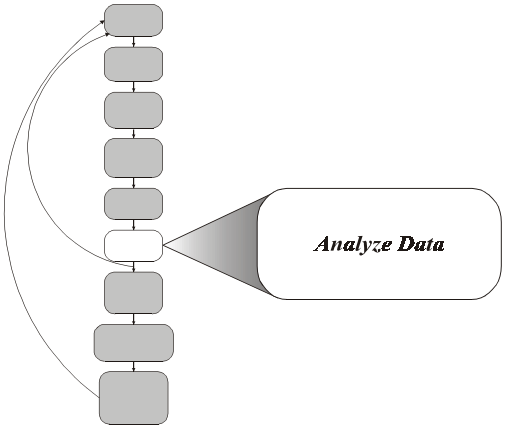
Chapter 6.
Analyze Data



6.1 INTRODUCTION

Data analysis is critical to nutrient criteria development. Proper analysis and interpretation of data determines the scientific defensibility and effectiveness of the criteria. Therefore, it is important to re-evaluate short and long-term goals for stream systems within the ecoregion of concern. These goals should be addressed when analyzing and interpreting nutrient and algal 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. Included are techniques that link relationships between nutrient loading and algal biomass, statistical analyses to evaluate compiled data, and a discussion of computer simulation models.

The difficulty associated with understanding predictive relationships between nutrient loading and algal biomass is perhaps the biggest challenge to establishing meaningful nutrient criteria. Several relatively simple methods of making this link for a variety of stream systems are discussed in this chapter. This chapter also presents more in-depth methods to use when simpler techniques prove inadequate.

Macrophytes depend primarily on sediments for nutrient uptake, and are relatively unaffected by nutrient water column concentrations. However, attempts to relate macrophyte growth or biomass with sediment nutrient content have been largely unsuccessful (Chambers et al. 1999). Links between macrophytes and nutrient enrichment are more indirect than with algae, and are therefore not considered here. A review of macrophytes and the current state of the science can be found in Chambers et al. (1999).

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. The type of statistical analysis required for criteria development will be determined by the source, quality, and quantity of data being analyzed. Concerns to be aware of during statistical analyses are discussed in this chapter. Specific statistical tests that may be useful in criteria development are described in Appendix C.

Models are abstractions designed to represent something real. In this sense, models can be anything from a representation of the human form in plaster, or a statistical equation expressing assumed relationships between parameters of interest. This chapter discusses modeling as mathematical abstractions for the purposes of analyzing data to derive nutrient criteria. Mathematical models can be categorized as process-based or empirical, and are used for different purposes. This guidance focuses on empirical models that serve to illuminate the relationship between the behavior of the system and measurements of one or many attributes of the system. Empirical models identify patterns but do not explain them. In contrast, process-based models are explanatory, and are built of equations that contain directly definable, observable parameters. The rules used for process-based models invoke levels of organization other than the components being modeled (Wiegert 1993).

Empirical models can be simple, statistical models or more complex simulation models. A linear regression of chlorophyll and P (phosphorus) data from a population of streams is a simple empirical model, in that it elucidates the relationship between chlorophyll and P in a single equation represented by a line. A more complex empirical model is the computer simulation model CE-QUAL-RIV1, which is comprised of a set of equations that predicts a constituent concentration over time. Prediction by both linear regression and computer simulation are based on empirical observations of a stream or population of streams. The linear regression described above is an example of a static model; static models do not represent changes over time. Dynamic models, such as CE-QUAL-RIV1, represent changes in system constituents over time (Wiegert 1993).

6.2 LINKING NUTRIENT AVAILABILITY TO ALGAL RESPONSE

When evaluating the relationships among nutrients and algal response within stream 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 and algal biomass relationships can be examined to identify potential criteria to avoid nuisance algal levels. This section will discuss defining the limiting nutrient, establishing predictive nutrient-algal relationships, analysis methods for establishing nutrient-algal relationships, analysis of algal species composition for system response to nutrients, characterizing biotic integrity and response to nutrients, developing a multimetric index of trophic status, assessing nutrient-algal relationships using experimental procedures, and a few other issues to keep in mind while analyzing data.

DEFINING THE LIMITING NUTRIENT

Defining the limiting nutrient is the first step in identifying nutrient-algal relationships. Nuisance levels of algal biomass are common in areas with strong nutrient enrichment, ample light, and stable flow regime. Experimental data have demonstrated that given optimum light, non-scouring flow, and modest to low grazing, enrichment of an oligotrophic stream will usually increase algal biomass and even secondary production (Perrin et al. 1987; Slaney and Ward 1993; Smith et al. 1999). Identification of the limiting nutrient is the first step in controlling nutrient enrichment and algal growth (Smith 1998; Smith et al. 1999). Criteria will be set for both TN and TP, but it is often more cost-effective to reduce the loading of one nutrient (N or P) to achieve reduction of nuisance algal growths.

Nitrogen frequently limits algal growth in streams and some have argued that this might be more common in streams than it is in lakes (Grimm and Fisher 1986; Hill and Knight 1988; Lohman et al. 1991; Chessman et al. 1992; Biggs 1995; Smith et al. 1999). However, there is evidence that P still often limits stream algae (Dodds et al. 1998; Welch et al. 1998; Smith et al. 1999). If nonpoint sources of nutrients predominate (assuming relatively high background levels of N), then N control may be a more important issue than control of P. However, if N limits growth in a stream due to point source discharges such as wastewater with low N:P, then the logical, cost-effective measure to control nuisance biomass is to reduce P input, because N:P should then increase and cause P limitation (see Section 3.3 Secondary Response Variables). If N and P are co-limiting, increasing the concentration of one nutrient will result in the other nutrient becoming limiting (e.g., an increase in N concentrations will result in P becoming limiting). The most prudent approach to controlling nutrient enrichment, regardless of the limiting nutrient, is to set criteria for maxima of N and P, and try to limit inputs of both.

Nitrogen usually becomes more limiting as enrichment increases because (1) wastewater N:P ratios are low, (2) N is increasingly lost through denitrification; (3) P is more easily sorbed to sediment particles than N and, thus, tends to be deposited in the sediment (in a waterbody with enough residence time to allow sedimentation) more effectively than does N (Welch 1992); and (4) P is released from high P-yielding bedrock. However, N lost through anaerobic denitrification may be limited by streamflow aeration, although denitrification rates may still be relatively high if the subsurface (hyporheic and parafluvial) components of the stream ecosystem are considered (see Holmes et al. 1996). Furthermore, P dissolved from bedrock or soil, whether complexed or not, is apt to remain in the water until it reaches a waterbody with enough residence time to allow sedimentation, therefore loss of nutrients via sedimentation is not usually important in most streams.

Although N may be a relatively more important controlling factor for growth in streams than lakes, there is evidence that P can limit stream algae. For instance, ratios of soluble N:P averaged 90:1 (by weight) in seven western Washington streams draining both forested and urbanized watersheds (Welch et al. 1998). Soluble N:TP ratios averaged 13:1 in three other western Washington streams (Welch et al. in press). Even more convincing evidence for a greater prevalence for P limitation in streams comes from the large data set discussed later in this chapter (Dodds et al. 1998). These data show that: 1) TN:TP ratios are nearly all >10:1, and 2) TN:TP ratios declined as enrichment increased from 24:1 (10% of data; TN = 316 and TP = 13 μg/L) to 20:1 (50% of data; TN = 1000 and TP = 50 μg/L) to 12.6:1 (90% of data; TN = 2512 and TP = 100 μg/L). The second point indicates that TN:TP in streams behaves similarly to that in lakes as enrichment increases, i.e., as enrichment increases, the ratio of water column TN:TP declines. An important cause for this may be the high concentration of P in wastewater (N:P = 3:1; Welch 1992) and in the runoff from applied animal manure (N:P $\leq$ 3:1; Daniel et al. 1997). As an in-stream example, DIN to SRP ratios in seven New Zealand streams receiving wastewater averaged 57:1 upstream and 13:1 downstream from effluent inputs (Welch et al. 1992).

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 and revealing the presence of chemical inhibitors (USEPA 1971). Yet, results from such assays usually agree with what would have been predicted from N:P ratios in the water or, especially N:P in biomass. While limiting nutrient-potential biomass relationships from AGP bottle tests are useful in projecting maximum potential biomass in standing or slow-moving water bodies, 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 natural species assemblage response.

Limitation may be detected by other means, such as alkaline-phosphatase activity, to determine if N is actually limiting in spite of a high N:P ratio. Alkaline phosphatase is an enzyme excreted by some algal species in response to P limitation. This enzyme hydrolyzes phosphate ester bonds, releasing orthophosphate (PO_4) from organic phophorus compounds (Steinman and Mulholland 1996). Therefore, the concentration of alkaline phosphatase in the water column can be used to assess the degree of P limitation. Alkaline phosphatase activity, monitored over time in a waterbody, can be used to assess the influence of P loads on the growth limitation of algae (Smith and Kalff 1981).

Periphyton biomass accrual experiments using nutrient-diffusing substrata (Pringle and Triska 1996) are useful for determining the limiting nutrient for a mixed species assemblage in running water and include the important factors of velocity-enhanced, nutrient uptake as well as constraints imposed by mat thickness that are nonexistent with bottle tests (Grimm and Fisher 1986b; Lohman et al. 1991; Pringle and Triska 1996). However, the existing ambient nutrient concentrations produced from the nutrient diffusing substrata and available for algal uptake are largely unknown with such tests.

Another experimental technique to determine ambient nutrient-maximum periphyton biomass potential in running water is with constructed channels, either with controlled light and temperature in the laboratory (Horner et al. 1983) or with natural light and temperature outdoors, along side natural streams (Stockner and Shortreed 1976; Bothwell 1985, 1989; Pringle and Triska 1996). Pringle and Triska (1996) describe methodologies for both nutrient diffusing substrata and in-stream channels.

Correlations between algal biomass and TN and TP (Dodds et al. 1997) indicate that N explains more of the variance than does P, although P may frequently be the limiting nutrient in stream systems. However, these results may be biased by the stream data used in correlation analyses. That is, the systems where nuisance algal biomass has been measured may be primarily N limited, although this may not be a reflection of a tendency for N limitation in all stream systems generally. In addition, sediment-bound particulate P may remain suspended in streams, confounding the relationship between P and algal biomass. Finally, the nutrient that limits growth in the short term may not always be the most cost-effective nutrient to control. Therefore, careful evaluation of nutrient limitation should be undertaken prior to criteria development and restoration efforts.

ESTABLISHING PREDICTIVE NUTRIENT-ALGAL RELATIONSHIPS

Once the limiting nutrient has been identified, the data need to be analyzed to characterize nutrient-algal relationships and patterns that clarify those relationships. Data analyses can provide mathematical approximations of the relationships that will allow prediction of algal biomass as a function of nutrient concentration. Predictive relationships between nutrients and periphyton (or phytoplankton) biomass are required to identify the critical or threshold concentrations that produce a nuisance algal biomass.

Relationships between TP and/or TN and periphytic biomass in streams have relatively low r^2 values on the order of 0.4-0.6 (Lohman et al. 1992; Dodds et al. 1997). Therefore, the following considerations need to be taken into account when establishing predictive nutrient-algal relationships. Critical and

highly variable factors other than nutrients – shading, type of attachment surfaces, scour, water level fluctuations that result in dessication, grazing intensity – have major effects on algal biomass levels and may provide an explanation for the weakness of the predictive relationships in streams. In addition, TP in the stream water column contains more sediment-and detrital-bound P than observed in lakes, and sediment-bound P is not necessarily available for algal uptake. The high detritus level in streams is indicated by TP versus chl *a* per volume (i.e., seston) relationships in streams where chl *a*/TP ratios ranged from 0.08 to 0.22 (Van Nieuwenhuyse and Jones 1996). These ratios suggest that the high detritus levels in streams are indicative of high proportions of water-column P bound to sediment or heterotrophic components of detrital material. Finally, inorganic nutrient species (PO_4 and NO_3) are frequently more available for uptake, and may need to be considered in instances where small scale effects from specific point and nonpoint sources are an important issue.

There are few existing relationships that predict algal biomass as a function of TN and TP. Dodds et al. (1997) compiled and analyzed the largest and broadest dataset (approximately 200 sites) in the literature that predicts relationships for benthic algal biomass. The best general approach for predicting mean suspended chlorophyll was developed using data from 292 temperate streams (Van Nieuwenhuyse and Jones 1996). The equations derived from these analyses are presented for use with periphyton-dominated and plankton-dominated systems, respectively.

The equations suggested by Dodds et al. (1997) are recommended to predict benthic algal biomass if more local, ecoregion-specific relationships are unavailable:

$$\log (\textbf{\textit{mean chl a}}) = 1.091 + \log (TP) * 0.2786 \quad (r^2 = 0.089)$$

$$\log (\textbf{\textit{mean chl a}}) = 0.01173 + \log (TN) * 0.5949 \quad (r^2 = 0.35)$$

$$\log (\textbf{\textit{maximum chl a}}) = 1.4995 + \log(TP) * 0.28651 \quad (r^2 = 0.071)$$

$$\log (\textbf{\textit{maximum chl a}}) = 0.47022 + \log (TN) * 0.60252 \quad (r^2 = 0.28)$$

where seasonal mean and maximum benthic chlorophyll are in mg/m^2 and TN and TP are in µg/L. The above equations are fairly simple and, although they have low r^2 values, are best suited for use with data having high TN and TP concentrations. Note that the graphical illustration of the relationships from which these equations were derived, shows a broad distribution of the data (Figure 7). This distribution suggests that periphytic algae tend to respond in a similar fashion to nutrients, regardless of location.

A second set of equations, also derived by Dodds et al. (1997), combines TN and TP measures resulting in higher r^2 values, but may be inaccurate in some high nutrient situations.

$$\log (\textbf{\textit{mean chl}}) = -3.233 + 2.826(\log TN) - 0.431(\log TN)^2 + 0.255(\log TP) \quad (r^2 = 0.43)$$

$$\log (\textbf{\textit{max chl}}) = -2.702 + 2.786(\log TN) - 0.433(\log TN)^2 + 0.306(\log TP) \quad (r^2 = 0.35).$$

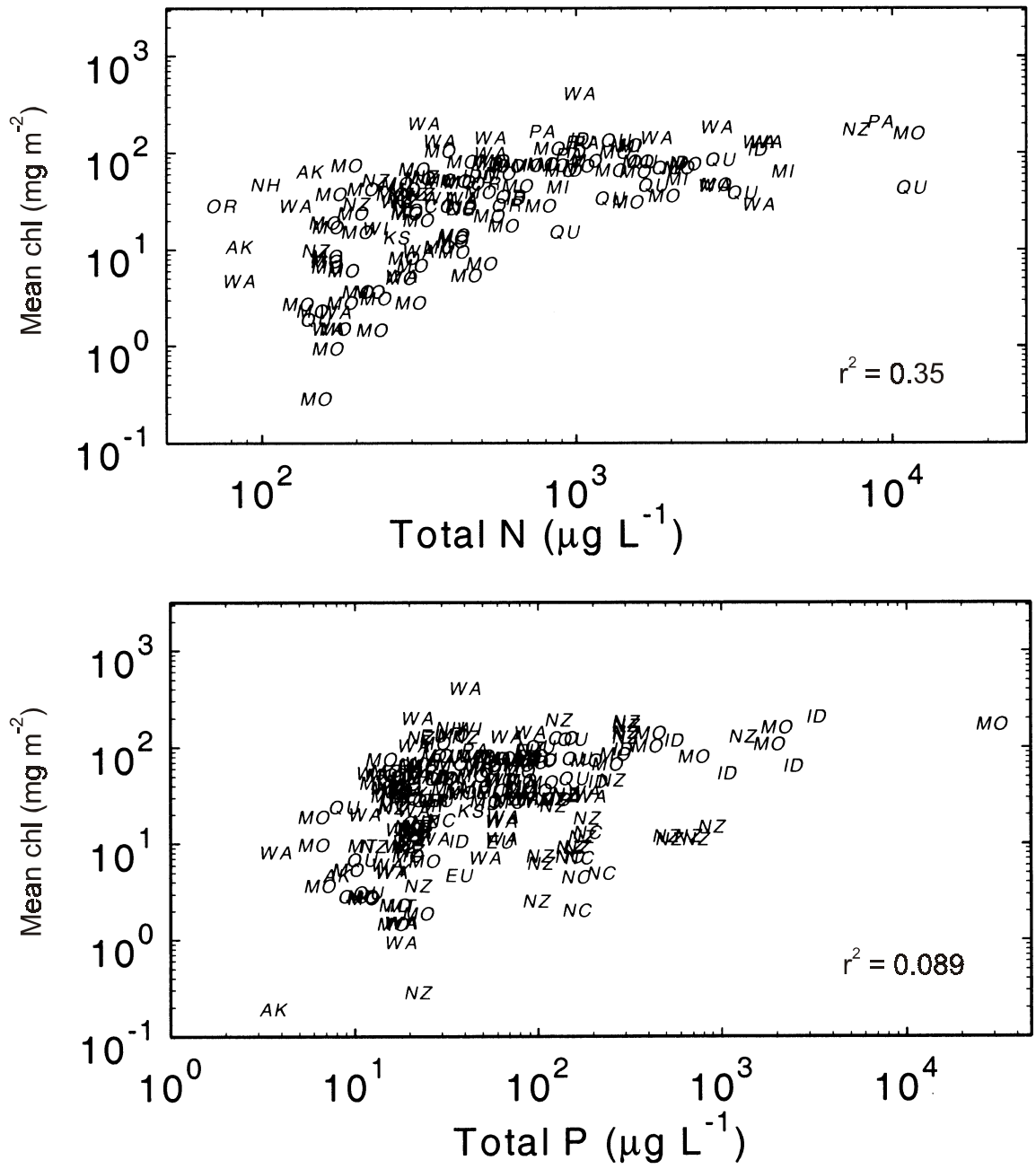


Figure 7. Relationships of log-transformed mean chlorophyll *a* as a function of TN and TP.

Data points are represented by abbreviations identifying the State or country of origin: AK- Alaska, ID- Idaho, MI- Michigan, MO-Montana, NH-New Hampshire, NC-North Carolina, OR-Oregon, PA- Pennsylvania, WA-Washington, QU-Quebec, EU-Europe, NZ-New Zealand.

It should be kept in mind that there is considerable variance in these relationships, and if extensive data for a single system are available, tighter predictive relationships may be constructed. More local, ecoregion-specific data sets should produce tighter relationships.

The equation suggested by Van Nieuwenhuyse and Jones (1996) is recommended to predict mean suspended chlorophyll if more local, ecoregion-specific relationships are unavailable:

$$\log Chl = -1.65 + 1.99(\log TP) - 0.28(\log TP)^2 \quad (r^2 = 0.67)$$

Where chl = summer mean chlorophyll and TP are expressed in mg/m^3.

Yields of algal biomass from given nutrient concentrations derived from regression models differ from the yield observed in controlled channel experiments. This discrepancy creates a problem when attempting to predict nutrient-periphyton chl *a* relationships in streams. For example, to produce a mean chl *a* of 100 mg/m^2 would require approximately 100-200 µg/L TP according to regression models of Lohman et al. (1992) and Dodds et al. (1997). Brezonik et al. 1999 used the equation from Van Nieuwenhuyse and Jones (1996) that includes the catchment size (basin area) to predict likely improvements in concentrations of growing season mean chl *a* that would occur with corresponding reductions in growing season mean TP.

$$\log Chl = -1.92 + 1.96(\log TP) - 0.30(\log TP)^2 + 0.12(\log A_c) \quad (r^2 = 0.73, n = 292)$$

Where A_c = stream catchment area.

They predicted that a reduction of streamwater TP from 125 to 100 µg/L would result in a chl *a* reduction of 18%, and a TP reduction from 50 to 25 µg/L would result in a chlorophyll *a* reduction of 52%. However, in-channel experiments have produced 600 to 1000 mg/m^2 chl *a* in a mixed algal assemblage using in-channel SRP and TP concentrations of 10-15 and 20-50 µg/L , respectively, a yield of ~10-50 chl *a*/TP (Horner et al. 1983, 1990; Walton et al. 1995; unpublished data). This seeming discrepancy may result from the nutrient demand by heterotrophic organisms in the detritus of natural streams. Residence time was short (16 minutes or less) in the above cited channel experiments, nutrient input was controlled to low levels, and velocity was usually constant with little sloughing during the growth period (Horner et al. 1990). Such characteristics would generate little detritus and low ambient TP and, hence, higher in-channel chl *a*/TP ratios than in natural streams sampled throughout the year.

The discrepancy in algal biomass yield between regression models and channel experiments may partly justify the use of regression models generated from large field data sets in recommending nutrient criteria. Channel data are not significantly confounded by the sloughed biomass that produces detrital material in natural streams and is unavailable for uptake and algal biomass increase. Although the correlation between chl *a* and nutrients in natural streams may be weakened (from the cause-effect standpoint) due to interference with detritus, the relations may nonetheless be useful for extrapolation and management because nutrient criteria must be applied where high detritus levels do exist.

Soluble nutrient concentrations determine periphytic growth rate and biomass; uptake is clearly saturated at very low (<10 µg/L SRP) concentrations (Bothwell 1985, 1989; Walton et al. 1995) and is independent

of TP concentrations. However, soluble nutrients are usually lowest when biomass is highest, due to depletion by algal uptake, similar to the situation in lakes. Therefore, estimates of inflow nutrient concentrations, in-stream concentrations during non-growth periods or at least annual mean concentrations are required to use soluble nutrients to set critical levels and relate soluble nutrients to algal biomass. These data/relationships are not currently available, but should be pursued in order to develop more direct, stronger nutrient-biomass relationships for streams.

ANALYSIS METHODS FOR ESTABLISHING NUTRIENT-ALGAL RELATIONSHIPS

The following analysis methods are suggested to develop predictive nutrient-biomass relationships in stream systems. These methods were primarily developed for gravel/cobble bed streams, but should function for other stream types with modifications. Intermittent and effluent-dominated streams will benefit from supplemental analysis methods specific to those stream types as the seasonal sampling discussed here may not be possible (see Appendix A). Samples for soluble and/or total N and P should be collected for at least one, preferably two or more years at sites with high as well as low summer biomass. Ideally, samples for periphyton biomass should be collected weekly or biweekly during summer low flow, beginning immediately after spring runoff or any subsequent high water, scouring event. Monthly data collection may be sufficient to define algal-nutrient relationships if supporting long-term trend data is available. Data can be analyzed using one or all of the following methods to establish predictive nutrient-biomass relationships in stream systems.

1. Relate the total concentration of a limiting nutrient (e.g., TN, TP) with the mean and maximum algal biomass as chl *a*; both data sets should be collected at the same time during summer (or season of maximum algal biomass). Such data were used by Dodds et al. (1997) to develop the relationship between nutrients and algal biomass discussed in the previous section. Relate the low/non-growing period mean concentration of limiting nutrient to summer maximum biomass as chl *a*.

2. It may also be possible to relate the pre-maximum growth period (usually spring, immediately following runoff) mean soluble limiting nutrient concentration to maximum algal biomass. Inorganic soluble N (ammonium and nitrate) should be used as the limiting nutrient if the N:P (soluble) is <10 (by weight) and SRP should be used if N:P >10. The threshold of 10 is chosen to simplify the assessment protocol, although N and P are known to be co-limiting over a rather wide range in N:P ratio (7-15) (Smith 1982; Welch 1992). Data should be stratified into discrete ranges of N:P ratios, if this approach does not produce sound relationships, in a manner similar to the methods used by Prairie et al. (1989).

   This analysis selects data that would most closely represent an "inflow concentration" of dissolved inorganic limiting nutrient because it utilizes the available form of the designated limiting nutrient during a period when algal nutrient uptake is minimal. The pre-growth period nutrient concentration should be analogous to the inflow limiting nutrient concentration (including groundwater) entering a continuous algal culture system, whether planktonic or periphytic, that yields a maximum steady-state biomass. Analysis of N and P loading could be used for this assessment in stream systems, though it has not been tested. However, because rivers, streams, lakes, and estuaries form a linked system in the context of a watershed, load analysis becomes

crucial at watershed scales. Relationships can be sought for TP and TN using this method and in method 3 below, which may be more appropriate for criteria throughout an ecoregion, although less specific for given streams.

3. Relate annual mean soluble nutrient concentration to the 75th percentile mean algal biomass. This approach does not provide sound continuous culture rationale like inflow concentration-maximum biomass relationships, but annual mean values for nutrients were used in the cellular N and flood frequency versus chl *a* relationship discovered by Biggs (1995), as well as soluble N and P concentrations versus maximum chl *a* for different accrual times (Biggs 2000). In instances where nutrient data are inadequate to provide distinct and reliable values used in method 2 above, an annual mean approach may offer a reasonable approximation of nutrient availability.

4. Another possibility for developing strong predictive relationships is the use of cellular concentrations of limiting nutrient (same ratio criterion used in 2 above) determined during the summer growth period, related to maximum algal biomass. This approach estimates the available nutrient directly from physiologically relevant data, as opposed to using the pre-growth soluble fractions in water to infer what is available for uptake. The validity of this approach is supported by a multiple relationship among cellular N, chl *a*, and flood frequency, in which cellular N content varied over a range of four-fold (Biggs 1995). A sound relationship between cellular nutrient content and periphytic algal biomass would, however, still require a link to the respective limiting nutrient concentration in water for management purposes. That could be accomplished by developing a relationship between cellular nutrient and ambient nutrient concentrations (either soluble or total) using constant flow laboratory channel experiments.

As further evidence for the potential of this approach, Wong and Clark (1976) described a direct relationship (r^2=0.80) between cellular P and ambient TP in six rubble-bed streams in Ontario, such that;

$$TP_w = 0.05\ P_t - 0.02$$

where P_t is tissue content, and TP_w is ambient water column TP. They determined further that photosynthetic rate of *Cladophora* at optimum light availability, decreased below 1.6 mg P/g dry weight, which was equivalent to 60 mg/L TP in the water. Nevertheless, this had no predictive value for maximum biomass. Development of a relation between cellular limiting nutrient and biomass, instead of productivity, would be necessary to back- calculate to ambient nutrient content, either soluble nutrient as in methods two or three above, or total nutrient as from method one and Wong and Clark (1976).

ANALYSIS OF ALGAL SPECIES COMPOSITION TO CLASSIFY STREAM RESPONSE TO NUTRIENTS

Differences in algal species composition among streams can identify important regional environmental gradients that may affect algal-nutrient relationships. Algal species composition should be used in data analysis to validate stream classification and enable development of indicators of nutrient conditions and the likelihood of nuisance algal blooms. Different classes of streams may require different nutrient criteria, depending upon algal responses to nutrients in different stream classes. For example, algal-nutrient problems may be related to proliferation of filamentous green algae *Cladophora* or *Spirogyra*, benthic or planktonic diatoms, dinoflagellates, or blue-green algae. Each of these problems may occur at

different nutrient concentrations, but will probably only occur in certain classes of streams during specific seasonally-optimum conditions (Biggs et al. 1998b).

Cluster analysis is used to identify groups of streams with similar algal assemblages. TWINSPAN (Two Way INdicator SPecies Analysis; Hill 1979) and UPGMA (Unweighted Pair Group Method using Arithmetic averages; Sneath and Sokal 1973) represent two examples of cluster analysis that are commonly used and differ in how results are generated. TWINSPAN employs a divisive approach in which all algal assemblages are initially grouped in one cluster and then that cluster is divided into two groups based on the greatest dissimilarities between the groups. Subsequently, each cluster is divided into two more clusters so that one cluster becomes two, two becomes four, four becomes eight, and eight becomes 16, etc. In contrast, UPGMA is an aggregational technique that begins with all algal assemblages separated into single assemblage clusters and builds clusters by aggregation of the most similar clusters. So N clusters becomes N-1 clusters, and N-1 clusters becomes N-2 clusters, and so on. At each step, one algal assemblage is grouped with another assemblage or group of assemblages. Results of both techniques can be used together by identifying groups of assemblages (and associated streams) that cluster the same in both analyses. These groups can be designated as core clusters. Assemblages that are not grouped in the same clusters in both analyses can be associated with core clusters based on some simple evaluation, such as percent similarity to assemblages in the core cluster.

Cluster analysis of algal assemblages can be used as one step in classifying streams based on their response to nutrients (e.g., Pan et al. in press). Habitat classification is based on assemblages in reference conditions, because human impacts may constrain species membership in assemblages and mask diversity among stream classes and impacts that nutrients have on that diversity. In addition, algal assemblages in different classes of streams may respond differently to nutrient addition (Biggs et al. 1998b). The number of stream classes that should be used depends on many factors, but the number should be limited based on practicality, utility in explaining algal responses to nutrient enrichment, and utility in explaining algal responses to remediation. In addition, statistical significance of clusters, based on discriminate analysis for example, can also form the basis for determining the number of stream classes. Algal assemblage clusters can be related to the physical classification (described in Chapter 2), to predict responses of similar stream classes to further enrichment or remediation (Biggs et al. 1998b).

The form of species composition data used in classification of stream algal assemblage, and other analyses as well, has a substantial effect on resolution of patterns that are related to the phenomena with which we are concerned. Algal species composition data based on species densities (cells/cm^2), relative abundance (% of assemblage), and presence/absence differ successively in sensitivity to diurnal and daily changes in environmental conditions. Both theoretically and in practice, species composition data based on species densities are more sensitive to small-scale spatial and temporal variability than are data based on species relative abundances and presence/absence data (Stevenson unpublished data). Most stream classification analyses should be done with relative abundances because they integrate over space and time and most results in the literature are presented in this form.

Ordination helps to visualize differences in species assemblages among classes of streams. When species composition is combined with environmental data or algal autecological characteristics, the important environmental factors affecting species composition in a region can be deduced. These environmental factors may be important for constraining algal response to nutrient concentration and may therefore be

important for identifying confounding factors in the relationship between algal assemblages and nutrient conditions. Caution should be exercised in using ordination to develop attributes of algal assemblages for use in establishing nutrient criteria. Ordination scores for taxa and classifications will change as new data are added and ordinations are recalculated. Therefore, ordinations should not be recalculated after a standard classification system or assessment system has been established. Species scores based on the original ordination should be used in subsequent classifications and assessments (Barbour et al. 1999).

CHARACTERIZING NUTRIENT STATUS WITH ALGAL SPECIES COMPOSITION

Theory and empirical evidence indicate that algal species composition may be a more precise indicator of nutrient status and the potential for nuisance algal problems than one-time sampling and assessment of nutrient concentrations and algal biomass. Shifts in algal species composition may be more sensitive to changes in nutrient concentrations and may therefore help define nutrient criteria. Many monitoring programs utilize multiple lines of evidence to increase the certainty of assessments. Algal species composition, as well as growth form and mat chemistry, can provide evidence of nutrient condition and a greater certainty of assessing nutrient conditions. This topic has been the subject of many recent reviews (McCormick and Cairns 1994; Kelly et al. 1995; Whitton and Kelly 1995; Lowe and Pan 1996; Stevenson 1998; McCormick and Stevenson 1998; Wehr and Descy 1998; Kelly et al. 1998; Ibelings et al. 1998; Stevenson and Pan 1999; Stevenson and Bahls 1999; Stoermer and Smol 1999; Stevenson in press).

Species composition and autecological characteristics of algae are commonly used to evaluate environmental conditions, ranging from organic (sewage) contamination to pH and nutrient conditions (Kolkwitz and Marsson 1908; Zelinka and Marvan 1961; Renberg and Hellberg 1982; Charles and Smol 1988; Whitmore 1989; Kelly and Whitton 1995; Pan et al. 1996). With diurnal and weekly variability in environmental concentrations within streams due to metabolic and weather-related factors or periodic releases of pollution from point sources, it is assumed that the biological assemblages that develop over longer periods of time are adapted to the average conditions in those habitats and tolerant to the environmental maxima and minima. Thus, if environmental tolerances and sensitivities of organisms are known, the physical, chemical, and potentially biological conditions for a habitat can be inferred if environmental effects differed among species.

Autecological characteristics, the environmental preferences for specific taxa, are frequently documented in the literature, particularly for diatoms (see van Dam et al. [1994] or Stevenson and Bahls [1999] for a literature list). Autecological characteristics have been compiled and summarized in several publications (Lowe 1974; Beaver 1981; Van Dam et al. 1994). Accuracy of the autecological characterizations in these compilations is limited to multi-category classification systems. For example, a categorical characterization of nutrient sensitivity might vary with the integers from 1-5, where 1 would be assigned to species least sensitive to low nutrients and 5 would indicate taxa most sensitive to low nutrients (van Dam et al. 1994). Thus, high abundance in a habitat of taxa classified as 5 would indicate highly eutrophic conditions. In contrast, more accurate characterizations of algal taxa have been achieved recently by using weighted averages of species relative abundances and a quantitative assessment of the environmental conditions in which they are observed (e.g., ter Braak and van Dam 1989; Birks 1988). The result is an accurate assessment of the specific environmental conditions in which a species will have its highest relative abundance (environmental optima). The weighted average approach assumes that species have optima along environmental gradients if each gradient (nutrients, pH, salinity, organic

contamination) includes a broad range of conditions that includes most of a species range. These weighted average descriptions of species autecologies have been developed for optimal total phosphorus concentrations in streams (Pan et al. 1996).

A trophic status indicator (TSI) can be calculated by summing the products of species relative (proportional) abundances (p_i, ranging from 0-1) and their autecological characterization for trophic status (Θ_i) for all *i* species:

$$TSI = \sum_{i=1,s} p_i \Theta_i$$

If all *i* species do not have autecological characteristics, normalize the index by adjusting description of the community to only those taxa that have autecological characteristics:

$$TSI = \sum_{i=1,s} p_i \Theta_i / \sum_{i=1,s} p_i$$

Weighted average indices can be calculated easily with a spreadsheet. The weighted average formula can be used with categorical or weighted average autecological characterizations; see Kelly and Whitton (1995) and Pan et al. (1996) respectively. When indices are used with the highly accurate environmental optima determined by weighted average regression, they actually infer the phosphorus concentration or nitrogen concentration in the stream (Pan et al. 1996). Comparisons of precision of inferring TP concentrations with weighted average indicators and one-time measurement of TP concentration in a stream show that diatom indices are more precise (Stevenson and Smol in press).

Kelly and Whitton (1995) make several adjustments to sample processing and index calculation that make processing easier while maintaining index performance and distinguishing between organic and inorganic nutrients. They make sample processing easier by only counting 200 diatoms and a single set of diatom taxa that are easy to identify and that are good indicators of nutrient condition (Kelly 1996). Weights of species can be added to this formula to decrease the importance of taxa that have a broad tolerance to trophic status (see formula in Kelly and Whitton 1995), but they may not improve precision of the indices (Pan et al. 1996). Finally, autecological information is also available for assessing organic (sewage) contamination in waters. This information can be used with a TSI to distinguish enrichment effects due to inorganic and organic pollution Kelly and Whitton (1995).

Most autecological characteristics of diatom taxa have been described from European populations. Further testing will be important to determine how well autecological characterizations of taxa found in Europe compare to those in North America. However, these autecological indices should be useful for general classification of relative trophic status in streams when reference conditions and relations between changes in species composition and nutrient concentrations have not been established. The relative benefits of more accurately defining autecological characteristics with weighted averages versus coarse scale categories have not been thoroughly evaluated. Investigations have shown that inferences of environmental conditions based on indices using weighted average autecologies are more precise than those using categorical autecologies (ter Braak and van Dam 1989; Agbeti 1992). Tradeoffs may exist between greater precision for indices that are calculated with weighted average autecologies when they are used in conditions similar to those where the autecologies were developed versus less error associated with categorical autecologies when indices are used across broad diverse regions. Details and references

to development of algal indices of environmental conditions can be found in recent reviews (Birks 1998; Stoermer and Smol 1999, Stevenson and Pan 1999; Stevenson and Smol in press).

DEVELOPING MULTIMETRIC INDICES TO COMPLEMENT NUTRIENT CRITERIA

Multimetric indices are valuable for summarizing and communicating results of environmental assessments and may be developed as an alternative to numeric 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 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). However, fish and macroinvertebrates do not directly respond to nutrients, and therefore may not be as sensitive to changes in nutrient concentrations as algal assemblages. It is recommended that relations between biotic integrity of algal assemblages and nutrients be defined and then related to biotic integrity of macroinvertebrate and fish assemblages in a stepwise, mechanistic fashion. This section provides an overview for developing a multimetric index that will indicate algal problems that are associated with trophic status in streams.

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 in streams. Attributes that respond to an increase in human disturbance are referred to as metrics. Six to ten 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 should also respond to the breadth of biological responses to nutrient conditions (see discussion of metric properties in McCormick and Cairns 1994; Stevenson and Smol in press).

Many structural and functional attributes of algal assemblages can be used to characterize the biotic integrity of algae (McCormick and Cairns 1994; Stevenson 1996; Kelly et al. 1998; Stevenson and Pan 1999). Biomass, species composition, species diversity, chemical composition, productivity, respiration, and nutrient turnover rates (spiraling distance) are examples of these attributes. All of these attributes are important and respond with different lag times to spatial and temporal variability in environmental conditions. Most monitoring programs measure structural attributes because structural characteristics vary less than functional characteristics on diurnal and daily time scales. For example, state monitoring programs (e.g., KY, MT) rely on changes in species composition, rather than biomass and chemical composition, to assess ecological conditions in streams because species composition is hypothesized to vary less. However, the relationship between all algal attributes, if characterized for an appropriate time and space, can be related to nutrient concentrations to determine the effect of nutrients on algal assemblages in streams.

Many algal metrics can be used to characterize the valued ecological attributes that we want to protect in a habitat or the nuisance problems that may develop as a result of nutrient enrichment. These are "response" or "condition" metrics (Paulsen et al. 1991; Barbour et al. 1999) and they should be distinguished from "stressor" or "causal" indicators, such as nutrient concentrations (water chemistry or periphyton chemistry) and biological indicators of nutrient concentrations. While both "response" and "stressor" metrics could be used in a single multimetric index, we recommend that separate multimetric

indices be used for "response" and "stressor" assessment. Distinguishing between "response" and "stressor" indices can be accomplished utilizing a risk assessment approach with separate hazard and exposure assessments that are linked with response-stressor relationships (USEPA 1996; Stevenson 1998; Barbour et al. 1999; Stevenson and Smol in press). A multimetric index that specifically characterizes "responses" can be used to clarify goals of management (maintainance or restoration of valued ecosystem attributes) and to measure whether goals have been attained with nutrient management strategies.

Measurements of nutrient concentrations and algal indicators of nutrients could be combined to develop a multimetric "stressor" index specifically for nutrient conditions. Metrics of nutrient concentrations such as water and mat chemistry (µg P/mg AFDM, µg N/mg AFDM) are described in Appendix C and are relatively straight forward. Biological indicators of nutrient concentrations are described in the above section, Characterizing Nutrient Status with Algal Species Composition. The following paragraphs discuss algal metrics that characterize valued ecological attributes and nuisances.

Algal metrics can be distinguished with respect to types of designated use that is being impaired. Algal biomass can be measured as percent cover by filamentous algae, turbidity, mg chl *a*/m^2, g AFDM/m^2. Determining when biomass becomes a nuisance will require relating biomass to designated uses, such as support of aquatic life (biotic integrity), or potability. Effects of nutrients on algal biomass and effects of algae 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). Potability can be impaired by algae that cause taste and odor problems and whose growth may be stimulated by nutrients. Thus, relationships should be developed between nutrients and taste and odor producing algae or nutrients and the frequency of taste and odor complaints to develop management plans and criteria to support potability as a designated use. Relative abundance or biomass of taste and odor algae (Palmer 1962) may be good indicators of the potential for potability problems. Percent toxic algae could provide indicators of potential for toxic algal blooms in streams at low flow in which wildlife and livestock could be endangered, although little is known about the effects of toxic algae in streams.

Biotic integrity of algal assemblages may be indicated by many quantitative attributes of algal assemblages (Stevenson 1996; Stevenson and Pan 1999). Attributes of species composition can be characterized at different levels of resolution, e.g., actual biomass (biovolume/cm^2), relative biovolume relative abundances, cell density, or presence/absence at each taxonomic level. Relative biovolume is usually used to characterize changes in functional groups (as defined by physiognomy and taxonomic division) of algae in assemblages because cell sizes vary so much among functional groups (e.g., filamentous cyanobacteria, colonial cyanobacteria, diatoms, and large cells of filamentous green algae). Relative abundances are usually used to characterize changes in species composition of specific groups of taxa, such as diatoms. Many environmental programs only evaluate diatom assemblages for species level indicators (e.g., Kentucky Division of Water 1993; Pan et al. 1996; Kelly et al. 1998).

Even though many taxonomic attributes of algal assemblages would be expected to change in response to increasing nutrient concentrations, analyses should be focused to some extent on variables that have intrinsic value. Thus, changes in relative biovolume from non-nuisance algae (e.g., diatoms) to filamentous green algae with nutrient addition may be an indicator of loss in biotic integrity, because habitat structure and food availability for invertebrates (e.g., Holomuzki et al. 2000). Loss of species may

be an issue: such as some macroalgae that are relatively sensitive to nutrient enrichment and overgrowth by diatoms (e.g., filamentous red algae or some nitrogen-fixing, blue-green algae such as *Nostoc*).

Another approach for characterizing biotic integrity of algal assemblages as a function of trophic status in streams is to calculate the deviation in species composition or algal growth forms at assessed sites from composition in 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 algal growth forms or species, or species presence/absence. One standard form of these indices is percent community similarity (PS_c, Whittaker 1952):

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

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

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

log-transformation of species relative abundances in these calculations can increase precision of metrics by reducing variability in the most abundant taxa. 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 of algal assemblages (see discussions in Stevenson and Smol accepted). High precision and sensitivity argues for including assessments of algal species composition and its response to nutrient conditions in the process of developing nutrient criteria. The response of algal species composition to increases in nutrient concentrations can be used as another line of evidence to develop a rationale for specific nutrient criteria in specific classes of streams.

To develop the multimetric index, metrics must 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 ten 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 stream. A high value of this multimetric index of trophic status would indicate high impacts of nutrients in a stream 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.

Arguments have been made for limiting membership of metrics in a mulitmetric index to only biological metrics and only biological metrics from one assemblage of organisms (Karr and Chu 1999). We

generally concur with that recommendation. More detailed descriptions of this multimetric index development can be found in Karr and Chu (1999), Barbour et al. (1999), and Hill et al. (2000)

ASSESSING NUTRIENT-ALGAL RELATIONSHIPS USING EXPERIMENTAL PROCEDURES

Management of nutrients to ensure high stream quality is greatly strengthened by examining relationships between the limiting nutrient and maximum algal biomass (i.e., potential) that will occur if/when other factors are optimum. Relationships between ambient nutrient content and existing biomass may not adequately predict maximum biomass potential for any single stream because other factors, such as light, high-flow scouring, and grazing often limit biomass accrual in natural streams. Experimental procedures are valuable for determining the maximum biomass potential of a system. However, physical constraints imposed in experimental setups are often unrealistic. Thus, the value of extrapolating results from laboratory experiments to natural conditions is often uncertain. There are many more experimental results reported to determine which nutrient (N, P, or carbon) limits algal growth, than to determine nutrient-biomass relationships. Experimental procedures to determine the limiting nutrient/s for algal growth are discussed earlier in this section (see Defining the Limiting Nutrient).

As indicated previously, biomass levels up to 1000 mg/m^2 chl *a* were accrued on stones of in-stream channels receiving as little as 10 mg/L SRP (Walton et al. 1995). Although *Cladophora* has not been grown in channels, other filamentous green algae (FGA) (*Mougeotia*, *Stigeoclonium*, *Ulothrix*) have dominated in such experiments. In contrast, bottle tests with unattached *Cladophora* have shown that growth/biomass is not saturated at such low SRP concentrations (Pitcairn and Hawkes 1973), indicating results from flowing-water channel experiments more closely represent natural systems. Nevertheless, Bothwell (1989) did show added accrual of diatom films from about 250 mg/m^2 chl *a* at an SRP of 5 µg/L, increasing to 350 mg/m^2 at about 50 µg/L.

There may be problems with achieving a species assemblage in channel experiments that is representative of the natural stream(s) in question. In fact, accurate prediction or even chatacterization of ambient assemblages in dynamic systems may be challenging. *Cladophora* has been difficult, if not impossible, to establish in such systems, and other FGA have not established on Styrofoam substrata (used by Bothwell 1985), even when abundant in the source stream. Diatoms are usually first to establish, with more time required for FGA to colonize due to their more complex reproduction requirements. Natural stones seem to be the most effective substratum for colonizing either diatoms or FGA in these systems, but resulting dominant taxa in channels may not replicate exactly as in natural streams, even though channels are inoculated from stream rocks. Moreover, diatoms may, in fact, dominate the biomass in channels even though FGA establishes and appears most abundant to the eye. Correctly predicting community composition in future stages of succession is very difficult, even in simple systems. Given the complexity inherent in dynamic ecosystems, only excessively broad predictions may be possible. Data gathered from channel experiments may be little better at characterizing process than a grab sample is at characterizing water chemistry. Only simple extrapolations can be made employing data gathered from simple systems.

Caution is recommended in applying nutrient-biomass relationships developed in channel experiments to natural streams, primarily for two reasons: (1) TP and TN content required to produce a maximum biomass will probably be higher in natural streams than in channels, as previously discussed, because more detrital TP and TN will accumulate in enriched natural streams than in short-detention time

channels. Hence, the yield (i.e., slope of regression line) of chl *a*/TP or TN in channels will be greater. (2) The more or less continual input of soluble nutrients from groundwater to the natural stream is usually unknown, so inflow soluble nutrient-maximum biomass relations from short-detention time channels may not be applicable to natural streams where in-stream soluble nutrients are low as a result of algal uptake during long travel times, yet may have a relatively high inflow concentration of soluble nutrients.

OTHER ISSUES TO KEEP IN MIND

Changes in certain physical factors including: (1) riparian vegetation; (2) total suspended solids (TSS); (3) reduced flow following scouring-flood conditions; (4) greatly reduced summer flow due to prolonged drought (somewhat common); or (5) reduced grazing may cause nuisance algal growths in stream systems. Identifying the controlling physical constraint(s), should be rather straightforward. If the stream is shaded, available light at the streambed should be measured to determine the extent to which photosynthesis is inhibited (Jasper and Bothwell 1986; Boston and Hill 1991). Shading can substantially reduce production (Welch et al. 1992), even though photosynthesis of periphyton is usually saturated at relatively low intensities (<25% full sunlight; Boston and Hill 1991). Turbidity can inhibit periphyton at relatively low levels (>10 NTU) (Quinn et al. 1992).

Biggs (1996) argued that flood disturbance is "perhaps the fundamental factor" determining the physical suitability for algal accrual in unshaded streams. Floods act as a "reset" mechanism, initiating a new cycle of accrual, succession, and loss due to grazing. Post-flood (scour) accrual rates are related to enrichment level (Lohman et al. 1992). The role of scouring high flow should be readily discernible from flow records and the seasonal pattern of periphyton accrual (Biggs 1996).

Flow can also regulate biomass. For example, *Cladophora* was observed to reach high biomass followed by senescence and detachment from substrata in enriched, unregulated northern California rivers, which experienced winter flooding and scour (Power 1992). In regulated rivers, where the flood, scour, and re-growth phenomenon did not occur, low biomass levels of *Cladophora* were maintained through grazing.

6.3 STATISTICAL ANALYSES

Statistical analyses are used to identify variability in data and to elucidate relationships among sampling parameters. Several statistical approaches for analyzing data are mentioned here. We advocate simple descriptive statistics for initial data analyses, i.e., calculating the mean, median, mode, ranges and standard deviation for each parameter in the system of interest. The National Nutrients Database discussed in Chapter 5 will calculate simple descriptive statistics for queried data. Creating a histogram or frequency distribution of the data for the class of streams of concern can identify the nutrient condition continuum for that class of streams. Specific recommendations for setting criteria using frequency distributions are discussed in Chapter 7, although the basis for the analysis is discussed here. Methods of statistical analyses are included in Appendix C to provide relevant references for the investigator if additional analyses are needed to understand and interpret data for criteria derivation.

FREQUENCY DISTRIBUTION

Frequency distributions can be used to aid in the setting of criteria. Frequency distributions do not require prior knowledge of individual stream condition prior to setting criteria. Criteria are based on and, in a sense, developed relative to the population of stream systems in the Region, State, or Tribe.

Data plotted on a scale of mean nutrient concentration versus frequency of occurrence in a specific stream class produces a frequency distribution of mean nutrient concentration. Plots of frequency distributions of mean TP, mean TN, mean chl *a*, and turbidity for the index period (discussed in Chapter 4) should be examined to determine the normalcy of the data in the distribution and to locate patterns for the class of streams being investigated. A sample size of thirty streams within a stream class is recommended for developing nutrient criteria. Smaller sample sizes will require more reference streams, more complete knowledge of the stream systems being investigated, more in-depth statistical analyses, and/or modeling to complete criteria derivation. Sample sizes smaller than thirty may be highly affected by extreme values in the dataset. Data that are not normally distributed are often transformed into a distribution more approximating the normal distribution by taking the logarithm of each value. Analysis of outliers may assist in explaining variability in small data sets. Additional analysis can be conducted to identify the statistical significance of population differences.

CORRELATION AND REGRESSION ANALYSES

The relationship between two variables may be of use in analyzing data for criteria derivation. Correlation and regression analyses allow the relationship to be defined in statistical terms. A correlation coefficient, usually identified as *r*, can be calculated to quantitatively express the relationship between two variables. The appropriate correlation coefficient is dependent on the scale of measurement in which each variable is expressed (whether the distribution of data is continuous or discrete) and, whether there is a linear or non-linear relationship. Results of correlation analyses may be represented by indicating the correlation coefficient, and represented graphically as a scatter diagram which plots all of the collected data, not just a measure of central tendency. The statistical significance of a calculated correlation coefficient can be determined with the *t* test. The *t* test is used to determine if there is a true relationship between two variables. Therefore, the null hypothesis states that there is no correlation between the data variables measured within the population. A critical α value is chosen as a criterion for determining whether to reject the null hypothesis. If the null hypothesis is rejected, the alternate hypothesis states that the correlation at the calculated *r* value between the two variables is significant.

Regression analyses provides a means of defining a mathematical relationship between two variables that permits prediction of one variable if the value of the other variable is known. In contrast to correlation analyses, there should be a true independent variable (a variable under the control of the experimenter) in regression analyses. Regression analyses establishes a relationship between two variables that allows prediction of the dependent variable (predicted variable) for a given value of an independent variable (predictor variable). However, scientists (other than statisticians) apply regression analyses to field data when a relationship is known to exist, even when there is no true independent variable (e.g., cell counts of algae and chlorophyll concentration; nutrient concentrations and chlorophyll concentration) (Ott 1988, 1995; Campbell 1989; Atlas and Bartha 1993).

TESTS OF SIGNIFICANCE

Various statistical tests are used to assess the hypotheses being tested. Statistical tests of significance differ in their applicability to the dataset of interest, and the power of the test (the ability of the test to detect a false null hypothesis). A parametric test of significance assumes a normal distibution of the population. Non-parametric analyses are valid for any type of distribution (normal, log-normal, etc.) and can be used if the data distribution is not normal or unknown. A parametric test has more power than a non-parametric test when its assumptions are satisfied. Two types of errors can be made when testing hypotheses: Type I–where a correct null hypothesis is mistakenly rejected, and Type II–when there is a failure to reject a false null hypothesis. The parametric test is less likely to make a Type II error, when the assumptions are met, than a non-parametric test. Therefore, if given a choice, the parametric test should be used rather than the non-parametric test when the assumptions of the parametric test are fulfilled. Less powerful, non-parametric tests of significance must be used in cases where the data do not fit the assumption of a normal distribution (Ott 1988; Campbell 1989; Atlas and Bartha 1993). Parametric tests include: the student *t* test, analysis of variance, multivariate analysis of variance, and multiple range tests. Non-parametric tests include: chi square, Mann Whitney U test; and the Kruskal - Wallis test (Ott 1988; Campbell 1989; Atlas and Bartha 1993) Detailed descriptions of these and other relevant statistical tests can be found in Appendix C.

6.4 USING MODELS AS MANAGEMENT TOOLS

Computer simulation modeling and probability testing can be used to predict responses to candidate criteria (i.e., numeric nutrient concentrations). Models that have been calibrated and verified can be used to extrapolate to a projected nutrient condition where existing data are either insufficient or unavailable. Data from the same system that is far removed from the present can be used if parameters can be adjusted to the present conditions. The model output can be compared to data from a similar stream system of the same class and in the same ecoregion for validation. Data from a similar system may also be used to extrapolate the nutrient condition when data for the system of interest are unavailable. In both cases, data are complemented by a set of clearly stated assumptions developed from data representing one point in time to estimate conditions in the future. In some instances, surrogate information such as turbidity and chl *a* concentration can be used to estimate nutrient concentrations.

Site-specific simulation models can also be developed for a system of interest, although this is frequently a time-consuming, expensive process. Site-specific computer simulation models should be solicited from the regional academic community, because they are more accurate for predicting specific waterbody concentrations and loadings. This section will not discuss site-specific model development, although several ecological and water quality modeling texts and articles can assist the investigator in developing such a model (see Fry [1993] and McIntire et al. [1996]). Appendix C provides information on several relevant stream water quality models.