A COMPARISON OF APPROACHES FOR ESTABLISHING NUTRIENT CRITERIA BASED ON ALGAL ATTRIBUTES IN MID-ATLANTIC STREAMS

R. Jan Stevenson,[1] Brian . Hill,[2] and Alan T. Herlihy[3]

[1]Department of Zoology, Michigan State University, East Lansing, Michigan 48824 USA

[2]Department of fisheries and Wildlife, Oregon State University, c/o United States Environmental Protection Agency, Corvallis, OR 97333 USA

[3] United States Environmental Protection Agency, NHEEL, Mid-Continent Ecology Division, Duluth, MN 55804-2595

Abstract. We compared nutrient criteria delineated by frequency distributions and stressor-response relationships between nutrient concentrations and attributes of benthic algal assemblages. Our objectives were to evaluate the predictability of progressive degradation in streams impacted by nutrients and to integrate different approaches for developing criteria into a single framework. Benthic algal attributes, water chemistry, physical habitat, and riparian disturbance by human activities were determined in Mid-Atlantic streams from 1993-1997 by the Environmental Monitoring and Assessment Program (EMAP). Streams types were classified based on differences in diatom species composition among reference sites (those with minimal evidence of human activity) to increase precision in assessments. Factors related to buffering capacity explained most of the variability among diatom assemblages at reference sites, so well-buffered streams were selected from the complete dataset for detailed analysis. Changes in diatom species composition, numerous biotic indices, phosphatase activity, and biomass of benthic algae were related to TP and TN concentrations. Relationships between TP and TN concentration and diatom species composition and biotic indices had much higher r^2 than measures of phosphatase activity and algal biomass. Nutrient and biological criteria were based on quartiles of frequency distributions for reference sites defined two ways and for all sites. Criteria based on quartiles of frequency distributions (either 25th or 75th percentiles, depending upon positive or negative relation between ecosystem characteristics and human disturbance) were more conservative (e.g., lowest for attributes increasing with human disturbance) when based on frequency distributions from all sites versus from reference sites defined by Waite et al. (2000) standards or by 0 riparian disturbance. Restricting evaluation of stressor-response relationships to well-buffered streams increased precision of relationships. Candidate nutrient criteria were established three ways with stressor-response relationships. Thresholds along TP and TN gradients were sought to justify criteria by using regression tree and lowess regression. In addition, candidate TP and TN criteria were calculated by relating nutrients to biocriteria (defined with frequency distributions of algal attributes at reference sites) with linear regression models. Criteria based on response change points along nutrient gradients defined by lowess regression were generally the most conservative. Evidence was observed for predictable progressive degradation from nutrient-limited streams to greater signs of degradation associated with nuisance algal blooms or other stressors that often coincide with higher nutrient loading (e.g., sediments and low oxygen). Multiple lines of evidence indicated that nutrient concentrations below 10 μg TP/L and 300 μg TN/L would maintain moderate nutrient limitation in streams. Nutrient concentrations below 700 μg TN/L would protect streams from greater symptoms of degradation. Frequency distributions were valuable for establishing stream conditions with low levels of human impact that provide goals for protection and restoration. Stressor-response relationships defined loss of valued ecological attributes with specific increases in stressors. Both approaches provided valuable information for delineating two levels of protection for ecological integrity along gradients of nutrient enrichment in streams.

Key words: algae, biotic indices, criteria, diatoms, frequency distributions, Mid-Atlantic Region, nitrogen, nutrients, phosphorus, species composition, streams, stressor-response relationships.

INTRODUCTION

Determining the ecological conditions necessary to protect or restore valued ecological attributes is a key component of managing ecological systems. These specific ecological conditions, or criteria, are one part of regulatory standards that state agencies in the U.S. establish to protect and restore ecosystems. The designated use and an anti-degradation rule are two other official components of regulatory standards. Different criteria are established for the designated uses of ecosystems. U.S. Environmental Protection Agency (USEPA) recently initiated an effort to develop "tiered uses" for ecosystems, which would establish multiple criteria along a gradient of human disturbance for different designated uses for a specific type of ecosystem (e.g., streams, wetlands, or forests). Tiered uses could facilitate management of individual ecosystems for specific goals, help prevent degradation by having criteria closer to existing conditions, and help prioritize and achieve restoration goals.

Several approaches are commonly used to establish environmental criteria. Frequency distributions of ecological conditions at either reference sites or all sites characterize the central tendency and dispersion in ecological attributes (USEPA 2000a). A criterion delineated with a frequency distribution is either the maximum or minimum ecological condition for a specific percentage of sites. For example, biological criteria could be established at the 5th, 25th, or 33rd percentile of a frequency distribution of a biotic index at reference sites (assuming that the biotic index increases with ecological integrity). Alternatively, the criterion could be the 75th percentile of the frequency distribution based on all sites, representing the best 25% of conditions in the region. Another approach for establishing criteria is based on quantitatively understanding relationships

between human activities, stressors, and valued ecological attributes. Criteria are established with this "stressor-response" approach at levels of stressors and valued attributes that protect the desired level of the valued ecological attribute. Human activities are then managed to reduce stressors (abiotic or biotic factors having relatively direct effects on value attributes) to protect and restore valued ecological attributes. The stressor-response approach is the foundation of risk assessment (USEPA 1998a, Suter 1993) and has the potential for providing justifiable criteria that protect valued ecological attributes (Muradian 2001).

Efforts for developing a common approach for establishing environmental criteria could be thwarted by regulatory agencies having different methods for setting criteria. However, interest in sustainable management by all types of stakeholders is requiring agencies to develop the most scientifically defensible approach for managing natural resources. The frequency distribution and stressor-response approaches are complementary approaches, each with strengths that can complement the other. For example, the problem with setting criteria with frequency distributions is that the actual percentile used is difficult to justify. In addition, the level of protection achieved may vary among agencies depending on the quality of their reference sites used to set criteria. Linear stressor-response relationships can identify contaminating levels of stressors that cause undesirable conditions and levels of human activities that generate those levels of stressors. Non-linear relationships, commonly predicted for ecological interactions (Odum et al. 1979), provide change points along continuous gradients in conditions that can be particularly valuable for justifying establishment of criteria at specific levels (Muradian 2001).

Nutrient enrichment by humans is recognized as a leading cause of degradation of the ecological integrity of streams in the U.S. (Vitousek et al. 1997, Carpenter et al. 1998, Smith et al. 1999, USEPA 2000b). It has become the focus of a national effort to establish nutrient criteria for all water body types in all regions of the U.S. (USEPA 1998b). Nutrient enrichment should cause a predictable, subsidy-stress (Odum et al. 1979) progression in degradation of ecosystems from natural condition and the ecological integrity protected in the Clean Water Act (section 101(a)). Productivity is nutrient limited in the natural condition of many streams. Although ecosystem services such as fishery production and support of high species richness may be enhanced by the subsidy of low levels of nutrient enrichment, the natural condition of streams has been altered by release of the ecosystem from nutrient limitation. At higher levels of nutrient enrichment, nuisance algal blooms can alter habitat and affect invertebrate assemblages (Dudley et al. 1986), may contribute to de-oxygenation, and can produce foul taste, odor, and toxicity (Palmer 1969). Recommendations for characterizing the trophic status of streams with nutrient concentrations and algal biomass have been proposed (Dodds et al. 1998), but they are based on relationships with high variability. Identifying the specific nutrient concentrations causing the problems has been a challenge because of the high spatial and temporal variability in algal biomass and nutrient concentrations in streams (e.g., Biggs 2001). Complementary use of biological indices based on algal species composition and species environmental tolerances shows great promise for sensitively monitoring and assessing biological response to environmental change and particularly, respond to nutrient enrichment (Kelly and Whitton 1995, Pan et al. 1996, McCormick and Stevenson 1998).

The objective of our research was to evaluate linkages among the frequency distribution and stressor-response approaches for establishing criteria and demonstrate a common framework for developing environmental criteria. We also wanted to develop recommendations for nutrient criteria for streams in the Mid-Atlantic region of the US. In this project, we analyze data from the Environmental Monitoring and Assessment Program (EMAP) of the USEPA and test hypotheses to address a series of questions. What changes in algal assemblages are related to nutrient concentrations? How do criteria nutrient and biocriteria established by different approaches compare? Do multiple lines of evidence converge and identify one or more specific levels of nutrient concentrations that have distinctive effects on algal assemblages? Do these levels represent predictable stages in progressive ecosystem degradation along nutrient gradients?

METHODS

Sample Collection and Analysis

From 1993-1998, 1185 periphyton samples were collected in 607 streams throughout the Mid-Atlantic region as part of the EMAP program of the USEPA. This region covers Maryland, Pennsylvania, West Virginia, Virginia, and Delaware and includes seven ecoregions (Omerick 1987, Pan et al. 1996) and five major watersheds (Alleghany, Kanawha, Monongehela, Potomac, and Susquehanna). Periphyton was collected by scraping and brushing organic material from a known area of natural substrate at a random location on each of nine transects of a reach (Pan et al. 1996, see Lazorchak and Klemm 1993 for details of all methods). Samples from riffles and pools were separated into two composite samples from a stream, if both habitats existed in the reach. Volume of each sample was measured in the field and then split into four subsamples for assays of chlorophyll a (chl a), ash free dry mass (AFDM), algal cell numbers and species composition, and acid and alkaline phosphatase activity. Chlorophyll a was measured with a spectrophotometer using standard methods (American Public Health Administration 1992) with acidification to determine the phaeophytin degradation portion of uncorrected chlorophyll a estimates. AFDM was assayed by drying and ashing samples according to standard methods (American Public Health Adminstration 1992). Algal cell numbers and species composition of non-diatom algae were determined in two steps: 1) by identification and counting 300 cells at 400X in a Palmer counting chamber and 2) by identification and counting at least 500

diatom valves at 1000X after cleaning and mounting diatoms on slides in a high refractive index mounting medium (HYRAX® or NAPHRAX®). Acid and alkaline phosphatase were assayed with standard methods.

Water chemistry, physical habitat, and riparian characteristics in watersheds were also assessed at most sites. A 4-L cubitainer and 4 60-mL syringes of stream water (sealed with Luer-lock valves for transport) were collected from each stream, stored on ice, and sent by overnight courier to the analytical laboratory. A list of sample preparation and preservation protocols for water chemistry can be found in Pan et al. (1996). TP and TN were analyzed spectrophotometrically after persulfate digestion. Soluble reactive phosphorus (SRP) was not measured but NO_3 and NH_4 were. Water chemistry characteristics, such as nutrients, Cl, pH, sulfate, and measures in Table 1, were analyzed using procedures outlined in USEPA (1987). Physical habitat characteristics measured in streams included thalweg depth, mean wetted width, mean reach cross section, slope, residual pool area, substrate size, embeddedness and other measurements listed in Table 1. Riparian evidence of human disturbance was assessed in the field at the ends of 11 transects throughout the reach with the presence and proximity of row crops, pasture, dams, buildings, pavement, roadways, pipes, landfill or trash, parks or lawns, logging, or mining operations. Summaries and details of these methods can be found in Pan et al. (1996) and Kaufmann and Robison (1994).

Data Analysis

Data were gathered from the EMAP web site (www.epa.gov/emap), Brian Hill (U.S. Environmental Protection Agency, Duluth, Minnesota), and Alan Herlihy (U.S. Environmental Protection Agency, Corvallis, Oregon). Data were transformed from comma-delimited text files to Excel® files and then imported into an Access® database. Analysis of species composition of algal assemblages was restricted to diatoms because we wanted to compare results of indicators based on European diatom information. We also restricted analysis to diatoms in riffle samples to minimize effects of natural variation in assessment of algal attributes. Pan et al. (1996) showed that diatoms in riffles and pools during 1993-1994 responded similarity to water chemistry in Mid-Atlantic streams. Diatom relative abundances (proportions of diatoms counted) in samples from Mid-Atlantic streams were calculated by dividing cell densities of each species by sums of cell densities of all diatoms for respective samples.

The first step in data analysis was to classify streams based on similarity in diatom species composition among reference sites. Reference sites (referred to as EMAP reference sites) were initially chosen for consistency with other EMAP data analyses of Mid-Atlantic streams by using selection criteria described in Waite et al. (2000). These criteria were > 50 eq/L acid neutralizing capacity (ANC), < 100 mg Cl/L, < 20 µg TP/L, < 750 µg TN/L, and < 400 eq SO_4/L. These criteria eliminated sites with evidence of stressors from mining, agriculture, and urban development. To evaluate effects of using nutrient criteria in a definition of reference that then was used to determine effects of nutrients, the variability in nutrients at sites with 0 (zero) riparian disturbance was compared to the EMAP reference condition. Only one sample from a site was used in an analysis (since some sites were sampled more than once during the year). Two-sample indicator species analysis (TWINSPAN, Hill et al. 1975) was used to group sites with similar diatom assemblages. Discriminant analyses and descriptive statistics (SYSTAT 10.0®) were used to determine the environmental variables that could best predict membership of sites in different TWINSPAN groups. Initial analyses indicated that the person counting samples and year of sampling had a significant effect on membership of sites in TWINSPAN groups. The list of diatom taxa was reviewed to determine the likely differences in how analysts identified taxa differently. Taxa evident in large numbers of samples evaluated by one analyst but not another were selected to determine if they could be grouped with a morphologically similar taxon identified by other analysts. Groups of taxa with similar morphology, like varieties within the same species and groups of small taxa with very similar morphology, were also considered for combination. After "lumping" taxa (grouping according to the preceding criteria), 1997 and 1998 samples from reference sites were still distinguishable from sites sampled from 1993-1996, but other environmental factors that strongly affect diatoms species composition became important for distinguishing groups of reference sites. Discriminant function analyses with forward and backward selection were used to identify environmental variables that best distinguished membership of sites in TWINSPAN groups. Most analyses of nutrient and algal assemblage attributes were based on one TWINSPAN-defined class of streams. The class chosen, well-buffered streams, had the most sites sampled during 1993-1996 in EMAP.

Similarity of species composition at test sites and reference sites was determined after calculating average species composition of diatom assemblages at reference sites in well-buffered streams. Proportional similarity (PS, similarity) in relative abundances between two samples was calculated with the following equation, which was modified from the description of percent similarity in Pielou (1984): $PS = \Sigma (\min(p_{i1}, p_{i2}))$. p_{i1} and p_{i2} are the proportional relative abundances (range 0-1) of the ith taxon in average reference assemblages and in test sites, respectively. PS varies from 0 when no individuals of the same species are found in the compared assemblages to 1, when all taxa are the same.

Percent "native" taxa can be indicated by the ratio of the observed number of native taxa at test sites versus the number expected to occur at a reference site (e.g., the O/E ratio, Wright et al. 1993, Hawkins et al. 2000). "Native" taxa were defined with a list of taxa known to occur at reference sites in well-buffered streams. The probability of occurrence of each taxon at reference sites in well-buffered streams was determined by dividing the number of reference sites in which it was observed by the total number of reference sites. The sum of these probabilities provided the expected number of taxa to occur at test sites. The observed number of taxa was simply the number of taxa at a test site that had been observed at reference sites.

The O/E ratio varies from 0 when no taxa from reference sites were observed at test sites to greater when 1.0 when more reference taxa are observed at test sites than at reference sites. O/E ratios should be close to 1.0 for high quality sites and less than 1.0 when native taxa are lost. Values greater than 1.0 are commonly associated with nutrient enrichment.

Autecological indices for assemblages were calculated based on their species composition and the environmental preferences, sensitivities, and tolerances of taxa (i.e., the autecological characteristics or autecologies of taxa). An autecological database for taxa was developed by augmenting the data assembled in electronic form by van Dam et al. (1994). These data were based on occurrences of taxa in different environments as reported in the literature. Almost all of these reports are from Europe, but habitats world-wide are included. We augmented the data by adding species nutrient preferences from a databases of algal species composition and nutrient concentrations in Florida streams (from a previous, unpublished study) and from Mid-Atlantic streams sampled by EMAP during 1997 and 1998. Species TP and TN optima (the nutrient concentration in which they had the highest relative abundances) were determined by weighted average regression using CALIBRATE v 0.6® (Juggins and ter Braak 1992), if inference models explained significant amounts of variation in TP or TN concentrations among habitats. Species nutrient optima were then transformed into 8 categories so that they could be combined with the van Dam's categorical system. These 8 categories grouped taxa with similar TP optima and ranged from low to high nutrient concentration (1-8). The categorical autecologies from Florida and Mid-Atlantic streams were then averaged with trophic status categories in the van Dam database.

Autecological indices were calculated for the proportion of sensitive individuals, proportion of tolerant individuals, MAIA trophic status index (TSI), van Dam's TSI, a weighted average inference model for nutrient concentrations (TP or TN) in streams, the van Dam saprobian index (organic pollution), and the van Dam oxygen tolerance index. All these indices were calculated as: $\Sigma\ p'_i\Theta_i/\Sigma\ p'_i$, where p'_i is the proportion of the ith species in the sample for species with autecological information and Θ_i is the autecological category or environmental optimum of the ith taxon (Zelinka and Marvin 1961). Dividing by $\Sigma\ p'_i$ redefines the assemblage to those organisms for which autecological information is known and scales all indices to the full range of autecological values. Sensitive and tolerant taxa were defined as those taxa with autecologies in the lowest or highest two trophic ranks, respectively, and with little variability between classifications from different sources of data. Θ_i took values of either 0 or 1 for the sensitive and tolerant categorizations. The TSI categories ranged from 1-8, van Dam's TSI categories ranged from 1-6, and the saprobic and oxygen tolerance categories both ranged from 1-5. High values indicated the most polluted sites.

Relationships between algal attributes and TP and TN concentrations were evaluated with linear regression to determine which algal attributes changed with nutrient concentrations. Algal attributes evaluated were similarity to reference condition, O/E ratio, the autecological indices in the previous paragraph, AFDM, chl a, phaeophytin, the ratio of chl a/AFDM (the autotrophic index, Stevenson and Lowe 1986), total uncorrected chlorophyll (chl a_{UC} = chl a + phaeophytin), chl a/total chlorophyll ratio (a periphyton health index), filamentous algal cover in habitats, and acid and alkaline phosphatase activity. Many variables, including TP and TN concentrations, were log-transformed to reduce the skewed distribution of values. Transformations of algal attributes are clearly reported in tables of results. NO_3 and NH_4 were not included in analyses because high N:P ratios in streams indicated algae were P-limited. SRP was not measured. The effects of stream classification on precision of relations between algal attributes and TP were evaluated by comparing the correlation coefficients when data from all streams were included in the analysis and data from well-buffered streams only (R_{wb}-R_{all}). A positive classification effect indicated that classification increase the correlation.

Candidate values of TN and TP for nutrient criteria were determined with frequency distribution and stressor-response approaches. Three distinct frequency distribution approaches were used. Candidate values of nutrient criteria were defined at the 25th percentile of frequency distributions of nutrients at all sites and at the 75th percentile for reference sites as defined by EMAP (Waite et al. 2000) and by 0 riparian disturbance. Biocriteria with algal attributes were also developed using these frequency distribution methods. Since expected reference conditions were high values of some algal attributes and low values of others, the relative expected condition (REC) was defined as high or low for each algal attribute. Selecting use of the 25th or 75th percentiles of frequency distributions for all sites and reference sites were based on relative expected condition.

Candidate nutrient criteria were also determined with stressor-response relations with lowess regression, regression tree analysis, and with the predicted values of TP and TN that would produce each biocriterion. Lowess regression and regression tree analysis identify change points in patterns along environmental gradients and were performed with SYSTAT 10.0®. Both techniques order paired stressor-response observations (i.e., independent and dependent variables) from a site by stressor values. Lowess regression involves development of a locally weighted regression based on a set of similar stressor values and provides a functional relationship without assuming shape of stressor-response patterns before analysis. Regression tree analysis identifies change points along the stressor gradient where the mean values of responses differ along a local stressor gradient (Fig. 1B). A regression tree is produced in which clusters of observations along the stressor gradient have different mean values. It also provides an analysis of the stress-response pattern without the a priori assumption of a specific non-linear pattern, but it is constrained to step-like functions in which predicted responses do not change between change points along the stressor-axis. Predicted values of TP and TN for biocriteria defined by frequency distributions (FD) of algal attributes at EMAP-defined reference sites (P* and N*, respectively) were determined with linear regression models of relations between each nutrient and individual algal attributes using data from all sites. These models were solved for the TP (P*) and TN (N*) values that produced each FD-defined biocriterion (Fig. 1A).

Differences in diatom species composition in reference streams indicated that factors associated with conductivity and alkalinity had strong effects on expected species composition of reference streams. 109 taxa from 120 reference sites were included in this analysis because they had an average relative abundance greater than 1.0 % of samples in which they were observed. Two groups of sites were identified by TWINSPAN. One group was characterized by common occurrence of diatoms in the genus Eunotia, which are commonly found in low alkalinity waters. Discriminant functions, based on both forward and backward selection, identified DOC, conductivity, alkalinity, silica and sulfate as important factors affecting species composition in reference streams. More streams in the dataset were classified as "well-buffered" than "poorly-buffered," so the former was chosen for further analyses. "Well-buffered" streams were defined by meeting criteria for 2 discriminant functions (based on forward and backward selection) and constrained by having little evidence of mining impacts (ANC>12 and SO_4<2000 μg/L).

Weighted average regression models for TP were statistically significant for well-buffered streams sampled in 1997 and 1998. TP optima for the 100 diatom taxa included in the analysis ranged from 1.3 to 109.7 μg P/L. Inferred TP based on diatom TP optima explained 26.9 and 8.8% of the variation in measured TP concentrations before and after cross-validation. Weighted average regression models for log(TP) explained 45.5 and 34.5% of the variation in measured log(TP) concentrations before and after cross-validation. Species TP optima were grouped into eight categories based on the following benchmarks, 5, 10, 15, 20, 25, 30, and 35 μg TP/L.

Phosphorus and nitrogen concentrations in streams were related poorly to the riparian disturbance metric. TP concentrations in streams with 0 riparian disturbance (N=22) ranged from 2 to 80 μg/L, with a mean of 17.7 (Fig. 2); in streams with riparian disturbance (N=157) TP ranged from non-detectable concentrations to 145 μg/L, with a mean of 28. TN concentrations in streams with 0 riparian disturbance ranged from 63 to 1319 μg/L, with a mean of 504.6; in streams with riparian disturbance TN ranged from 69 to 16260 μg/L, with a mean of 1356. Linear regression indicated riparian disturbance did not affect TP ($P = 0.079$, $r^2 = 0.017$, N = 178) and explained little variation in TN ($P = 0.002$, $r^2 = 0.059$, N = 178).

Most species-level attributes of diatom assemblages were significantly related to both TP and TN concentrations ($P <$ 0.05, Table 2). The autecological indices (percent sensitive and tolerant individuals, the TSI, van Dam's TSI, the WA TP inference model, and saprobic and oxygen tolerance indices) were the most precisely correlated to TP and TN concentrations with 10-25% of variation in the indices explained either by TP or TN concentration (r^2). Similarity of assemblages to reference assemblages was correlated well to both TP and TN ($r^2 > 0.10$), decreasing from about 0.5 to 0.25 with increasing TP and TN. The O/E taxa ratio had the lowest correlation with nutrient concentration of species-level attributes, and it was positively related to nutrient concentrations. All other indices were related to nutrient concentrations as predicted (all positive, except similarity to reference assemblage and percent sensitive species).

The MAIA TSI was better correlated to TP concentrations than the van Dam TSI ($r = 0.531$ and 0.417, respectively), but both indices were equally well correlated to TN (Table 2). Inferred log(TP) concentrations in streams during 1993-96 were well predicted by the weighted average model for log(TP) concentration developed with data from Mid-Atlantic streams during 1997-98 ($r = 0.528$, Table 1). Bias was evident in the latter model with a=0.792 and coefficient=0.140 indicating inferred log(TP) concentration was overestimated in low TP streams and underestimated in high TP streams.

Biomass of periphyton was poorly related to nutrient concentrations (Table 2). Although AFDM, phaeophytin, and uncorrected chlorophyll were significantly related to TP and TN, r^2 for these relations was usually less that 0.05. Chlorophyll a concentrations were significantly related to TN concentration, but not TP concentration. AFDM increased from less than 10 to as high as 100 g/m^2 with increasing nutrients. Filamentous algal cover and ratios of chl a/AFDM and chl a/uncorrected chlorophyll were not related to TP and TN concentration. However, it should be noted that no filamentous algal cover was recorded in reference conditions.

Phosphatase activity, however, was related to significantly to nutrient concentrations (Table 2). Acid-phosphatase activity decreased from about 10000 to 2000 nmoles activity g AFDM^{-1} h^{-1} from low TP to 30 μg/L. Alkaline phosphatase activity decreased from about 3000 to about 500 nmoles activity g AFDM^{-1} h^{-1} with increasing TP. Correlations were higher between phosphatase activity and TP than with TN. Periphyton biomass (AFDM) was negatively related to phosphatase activity in linear regression models predicting phosphatase activity as a function of both TP and AFDM ($r^2 = 0.436$ and −0.392 for acid and alkaline phosphatase activity, respectively).

Classification generally increased the proportion of variation in algal attributes explained by TP concentration (Table 3). On average the classification effect (difference in r^2) was 0.03 (r is shown in Table 3). Classification had the greatest positive effect on precision in relations between species composition similarity to reference assemblages and van Dam's TSI, with increases in r due to classification of 0.289 and 0.233, respectively. r for O/E ratios increased due to more low ratios in reference assemblages in streams that had low ANC. Many attribute relations with TP were only slightly affected by classification. Except for van Dam's TSI, most relations between autecological indices and TP were not improved greatly by stream classification. The WA TP inference model was notably unaffected by effects of classification. TP relations with

biomass attributes were not improved much by classification. Relations between TP and alkaline phosphatase were improved more by stream classification than TP relations with acid-phosphatase.

Nutrient criteria derived from frequency distributions differed depending upon the whether reference or all sites were used and with different definitions of reference conditions (Figs. 2-3, Table 4). Criteria ranged from 10-22 µg TP/L and from 350-684 µg TN/L. Nutrient concentrations at the 25th percentile of concentrations at all sites (10 µg TP/L and 350 µg TN/L) were lower than the 75th percentile concentrations for reference sites defined by both EMAP and 0 riparian disturbance. Nutrient criteria defined by the 75th percentile of reference sites with 0 riparian disturbance were twice as high as those defined the 25th percentile of all sites.

Biocriteria derived from frequency distributions were most conservative when based on the 25th percentile of frequency distributions of attributes at all sites (Table 4). Based on species-level biocriteria derived from the 25th percentile for all sites: at least 52% similarity in species composition between test and reference assemblages and 34% of individuals should be sensitive taxa; no more than 0.5% of individuals should be tolerant taxa; and the TSI, saprobic index, and oxygen tolerance index should be less than 3.5, 1.6, and 1.4, respectively. Biocriteria derived from all sites indicate algal biomass should be less than 4.5 mg AFDM/cm^2 and 12.2 µg chl a/cm^2 (log(4.5) = 1.5, log(12.2) = 2.5, Table 4) and no filamentous algal cover should be present. Biocriteria for acid and alkaline phosphatase activity (log-transformed) were highest when based on all sites, 8.6 and 7.5 nmole·L^{-1}·h^{-1}.

Biocriteria based on attributes from sites with 0 riparian disturbance were the least conservative (Table 4) of the three approaches using frequency distributions. For example, 35% similarity in species composition between test and reference assemblages, 12% individuals from sensitive taxa, and 6.1% individuals in tolerant taxa were biocriteria based on 0 riparian disturbance. Periphyton biomass could be as high as 9.2 mg AFDM/cm^2 and 47.8 µg chl a/cm^2 and phosphatase activity could be as low as 7.6 and 5.7 nmole·L^{-1}·h^{-1} (acid and alkaline, respectively; log-transformed) when defining biocriteria with reference sites having 0 riparian disturbance.

Candidate criteria based on biological responses to nutrients were generally lowest when defined by lowess regression, but a consistent pattern was not as clearly evident as in comparisons of criteria based on different frequency distribution methods. Several rationale were used to set nutrient criteria with lowess regression and each was related to different responses of algae to nutrients. No detectable lowess change points were observed for some attributes with linear responses to nutrients. % sensitive individuals and the TSI were linearly related to TP and TN concentration. Chl a$_{UC}$ was poorly related to TN. Similarity of assemblages between test and reference sites had a threshold response near 10 µg TP/L and 400-500 µg TN/L (Figs. 3-4); thus candidate criteria were below the thresholds at 8 and 350 µg/L TP and TN, respectively. Threshold responses in saprobic and oxygen tolerance indices were weak, but interesting. Both indices gradually increased until one threshold in nutrients (TP & TN) occurred, then these indices increased faster (an acceleration) with increasing nutrient concentrations until reaching a saturation TN concentration. Above that concentration indices did not change with TN. Saturation was not an evident response for saprobic and low oxygen indices along the TP gradient. Candidate TP and TN criteria were proposed below the lowest nutrient threshold to protect the system from accelerating degradation. TN criteria were also proposed below the upper threshold to protect the system from saturation with organic enrichment and reaching highest levels of oxygen stress (Fig. 4). Candidate criteria were established before change points and degradation for attributes that had subsidy-stress relations with nutrient concentrations, such as AFDM and chl a with TP (Fig. 3) and O/E, AFDM, and phaeophytin with TN (Fig. 4). Phosphatase activity decreased with TP concentration and showed signs of reaching a stable minimum activity at high nutrient concentrations (Fig. 3). Criteria were set at TP concentrations below the change point to maintain nutrient limitation in ecosystems. A second criterion was established for TP and phosphatase response at the change point in response to protect algal assemblages from over saturation with nutrients. No criteria were established based on the increase in O/E ratio with phosphorus, even though it had a similar saturation point because an increase in diversity was not considered a problem.

Little relation was observed in specific nutrient criteria among methods. This was observed at the fine scale in the lack of correlation between nutrient criteria for the same attribute between methods (Table 7). P* and N* were highly positively correlated, but P* was not well correlated with P criteria defined by regression tree analysis; N* was not well correlated with N criteria by regression tree analysis. Although P* and N* were generally higher for biomass than for phosphatase activity and species composition indices, the opposite was somewhat true for criteria based on change point analysis. Concentrations between 8.03 and 18.27 µg TP/L were predicted to cause species-level biological responses corresponding to the 25th percentile of the EMAP reference sites (P*), except for the P* of 53 µg TP/L for % sensitive individuals. Chl a$_{UC}$ was the only biomass parameter correlated well to TP, and its P* was 646 µg/L. Change points indicated by regression tree analysis were usually higher for species-level responses than biomass. TP criteria for species-level indices were between 12 and 27 µg/L and the criterion was 8 µg/L for AFDM. They were 8 and 17 µg TP/L for acid and alkaline phosphatase activity. TN criteria for species-level indices ranged from 498 to 1125 µg/L and from 217 to 1170 for biomass indicators (AFDM, chl a, chl a$_{UC}$).

DISCUSSION

Human activities are well-known causes of nutrient enrichment of surface waters (Vitousek et al. 1997, Carpenter et al. 1998). Land cover/use indicators of human activities and related water quality models are commonly correlated with nutrient

concentrations in surface waters. Herlihy et al. (1998) found TP, TN, and NO_3 concentrations to be related to % forest in the watersheds of Mid-Atlantic streams. However, we did not find TP or TN to be strongly related to riparian disturbance in watersheds. The lack of highly significant relations between riparian disturbance indicators of human activities and nutrient concentrations in Mid-Atlantic streams may be due to nutrient sources in other regions of watersheds. It may also be due to the complex relations between nutrient loading, algal and bacterial uptake of nutrients, and nutrient concentrations in streams. Relations between nutrient loading and nutrient concentrations could be masked by microbial uptake of nutrients, which is known to be an important sink of dissolved nutrients in streams (Grimm et al. 1981, Triska et al. 1989, Mulholland 1996).

Changes in species composition, biotic indices, enzyme activity, and biomass of algae were related to nutrient concentrations in streams of the Mid-Atlantic region. Although some of these observations are common in studies of algal assemblages, seldom are all attributes measured in the same study. These results represent the largest assessment of relations between environmental conditions and multiple periphyton responses that has been published to date. Biotic indices based on species composition and species autecologies were much more precisely related to nutrient concentrations ($r^2 \approx 0.10\text{-}0.30$) than algal biomass and enzyme activities in Mid-Atlantic streams ($r^2 \approx 0.02\text{-}0.10$).

Multimetric indices of biotic integrity are commonly used to summarize effects of human activities on species composition of fish and invertebrates in streams (Karr 1981, Kerans and Karr 1994, Miltner and Rankin 1998, Barbour et al. 1999). Deviation in species composition from reference assemblages as assessed in this study is not commonly used as a variable. It represents a sensitive, individual-based characterization of changes in species composition of assemblages as compared to multimetric indices, which commonly are based on functional groups and higher-level taxa (families and orders). Conceptually, comparison of similarity in individuals by species should be more sensitive than loss of native taxa (presence/absence of species) from a region because relative abundance of taxa should change before they are lost from a habitat. However, variability in assessments could mask those differences. In this study, deviations in diatom species composition from reference assemblages closely corresponded to decreases in nutrient limitation as indicated by phosphatase activity.

Our attempt to quantify loss of native taxa (O/E ratios) with increasing nutrients was not successful. We observed a slight increase in diatom O/E ratios with increasing nutrients, which has also been observed with invertebrate O/E ratios. We expected a decrease in native taxa with increases in nutrient enrichment due to competitive exclusion for other limiting resources, increased predation, or alteration of other abiotic factors through indirect effects of nutrients (e.g., dissolved oxygen concentration, microbial interactions, or habitat structure). Increases in native taxa with enrichment may be due to increased species packing as resource availability increases. Increasing O/E may be related to poor estimates of species membership in assemblages when billions of organisms are present but only 500 valves (halves of organisms) are counted. In addition, our analysis did not thoroughly examine the potential loss of rare sensitive taxa, which may be the must vulnerable group and would not have been evaluated well with 600 valve counts.

Autecological indices are commonly used in assessments of ecological conditions in streams, lakes, and wetlands (Kolkwitz and Marsson 1908, Kelly et al. 1995, McCormick and Stevenson 1998, Dixit et al. 1999, Stevenson and Smol in press). Generating and gathering autecological information is a challenge. In this study, characterizations of the TP "optima" for only 100 taxa were possible and they were integrated into a global database of autecological characterizations of diatoms. Abundances of other taxa were too low or detected in too few samples to characterize autecologies. Several sources of compiled autecological information exist (e.g., Lowe 1974, Beaver 1981, van Dam et al. 1994). Many other region-specific sources are scattered throughout the literature (e.g., Hustedt 1939, Pan et al. 1996). Electronic databases with species autecological characteristics were developed primarily with ecological distributions of taxa in Europe (van Dam et al. 1994).

Species autecologies could differ greatly between the US and Europe. Recent observations about endemic diatom taxa have raised concerns about the transferability of autecological information for taxa among regions and the likelihood that morphologically similar diatoms in Europe and North America may be very different phenotypes (Kociolek and Stoermer 2001). However, van Dam's TSI, using taxon-specific autecological information based on observations in Europe, was correlated well to TP and TN concentrations in Mid-Atlantic streams. The WA inference model for TP and the TSI with autecological information from Europe revised with data for diatoms in Florida and the Mid-Atlantic streams were better correlated to TP than van Dam's TSI, but both were similarly correlated to TN. The ability to use autecologies of taxa from Europe in ecological assessments of North American streams indicates that variability in species autecologies between regions was not enough to counteract the effect of having many species in a single assessment and the random variation in changes in autecologies, if they occurred. A rigorous comparison of regional variability in species environmental preferences was beyond the scope of the research presented here, but it is important to address in future projects.

Algal biomass is complexly related to environmental conditions in streams and is notoriously challenging to relate to nutrient concentrations in the water column (Stevenson 1997, Dodds and Welch 2000). Time since recent scouring by storms, shading, grazers, patchiness due to macroalgal colonization, current velocity, and many other factors can also affect algal biomass and its estimation in streams (Stevenson et al. 1996, Kohler and Wiley 1997, Biggs 2000). Algae remove nutrients from the water column and could be expected in the most extreme case to cause a negative relation between algal biomass on substrata and nutrients in the water column. AFDM and uncorrected chl a increased with nutrients, slightly, but seldom reached nuisance levels (> 100-200 mg chl a/m², Welch et al. 1988, 1989) in these streams during the summer, low flow periods. Nuisance blooms of <u>Cladophora</u>, a common filamentous green macroscopic alga, usually occur during spring in this climate and

streams were sampled during late spring and summer. Therefore, the poor correlation between nutrients and algal biomass certainly does not indicate that nutrients have no effect on algal biomass.

Phosphatase activity decreased with nutrients and decreased relatively rapidly at low nutrient concentrations in streams. Phosphatase activity is seldom measured in field ecological assessments. In the Everglades, a large freshwater marsh, phosphatase activity decreased with phosphorus enrichment to background activities levels at TP concentrations greater than 10-15 µg/L (Richardson and Qian 1999). The high variability in relations between phosphatase activity and TP in streams is surely due to many factors. Periphyton biomass could be a confounding factor because higher biomasses could generate nutrient limitation within periphyton mats, due to higher nutrient uptake rates within mats than transport rates into the mat (Stevenson and Glover 1993). However, a review of our data shows phosphatase activity was negatively related to AFDM, even when taking into account the negative effect of TP. The role of AFDM as a complementary measure of nutrient enrichment must have out-weighted the more small-scale effects localized, within mat nutrient limitation.

Classification of streams was important for increasing precision of stressor-response relationships for species similarity to reference conditions, but not all other attributes. Classification of habitats types should increase the precision of ecological assessments because the expected condition and deviation from expected condition should be more narrowly defined (Hawkins et al. 2000). Species composition of diatom assemblages in all freshwater systems is greatly affected by ANC-related factors (pH, conductivity, etc., Hustedt 1939, Dixit et al. 1993, Pan et al. 1999, Stevenson et al. 1999). Thus, metrics particularly sensitive to changes in species composition alone, without weighting effects by autecologies, should be most improved by classification. The lack of classification effects on TP relations with biomass attributes may be due to functional redundancy in species. The difference in effects of stream classification on TP relations with acid and alkaline phosphatase activity may be due to the latter being much less effective than acid phosphatase in low pH, low ANC habitats.

Many algal attributes changed linearly with increases in nutrient concentration, but some had non-linear responses. Linear responses were indicated by patterns in lowess regression for % sensitive species and the TSI. The Van Dam indices for saprobic and low oxygen conditions were relatively linear, but some evidence for non-linear response was evident. More evident non-linear responses were observed with similarity of species composition to reference assemblages and AFDM along P and N gradients and in O/E ratios along N gradients. Whereas similarity in species composition showed a sigmoid threshold response, AFDM and O/E ratios had hyperbolic, subsidy-stress responses. Non-linear stressor-response relations have great potential for justifying criteria and setting management goals (Fig. 6, Muradian 2001), however they may not be the best indicators of environmental change. Ecological attributes that respond linearly to stressors and human disturbance can provide a valuable early warning for degradation or indications of restoration.

Predictable, progressive degradation of periphyton assemblages was not evident in relations between change points (criteria) defined by regression tree analysis or by lowess regression. However, a coarser-scale prediction of degradation was evident in the general pattern of results and was probably related to two or more processes causing degradation. At relatively low nutrient concentrations, greater than 10 µg TP/L and 300 µg TN/L, algal assemblages seem to be released from constraints of nutrient limitation. In ecosystems where human disturbance has generated high nutrient concentrations (e.g., >700 µg TN/L), algal assemblages are probably affected by multiple stressors, such as sediment loading, pesticides, and low oxygen concentrations.

Experimental research indicates low P concentrations, as reflected by 10 µg TP/L, are sufficient to relieve periphyton from severe nutrient constraints. 1.0 µg PO_4-P/L relieves periphytic diatoms of nutrient limitation (Bothwell 1989). Although SRP/TP ratios vary greatly in streams and depend on a variety of factors, TP concentrations 10 times as high as limiting SRP ratios are very reasonable indicators that bioavailable P is sufficient to relieve periphyton of severe nutrient constraint. 10 µg TP/L is the nutrient criterion established for waters entering the Everglades to preserve the natural algal assemblages in this P-limited marsh.

The role of N regulation in Mid-Atlantic streams is questionable, and thus, so is an N criterion of 300 µg/L to protect these streams from release from nutrient limitation. Experimental studies have not been published for effects of dissolved nitrogen to help us in this assessment. We do know that N:P ratios can be some help for evaluating the relative importance of nutrients in nutrient limitation. In Mid-Atlantic streams, most N:P ratios indicated P limitation because ratios were much higher than the 7:1 mass ratio that indicates a balance in N and P availability. However, the 300 µg TN/L criterion could be a valuable indicator of general nutrient enrichment in the ecosystem. Many streams may be N-limited (Franceour 2001). The 300 µg TN/L criterion may not protect N-limited systems from N saturation.

Alteration of stream ecosystems with higher nutrient concentrations (30 µg TP/L and 700 µg TN/L) is likely due to multiple stressors associated with the more intense human activity. The TN criterion that emerged from the Mid-Atlantic assessment of periphyton is very close to the 610 µg total inorganic nitrogen concentration that Miltner and Rankin (1998) identified as affecting fish in Ohio streams. Nutrients certainly do not have negative direct effects on algae or fish at these concentrations, but may covary with excess sediment loading, pesticides, heavy metals, and hydrologic alterations caused by a variety of human activities. Nutrients may negatively affect the ecological integrity of biota indirectly by stimulating filamentous algal blooms that can alter habitats and generate oxygen demand. Nuisance growths of Cladophora can accrue in waters with > 20 µg TP/L (Chetelat et al. 1999). Dodds et al. (1997) suggest nuisance algal growths develop in 38-90 µg TP/L and 275-600 µg TN/L. High ammonia concentrations could generate oxygen demand by nitrifying bacteria. The relative importance of

different demands for oxygen and relations to nutrients has been related to point sources of discharge in single streams, but is poorly understood at these larger spatial scales and in association with non-point sources of contaminants.

Protocols for establishing criteria to protect aquatic ecosystems should follow a logical order. The "physical, chemical, and biological integrity" protected by the US Clean Water Act (section 101(a)), has been defined many ways (Ballentine and Guarraia 1977, Karr and Dudley 1981), but it is usually related to natural conditions. Thus, we first should estimate the natural condition by direct assessment of streams in reference watersheds with minimal human activity, contamination, and alteration. In addition, we must predict ecological condition with no human activity and stressors to characterize the quality of reference sites. Relating assessments to natural condition provides a standard reference condition, quantifies ecosystem alteration by humans, and indicates the potential result of ecosystem restoration. Second, specific effects of stressors on valued ecological attributes should be understood so that costs and benefits of different criteria can be justified. For example, some increase in stressors (such as nutrients) may not result in "deleterious" effects and ecosystems may demonstrate all attributes associated with ecological integrity. Relative abundances of organisms could change without loss of native taxa and with little change in ecosystem function. Slight increases in productivity by algae and bacteria in streams may not result in major changes in decomposition rates, habitat structure, or loss of native taxa of algae, microbes, or invertebrates. In this example, changes in stressors have occurred with minimal change in structure and function of the ecosystem.

Although restoration of ecosystems to natural condition may not be practical, natural condition establishes a common benchmark for comparing definitions of reference condition. If natural conditions do not occur in a region because of extensive (not necessarily intensive) alterations, the natural conditions could be predicted with relations between stressors and ecological conditions developed in other regions. For nutrient concentrations in streams in the Mid-Atlantic region, we could assume that most watersheds were forested before humans prepared them for agriculture. Forested watersheds leak very few nutrients, so we could assume that nutrient concentrations were very low in streams of the Mid-Atlantic region before humans altered watersheds. Thus ecological integrity should be defined relative to conditions expected in streams with riparian canopy and low nutrients. This represents the natural condition. Best attainable reference conditions would represent the response of this ecosystem to stressor levels that result from implementing the best management practices available. Best-available reference conditions refers to the sites with least human disturbance and lowest levels of stressors in a region and the most similar to natural condition. Because near-natural ecosystems exist in the Mid-Atlantic, we can relate ecosystem condition to stressors caused by human activities and develop regional predictive models for conditions without human disturbances.

Nutrient criteria that would protect some of the nutrient-limited state of Mid-Atlantic streams according stressor-response relations (10 µg TP/L and 300 µg TN/L) were more similar to criteria developed with frequency distributions using all sites than to criteria developed with frequency distributions from reference conditions. We'd argue that this result has little conceptual rationale and is probably a coincidence rather than a confirmation of multiple lines of evidence. This should not be considered justification for using best available (minimally impacted of all sites) reference conditions alone to set criteria. Conceptually nutrient criteria defined by P* and N* should closely correspond to nutrient criteria defined by the 75th percentile of a frequency distribution using any kind of reference sites. The level of a valued ecological attribute defined by the 75th percentile of a frequency distribution should correspond to stressor levels defined by that same percentile. Dodds et al. (1998) developed a trophic status classification system for streams based on comparison of cumulative frequency distributions. They related nuisance algal biomasses to a specific percentile of stream site and then identified the nutrient concentrations for corresponding percentile of stream sites. Thus, nutrient criteria estimated by solving regression models relating nutrients and valued ecological attributes for the 75th percentile of those attributes (P* or N*) should be the same as the 75th percentile of N and P in reference conditions. Based on this rationale alone, the nutrient criteria recommended to protect a nutrient-limited state in Mid-Atlantic streams are lower than would be expected for the natural condition. However, many reference sites, defined either as Waite et al. (2000) or 0 riparian disturbance, probably had human activities high enough in the watershed to cause higher nutrient concentrations than expected from a forested watershed. These reference sites probably did not represent natural conditions.

Signs of ecosystem degradation that substantially affect invertebrate assemblages and fish are good indicators of more substantial changes in structure and ecosystem function than changes in algal species composition and release from nutrient enrichment. These successive changes in ecosystem response along a human disturbance gradient, here reflected in increasing nutrient concentrations, represent a predictable progression in ecosystem degradation. Algal species composition should respond at lower nutrient levels than invertebrate and fish assemblages, because of direct effects of nutrients on algae. Nuisance algal growths and oxygen depletion are deleterious effects probably related to even higher nutrient enrichment that was not documented well in this survey of Mid-Atlantic streams. Thus four categories of ecological condition could be expected along gradients of nutrient condition. Near-natural, nutrient-limited conditions in well-buffered Mid-Atlantic streams had TP < 10 µg/L and TN < 300 µg/L. Based on frequency distributions of nutrient data from more that 200 sites in temperate regions of North America and New Zealand, Dodds et al. (1998) suggest nutrient criteria of 25 µg TP/L and 700 µg TN/L to distinguish oligotrophic and mesotrophic streams and 75 µg TP/L and 1500 µg TN/L to distinguish mesotrophic and eutrophic streams. The near-natural, nutrient-limited conditions in the Mid-Atlantic would be relatively ultraoligotrophic. The oligotrophic-mesotrophic boundary of Dodds et al. was very similar to the upper limit of nutrient concentrations for well-buffered Mid-Atlantic streams in which some level of nutrient limitation was still detected. Thus, mesotrophic streams in the mid-Atlantic region had indications of degradation and eutrophic streams were not detected in abundance.

By integrating frequency distribution and stressor-response approaches for setting ecological criteria, we bring together two valuable tools in ecological assessment. Frequency distributions characterize natural or a type of reference condition. Stressor-response approaches facilitate development of cost-benefit justifications for establishing criteria at specific levels. They also help develop criteria at multiple levels of ecological condition and human disturbance to prevent degradation and to provide incremental restoration goals. Future research could improve ecosystem management by exploring ecological responses to nutrient concentrations in wetlands and lakes and better relating nutrient levels to nutrient loading, human activities, and best management practices.

LITERATURE CITED

American Public Health Association. Standard methods for the evaluation of water and wastewater. 18th edition. American Public Health Association, Washington, DC.

Barbour, M. T., J. Gerritsen, and B. D. Snyder. 1999. Rapid bioassessment protocols for use in wadeable streams and rivers. Second Edition. United States Environmental Protection Agency. **EPA 841-B-99-002.**

Ballentine, R. K. and L. J Guarraia. 1997. The integrity of water. U. S. Environmental Protection Agency, Office of Water and Hazardous Substances, Washington, DC.

Beaver, J. 1981. Apparent ecological charateristics of some common freshwater diatoms. Ontario Ministry of the Environment.

Biggs, B. J. F. 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society **19**:17-31.

Bothwell, M. L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomas and cellular growth rate responses. Canadian Journal of Fisheries and Aquatic Sciences **46**:1293-1301.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559-568.

Chetelat, J., F. R. Pick, and A. Morin. 1999. Periphyton biomass and community composition in rivers of different trophic status. Canadian Journal of Fisheries and Aquatic Sciences **56**:560-569.

Dent, C. L., N. B. Grimm, and S. G. Fisher. 2001. Multiscale effects of surface-subsurface exchange on stream water nutrient concentrations. Journal of the North American Benthological Society **20**:162-181.

Dixit, S. S., B. F. Cumming, J. C. Kingston, J. P. Smol, H. J. B. Birks, A. J. Uutala, D. F. Charles, and K. Camburn. 1993. Diatom assemblages from Adirondack lakes (N.Y., USA) and the development of inference models for retrospective environmental assessment. Journal of Paleolimnoogy **8**:27-47.

Dixit, S. S., J. P. Smol, D. F. Charles, R. M. Hughes, S. G. Paulsen, and G. B. Collins. 1999. Assessing water quality changes in the lakes of the northeastern United States using sediment diatoms. Canadian Journal of Fisheries and Aquatic Sciences **56**:131-152.

Dodds, W. K., J. R. Jones, and E. B. Welch. 1998. Suggested classifications of stream trophic state: distributions of temperature stream types by chlorophyll, total nitrogen, and phosphorus. Water Research.

Dodds, W. K., V. H. Smith, and B. Zander. 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: a case study of the Clark Fork River. Water Research **31**:1738-1750.

Dodds, W. K., and E. B. Welch. 2000. Establishing nutrient criteria in streams. Journal of the North American Benthological Society **19**:186-196.

Dudley, T. L., S. D. Cooper, and N. Hemphill. 1986. Effects of macroalgae on a stream invertebrate community. Journal of the North American Benthological Society **5**:93-106.

Francoeur, S. N. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. Journal of the North American Benthological Society **20**:358-369.

Grimm, N. B., S. G. Fisher, and W. L. Minckley. 1981. Nitrogen and phosphorus dynamics in hot desert streams of the southwestern U.S.A. Hydrobiologia **83**:303-312.

Hawkins, C. P., R. H. Norris, J. N. Hogue, and J. W. Feminella. 2000. Development and evaluation of predictive models for measuring the biological integrity of streams. Ecological Applications **10**:1456-1477.

Herlihy, A. T., J. L. Stoddard, and C. B. Johnson. 1998. The relationship between stream chemistry and watershed land cover data in the mid-Atlantic region, U.S. Water, Air, and Soil Pollution **105**:377-386.

Hill, B. H., A. T. Herlihy, P. R. Kaufmann, R. J. Stevenson, and F. H. McCormick. 2000. The use of periphyton assemblage data as an index of biotic integrity. Journal of the North American Benthological Society **19**:50-67.

Hill, M. O., R. G. H. Bounce, and M. W. Shaw. 1975. Indicator species analysis, a divisive polythetic method of classification

and its application to a survey of native pinewoods in Scotland. Journal of Ecology **63**:597-613.

Hughes, R. M. 1995. Defining acceptable biological status by comparing with reference conditions. Pages 31-47 *in* W. S. Davis and T. P. Simon, editors. Biological assessmentand criteria: tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.

Hughes, R. M., D. M. Larsen, and J. M. Omernik. 1986. Regional reference sites: a method for assessing stream potentials. Environmental Management **10**:629-635.

Hustedt, F. 1937-1939. Systematische and Ökologische Untersuchungen über die Diatomeenflora von Java, Bali, und Samatra. Archiv für Hydrobiologie **15**:131-177, **16**:187-295, **16**:393-506.

Juggins, S., and C. J. F. ter Braak. 1992. CALIBRATE - A Program For Species-Environment Calibration By [Weighted Averaging] Partial Least Squares Regression. Environmental Change Research Center, University College, London.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Karr, J. R., and D. R. Dudley. 1981. Ecological perspective on water quality goals. Environmental Management 5:55-68.

Kelly, M. G., and B. A. Whitton. 1995. A trophic diatom index: a new index for monitoring eutrophication in rivers. Journal of Applied Ecology 7:433-444.

Kaufmann, P. R., and E. G. Robison. 1994. Physical habitat assessment. Pages 1-38 *in* D. J. Klemm and J. M. Lazorchak, editors. Environmental monitoring and assessment program: 1994 field operations manual for streams. United States Environmental Protection Agency, Cincinnati, Ohio.

Kerans, B. L., and J. R. Karr. 1994. A benthic index of biotic integrity (B-IBI) for rivers of th Tennessee Valley. Ecological Applications 4:768-785.

Kociolek, J. P., and E. F. Stoermer. 2001. Taxonomy and ecology: a marriage of necessity. Diatom research **16**:433-442.

Kohler, S. L., and M. J. Wiley. 1997. Pathogen outbreaks reveal large-scale effects of competition in stream communities. Ecology **78**:2164-2176.

Kolkwitz, R., and M. Marsson. 1908. Ökologie der pflanzliche Saprobien. Berichte der Deutschen Botanischen Gesellschaft **26**:505-519.

Lowe, R. L. 1974. Environmental requirements and poolution tolerance of freshwater diatoms. United States Environmental Protection Agency, Cincinnati, Ohio. **EPA-670-4-74-005.**

McCormick, P. V. and R. J. Stevenson. 1998. Periphyton as a tool for ecological assessment and management in the Florida Everglades. Journal of Phycology **34**:726-733.

Miltner, R. J., and E. T. Rankin. 1998. Primary nutrients and the biotic integrity of rivers and streams. Freshwater Biology **40**:145-150.

Mulholland, P. J. 1996. Role in nutrient cycling. Pages 609-639 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, editors. Algal ecology, freshwater benthic ecosystems. Academic Press, San Diego, California.

Muradian, R. 2001. Ecological thresholds: a survey. Ecological Economics **38**:7-24.

Odum, E. P., J. T. Finn, and E. H. Franz. 1979. Perturbation theory and the subsidy-stress gradient. BioScience **29**:349-352.

Omernik, J. M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers **77**:118-125.

Palmer, C. M. 1969. A composite rating of algae tolerating organic pollution. Journal of Phycology 5:78-82.

Pan, Y., R. J. Stevenson, B. H. Hill, A. T. Herlihy, and G. B. Collins. 1996. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. Journal of the North American Benthological Society **15**:481-495.

Pan, Y., R. J. Stevenson, B. Hill, P. R. Kaufmann, and A. T. Herlihy. 1999. Spatial patterns and ecological determinants of benthic algal assemblages in Mid-Atlantic Highland streams. Journal of Phycology **35**:460-468.

Pielou, E. C. 1984. The interpretation of ecological data. John Wiley & Sons, Inc., New York.

Richardson, C. J., and S. Qian. 1999. Long-term phosphorus assimilative capacity in freshwater wetlands: a new paradigm for maintaining ecosystem structure and function. Environmental Science and Technology **33**:1545-1551.

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution **100**:179-196.

Stelzer, R. S., and G. A. Lamberti. 2001. Effects of N:P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnology and Oceanography **46**:356-367.

Stevenson, R. J., M. Bothwell, and R. L. Lowe, eds. 1996. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego, CA.

Stevenson, R. J. 1997. Resource thresholds and stream ecosystem sustainability. Journal of the North American Benthological Society **16**:410-424.

Stevenson, R. J. and R. Glover. 1993. Effects of algal density and current on ion transport through periphyton communities. Limnology and Oceanography **38**:1276-1281.

Stevenson, R.J., and R. L. Lowe. 1986. Sampling and interpretation of algal patterns for water quality assessment. In: B.G. Isom, ed., Rationale for Sampling and Interpretation of Ecological Data in the Assessment of Freshwater Ecosystems. ASTM STP 894. American Society for Testing and Materials Publication, Philadelphia, PA. pp. 118-149.

Stevenson, R. J., P. R. Sweets, Y. Pan, and R. E. Schultz. 1999. Algal community patterns in wetlands and their use as indicators of ecological conditions. In: A. J. McComb and J. A. Davis, eds. Proceedings of INTECOL's Vth International Wetland Conference. Pp.517-527. Gleneagles Press, Adelaide, Australia.

Suter, G. W. 1993. Ecological risk assessment. CRC Press, LLC, Boca Raton, Florida.

Triska, F. J., V. C. Kennedy, R. J. Avanzino, G. W. Zellweger, and K. E. Bencala. 1989. Retention and transport of nutrients in a third-order stream: channel processes. Ecology **70**:1877-1892.

United States Environmental Protection Agency (USEPA). 1998a. Guidelines for ecological risk assessment. United States Environmental Protection Agency. **EPA-630-R-95-002F.**

United States Environmental Protection Agency (USEPA). 1998b. National strategy for the development of regional nutrient criteria. United States Environmental Protection Agency. **EPA-822-R-98-002.**

United States Environmental Protection Agency (USEPA). 2000. Nutrient critieria technical guidance manual. Rivers and streams. United States Environmental Protection Agency. **EPA-822-B-00-002.**

van Dam, H., A. Mertens, and J. Sinkeldam. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands Journal of Aquatic Ecology **28**:117-133.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications **7**:737-750.

Waite, I. R., A. T. Herlihy, D. P. Larsen, and D. J. Klemm. 2000. Comparing the strengths of geographic and nongeographic classifications of stream benthic macroinvertebrates in the Mid-Atlantic Highlands, USA. Journal of the North American Benthological Society **19**:429-441.

Welch, E. B., J. M. Jacoby, R. R. Horner, and M. R. Seeley. 1988. Nuisance levels of periphytic algae in streams. Hydrobiologia **157**:161-168.

Welch, E. G., R. R. Horner, and C. R. Patmont. 1989. Prediction of nuisance periphytic biomass: a management approach. Water Research **23**:401-405.

Wright, J. F., M. T. Furse, and P. D. Armitage. 1993. RIVPACS: a technique for evaluating the biological quality of rivers in the UK. European Water Pollution Control **3**:15-25.

Zelinka, M., and P. Marvan. 1961. Zur Prazisierung der biologischen Klassifikation des Reinheit fliessender Gewässer. Archiv für Hydrobiologie **57**:389-407.

Table 1. List of physical, chemical, and environmental variables included in the data analysis to determine factors affecting similarity in diatom species composition among reference sites, and thereby, classify stream types with similar expected diatom species composition.

Chemical	Physical
Alkalinity	mean depth
Acid Neutralizing Capacity	maximum depth in reach
Calcium	number of pools > 75 cm deep in reach
Chloride	area of pools
Carbonate	mean depth/width ratio
Conductivity	mean wetted width
Dissolved Organic Carbon	mean width*depth product
Dissolved Inorganic Carbon	standard deviation of thalweg depth
pH	standard deviation of width*depth product
Sulfate	channel sinuosity
Total Suspended Solids	slope
Turbidity	stream order
	latitude
	longitude

Table 2. Linear regression statistics indicating the relationships between algal assemblage attributes and TP and TN concentrations in streams of the Mid-Atlantic Region; where a is the constant and coeff is the coefficient in a regression equation of the form Y=a+coeff(X), N is the number of streams in the analysis, r is the Pearson correlation coefficient, and P is the attained significance of the regression coefficient.

Attribute	Log(TP)					Log(TN)				
	a	coeff	N	r	P	a	coeff	N	r	P
Spp Similarity to Reference	0.591	-0.066	204	-0.398	<0.001	0.74	-0.053	205	-0.343	<0.001
Observed/Expected Taxa Ratio	0.835	0.136	204	0.286	<0.001	0.814	0.066	205	0.147	0.035
Percent Sensitive Individuals	43.19	-6.87	242	-0.421	<0.001	54.19	-4.76	243	-0.311	<0.001
Percent Tolerant Individuals (log)	-1.257	0.77	204	0.496	<0.001	-2.922	0.604	205	0.419	<0.001
MAIA Trophic Status Index	3.015	0.304	242	0.531	<0.001	2.165	0.267	243	0.498	<0.001
van Dam Trophic Status Index	2.755	0.31	250	0.417	<0.001	1.423	0.344	251	0.501	<0.001
Weighted Average TP model (log)	0.792	0.140	250	0.528	<0.001					
Saprobic Index	1.207	0.266	242	0.515	<0.001	0.518	0.225	243	0.466	<0.001
Oxygen Tolerance Index	0.976	0.305	242	0.548	<0.001	0.221	0.253	243	0.485	<0.001
Ash-Free Dry Mass (log)	1.404	0.289	210	0.241	<0.001	0.764	0.229	211	0.191	0.005
Chlorophyll a (log)	3.056	0.109	211	0.085	0.218	1.336	0.314	212	0.241	<0.001
Phaeophytin (log)	1.64	0.265	187	0.179	0.014	0.23	0.337	188	0.23	0.001
Chl/AFDM (log)	1.768	-0.177	205	-0.131	0.062	0.748	0.076	206	0.055	0.428
Acid Phosphatase Activity (log)	9.015	-0.504	195	-0.25	<0.001	9.585	-0.32	196	-0.156	0.029
Alkaline Phosphatase Activity (log)	7.946	-0.51	201	-0.317	<0.001	7.928	-0.229	202	-0.142	0.044
Total Chlorophyll (log)	3.266	0.178	216	0.152	0.026	1.541	0.346	217	0.293	<0.001
Chl a/Tot Chlorophyll	0.783	-0.03	216	-0.102	0.135	0.702	-0.001	217	-0.004	0.953
Filamentous Algal Cover	0.053	-0.006	179	-0.054	0.471	-0.035	0.011	180	0.113	0.132

Table 3. Effect of stream classification on variance in algal attributes explained by TP. r (all) is the pearson correlation coefficient for relations between algal-related attributes and log(TP) concentration at sites when all sites samples were included in the analysis, not just well-buffered streams as in Table 2. The classification effect is the difference in the absolute value in R with and without classification.

Attribute	R (all)	Classification Effect
Spp Similarity to Reference	-0.109	0.289
Observed/Expected Taxa	0.400	-0.114
Percent Sensitive Individuals	-0.439	-0.018
Percent Tolerant Individuals (log)	0.444	0.052
MAIA Trophic Status Index	0.488	0.043
van Dam Trophic Status Index	0.184	0.233
Weighted Average TP model (log)	0.489	0.039
Saprobic Index	0.388	0.127
Oxygen Tolerance Index	0.522	0.026
Ash-Free Dry Mass (log)	0.188	0.053
Chlorophyll a (log)	0.152	-0.067
Phaeophytin (log)	0.176	0.003
Chl/AFDM (log)	-0.040	0.091
Acid Phosphatase Activity (log)	-0.223	0.027
Alkaline Phosphatase Activity (log)	-0.220	0.097
Total Chlorophyll (log)	0.190	-0.038
Chl a/Tot Chlorophyll	-0.048	0.054
Filamentous Algal Cover	-0.056	-0.002

15

Table 4. Candidate nutrient and biological criteria for streams based on frequency distributions of attributes at reference sites as defined by Waite et. al. (2000, EMAP Ref) and 0 riparian disturbance (RD Ref) and at all sites (both reference and non-reference). All values that were log-transformed for data analysis and untransformed for presentation in this table are labeled with "(log)". Nutrient concentrations are presented as both untransformed and transformed. REC indicates "Relative Expected Condition" as relatively low or high for reference condition when compared to impacted condition. RES indicates an "a priori" prediction of relative sensitivity of attributes to nutrient enrichment.

Attribute	Waite Ref	RD Ref	All Sites	REC	RES
TN	515	684	350	low	
TP	12	22	10	low	
Log(TN)	6.245	6.528	5.859	low	
Log(TP)	2.485	3.091	2.303	low	
Spp Similarity to Reference	0.468	0.350	0.519	high	2
Observed/Expected Taxa	1.200	1.200	0.900	low	3
Percent Sensitive Individual	15.8	11.6	34.0	high	1
Percent Tolerant Individuals (log)	2.3	8.2	1.1	low	2
MAIA Trophic Status Index	3.689	3.920	3.497	low	2
Saprobic Index	1.835	2.169	1.601	low	3
Oxygen Tolerance Index	1.714	1.954	1.436	low	3
Ash-Free Dry Mass (log)	11.752	9.244	4.531	low	4
Chlorophyll a (log)	68.9	47.8	12.5	low	4
Phaeophytin (log)	22.6	15.5	4.7	low	4
Total Chlorophyll (log)	82.9	47.8	21.6	low	4
Acid Phosphatase Activity (log)	2100.6	2040.6	5756.0	high	2
Alkaline Phosphatase Activity (log)	364.3	301.9	1833.5	high	2
Filamentous Algal Cover	0	0	0	low	4
Chl a/Tot Chlorophyll	0.597	0.757	0.908	high	2

Table 5. Candidate TP criteria derived from biological responses (attributes) to nutrient enrichment and 4 approaches for delineating the nutrient concentration generating the response. The 3 approaches were the TP concentration: predicted by linear regression to cause the 25th percentile condition for EMAP-defined reference sites (P*); indicated by regression tree analysis (P RT); and defined by lowess regression. Two values for criteria from lowess regression indicate two changes in patterns in response along stressor gradient. AS indicates attained significance of linear regression (Table 2), with * indicating p<0.001, ** indicating p<0.001, * indicating p=0.001-0.01, * indicating p=0.01-0.05, > indicating p=0.05-0.15. No AS designation indicates p>0.15. nd indicated that no change was detected.

Attribute	AS	P*	P RT	Lowess	Lowess Rationale
Spp Similarity to Reference	***	8.03	27.00	8/30	Threshold Response
Observed/Expected Taxa	***	18.27	12.00	nd	
Percent Sensitive Individual	***	53.04	18.99	nd	
Percent Tolerant Individuals	***	5.64	18.99	nd	
Percent Tolerant Individuals (log)	***	15.15	27.00	nd	
MAIA Trophic Status Index	***	9.18	18.99	nd	
Saprobic Index	***	10.60	12.00	6	Below acceleration point
Oxygen Tolerance Index	***	11.25	20.01	6	Below acceleration point
Ash-Free Dry Mass (log)		39.17	8.00	nd	
Chlorophyll a (log)	*	48484.53	nd	nd	
Phaeophytin (log)	>	263.49	nd	nd	
Total Chlorophyll (log)	***	646.78	nd	30	Degradation
Acid Phosphatase Activity (log)	***	15.00	17.00	8	Below limitation release
Alkaline Phosphatase Activity (log)	*	55.48	8.00	7	Below limitation release
Filamentous Algal Cover		6856.83	nd	nd	
Chl a/Tot Chlorophyll	>	492.75	nd	nd	

Table 6. Candidate TN criteria derived from biological responses (attributes) to nutrient enrichment and 4 approaches for delineating the response. The 3 approaches were the TN concentration: predicted by linear regression to cause the 25th percentile condition for EMAP-defined reference sites (N*); indicated by regression tree analysis (N RT); and defined by lowess regression. Two values for criteria from lowess regression indicate two changes in patterns in response along stressor gradient. AS indicates attained significance of linear regression (Table 2), with * indicating $p<0.001$, ** indicating $p=0.001-0.01$, * indicating $p=0.01-0.05$, > indicating $p=0.05-0.15$. No AS designation indicates $p>0.15$. nd indicated that no change was detected. vh indicates the predicted value was so high that the number would not fit in the table.

Attribute	AS	N*	N RT	Lowess	Low Rationale
Spp Similarity to Reference	***	169.36	971.65	350	Threshold Response
Observed/Expected Taxa	*	346.54	498.20	1000	Degradation
Percent Sensitive Individual	***	2980.96	1125.52	nd	
Percent Tolerant Individuals	***	203.57	485.90	nd	
Percent Tolerant Individuals (log)	***	503.71	nd	nd	
MAIA Trophic Status Index	***	301.27	990.29	nd	
Saprobic Index	***	348.28	673.84	300/800	
Oxygen Tolerance Index	**	365.40	673.84	300/700	Acceleration/Saturation
Ash-Free Dry Mass (log)	***	1675.72	217.89	1500	Acceleration/Saturation
Chlorophyll a (log)	**	10127.40	1170.28	nd	Degradation
Phaeophytin (log)		5255.34	317.98	nd	
Total Chlorophyll (log)	*	4084.69	313.88	nd	
Acid Phosphatase Activity (log)	*	422.84	nd	nd	
Alkaline Phosphatase Activity (log)	***	7072.72	nd	nd	
Filamentous Algal Cover	>	24.09	nd	nd	
Chl a/Tot Chlorophyll		vh	nd	nd	

Table 7. Spearman rank correlation coefficients between N and P criteria using the 3 of 4 statistical approaches for defining criteria that provided sufficient
information for correlations. See Tables 6 and 7 for meaning of column headings.

	P*	P RT	N*
P RT	-0.466		
N*	0.905	-0.43	
N RT	0.06	0.55	0.036

Figure Captions

Figure 1. Two approaches that use stressor-response relations for determining candidate TP and TN criteria for protecting valued ecological attributes. Fig. 1A shows how P* and N* (marked by arrow) are determined by solving the linear regression model for TN or TP given the biocriterion determined with specified percentiles of a frequency distribution of algal attributes. The solid line shows the linear relation between an attribute measuring biological condition (Bio Cond) and a stressor (TP or TN). The dotted line marks the number of reference sites (N Ref Sites) with specific values of biological condition. The dashed line shows value of the algal attribute the 25th percentile of the frequency distribution. Fig. 1B shows candidate nutrient criteria (vertical dashed line) indicated by regression trees and predicted changes in means of an algal attribute (e.g., similarity to reference condition, horizontal lines) along a stressor gradient.

Figure 2. Frequency distributions of TP and TN concentrations in Mid-Atlantic streams for reference sites (figures on right) and all sites (figures on left).

Figure 3. Changes in algal attributes in Mid-Atlantic streams as a function of TP concentration. Solid lines mark the smoothing line created by lowess regression. Vertical lines indicate candidate nutrient criteria. Rationale for selecting candidate nutrient criteria and meanings of abbreviations are provided in the results and methods, respectively.

Figure 4. Changes in algal attributes in Mid-Atlantic streams as a function of TN concentration. Solid lines mark the smoothing line created by lowess regression. Vertical lines indicate candidate nutrient criteria. Rationale for selecting candidate nutrient criteria and meanings of abbreviations are provided in the results and methods, respectively.

Figure 5. Changes in acid and alkaline phosphatase activity (nmoles activity g AFDM^{-1} h^{-1}) in Mid-Atlantic streams as a function of TP concentration. Solid lines mark the smoothing line created by lowess regression. Vertical lines indicate candidate nutrient criteria. Rationale for selecting candidate nutrient criteria are provided in the results.

Figure 6. Changes in biological indicators and valued ecological attributes with increasing TP and TN concentrations. Non-linear ecological responses to stressors and human activities provide justification for specific levels of criteria. Linear ecological responses provide good indicators of change because they respond sensitively and uniformly along the entire gradient of disturbance.