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Nutrient Criteria Technical Guidance Manual

Wetlands

Full Guidance Document available in full at:
http://www.epa.gov/waterscience/criteria/nutrient/guidance/

Chapter 7 Data Analysis

7.1 INTRODUCTION

Data analysis is critical to nutrient criteria development. Proper analysis and interpretation of data determine the scientific defensibility and effectiveness of the criteria. Therefore, it is important to evaluate short- and long-term goals for wetlands of a given class within the region of concern. These goals should be addressed when analyzing and interpreting nutrient and response data. Specific objectives to be accomplished through use of nutrient criteria should be identified and revisited regularly to ensure that goals are being met. The purpose of this chapter is to explore methods for analyzing data that can be used to develop nutrient criteria consistent with these goals. Included are techniques to evaluate metrics, to examine or compare distributions of nutrient exposure or response variables, and to examine nutrient exposure-response relationships.

Statistical analyses are used to interpret monitoring data for criteria development. Statistical methods are data-driven and range from very simple descriptive statistics to more complex statistical analyses. Generally, the type of statistical analysis used for criteria development is determined by the source, quality, and quantity of data available.

7.2 FACTORS AFFECTING ANALYSIS APPROACH

Wetland systems should be appropriately classified *a priori* for nutrient criteria development to minimize natural background variation (see Chapter 3). This section discusses some of the factors that should be considered when classifying wetland systems and in determining the choice of predictor (causal) and response variables to include in the analysis.

Wetland hydrogeomorphic type http://el.erdc.usace.army.mil/wrap/wrap.html may determine the sensitivity of wetlands to nutrient inputs, as well as the interaction of nutrients with other driving factors in producing an ecological response. Hydrogeomorphic types differ in landscape position, predominant water source, and hydrologic exchanges with adjacent water bodies (Brinson 1993). These factors, in turn, influence water residence time, hydrologic regime, and disturbance regime. In general, isolated depressional wetlands will have greater residence times than fringe wetlands, which, in turn, will have greater residence times than riverine wetlands. Systems with long residence times are likely to behave more like lakes than flow-through systems and may show a greater response to cumulative loadings. Thus, nutrient loading rates or indicators thereof are likely to be a more sensitive predictor of ecological effects for depressional wetlands, while nutrient water column or sediment concentrations are likely to be a more sensitive predictor of responses for riverine wetlands. Water column concentrations will influence the response of algal communities, while macrophytes derive nutrients from both the water column and sediments. Fringe wetlands are likely to be influenced both by concentration of nutrients in the adjacent lake or estuary as well as the accumulation of nutrients within these systems from groundwater inflow and, in some cases, riverine inputs. The relative influence of these two sources will depend on the exchange rate with the adjacent lake, e.g., through seiche activity (Keough et al., 1999; Trebitz et al., 2002). In practice, it is difficult to measure loadings from multiple sources including groundwater and exchange with adjacent water bodies. If sediment concentrations are shown to be a good indicator of recent loading rates, then sediment concentrations might be the best predictor to use across systems.

It may be important to control for ancillary factors when teasing out the relationship between nutrients and vegetation community response, particularly if those factors interact with nutrients in eliciting responses. For example, riverine and fringe wetlands differ from basin wetlands in the frequency and intensity of disturbance from flooding events or ice. Day et. al. (1988) describe a fertility-disturbance gradient model for riverine wetlands describing how the relative dominance of plant guilds with different growth forms and life history strategies depends on the interactive effects of productivity, fertility, disturbance, and water level. In depressional wetlands, the model could be simplified to include only the interaction of fertility with the hydrologic regime. Disturbance regimes and water level could be incorporated into analysis of cause-effect relationships either as categorical factors or as covariates.

The selection of assessment and measurement of response attributes for determining ecological response to nutrient loadings should depend, in part, on designated uses assigned to wetlands as part of standards development. Designated uses such as recreation (aesthetics and contact) or drinking water are not typically assigned to wetlands; thus, defining nuisance algal blooms in terms of taste or odor problems or aesthetic considerations may not be appropriate for wetlands. Guidance for the definition of aquatic life use is currently being refined to describe six stages of impact along a human disturbance gradient, from pristine reference condition to heavily degraded sites (Figure 7.1, Stevenson and Hauer 2002, Davies and Jackson 2006). The relative abundance of sensitive native taxa is expected to shift with relatively minor impacts, while organism condition or functional attributes are relatively robust to altered loadings. However, if maintenance of ecological integrity of sensitive downstream systems is of concern, then it may be important to measure some functional attributes related to nutrient retention. Stevenson and Hauer (2002) have suggested a series of "resource condition tiers" analogous to those defined for biological condition but related to ecosystem functions. Tier 1 requirements are proposed as: "Native structure and function of the hydrologic and geomorphic regimes and processes are in the natural range of variation in time and space." Thus maintenance of structure and function of upstream processes should be protective of downstream biological conditions.

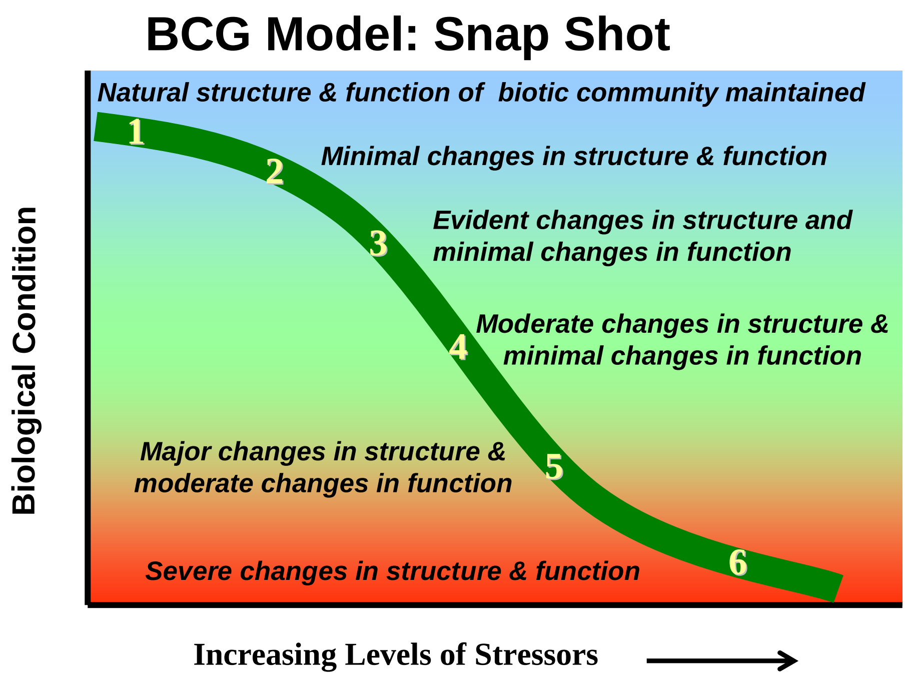

Figure 7.1. Biological condition gradient model describing biotic community condition as levels of stressors increase.

7.3 DISTRIBUTION-BASED APPROACHES

Frequency distributions can aid in the setting of criteria by describing central tendency and variability among wetlands. Approaches to numeric nutrient criteria development based on frequency distributions do not require specific knowledge of individual wetland condition prior to setting criteria. Criteria are based on and, in a sense, developed relative to the conditions of the population of wetlands of a given class.

The simplest statistic describing the shape of distributions refers to *quartiles*, or the 25th and the 75th percentile. These can be defined as the observation which has 25% of the observations on one side and 75% on the other side in the case of the first quartile (25th percentile), or vice versa in the case of the third quartile (75th percentile). In the same manner, the median is the second quartile or the 50th percentile. Graphically, this is depicted in boxplots as the box length, the lower extreme represents the first quartile, and the upper extreme represents the third quartile, the area inside the box encompassing 50% of the data.

Distributions of nutrient exposure metrics or response variables can be developed to represent either an entire population of wetlands or only a subset of those considered to be minimally impacted. In either case, a population of wetlands should be defined narrowly enough through classification so that the range in attributes due to natural variability does not equal or exceed the range in attributes related to anthropogenic effects. The effects of natural variability can be minimized by classifying wetlands by type and/or region. Nutrient ecoregions define one potential regional classification system (USEPA 2000). Alternatively, thresholds in landscape or watershed attributes defining natural breakpoints in nutrient concentrations can be determined objectively through procedures such as classification and regression tree (CART) analysis (Robertson et. al., 2001). If a distribution-based approach is used, periodic reviews using empirical data that relate a measured value to an ecological attribute or ecosystem function can validate the assumptions of the chosen percentiles.

7.4 RESPONSE-BASED APPROACHES

Indicators characterized as “response” or “condition” metrics should be distinguished from “stressor” or “causal” indicators, such as nutrient concentrations (Paulsen et al., 1991; USEPA 1998a; Stevenson 2004a). While both “response“ and “causal” indicators could be used in a single multimetric index, it is recommended that separate multimetric indices be used for “response” and “causal” assessment. Distinguishing between “response” and “causal” indices can be accomplished utilizing a risk assessment approach with separate hazard and exposure assessments that are linked to response-stressor relationships (USEPA 1996, 1998a; Stevenson 1998; Stevenson et al., 2004a, b). A multimetric index that specifically characterizes “responses” can be used to clarify goals of management (maintenance or restoration of ecological attributes) and to measure whether goals have been attained with nutrient management strategies. Response-based multimetric indices can also be used more directly for natural resource damage assessments than multimetric indices with response and causal variables.

Factors that should be considered in selecting indicators include conceptual relevance (relevance to the assessment and ecological function), feasibility of implementation (data collection logistics, information management, quality assurance, cost), response variability (measurement error, seasonal variability, interannual variability, spatial variability, discriminatory ability), and interpretation and utility (data quality objectives, assessment thresholds, link to management actions) (Jackson et al., 2000). Of these factors, cost, response variability, and ability to meet data quality objectives can be assessed through quantitative methods. An analytical understanding of the factors that affect wetlands the most will also help States develop the most effective monitoring and assessment strategies.

Designated uses such as contact recreation and drinking water may not be applicable to wetlands, hence, it may not be readily apparent what the relative significance of changes in different primary producers is for organisms at higher trophic levels. Wetland food webs have traditionally been considered to be detritus-based (Odum and de la Cruz 1967; Mann 1972, 1988). However, more recent research on wetland food webs utilizing stable isotope analysis have identified the importance of phytoplankton, periphyton, or benthic algae as the base of the food chain for higher trophic levels (Fry 1984, Kitting et al., 1984, Sullivan and Moncreiff 1990, Hamilton et al., 1992, Newell et al., 1995, Keough et al., 1996); in these cases, it would be particularly important to monitor shifts in algal producers.

Empirical relationships can be derived directly between water quality parameters such as total P or transparency and wetland biological responses. Unlike lakes or streams, the level of algal biomass corresponding to aesthetic problems or ecological degradation in wetlands is not readily defined, so that defining a TP-chlorophyll *a* relationship based on water column measurements is not likely to be useful. However, in some wetlands such as coastal Great Lakes, the loss of submerged aquatic vegetation biomass and/or diversity with increased eutrophication provides an ecologically significant endpoint (Lougheed et al., 2001). Reductions in submerged plant species diversity was associated with increases in turbidity, total P, total N, and chlorophyll *a*, suggesting that a trophic state index incorporating multiple parameters might be a better predictor than a single variable such as total P (Carlson 1977).

Models describing empirical relationships can include linear or nonlinear univariate forms with a single response metric, multivariate with multiple response metrics, a series of linked relationships, and simulation models. The simplest forms of linear univariate approaches are correlation and regression analyses; these approaches have the advantage that they are simple to perform and transparent to the general public. When assessment thresholds can be determined based on severity of effect or difference from reference conditions such that associated exposure criteria can be derived, linear forms should be adequate. In the case of nonlinear relationships, data can generally be transformed to linearize the relationship. However, if it is desired to identify the inflection point in a curvilinear relationship as an indicator of rapid ecological change, alternative data analysis methods are available, including changepoint analysis (Richardson and Qian 1999) and piecewise iterative regression techniques (Wilkinson 1999).

Multivariate models are useful for relating nutrient exposure metrics to community-level responses. Both parametric and nonparametric (nonmetric dimensional scaling or NMDS) ordination procedures can be used to define axes or gradients of variation in community composition based on relative density, relative abundance, or simple presence-absence measures (Gauch 1982, Beals 1984, Heikkila 1987, Growns et al., 1992). Ordination scores then can be regressed against nutrient exposure metrics as an indicator of a composite response (McCormick et al., 1996). Direct gradient analysis techniques such as canonical correspondence analysis can be used to determine which combination of nutrient exposure variables predict a combination of nutrient response variables as a first step in deriving multimetric exposure and response variables (Cooper et al., 1999). Indicator analysis can be used to determine which subset of species best discriminate between reference sites with low nutrient loadings versus potentially impacted sites with high loadings, or weighted averaging techniques can be used to infer nutrient levels from species composition (McCormick et al., 1996, Cooper et al., 1999, Jensen et al., 1999). In the latter case, paleoecological records can be examined to infer historic changes in total P levels from macrophyte pollen or diatom frustrules, which will be particularly valuable in the absence of sites representing reference condition (Cooper et al., 1999, Jensen et. al., 1999).

Some ecohydrological models have been derived that incorporate the effect of multiple stressors (hydrology, eutrophication, acidity) on wetland vegetation, thus providing a link between process-based models and community level response (see Olde Venterink and Wassen 1997 for review). These models are based on: 1) a combination of expert opinion to estimate species sensitivities, supplemented by multivariate classification of vegetation and environmental data to determine boundaries of species guilds; or, 2) field measurements used to derive logistic models to quantify dose-response. These approaches could be used to derive wetland nutrient criteria for the U.S. provided that models could be calibrated using species and response curves developed using data for the U.S. Most multiple-stressor models for wetland vegetation have been calibrated using data from Western Europe (Olde Venterink and Wassen 1997). Latour and colleagues (Latour and Reiling 1993, Latour et al., 1994) have suggested a mechanism for setting nutrient standards using the occurrence probability of species along a trophic gradient to extrapolate maximum tolerable concentrations that protect 95% of species.

A series of linked empirical relationships for wetlands may be most effective for developing nutrient criteria. Linked empirical relationships may be most useful in cases where integrative exposure measurements such as sediment nutrient concentrations are more sensitive predictors of shifts in community composition, or algal P limitation, or other ecological responses (phosphatase enzyme assays; Qian et al., 2003) than are spatially and temporally heterogeneous water column nutrient concentrations. In these cases, it may be important to develop one set of relationships between nutrient loading and exposure indicators for a subset of sites at which intensive monitoring is done, and another set of relationships between nutrient exposure and ecological response indicators for a larger sample population (Qian et al., 2003).

7.5 PARTITIONING EFFECTS AMONG MULTIPLE STRESSORS

Changes in nutrient concentrations within or loadings to wetlands often co-occur with other potential stressors such as changes in hydrologic regime and sediment loading. In a few cases, researchers have been able to separate the simple effects of nutrient addition through manipulations of mesocosms (Busnardo et al., 1992, Gabor et.al., 1994, Murkin et al., 1994, McDougal et al., 1997, Hann and Goldsborough 1997), segments of natural systems (Richardson and Qian 1999, Thormann and Bayley 1997), or whole wetlands (Spieles and Mitsch 2000). In other cases, both simple and interactive effects have been examined experimentally, e.g., to separate effects of hydrologic regime from nutrient loading (Neill 1990a, b; Neill 1992, Bayley et al., 1985). If nutrient effects are examined by comparing condition of natural wetlands along a loading or concentration gradient, effects of other driving factors can be minimized by making comparisons among wetlands of similar hydrogeomorphic type and climatic regime within a well-defined sampling window. In addition, multivariate techniques for partitioning effects among multiple factors can be used, such as partial CCA or partial redundancy analysis (Cooper et al., 1999, Jensen et al., 1999).

7.6 STATISTICAL TECHNIQUES

Quantitative methods can be used to assess metric cost, evaluation, response variability, and ability to meet data quality objectives. The most appropriate method varies with respect to the indicator or variable being considered. In general, statistical techniques are aimed at making conjectures or inferences about a population's values or relationships between variables in a sample randomly taken from the population of interest. In these terms, population is defined as all possible values that a certain parameter may take. For example, in the case of total phosphorus levels present in marsh sediments in nutrient ecoregion VII, the total population would be determined if all the marshes in that ecoregion were sampled, which would negate the need for data analysis. Practically, a sample is taken from the population and the characteristics associated with that sample (mean, standard deviation) are "transferred" to the entire population. Many of the basic statistical techniques are designed to quantify the reliability of this transferred estimate by placing a confidence interval over the sample-derived parameter. More complex forms of data analysis involve comparisons of these parameters from different populations (for example, comparison between sites) or the establishment of complex data models that are thought to better describe the original population structure (for example, regression). They are still basic inference techniques that utilize sample characteristics to make conjectures about the original population.

A basic and typical issue facing any type of sampling design is the number of samples that should be taken to be confident in the translation from samples to population. The degree of confidence required should be defined as data quality objectives by the end-user and identify the expected statistical rigor for those objectives to be met. There are extensive texts on types and manners of sampling schemes; these will not be discussed here. This section is geared to determining the minimum data set recommended to work with subsequent sections of the data analysis chapter. In interpreting the results of various forms of data analysis, an acceptable level of statistical error is formulated; this is called Type I error, or alpha (α). Type I error can be defined as the probability of rejecting the null hypothesis (H_0) when this is actually true. In setting the Type I error rate, the Type II error rate is also specified. The Type II error rate, or beta (β), is defined as failing to reject the null hypothesis when it is actually false, i.e., declaring that no significant effect exists when in reality this is the case. In setting the Type I error rate, an acceptable level of risk is recommended; the risk of concluding that a significance exists when this is not the case in reality, i.e., the risk of a "false positive"(Type I error) or "false negative" (Type II error). The concepts of Type I and Type II errors are introduced in Chapter 4 with reference to sampling design and monitoring, and more fully discussed in Chapter 8 with reference to criteria development.

In experimental or sampling design, of greater interest is a statistic associated with beta (β), specifically $1 - \beta$, which is the power of a statistical test. Power is the ability of the statistical test to indicate significance based on the probability that it will reject a false null hypothesis. Statistical power depends on the level of acceptable statistical significance (usually expressed as a probability 0.05 – 0.001 (5% -1%) and termed the α level); the level of power dictates the probability of "success," or identifying the effect. Statistical power is a function of three factors: effect size, alpha (α), and sample size, the relationship between the three factors being relatively complex.

1. Effect size is defined as the actual magnitude of the effect of interest. This could be the difference between two means or the actual correlation between the variables. The relationship between the effect size and power is intuitive; if the effect size is large (for example, a large difference between means) this results in a concomitantly large power.

2. Alpha is related to power; to achieve a higher level of significance, power decreases if other factors are kept constant.

3. Sample size. Generally, this is the easiest factor to control. If the two preceding factors are set, increased sample sizes will always result in a greater power.

As indicated before, the relationship between these three factors is complex and depends on the nature of the intended statistical analysis. An online guide for selecting appropriate statistical procedures is available at: http://www.socialresearchmethods.net/. Software packages for performing power analysis have been reviewed by Thomas and Krebs (1997). Online power calculations have been made available by several statistical faculty and are available at these Web sites: http://www.math.yorku.ca/SCS/, http://calculators.stat.ucla.edu/powercalc/, http://www.surveysystem.com/sscalc.htm, http://www.health.ucalgary.ca/~rollin/stats/ssize/index.html, http://www.stat.ohio-state.edu/~jch/ssinput.html, and http://www.stat.uiowa.edu. Additional Web sites are listed in Chapter 4 that emphasize designs for monitoring with statistical rigor.

Metric response variability can be evaluated by examining the signal to noise ratio along a gradient of nutrient concentrations or loading rates (Reddy et.al., 1999). The power of regression analyses can be determined using the power function for a t-test. Optimization of the design, such as the spacing, number of levels of observations, and replication at each level, depend on the purpose of the regression analysis (Neter et.al., 1983).

Multiple correlation analysis can compound uncertainty and in some instances misidentify correlations due to chance as relevant. Appropriate corrections (e.g., Bonferroni) should be applied to avoid these errors (Rice 1989).

MULTIMETRIC INDICIES

Multimetric indices are valuable for summarizing and communicating results of environmental assessments. Use of multimetric indices is one approach in developing criteria. Furthermore, preservation of the biotic integrity of algal assemblages, as well as fish and macroinvertebrate assemblages, may be an objective for establishing nutrient criteria. Multimetric indices for stream macroinvertebrates and fish are common (e.g., Kerans and Karr 1994, Barbour et.al., 1999), and multimetric indices with benthic algae have recently been developed and tested on a relatively limited basis (Kentucky Division of Water 1993; Hill et.al., 2000). Efforts are underway to develop multi-metric indices of biotic integrity for wetlands, and methods modules are available for characterizing wetland algal, plant, macroinvertebrate, amphibian, and bird communities (http://www.epa.gov/waterscience/criteria/wetlands/). Methods for multi-metric indices are well developed for streams and are readily transferable to wetlands. However, higher trophic levels do not often directly respond to nutrients and therefore may not be as sensitive to relatively small changes in nutrient concentrations as algal assemblages. It is recommended that relations between biotic integrity of algal or vegetation assemblages and nutrients be defined and then related to biotic integrity of macroinvertebrate and fish assemblages in a stepwise, mechanistic fashion. The practitioner should realize, however, that wetlands with a history of high nutrient loadings have often lost the most sensitive species and in these cases higher trophic level species may prove to be the best indicators of current nutrient loadings and wetland nutrient condition.

This section provides an overview for developing a multimetric index that will indicate shifts in primary producers that are associated with trophic status in wetlands. The first step in developing a multimetric index of trophic status is to select a set of ecological attributes that respond to human changes in nutrient concentrations or loading. Attributes that respond to an increase in human disturbance are referred to as metrics. Six to 10 metrics should be selected for the index based on their sensitivity to human activities that increase nutrient availability (loading and concentrations), their precision, and their transferability among regions and habitat types. Selected metrics also should respond to the breadth of biological responses to nutrient conditions (see discussion of metric properties in McCormick and Cairns 1994).

Effects of nutrients on primary producers and effects of primary producers on the biotic integrity of macroinvertebrates and fish should be characterized to aid in developing nutrient criteria that will protect designated uses related to aquatic life (e.g., Miltner and Rankin 1998, King and Richardson 2002).

Another approach for characterizing biotic integrity of assemblages as a function of trophic status is to calculate the deviation in species composition or growth forms at assessed sites from composition in the reference condition. Similarity or dissimilarity indices can be used for the determining the differences in biotic integrity of a wetland in comparison to the reference condition. Multivariate similarity or dissimilarity indices need to be calculated for multivariate attributes such as taxonomic composition (Stevenson 1984; Raschke 1993) as defined by relative abundance of different growth forms or species, or species presence/absence. One standard form of these indices is percent community similarity (PS_c, Whittaker 1952):

$$PS_c = \Sigma_{i=1,s} \min(a_i, b_i)$$

Here a_i is the percentage of the i^{th} species in sample a, and b_i is the percentage of the same i^{th} species in a subsequent sample, sample b.

A second common community similarity measurement is based on a distance measurement (which is actually a dissimilarity measurement, rather than similarity measurement, because the index increases with greater dissimilarity, Stevenson 1984; Pielou 1984). Euclidean distance (ED) is a standard distance dissimilarity index, where:

$$ED = \sqrt{(\Sigma_{i=1}(a_i - b_i)^2)}$$

Log-transformation of species relative abundances in these calculations can increase precision of metrics by reducing variability in the most abundant taxa. However, the practitioner should also be aware that transformation, while reducing variability, often decreases sensitivity and the ability to distinguish true fine scale changes in community and species composition. Theoretically and empirically, we expect to find that multivariate attributes based on taxonomic composition more precisely and sensitively respond to nutrient conditions than do univariate attributes, for instance multimetric algal assemblages (see discussions in Stevenson and Pan 1999).

To develop the multimetric index, metrics should be selected and their values normalized to a standard range such that they all increase with trophic status. Criteria for selecting metrics can be found in McCormick and Cairns (1994) or many other references. Basically, sensitive and precise metrics should be selected for the multimetric index and selected metrics should represent a broad range of impacts and, perhaps, designated uses. Values can be normalized to a standard range using many techniques. For example, if 10 metrics are used and the maximum value of the multimetric index is defined as 100, all 10 metrics should be normalized to the range of 10 so that the sum of all metrics would range between 0 and 100. The multimetric index is calculated as the sum of all metrics measured in a system. A high value of this multimetric index of trophic status would indicate high impacts of nutrients and should be a robust (certain and transferable) and moderately sensitive indicator of nutrient impacts in a stream. A 1-3-5 scaling technique is commonly used with aquatic invertebrates (Barbour et.al., 1999; Karr and Chu 1999) and could be used with a multimetric index of trophic status as well. Using the 95^{th} percentile when developing metrics is an approach that may decrease the influence of outliers (Mack 2004).

7.7 LINKING NUTRIENT AVAILABILITY TO PRIMARY PRODUCER RESPONSE

When evaluating the relationships between nutrients and primary producer response within wetland systems, it is important to first understand which nutrient is limiting. Once the limiting nutrient is defined, critical nutrient concentrations can be specified and nutrient-response relationships developed.

DEFINING THE LIMITING NUTRIENT

The first step in identifying nutrient-producer relationships should be to define the limiting nutrient. Limiting nutrients will control biomass and productivity within a system. However, non-limiting nutrients may have other impacts, e.g., toxicological effects related to ammonia concentrations in sediments or effects on competitive interactions that determine vegetation community composition (Guesewell et.al., 2003). A review of fertilization studies indicated that vegetation N:P mass ratios are a good predictor of the nature of nutrient limitation in wetlands, with N:P ratios > 16 indicating P limitation at a community level, and N:P ratios < 14 indicative of N limitation (Koerselman and Meuleman 1996). Guesewell et.al., (2003) found that vegetation N:P ratios were a good predictor of community-level biomass response to fertilization by N or P, but for individual species were only predictive of P-limitation and could not distinguish between N-limitation, co-limitation, or no limitation. Likewise, N, P, and K levels in wet meadow and fen vegetation were found to be correlated with estimated supply rates or extractable fractions in soils (Odle Venterink et.al., 2002). A survey of literature values of vegetation and soil total N:P ratios by Bedford et.al., (1999) indicated that many temperate North American wetlands are either P-limited or co-limited by N and P, especially those with organic soils. Only marshes have N:P ratios in both soils and plants indicative of N limitation, while soils data suggest that most swamps are also N-limited.

Many experimental procedures are used to determine which nutrient (N, P, or carbon) limits algal growth. Algal growth potential (AGP) bioassays are very useful for determining the limiting nutrient (USEPA 1971). Yet, results from such assays usually agree with what would have been predicted from N:P biomass ratios, and in some cases N:P ratios in the water. Limiting nutrient-potential biomass relationships from AGP bottle tests are useful in projecting maximum potential biomass in standing or slow-moving water bodies. However, they are not as useful in fast-flowing, and/or gravel or cobble bed environments. Also, the AGP bioassay utilizes a single species, which may not be representative of the response of the natural species assemblage.

Limitation may be detected by other means, such as alkaline-phosphatase activity, to determine if phosphorus is limiting. Alkaline phosphatase is an extracellular enzyme excreted by some algal species and from roots in some macrophytes in response to P limitation. This enzyme hydrolyzes phosphate ester bonds, releasing orthophosphate (PO_4) from organic phosphorus compounds (Mullholland et.al., 1991). Therefore, the concentration of alkaline phosphatase in the water can be used to assess the degree of P limitation. Alkaline phosphatase activity, monitored over time in a wetland, can be used to assess the influence of P loads on the growth limitation of algae (Richardson and Qian 1999).

There have been no empirical relationships published relating nutrient concentrations or inputs to wetland chlorophyll *a* or productivity levels as there have been for streams and lakes. This is likely due to the large number of factors interacting with nutrients that determine net ecological effects in wetlands. For example, eutrophication of Great Lakes coastal wetlands and increases in agricultural area in upstream watersheds have been correlated with decreases in diversity of submerged aquatic vegetation, yet researchers were unable to uncouple the effects of nutrients from those of turbidity (Lougheed et.al., 2001). Even in experimentally controlled settings, where it is possible to separate increased suspended solids loadings from nutrient loadings, effects of nutrients depend heavily on other factors such as periodicity of nutrient additions (pulse vs. press loadings; Gabor et.al., 1994, Murkin et al., 1994, Hann and Goldsborough 1997, McDougal et.al., 1997), water regime (Neill 1990a, b; Thormann and Bayley 1997), food web structure (Goldsborough and Robinson 1996), and time lags (Neill 1990a, b). It is important in experimental settings to utilize adequate controls for water additions that may accompany nutrients (Bayley et.al., 1985); in empirical comparisons from field data, it may be difficult if not impossible to separate out these effects. Day et.al., (1988) propose a general conceptual model describing responses of different wetland plant guilds in riverine wetlands based on a combination of disturbance regime, hydrologic regime, and nutrients. In the latter case, proper classification of sites based on disturbance and hydrologic regime prior to describing reference condition help to adequately separate out nutrient-related effects and explain differences in response.

The significance of food web structure in determining nutrient effects does not preclude deriving predictive nutrient-primary producer relationships or minimize the importance of describing significant impacts. However, it does highlight the importance of adequately characterizing the trophic structure of wetlands prior to comparison, especially the number of trophic levels (e.g., presence or absence of planktivorous fish), and examining interactive effects on multiple classes of primary producers: phytoplankton, epipelon, epiphytic algae, metaphyton, and macrophytes (Goldsborough and Robinson 1996, McDougal et al., 1997). In some cases, addition of nutrients may have little or no effect on some components such as benthic algae, but can create significant shifts in primary productivity among others, such as a loss of macrophytes and associated epiphytes with an increase in inedible filamentous metaphyton and shading of the water column (McDougal et al., 1997).