wetted channel width) was

based on studies of the relationship between fish species richness and stream size (Fausch et al. 1984). Algal species richness has been related to ecosystem area in several ecosystems (reviewed in Smith et al. 2005), but the appropriateness of the reach length used in the EMAP approach for diatoms, which respond to environmental conditions at much smaller scales than fish do, has not been tested.

To our knowledge, no published studies have determined the best way to sample a stream reach to characterize its diatom assemblage adequately. European stream ecologists suggest collecting and pooling material from  $\geq 5$  stones when sampling benthic diatoms (Kelly et al. 1998). Several studies have demonstrated that the perceived composition and richness of macroinvertebrate and fish assemblages change as more subsamples are collected within a reach (e.g., Cao et al. 1997, 2002), but no well-developed method exists to determine when enough subsamples have been collected. Diatom-based stream bioassessment would benefit greatly from studies on the number of samples needed to sample periphyton adequately in stream reaches of various lengths and habitat heterogeneities. For example, multiple samples from different habitats within a reach could be collected and analyzed separately to determine how habitat heterogeneity influences descriptors of diatom assemblages. Such data could be used to explore the relationship between the number of samples collected and diatom species richness to provide insight into the number of samples necessary to characterize the stream diatom assemblage. Cao et al. (2002) used a simulation approach as a surrogate for actual sampling to examine how sample representativeness changes with the number of samples collected. Sample representativeness tended to increase with the number of samples pooled, but the stabilization point varied among organisms and habitat types.

For individual bioassessment projects, the choice of reach-scale or targeted-habitat sampling may well depend on the study objectives and the endpoint of interest. The reach-scale protocol has the potential to yield diatom samples collected from all habitats within the reach and eliminates subjectivity on the part of the researcher in terms of habitat selection. However, using constant sampling effort regardless of stream size may not be appropriate for larger streams. The targeted-habitat protocol reduces the noise introduced by habitat heterogeneity in larger streams by sampling only one habitat type. However, this method may not accurately characterize periphyton growing in all habitats throughout the reach.

Our results suggest that large-scale diatom data sets generated from different sampling protocols, such as

NAWQA and EMAP, may be merged to allow a national synthesis of stream ecological condition. However, scientists interested in combining data sets collected with reach-scale and targeted-habitat approaches should pay particular attention to streams where reach length  $> 300$  m because greater habitat heterogeneity may cause divergence in diatom counts between reach-scale and targeted-habitat sampling.

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