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Development of diatom indicators of ecological conditions for streams of the western US

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Abstract. The species composition of benthic diatoms was related to environmental conditions in streams throughout the western US to develop diatom traits, indicators for assessment of biological condition and indicators for diagnosing stressors. We hypothesized that indicators based on species traits determined for subsets of streams with similar natural landscape features would be more precisely related to environmental conditions than would be indicators calculated based on species traits for all streams in the data set. The ranges of many environmental conditions were wide among western streams, and these conditions covaried greatly along a major environmental gradient characterized by positive correlations among % watershed disturbed by agricultural and urban land uses (% WD), conductivity, total N, total P, and % fine sediments. Species traits were calculated for 242 diatom taxa. Weighted average (WA) methods were used to define species environmental optima, and regression approaches were used to determine whether species were sensitive or tolerant to environmental conditions indicated by % WD, total P, total N, a nutrient multivariate index, pH, conductivity, % fine sediments, % embeddedness, and a watershed disturbance multivariate index. Indicators based on WA optima and sensitive/tolerant traits were highly correlated with these environmental conditions. Natural and anthropogenic conditions varied greatly among classes of streams grouped by climate regions, but indicators developed for the entire western US were consistently more accurate than were regional indicators. Indicators for individual stressors, such as total P, conductivity, and % embeddedness, were highly correlated with values of respective stressors, but covariation among all indicators and stressors indicated that only 1 environmental gradient was reliably reflected by the indicators. Thus, robust indicators of the biological condition of diatom assemblages were developed for streams of the western US, but development of stressor-specific indicators will require application of additional analytical approaches.

Key words: diatoms, indicators, conductivity, stressors, nutrients, sediments, streams, western US.

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The challenges of managing aquatic ecosystems will increase as use of those ecosystems and surrounding landscapes intensifies during the next century (Millennium Ecosystem Assessment 2005). Resource use will increase with human population and standard of living. Intensification of agriculture for food and fuel production conflicts with the demand for clean water for irrigation and drinking water (Postel 1998). This problem will be particularly great in regions, such as the western US, where demand for water far exceeds supply. Management of aquatic ecosystems will require development of both policy and technical infrastructure to meet these challenges. Our paper describes development of diatom indicators of ecological condition that can support that infrastructure.

Diatoms have been used for aquatic ecosystem assessment around the world (Watanabe et al. 1986, Kelly et al. 1998, Wang et al. 2005, Chessman et al. 2007, Taylor et al. 2007, Porter et al. 2008). Diatoms most often have been used to diagnose levels of stressors, such as organic contamination, lake acidification, climate change, and nutrient concentrations (Slàdecek 1973, Dickman et al. 1984, Fritz et al. 1991, Potapova et al. 2004). We define stressors as the habitat alterations and contaminants that are managed to protect and restore valued ecological attributes (sensu Stevenson et al. 2004). Diatom indicators of stressors complement actual measurement of stressors by providing another perspective on stressor condition. For some highly variable stressors (e.g., nutrient concentrations), diatom indicators can be more precise than a 1-time measurement of water chemistry because they integrate stressor effects over time (Stevenson 2006).

In recent studies, the biological condition of diatoms has been related to nutrient concentrations to justify establishment of nutrient criteria (Wang et al. 2005, Stevenson et al. 2008). Biological condition is a measure that compares species composition, biomass, and function of organisms at the assessed site to natural or reference conditions (Davies and Jackson 2006, Stoddard et al. 2006). Thus, biological condition reflects valued natural capital and ecosystem services as broadly defined in the Millennium Ecosystem Assessment (2005). Some researchers (Karr 1991, Stevenson et al. 2004) would argue that biological condition is an ultimate management endpoint. Thus, diatom species composition and biomass can be used as indicators of biological condition because diatoms themselves are important elements of aquatic food webs and biogeochemical processes. Diatom diversity probably is important for supporting diatom functions in ecosystems (Cardinale et al. 2006). Diatoms also might provide a better estimate of the biological condition of other algae and heterotrophic microbes than other commonly used biological indicators because of their similarity to other algae and microbes with respect to their size, unicellular organization, metabolic rates, nutritional requirements, and sensitivities to abiotic and biotic factors.

The analytical distinction between diatom indicators that measure stressors and those that measure biological condition is small, but the difference in the meanings of the information for management is great (Stevenson and Smol 2002, Stevenson 2006). Both types of indicators require measures of the abundance and traits of taxa. Abundance measures can be presence/absence, abundance relative to other organisms in the habitat, or absolute density. Diatom traits could be calculated as weighted average (WA) optima of taxa on a continuous scale (ter Braak and van Dam 1989), assigned to ranks on an ordinal scale (van Dam et al. 1994), or simply characterized as sensitive or tolerant to changes associated with human alterations of watersheds (Fore and Grafe 2002) (see *Diatom trait development and indicator evaluation* in Methods for our rationale for using the terms *indicators* and *traits*). To infer stressors, WA indicators are calculated from the relative abundance and either WA or rank traits of all taxa in the assemblage (Zelinka and Marvan 1961, ter Braak and van Dam 1989). The number of taxa, percentage of taxa, or percentage of individuals within the sensitive or tolerant groups are more appropriate indicators for characterizing biological condition. When the sensitivity and tolerance is related to a human disturbance gradient, these groups of taxa are reference and nonreference or native and nonnative taxa. Changes in sensitive and tolerant taxa (or individuals) enable a more accurate (less ambiguous) indication of changes in biological condition, such as a loss of sensitive species or an increase in nonnative species (Davies and Jackson 2006), than do WA models inferring total P concentration or relative sediment impacts. The WA models infer stressor conditions, which is valuable, but they use all taxa, so it is not clear whether we have increases in sensitive taxa or decreases in tolerant taxa. However, indicators that use only a subset of species might be less precise than those that use all species because less information is used to calculate the indicator. Thus, slight differences in trait characterization and indicator calculation affect application of indicators. Moreover, tradeoffs might exist between accuracy (closeness in meaning) of indicators for characterizing biological condition and precision (repeatability) of those indicators.

Therefore, accurate characterizations of diatom traits are important for assessing biological conditions and diagnosing stressors in aquatic ecosystems. Characterizations of diatom species traits are available, but many of these are global- or continental-scale summaries and tests of traits (Lowe 1974, van Dam et al. 1994, Porter et al. 2008). Potapova and Charles (2002) observed regional variation in species traits within the US. Regional variation in species traits might arise from interpopulation divergence (Gallagher 1982), interactions with environmental conditions, or as perceived differences when calculations are based on relative abundances (because changes in abundances of some taxa affect relative abundances of all taxa)

(Austin 2002). Diatom traits and indicators have not been evaluated widely in the western US.

The goal of our study was to characterize traits of diatoms that could be used to assess biological condition and to diagnose stressors of streams in the western US (West). First, we characterized major environmental gradients in the West to ensure that environmental variation was sufficient to affect diatom species composition and to characterize traits. Next, we characterized diatom traits and determined whether indicators based on them were sufficiently accurate to explain variation in biological condition among streams and to diagnose stressors. Last, we compared performances of indicators developed for the West and western climate regions to determine whether different diatom traits and indicators should be used in different types of streams. The West provided an excellent region to assess sources of variation in biological indicators because of the great variability in environmental conditions caused by both natural and anthropogenic processes.

Methods

Sampling and sample analysis

The sampling and sample analysis were conducted as part of the US Environmental Protection Agency (EPA) Environmental Monitoring and Assessment Program Western Pilot Survey (EMAP-West). Ecological conditions, water chemistry, in-stream habitat, riparian habitat, watershed land use, and geomorphic features were characterized for perennial wadeable streams and boatable rivers in the 12 western states of the US. Sites were selected throughout the study area using a spatially balanced probabilistic design (Stevens and Olsen 2004). A subset of 1203 of these streams and rivers in which benthic algae had been sampled was selected for the analyses in our paper. These sites spanned 1st-order streams to 8th-order rivers (Strahler 1952) and 3 climate regions, Mountain, Xeric, and Plains (Omernik 1987). Streams were sampled with wadeable-stream protocols (Peck et al. 2006), whereas rivers were sampled with rafts and boatable-river protocols (Peck et al., in press).

Watersheds were delineated for each site from US Geological Survey (USGS) 1:24,000 topographic maps. Stream order was determined with 1:100,000 USGS digital hydrography (Strahler 1952). Watershed conditions were characterized from the USGS 1992 National Land Cover Dataset, USGS runoff contour maps, and the 1994 parameter-elevation regressions on independent slopes model (PRISM) precipitation and air temperature database (http://www.prism.oregonstate.edu/docs/przfact.html). Conditions included watershed area, stream order, mean slope, mean annual temperature and precipitation, elevation, latitude, longitude, and landuse attributes, such as % agricultural and urban land use, % forest, and road and population density. Percent watershed disturbed (% WD) was calculated as the percentage of land in some form of urban or agricultural land use based on Anderson level 1 designations (Anderson et al. 1976).

The sites were visited during extended summers (May–October) from 2000 to 2004. The length of the reach studied was defined as 40× the mean wetted width of the channel or a minimum length of 150 m. Channel depth and alterations, embeddedness, % sand and fines, current velocity, and substratum roughness were determined using methods described in Kaufmann et al. (1999) and Peck et al. (2006). Water samples were collected in one 4-L cubitainer and 2 sealed 60-mL syringes near the middle of the stream in a flowing-water section for determination of water-chemistry attributes. Samples were kept on ice in the dark and shipped by overnight courier to a central processing laboratory where they were divided into aliquots and preserved within 72 h of collection. Base cations and anions were determined by atomic absorption and ion chromatography, respectively. Total N (TN) and total P (TP) were determined spectrophotometrically after persulfate digestion. pH was determined with a pH probe using closed headspace techniques and 1 sample from a sealed syringe. Details of water-chemistry analysis can be found in USEPA (1987).

Benthic algae were sampled at 1 of 3 locations along each of 11 evenly spaced transects in a study reach. The transects were limited to the wadeable shore area in nonwadeable rivers, but otherwise extended across wadeable streams. At each location, benthic algae were scraped from a 12-cm^2 area of substratum with a toothbrush if substrata were firm and large enough to hold. Otherwise, fine sediments were collected into a 60-mL syringe. All 11 benthic algal subsamples, whether from erosional or depositional habitats, were combined into 1 sample for the site.

Benthic algal samples were subsampled and acid cleaned for determination of diatom relative abundances at sites. Subsamples of cleaned diatoms were mounted on microscope slides using ZRAX® or NAPHRAX® (The Biology Shop, Hazelbrook, New South Wales, Australia; http://mywebsite.bigpond.com/thebiologyshop) as mounting medium. Six hundred diatom valves were identified and counted at 1000× with Leica DMLB microscopes and differential interference contrast optics (Leica Microsystems, Inc., Bannockburn, Illinois). Diatoms were identified primarily with keys provided in Krammer and Lange-

Bertalot (1986, 1988, 1991a, b), Patrick and Reimer (1966, 1975), and more recent references. Consistency in diatom identification among several technicians was maintained by regular communications, exchange of digital images of specimens, and taxonomic workshops. Taxonomic composition and density of non-diatom algae were determined, but those results were not used in our paper.

Data analysis

Relationships among land use and environmental factors.—Principal components analysis (PCA) was done to determine which proximate environmental factors, i.e., those factors that directly affect diatom species composition (sensu Stevenson 1997), varied most among streams in the West. This analysis also was done to identify the land use and natural landscape factors that probably regulated the proximate environmental factors. Regional studies show that ionic factors, such as conductivity and pH and nutrient concentrations, affect diatom species composition in streams (Pan et al. 1996, Potapova and Charles 2002, Acs et al. 2004). We used 36 of the environmental factors characterized during the EMAP-West survey for each stream in the PCA because of their probable indirect or direct effect on diatom species composition (Stevenson 1997). Relationships between selected proximate environmental factors and % WD were established with linear regression to confirm that human activities were a likely determinant of these factors in streams of the West.

Diatom indicators were developed for 6 proximate factors that were selected because they are important determinants of diatom species composition and are common stressors in US streams. TP, TN, % fines, and % embeddedness were selected because sediments and nutrients are among the most common causes of impairment of biological condition in US streams (USEPA 2007). In the West, pH and conductivity levels vary over 3 orders of magnitude and are strongly determined by local geology. In addition, pH and conductivity can be affected by mining, agriculture, and urbanization. Diatom species composition in streams is highly correlated with these variables (Pan et al. 1996, Potapova and Charles 2002).

Two multivariate indicators (MVIs) of environmental conditions were calculated to provide more robust indicators of nutrients and watershed disturbance by humans. The MVIs were calculated by standardizing stressor or landuse variables (i.e., dividing the difference between observed and average stressor values by the standard deviation in stressor values); the MVI was calculated as the average of the standardized variables. TP and TN were included in the nutrient MVI because these variables tend to covary, and the limiting nutrient can be depleted when high algal biomasses accumulate. TN, TP, conductivity, and % WD were included in the watershed disturbance MVI (WD MVI). Use of both stressors and land use in a watershed disturbance index can correct for some agricultural land use classes that have relatively low impact.

Diatom trait development and indicator evaluation.—In our paper, the term *trait* refers to an attribute of individual species that reflects its fitness (performance, both absolute and relative to other species) in different environmental conditions. Traits could include environmental optima calculated by WA or generalized additive models (ter Braak and van Dam 1989, Yuan 2004) and sensitivity and tolerance to different environmental stressors determined by regression (our paper). Traits also could refer to possession of a keeled raphe (assumed to confer fitness in fine sediments), mucilaginous stalks, endosymbiotic cyanobacteria, quantitative measures of metabolic parameters, and size. In our paper, the term *indicator* refers to a measure of ecological condition that uses species traits and species abundances. The indicator reflects a shift in species composition that either is or is not correlated with some measure of human activities and includes all kinds of metrics, which then must be related to human activities (sensu Karr and Chu 1999) or WA inference models (ter Braak and van Dam 1989).

A variety of diatom indicators of ecological condition that were expected to vary in their accuracy for inferring stressors and biological condition, precision, and ease of explanation to public audiences (Table 1) were tested. Traits and indicators were calculated in 2 fundamentally different ways. Traits were calculated with either WAs to determine environmental optima or regression to determine sensitivity or tolerance to an environmental gradient. Indicators were calculated using WA models (ter Braak and van Dam 1989) or from the number of species, percentage of individuals, or percentage of species that were either sensitive or tolerant. Environmental gradients were defined by stressors, % WD, or MVIs of these variables. WA indicators were expected to be the most precise indicators of both stressors and environmental condition, but to be least accurate for characterizing biological condition because they use both relatively sensitive and relatively tolerant taxa (Table 1). Species indicators based on the presence and absence of sensitive and tolerant taxa were expected to be least precise of all indicators because fewer taxa in samples are used in the indicator calculation than are used in calculation of WA indicators.

Table 1. The expected precision and accuracy of diatom indicators calculated in this study. Sensitive (S) and tolerant (T) indicator species traits were determined by regression to be sensitive or tolerant to an environmental gradient. WA = weighted average, WAI = weighted average indicator, HA = highest accuracy, ≈HA = relatively high accuracy, ≈LA = relatively low accuracy, LA = lowest accuracy, HP = highest precision, ≈HP = relatively high precision, ≈LP = relatively low precision, and LP = lowest precision.

Indicator calculation and trait type	Stressors	Biological condition
Number of species either S or T	LA, LP	HA, LP
% species either S or T	≈LA, ≈LP	≈HA, ≈LP
% individuals either S or T	≈HA, ≈HP	≈LA, ≈HP
WAI using all taxon relative abundances weighted by WA optimum	HA, HP	LA, HP

WA optima (ter Braak and van Dam 1989) were calculated for taxa using an Access (Microsoft Office 2003, Redmond, Washington) database. WA optima were calculated for taxa that were observed at ≥40 sites. The number of sites used was 1203 for all stressors except % embeddedness, for which 1061 sites were used because of missing data at large river sites where % embeddedness was not determined per protocol. Species optima were calculated for subsets of samples from climate regions if the species were observed in ≥10 samples.

WA indicators were calculated with the optima and relative abundances of taxa in samples (ter Braak and van Dam 1989). WA indicators were tested by cross validation. Samples were randomly assigned to 2 groups, A and B. Indicators for samples in group A were calculated with optima derived from sample group B and vice versa. The correlation coefficients (r^2) for relationships between measured stressor conditions in streams and diatom-inferred stressor conditions were used to evaluate precision of WA indicators and to test for statistically significant relationships between indicators and measured stressor conditions. In addition, WA indicators were recalculated using the classical deshrinking method (Birks et al. 1990), plotted against measured values of stressors, % WD, and multivariate indices of stressors, and evaluated for bias in inferred condition.

The process of determining WA optima and testing WA indicators was repeated for each climate region to test the hypothesis that indicators based on species traits determined for subsets of streams with similar natural landscape features (climate regions) would be more precisely related to environmental conditions than would be indicators based on species traits determined for all streams in the data set. Climate region accounts for great variation in diatom species composition and environmental factors in streams of the West (YP, RJS, C. L. Weilhofer, Portland State University, CAP, ATH, P. K. Kaufmann, US EPA, and DPL, unpublished data). Covariance among indicators and all stressors was analyzed to determine their independence.

Sensitivity and tolerance (S/T) of taxa to different stressors were characterized by using linear regression to relate individual stressors to relative abundances of individual taxa. Simple linear regression was used rather than WA categories, indicator species analysis (Dufrene and Legendre 1997), or generalized additive models (Yuan 2004) because simple linear regression is easier to explain to the public and interpretation of results is straightforward. Future analyses should be conducted to determine whether other S/T trait calculation methods improve performance of indicators. Taxa that were significantly ($p < 0.05$) negatively or positively related to stressors were characterized as sensitive or tolerant, respectively, to that stressor. S/T traits were evaluated for all taxa observed in ≥40 samples. Six indicators based on S/T classification of taxa were calculated: the number of sensitive taxa, % sensitive taxa, % sensitive individuals, the number of tolerant taxa, % tolerant taxa, and % of tolerant individuals. These indicators were tested by cross validation with sample groups A and B, as for WA indicators.

Results

Relationships among land use and environmental factors

PCA of environmental variables indicated 1 relatively dominant gradient and a 2nd subdominant gradient that explained 32 and 10%, respectively, of the variation in the correlation matrix (Table 2). PCA axis 1 was strongly related to human activities in watersheds and associated stressors and, thus, represented a major environmental gradient. PCA axis 1 was positively correlated with % WD, % agricultural land use, conductivity, % embeddedness, and concentrations of TN, TP, and Cl^- and negatively correlated with % forest cover, watershed slope, precipitation, and longitude. PCA axis 2 was strongly positively correlated with temperature and negatively correlated with latitude and elevation.

TP (Fig. 1A), TN (Fig. 1B), conductivity (Fig. 1C), pH (Fig. 1D), % fines (Fig. 1E), and % embeddedness (Fig. 1F) were significantly ($p < 0.001$) related to land use (Table 3). Upper and lower quartiles for these stressors for all sites in the study were 7 and 66 μg TP/L, 98 and 529 μg TN/L, pH 7.6 and 8.3, 83 and 584 μS/cm, 1 and

29% fines, and 38 and 77% embeddedness. The median TN:TP molar ratio was 19.6 (minimum = 0.7, quartiles = 10.1 and 49.8, maximum = 15,287). Variation in the stressor variables explained by land use was highest for TN ($r^2 = 0.51$) and lowest for pH and % fines ($r^2 = 0.10$ and 0.19, respectively).

Percent WD and environmental stressors varied greatly among climate regions (Kruskal–Wallis, $p < 0.05$). Percent WD averaged 1.0% (±4.8% SD, $n = 699$) in the Mountain climate region, 4.8% (±14.0%, $n = 257$) in the Xeric climate region, and 44.7% (±34.3 %, $n = 247$) in the Plains climate region (Fig. 2). Environmental stressors also varied significantly among climate regions (Fig. 3A–F). In all cases, stressors were lower in the Mountain climate region than in the other climate regions. Among stressors, the magnitude of differences in pH among classes was less than the magnitude of differences for other stressors (Fig. 3D).

Diatom trait development and indicator evaluation

WA optima and S/T traits for 242 of the 1349 taxa were calculated for the 6 stressors and 2 MVIs of stressors (Appendix; available online from: http://dx.doi.org/10.1899/08-040.1.s). The precision of traits increased with average relative abundance of taxa in the data set, as illustrated by the negative relationship between taxon relative abundances and the standard deviation in the WA optima of taxa for the WD MVI between cross-validation data sets (Fig. 4). Fewer taxa were identified as sensitive than as tolerant for most environmental gradients. For example, 57 taxa were negatively related (sensitive) to the WD MVI, whereas 101 taxa were positively related (tolerant). The rest of the 242 taxa were not significantly related to that WD MVI.

On average, diatom taxa that were sensitive to WD MVI had higher maximum abundances (Fig. 5A, B), were observed at more sites (Fig. 5C, D), and had higher relative abundances (Fig. 5E, F) than diatom taxa that were tolerant to WD MVI. *Achnanthidium minutissimum* (Kützing) Czarnecki, a taxon defined as WD MVI sensitive, was observed in more samples and with higher relative abundance than any other taxon. *Cocconeis placentula* and its varieties and *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot were the 2nd and 3rd most abundant sensitive species. *Nitzschia inconspicua* Grunow, *Nitzschia frustulum* (Kützing) Grunow, and *Cocconeis pediculus* Ehrenberg were the most commonly observed WD MVI tolerant taxa, occurring in >500 samples with 3 of the 4 highest average west-wide relative abundances. *Nupela lapidosa* (Krasske) Lange-Bertalot, *Diatoma anceps* (Ehrenberg) Kirchner, *Gomphonema olivaceoides* Hustedt, *Karayevia suchlandtii* (Hustedt) Bukhtiyarova, *Achnanthes nodosa* Cleve, and *Diatoma mesodon* (Ehrenberg) Kützing had the lowest optima for the WD MVI. *Aulacoseira granulata* (Ehrenberg) Simonsen, *Stephanodiscus hantzschii* Grunow, *Cyclotella atomus* Hustedt, *Stephanodiscus medius* Håkansson, *Biremis circumtexta* (Meister ex Hustedt) Lange-Bertalot et Witkowski, and *Nitzschia desertorum* Hustedt had the highest optima for the WD MVI.

TABLE 2. Loadings of environmental variables on ordination axes from principal components analysis. L and L1 indicate variables were $\log_{10}(x)$ transformed or $\log_{10}(x + 1)$ transformed, respectively, for analyses.

Variable	Axis 1	Axis 2
pH	0.427	0.138
Conductivity (L)	0.832	0.214
Acid neutralizing capacity (L)	0.745	0.265
Total suspended solids (L1)	0.663	−0.143
Total P (L1)	0.724	−0.063
Se (L1)	0.325	0.029
NH_4^+ (L1)	0.631	−0.084
NO_3^- (L1)	0.349	0.028
Cl^- (L1)	0.770	0.413
Total N (L)	0.839	−0.141
Zn (L)	0.024	−0.035
SiO_2 (L)	−0.011	0.283
HCO_3^- (L)	0.729	0.278
% embeddedness	0.703	−0.286
Channel slope (L)	−0.610	−0.125
Channel depth (L)	0.347	0.114
% fines (L1)	0.499	−0.432
% sand (L1)	0.229	−0.092
% slow-current habitat	0.559	0.144
% urban land use (L1)	0.498	0.154
% agricultural land use (L1)	0.812	−0.264
% forest	−0.615	0.357
% watershed disturbed (L1)	0.843	−0.215
Stream order	0.523	0.171
Road density (L1)	0.490	0.252
Population density (L1)	0.575	0.308
Elevation	−0.425	−0.552
Watershed slope	−0.794	0.230
Roughness	−0.172	0.528
Watershed area	0.278	0.084
Water temperature	0.545	0.365
Channel alteration	−0.420	−0.088
Mean annual air temperature	0.121	0.897
Mean annual precipitation (L)	−0.707	0.261
Latitude	0.140	−0.582
Longitude	−0.640	0.519

WA indicators tested by cross validation were significantly related for all stressors and MVIs (Fig. 6A–I, Table 4). The WA indicators for conductivity (Fig. 6F) and % fines (Fig. 6H) were the most and least precise, respectively ($r^2 = 0.687$ and 0.314), for single stressor measures. The WA indicator for pH (Fig. 6G) also was relatively imprecise ($r^2 = 0.323$), compared to other indicators, which had r^2 values ranging from

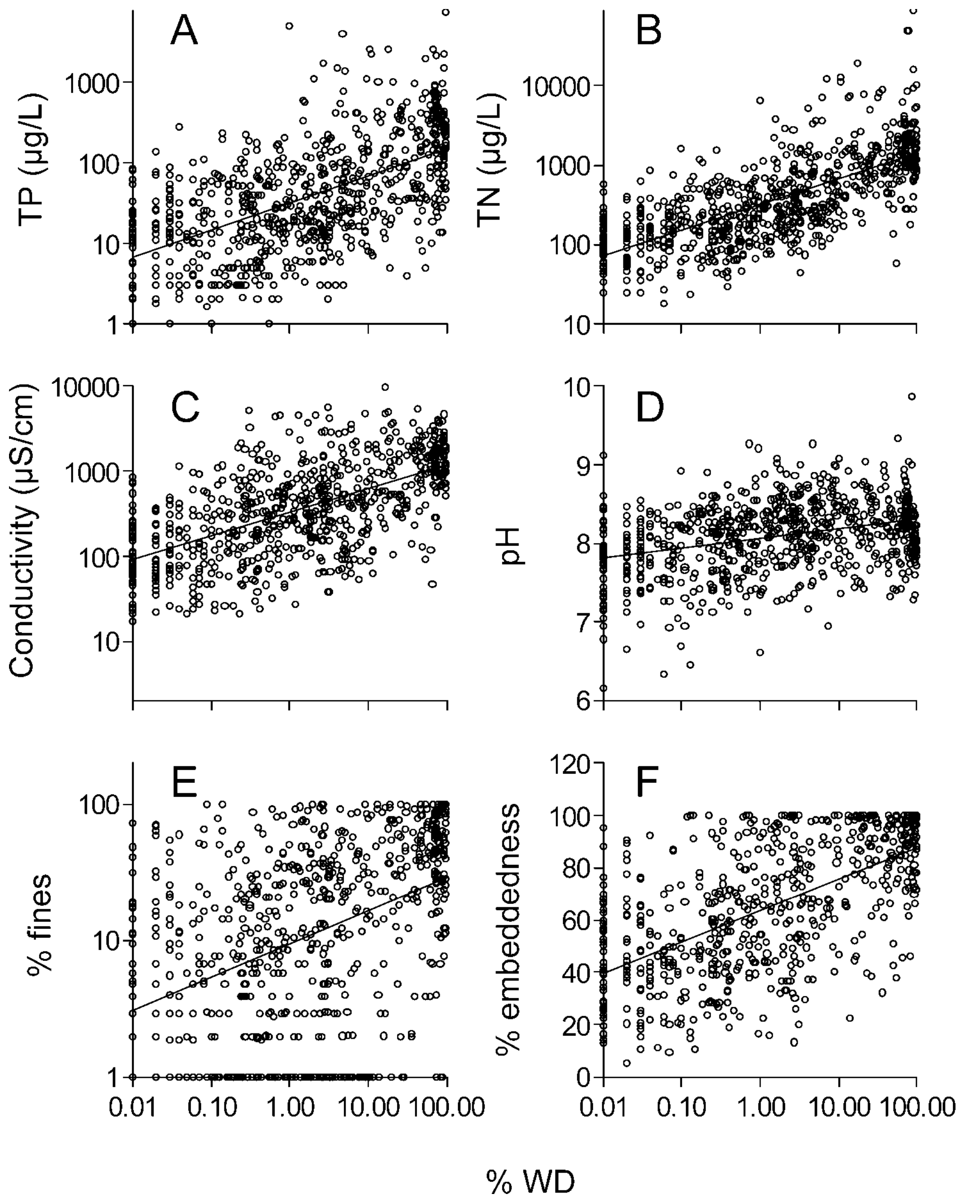

FIG. 1. Relationships among total P (TP) (A), total N (TN) (B), conductivity (C), pH (D), % fines (E), % embeddedness (F), and % watershed disturbed (% WD) by humans in watersheds of streams sampled in the western US. 1.0 was added to values of TP, TN, conductivity, and % fines so that all points could be plotted on a logarithmic scale.

TABLE 3. Correlations among stressors in streams of the western US. TP = total P, TN = total N, nutrient MVI = nutrient multivariate index, % WD = % watershed disturbed, WD MVI = watershed disturbance multivariate index.

Stressor	TP	TN	Nutrient MVI	% WD	WD MVI	pH	Conductivity	% embeddedness
TN	0.497							
Nutrient MVI	0.852	0.852						
% WD	0.342	0.513	0.496					
WD MVI	0.748	0.835	0.927	0.755				
pH	0.062	0.075	0.080	0.096	0.099			
Conductivity	0.300	0.437	0.429	0.373	0.473	0.284		
% embeddedness	0.310	0.361	0.401	0.312	0.430	0.057	0.349	
% fines	0.233	0.253	0.285	0.190	0.288	0.013	0.247	0.555

0.492 to 0.545 (TP, TN, % embeddedness; Fig. 6A, B, F, respectively). The precision (r^2) of WA indicators for the nutrient MVI (Fig. 6D) was higher, but not significantly higher, than WA indicators for TP or TN individually. Similarly, the WA indicator for the WD MVI (Fig. 6E) was more precise ($r^2 = 0.667$) than all individual indicators except conductivity.

Bias was relatively low for WA diatom indicators for TP (Fig. 6A), % WD (Fig. 6C), % fines (Fig. 6H), and % embeddedness (Fig. 6I) compared to other indicators. In general, the relationships predicted by least squares regression between diatom-inferred conditions based on WA indicators and measured values followed a 1:1 relationship (Fig. 6A–I), but nonlinear bias was observed for some indicators. Diatom-inferred TN (Fig. 6B) and conductivity (Fig. 6F) were overestimated at high levels of measured TN and conductivity. This bias resulted in slight overestimation of the diatom-inferred nutrient and WD MVIs at high levels of measured condition. The diatom-inferred pH indicator (Fig. 6G) was biased at both ends of the pH range and underestimated measured pH at low levels and overestimated pH at high levels.

Stressor variables and WA indicators were highly interrelated (Tables 3, 5). All correlations among stressor variables and among WA indicators were highly significant ($p < 0.001$). Correlations involving pH and other stressors or the WA pH indicator and other indicators were weaker than correlations for other stressors or indicators. The median correlation coefficient for all correlations among stressors was 0.346, whereas the median correlation coefficient for all correlations among indicators was 0.880 (Table 5). Factor analysis indicated that 65% of variation in the 8 stressors was explained by the 1st ordination factor, whereas 91% of the variation among indicators was explained by the 1st ordination factor.

WA indicators often were most strongly correlated with a stressor that had not been used to develop it (Table 6). In the worst of these cases, the % fines WA indicator was more strongly correlated with 7 of the 8 stressors other than % fines. The % fines WA indicator was significantly correlated with measured % fines ($r^2 = 0.314$) and with conductivity ($r^2 = 0.543$) and the WD MVI ($r^2 = 0.585$). The TP WA indicator was correlated with TP ($r^2 = 0.533$), WD MVI ($r^2 = 0.634$), the nutrient MVI ($r^2 = 0.585$), and conductivity ($r^2 = 0.533$). The TN WA indicator was correlated with TN ($r^2 = 0.548$), WD MVI ($r^2 = 0.663$), the nutrient MVI ($r^2 = 0.575$), and conductivity ($r^2 = 0.555$). Only the WA indicators for the WD MVI and conductivity were best correlated with the stressor with which they had been developed.

WA indicators developed independently for each climate region were not more precise than indicators

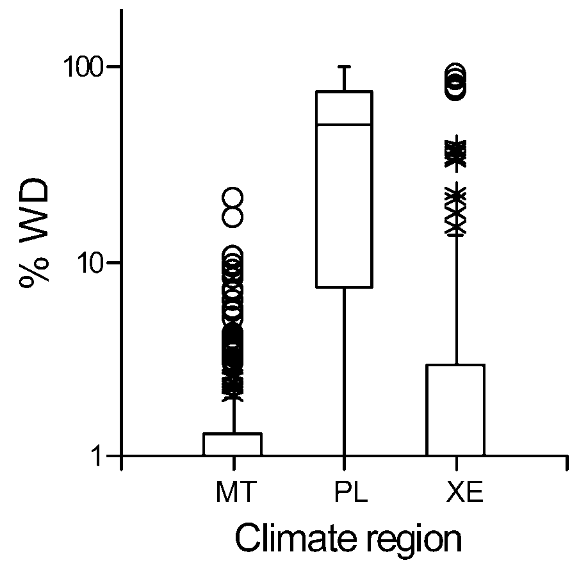

FIG. 2. Box-and-whisker plots for % watershed disturbed (% WD) by humans in streams in Mountain (MT), Plains (PL), and Xeric (XE) climate regions in the western US. 1.0% was added to values of % WD to enable plotting 0.0% WD on a logarithmic scale. Lines in boxes show medians, boxes show interquartile ranges, and whiskers show 2.5× the interquartile range. Near and far outliers are indicated by asterisks and circles, respectively.

developed for all the sites throughout the West (Table 4). On average, precision of indicators decreased 22 percentage points from $r^2 = 0.53$ to 0.41 when the climate region classification scheme was used rather than the west-wide scheme. Precision decreased most for % fines and % embeddedness indicators.

Indicators based on S/T traits of taxa were all significantly ($p < 0.001$) related to respective stressors (Table 7). The most precise S/T indicators were % taxa tolerant to conductivity ($r^2 = 0.671$) and % taxa tolerant to the WD MVI ($r^2 = 0.638$). The least precise indicator was the number of taxa sensitive to pH ($r^2 = 0.209$). Indicators based on tolerant taxa were consistently more precise than were indicators based on sensitive taxa. Indicators based on % sensitive taxa were consistently more precise than were indicators based on % sensitive individuals, and both of those indicators were more precise than indicators based on number of sensitive taxa. Precision of the S/T indicators was seldom as high as precision of WA indicators for the same stressor.

Discussion

Nutrient concentrations, conductivity, and % fine sediments varied greatly among streams in the West. Nutrient concentrations and % fine sediments, 2 of the leading causes of biological impairment of US waters

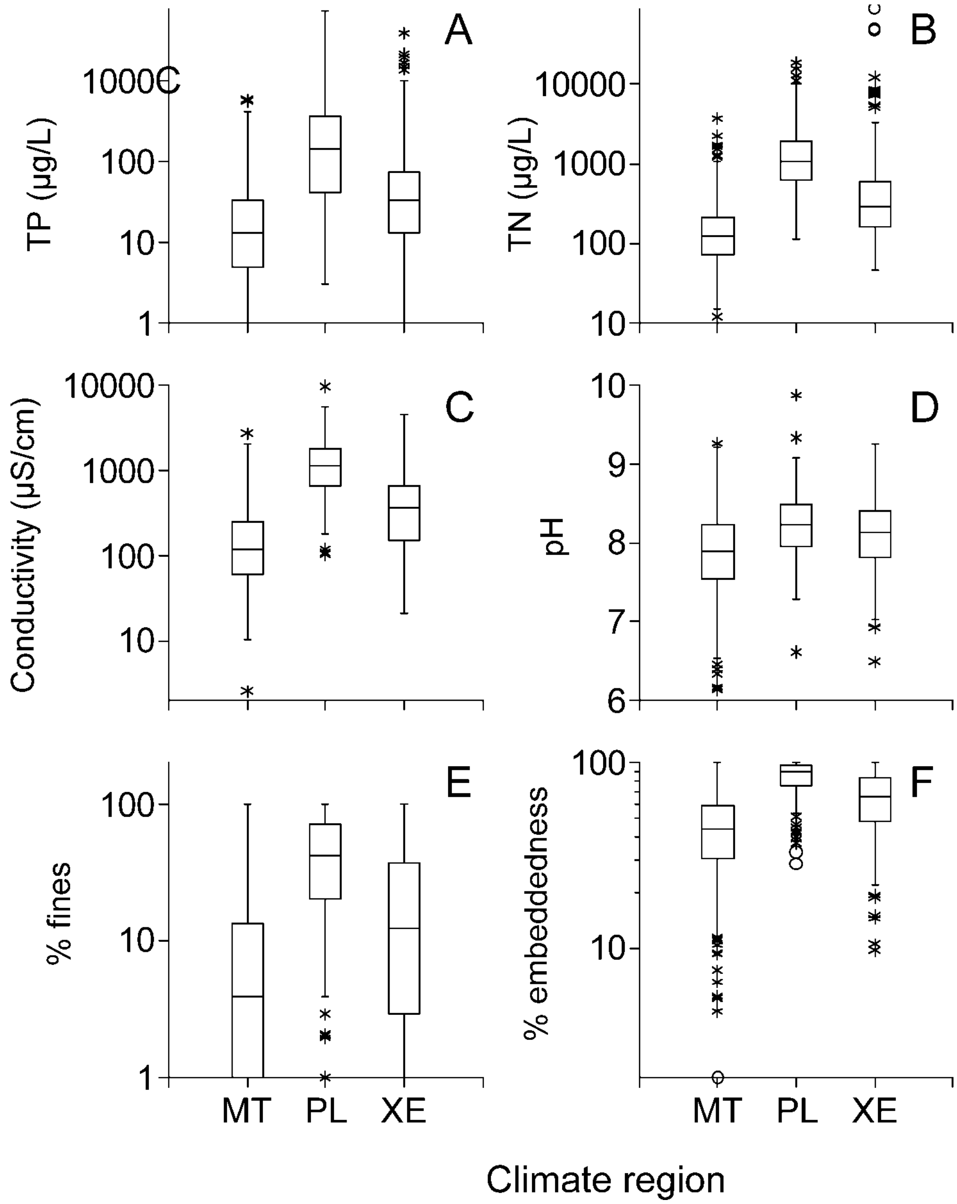

Fig. 3. Box-and-whisker plots for total P (TP) (A), total N (TN) (B), conductivity (C), pH (D), % fines (E), and % embeddedness (F) streams in Mountain (MT), Plains (PL), and Xeric (XE) climate regions in the western US. 1.0 was added to values for TP, TN, conductivity, and % fines so all points could be plotted on a logarithmic scale. Lines in boxes show medians, boxes show interquartile ranges, and whiskers show 2.5× the interquartile range. Near and far outliers are indicated by asterisks and circles, respectively.

(USEPA 2007), were highly correlated with human alteration of watersheds in the West. Conductivity, a variable that commonly is correlated with soil disturbance (Herlihy et al. 1998), also was strongly correlated with nutrients, % fine sediments, and % WD. The high variability in levels of correlation between the suite of 6 proximate environmental factors and % WD indicated that the 6 proximate environmental indicators also were affected by nonanthropogenic factors. Many of these abiotic factors also varied among climate regions because they are regulated by precipitation, soils, geology, and stream hydrogeomorphology (Welch et al. 1998, YP, RJS, C. L. Weilhofer, Portland State University, CAP, ATH, P. K. Kaufmann, US EPA, and DPL, unpublished data). However, the extent of human land use in watersheds also varied among climate regions. Accurate distinction between natural and anthropogenic sources of stressors will be important for assessment of stream condition and diagnosis of stressors (Omernik 1987, Wright et al. 1993, Hawkins et al. 2000, Stevenson et al. 2004).

The ranges of many selected stressors in western US streams were sufficient to affect diatom species composition. Sufficient range is needed when developing indicators of stressors. We based this conclusion on comparisons of ranges of stressors that cause changes in species composition in experiments to ranges of stressors in western US streams. The ranges of both N and P concentrations that affect biomass and species composition of diatom assemblages in experiments (Bothwell 1989, Rier and Stevenson 2006, Manoylov and Stevenson 2006) are within the ranges observed in western streams. We know relatively little from experimental research about the ranges of conductivity, % fines, and % embeddedness needed to affect diatom species composition. However, the observed ranges of these variables were very wide (0–100% for % fines and % embeddedness) and most probably encompass the ranges within which diatom responses are expected. The range of conductivity values (2 to 12,000 μS/cm) in western streams was greater than ranges in studies of lakes and other streams in which conductivity was implicated as a determinant of diatom species composition (Fritz et al. 1991, Pan et al. 1996). pH (6.1 to 9.9) is the least likely stressor to affect diatom species composition because its range did not span the acidic end of the scale (Lowe 1974, van Dam et al. 1994). Moreover, pH was not correlated well with other stressors. Therefore, we should be able to develop indicators of most stressors (expect pH), because ranges of stressor conditions were sufficient to affect diatom species composition in experiments in which cause–effect relationships were confirmed.

Diatom indicators were significantly and often strongly correlated with the stressors used to determine diatom traits in western US streams. However, indicators were often more highly correlated with other stressors (e.g., conductivity, WD MVI) than with the stressors from which they were developed, despite the fact that traits for diatoms were determined independently from WAs and regression models. This issue of covariation among multiple stressors and stressor indicators presents a problem for defining species traits and for diagnosing stressors with diatom indicators. Causal relationships should be evaluated thoroughly when developing biological indicators of stressor conditions (Yuan 2007). In addition, the high levels of covariation between indicators and multiple stressors prevented development of indicators that could have been used to diagnose specific stressors.

We expected that developing separate indicators for each climate region would minimize the confounding

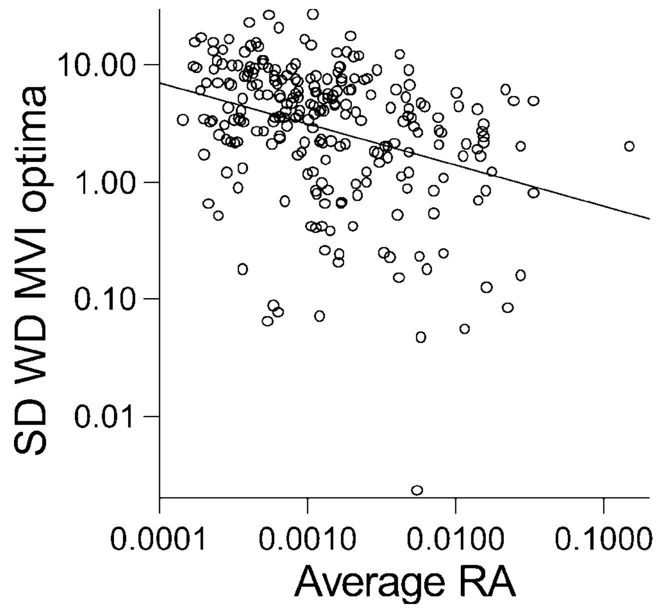

FIG. 4. The relationship between the average relative abundances (RA) of 242 taxa at all stream sites and the standard deviations (SD) of the weighted average (WA) optima for taxa that were calculated for the watershed disturbance multivariate index (WD MVI) the 2 cross-validation data sets.

effects of covariation among stressors and produce more accurate and precise indicators for individual stressors (Potapova and Charles 2002). Species traits can be affected by direct interactions among environmental factors, by historic exposure to different conditions that produce intraspecific variation in physiologies among populations, or by the presence of other species that affect relative performance (Gallagher 1982, Austin 2002). Therefore, refinement of species traits for classes of streams, with classes defined by climate region or hydrogeomorphic attributes, should have improved indicator performance. However, indicators developed for individual climate regions were not more precise than those developed for all sites in the West. The relatively poor performance of climate region–specific indicators might have been the result of shorter environmental gradients within climate regions than across the West or of smaller sample sizes in climate region–specific data sets. However, sample sizes were large, even within individual climate regions, and they were held constant in comparisons. We think it more likely that the limited variation in % WD within climate regions compared to % WD in the West probably was the reason that r^2 values for climate region–specific diatom indicators were lower than those for indicators based on all sites in the West.

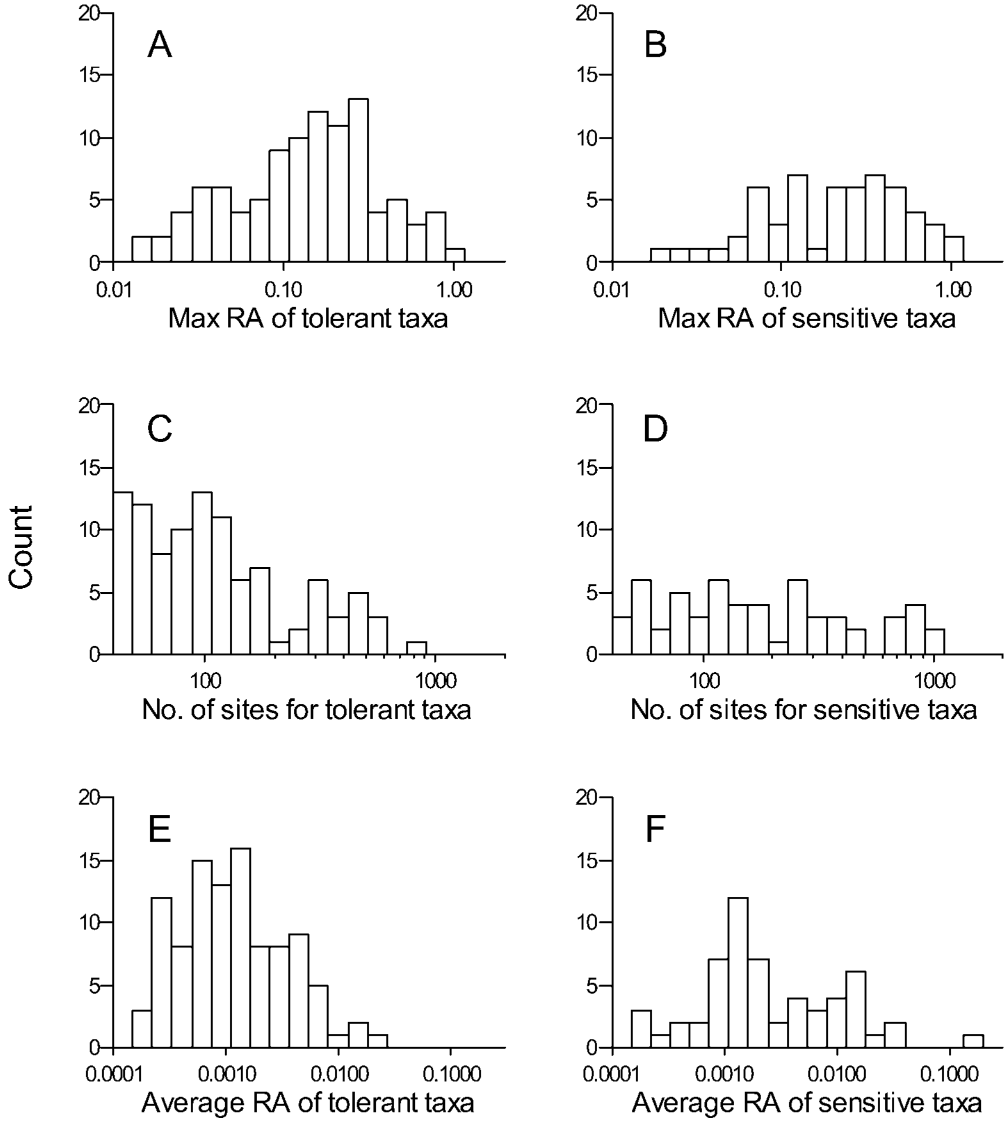

FIG. 5. Distributions of counts of tolerant and sensitive taxa as a function of their maximum (max) relative abundances (RA) at all sites in the western US (A, B, respectively), the number (No.) of sites at which they were observed (C, D), and their average RA at a site (E, F).

Diatom indicators based on the WD MVI will be the most valuable of the indicators developed for assessing biological condition of diatoms in western streams. Therefore, only WA optima and S/T traits for the WD MVI are listed in the Appendix. This disturbance gradient is characterized by a shift from streams with low conductivity and low nutrient concentrations to streams with high conductivity and high nutrient conditions. Percent fine sediments also was strongly correlated with WD MVI. The species, such as *A. minutissimum,* that are sensitive to this gradient probably are adapted to low conductivity and are capable of sequestering nutrients when concentrations are low (Manoylov and Stevenson 2006). In contrast, the tolerant taxa probably are adapted to high conductivity and require high nutrient concentrations. The WD MVI is highly correlated with % agricultural and urban land use and with many stressors in the West. Therefore, it measures conditions along a dominant environmental gradient that is common across western US streams. Thus, the defined species traits and WD MVI indicator are more comparable to the general pollution indicators developed by Descy (1979) and Lange-Bertalot (1979) than to stressor-specific indicators. Application of the indicator in assessment will require establishment of appropriate reference conditions (e.g., Cao et al. 2007, Kelly et al. 2008) and appropriate comparison with reference

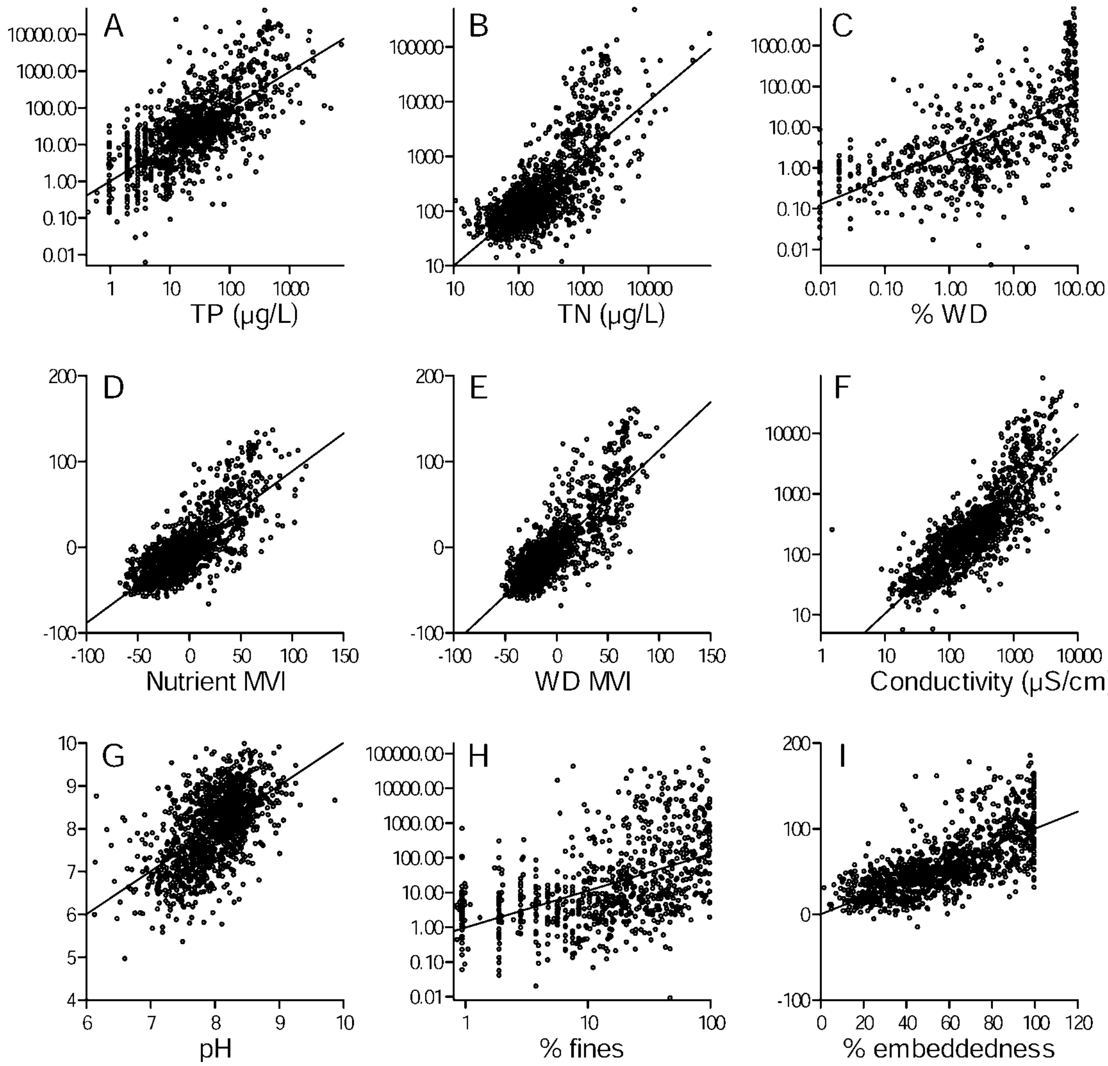

FIG. 6. Relationships between diatom-inferred condition for total P (TP) (A), total N (TN) (B), % watershed disturbance (% WD) (C), the nutrient multivariate index (MVI) (D), the watershed disturbance multivariate index (WD MVI) (E), conductivity (F), pH (G), % fines (H), and % embeddedness (I) and measured values of these conditions for streams throughout the western US. Lines show the 1:1 relationship between diatom-inferred and measured conditions.

conditions in climate regions with different extents of human activities (Davies and Jackson 2006, Stoddard et al. 2006).

Stressor-specific indicators developed from large data sets, such as EMAP-West data set, should be used with great caution. The EMAP-West TP indicator is strongly correlated with measured TP in Florida springs and in South Dakota streams, but so is the EMAP-West WD MVI indicator (Stevenson and Pinowska 2007, RJS, unpublished data). Large data sets offer much opportunity for harvesting information, but a new approach is needed for developing stressor-specific indicators from these data sets. Historically, correspondence analyses have been used to identify the water-chemistry variables most responsible for changes in diatom species composition and to limit development of indicators to only those variables that are most important (ter Braak 1995, Ponader et al. 2007). However, other approaches might enable development of indicators for subdominant factors. Multivariate maximum likelihood models might solve problems caused by covarying environmental factors (Yuan 2007). Our next steps will include stratifying the data set by stressors known to affect diatom species composition, randomly sampling streams from strata in which variation in nontarget stressors is controlled, characterizing taxon traits, and testing trait-based indicators in different settings.

The strong west-wide performance of diatom indicators should not be taken as an indication that

Table 4. Correlation coefficients (r^2) between diatom weighted average (WA) indicators for environmental conditions and measured environmental conditions when traits were determined for all sites and sites by climate region. Correlation coefficients were determined after indicators had been corrected by classical deshrinking. TP = total P, TN = total N, nutrient MVI = nutrient multivariate index, % WD = % watershed disturbed, WD MVI = watershed disturbance multivariate index.

WA indicator	All sites	Sites by climate region
TP	0.533	0.415
TN	0.548	0.314
% WD	0.587	0.425
Nutrient MVI	0.596	0.575
WD MVI	0.667	0.684
pH	0.323	0.289
Conductivity	0.687	0.678
% fines	0.314	0.040
% embeddedness	0.492	0.050

species traits do not vary among climate regions. That hypothesis was not tested directly. If species traits vary independently and without bias among stream types or biogeographically, then the mean indicator value across all species in a multispecies assemblage could remain the same when used in different regions. This central-limit-theorem property results from aggregating information from multiple sources (in this case, species). Thus, biological indicators using traits and abundance information for multiple species are "robust," "capable of performance under a wide range of conditions" (Merriam-Webster 2003). Robustness should be related to the number of species in the assemblages used in the multispecies indicator. Thus, the diatom indicators that commonly use information from ≥20 species in a sample tend to be correlated well with environmental conditions even when species traits are derived from other regions (e.g., Fore and Grafe 2002).

S/T indicators are useful in assessments because they characterize valued ecological attributes more accurately than do indicators predicting stressors (Stevenson and Smol 2002, Stevenson 2006) or indicators that use all species to assess biological condition. S/T indicators unambiguously quantify the changes in taxa that are or are not characteristic of reference conditions vs indicators based on all species. However, S/T indicators use many fewer species than do indicators that include all species, so statistical

Table 5. Correlations among diatom weighted average (WA) indicators of stressors in streams of the western US. TP = total P, TN = total N, nutrient MVI = nutrient multivariate index, % WD = % watershed disturbed, WD MVI = watershed disturbance multivariate index.

Indicator	TP	TN	Nutrient MVI	% WD	WD MVI	pH	Conductivity	% embeddedness
TN	0.891							
Nutrient MVI	0.970	0.972						
% WD	0.848	0.960	0.931					
WD MVI	0.941	0.984	0.992	0.970				
pH	0.524	0.473	0.513	0.461	0.503			
Conductivity	0.861	0.885	0.899	0.859	0.899	0.676		
% embeddedness	0.878	0.889	0.910	0.845	0.901	0.456	0.872	
% fines	0.867	0.872	0.895	0.819	0.882	0.361	0.830	0.931

Table 6. Correlations between diatom weighted average indicators (WAI) of environmental conditions and stressors. TP = total P, TN = total N, nutrient MVI = nutrient multivariate index, % WD = % watershed disturbed, WD MVI = watershed disturbance multivariate index.

	WAI								
Stressor	TP	TN	Nutrient MVI	% WD	WD MVI	pH	Conductivity	% embeddedness	% fines
TP	0.533	0.434	0.496	0.402	0.469	0.280	0.415	0.446	0.434
TN	0.465	0.548	0.521	0.513	0.527	0.268	0.466	0.493	0.465
Nutrient MVI	0.585	0.575	0.596	0.534	0.584	0.321	0.516	0.551	0.527
% WD	0.482	0.573	0.543	0.587	0.567	0.275	0.483	0.483	0.468
WD MVI	0.634	0.663	0.667	0.638	0.667	0.352	0.584	0.608	0.585
pH	0.123	0.110	0.120	0.102	0.115	0.323	0.177	0.104	0.088
Conductivity	0.533	0.555	0.560	0.518	0.554	0.507	0.687	0.566	0.543
% embeddedness	0.413	0.424	0.432	0.377	0.420	0.206	0.392	0.493	0.462
% fines	0.263	0.262	0.270	0.237	0.262	0.116	0.253	0.306	0.314

TABLE 7. Correlations between sensitive and tolerant (S/T) indicators and environmental factors. TP = total P, TN = total N, nutrient MVI = nutrient multivariate index, % WD = % watershed disturbed, WD MVI = watershed disturbance multivariate index.

S/T indicator	TP	TN	Nutrient MVI	% WD	WD MVI	pH	Conductivity	% fines	% embeddedness
Number of sensitive taxa	0.333	0.315	0.381	0.262	0.404	0.209	0.523	0.223	0.305
Number of tolerant taxa	0.399	0.425	0.469	0.539	0.558	0.222	0.490	0.306	0.425
% sensitive individuals	0.391	0.335	0.387	0.331	0.397	0.232	0.493	0.214	0.341
% tolerant individuals	0.436	0.498	0.520	0.569	0.604	0.250	0.608	0.259	0.416
% sensitive taxa	0.415	0.375	0.446	0.381	0.469	0.275	0.599	0.289	0.436
% tolerant taxa	0.452	0.529	0.554	0.601	0.638	0.288	0.671	0.335	0.437

precision and robustness could be sacrificed for more refined information. In the West, some S/T diatom indicators did almost as well as the indicators based on all species in assemblages. Indicators based on % S/T taxa were more precise than indicators based on % S/T individuals or number of S/T taxa. Relative abundances vs presence/absence of species is almost always used in diatom indicator development. Percent S/T taxa is a valuable indicator because it quantifies changes in biodiversity at the species level more directly than does % S/T individuals, but any inference about changes in number of S/T taxa is suspect because of the gross underestimation of the total number of species in assemblages when counts of only 600 valves are used (Patrick et al. 1954, Stevenson 2006). However, % S/T individuals or number of S/T taxa are precise indicators of environmental change.

We also observed that S/T indicators based on tolerant species were more precisely related to water chemistry and watershed disturbance than were S/T indicators based on sensitive species. Higher precision of indicators based on tolerant taxa than of indicators based on sensitive taxa also was observed with indicators based on invertebrates along nutrient gradients in the Mid-Atlantic Highlands (Yuan and Norton 2003). Stevenson et al. (2008) argue that, as a group, tolerant taxa should be more responsive than sensitive taxa along nutrient gradients because low nutrient availability should constrain species membership in assemblages more than should high nutrient availability. This relationship might explain the wider distribution of sensitive than of tolerant diatom taxa in western US streams.

In conclusion, land use and water chemistry varied greatly in streams in different climate regions in the West. A dominant stressor gradient in western streams was defined by increases in conductivity, nutrient concentrations, and % fine sediments as % WD. This dominant gradient enabled development of diatom indicators of this generalized stressor gradient (sensu Davies and Jackson 2006), but it complicated development of diatom indicators for specific stressor conditions. The problem of developing diatom indicators for specific stressors could be solved by using different analytical approaches to calculate species traits in large data sets. Future work to refine definitions of reference condition and stressor indicators will enable more accurate assessments of these microbial communities and diagnosis of the stressors that threaten or impair their biodiversity.

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