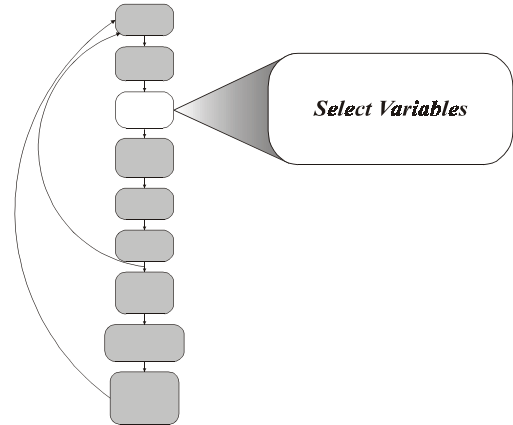# Chapter 3.
Select Variables

3.1 INTRODUCTION

Candidate variables, in the context of this document, are measurable water quality variables that can be used to evaluate or predict the condition or degree of eutrophication in a water body. Data that are most useful in determining river and stream trophic status are water column nutrient concentrations and algal biomass. Benthic and/or planktonic biomass can reach nuisance levels in many stream systems. Measurement of these variables provides a means to evaluate the current degree of nutrient enrichment, and can form the basis for establishing regional and waterbody-specific nutrient criteria. Numerous variables can potentially be used as part of nutrient surveys or eutrophication assessments including measures of water column nutrient concentrations (e.g., TP, SRP, orthophosphate, TN, total Kjeldahl nitrogen [TKN], NO_3^-, ammonia [NH_3]); dissolved organic carbon (DOC); water column and algal/macrophyte tissue N:P nutrient ratios; and algal biomass surrogates (e.g., chl *a*, ash-free dry mass [AFDM], turbidity, percent of benthic algal coverage, species composition).

Criteria development at the EPA Regional and National level will begin with nutrient data gleaned from EPA's STORET (STOrage and RETrieval) database. Primary nutrient parameters to be considered include water column concentrations of TN, TP, algal biomass as chl *a*, and turbidity or transparency. These four variables are considered a starting point for criteria development and their efficacy in controlling nutrient enrichment will be re-evaluated over time. Inorganic nutrient species (PO_4 and NO_3) are usually more biologically available, and may need to be considered in instances where small scale effects from specific sources are an important issue (e.g., point source impacts from outfall pipes, and non-point source impacts during rain events immediately following inorganic fertilizer application). STORET data on the primary parameters are the foundation of the dataset used at National and Regional levels for developing nutrient criteria. Supplemental data from other Federal agencies, State/Tribal agencies, and university studies will also be included as available. Sources of available data, the parameters included in the primary datasets, and the minimum data requirements for criteria development are discussed in Chapter 5.

Interpretation of parameter values and their cause-and-effect relationships depends on whether the data are from stream segments that are slow-moving with a depository substratum and plankton-dominated, or

that are fast-moving with an eroding (gravel/cobble) substratum and periphyton-dominated. Criteria for streams with intermediate characteristics, i.e., in which the bottom is not generally visible in slow-moving segments and is not likely to have algal biomass problems, may need to be developed primarily for fast moving stream segments. Hence, significance of each individual or group of variables is discussed for each extreme stream/river type; the reader, of course, realizes that flowing waters can be found along all points on the trophic continuum and parameter values can vary even within a stream reach. This chapter lists and describes (1) primary response variables that will be used by EPA to set default criteria and (2) secondary response variables (including sensitive variables, i.e., those likely to be most sensitive to enrichment as influenced by increased primary producer biomass and metabolic activity) that can be used to predict the enrichment status of stream systems.

3.2 PRIMARY VARIABLES

The primary variables considered for nutrient criteria development are water column concentrations of TN, TP, benthic and planktonic algal biomass as chl *a*, and turbidity or transparency. These variables will be used to set criteria ranges for each EPA ecoregion at the National level (see section 1.5). The primary causal variables, TN and TP, are closely related to the response variables, algal biomass as chl *a* and turbidity or transparency, although the relationships between these variables are not as tightly coupled in rivers and streams as they are in lakes. Concentrations of nutrients and algal biomass and measures of turbidity/transparency are more highly variable in rivers and streams because of fluctuating flow conditions. Therefore, knowledge of the flow conditions in the waterbody of concern will be used to help define the nutrient condition of that waterbody, and will be used in criteria development. Criteria will not be established for flow as a variable. Stream sampling should be conducted during periods of peak algal biomass or periods when problems related to algae may be greatest (e.g., low-flow or following rain events with high nonpoint source nutrient inputs). Subsequent sections of the chapter discuss other potential variables that may be useful in developing nutrient criteria. Methods for measuring and analyzing many of the variables discussed in this Chapter can be found in Appendix B.

NUTRIENTS

Nitrogen and phosphorus are the primary macro-nutrients that enrich streams and rivers and cause nuisance levels of algae. Conditions that allow periphyton/plankton biomass to accumulate (i.e., adequate light, optimum current velocity [periphyton], sufficient water detention time [plankton], as well as low loss to grazing) will not result in high biomass without sufficient nutrient supply. Nutrients, especially P, are frequently the key stimulus to increased and high algal biomass.

Phosphorus is the key nutrient controlling productivity and causing excess algal biomass in many freshwaters worldwide. However, nitrogen can become important in waters receiving agricultural runoff and/or wastewater with a low N/P ratio and in waters with naturally phosphorus-rich bedrock (Welch 1992). Nitrogen may have more importance as a limiting element of biomass in streams than in lakes (Grimm and Fisher 1986; Hill and Knight 1988; Lohman et al. 1991; Chessman et al. 1992; Biggs 1995; Smith et al. 1999). Lohman et al. (1991) reported low NO_3-N causing N limitation at sixteen sites in ten Ozark Mountain streams and cited sources for N limitation in northern California and the Pacific Northwest. Nitrogen was clearly the limiting nutrient in the upper Spokane River, Washington (Welch et al. 1989). Chessman et al. (1992) observed that N was more often limiting than P in Australian streams.

Analyses of data from 200 rivers suggests that TN is more closely correlated to mean benthic algal biomass than TP, and DIN is more closely correlated to biomass than SRP (Dodds et al. unpublished).

The directly available forms of N and P are mainly inorganic (NO_3^-, NH_4^+ and PO_4^{3-}), although many algae are able to use organic forms (Darley 1982). Total N and TP include these soluble fractions, as well as the particulate and dissolved organic fractions. Particulate and dissolved organic fractions are not immediately available and portions may be relatively refractory. Because soluble inorganic fractions are directly available, soluble inorganic N, P, or both may be low during active growth periods when demand is high and, therefore, may not be good predictors of biomass (Welch et al. 1988). Total N and TP are often good predictors of algal biomass in lakes and reservoirs, to a large extent because much of the particulate fraction is live algal biomass. That is not the case in fast-flowing, gravel/cobble bed streams where the total nutrient concentration includes detritus but not the living periphytic algae where biomass measurements are taken. In fast-flowing systems, water column nutrients flow past the living periphyton biomass before they can be completely assimilated. Therefore, the relationship between benthic chlorophyll and water column nutrients is weaker in fast-flowing versus standing water systems (Dodds et al. 1998).

ALGAL BIOMASS AS CHLOROPHYLL *a*

Algae are either the direct or indirect cause of most problems related to excessive nutrient enrichment; e.g., algae are directly responsible for excessive, unsightly periphyton mats or surface plankton scums, and may cause high turbidity, and algae are indirectly responsible for diurnal changes in DO and pH. Chl *a* is a photosynthetic pigment and sensitive indicator of algal biomass. It can be considered the most important biological response variable for nutrient-related problems. The following discussion of chl *a* as a primary variable includes information for both benthic and planktonic chl *a*. Benthic chl *a* can be difficult to measure reliably due to its patchy distribution and occurrence on non-uniform stream bottoms. Periphyton is often analyzed for AFDM, which includes non-algal organisms. Additional factors that can be used to determine which type of chlorophyll (benthic or planktonic) is most important in the system of interest can be found in Table 1, Section 2.2.

Unenriched, light-limited, or scour-dominated stream systems typically have benthic chl *a* values much less than 50 mg/m^2. Biggs (1995) reported the following range of chl *a* values from monthly observations over a one year period in 16 New Zealand streams: 1) unenriched streams in forested catchment (0.5-3 mg/m^2), 2) moderately enriched streams in catchments with moderate agricultural use (3-60 mg/m^2), and 3) enriched streams in catchments highly developed for agriculture and/or underlain with nutrient-rich bedrock (25-260 mg/m^2). Lohman et al. (1992) reported a range of 42 to 678 mg/m^2 chl *a* from over two years of spring to fall biweekly observations at 22 sites on 12 Missouri Ozark Mountain streams, with higher levels occurring at more enriched sites. Unenriched sites exhibited mean biomass values that did not exceed 75 mg/m^2. However, highly and moderately enriched sites exceeded a nuisance level mean biomass (150 mg/m^2) within 3 or 4 weeks, respectively, following flood-scour events. The highest maximum value observed at ten sites in late summer 1987 in the Clark Fork River, Montana, was approximately 600 mg/m^2 (Watson and Gestring 1996). Furthermore, values for benthic chl *a* as high as 1200 mg/m^2 have been observed in gravel/cobble bottom bed streams (Welch et al. 1992).

Planktonic chl *a* in deep, slow-moving rivers will have an upper limit determined by light attenuation, which increases with the suspended chl *a* concentration. Maximum chl *a* can be low (<10 μg/L) even if slow-moving systems are nutrient enriched because most flowing systems disperse phytoplankton before high algal biomass develops. However, under low flow conditions (accompanied by low mixing and shallow depth), large planktonic algal blooms often develop in slow-moving, nutrient enriched rivers. The theoretical maximum attainable before light limits photosynthesis in lakes (assuming light is attenuated by algae only) is about 250 mg/m^2. This theoretical maximum is equivalent to 25 mg/m^3 (μg/L) in a 10-m depth water column or 125 μg/L in a 2 m deep lake. Van Nieuwenhuyse and Jones (1996) compiled summer mean suspended chl *a* values for rivers, and found no values greater than 180 μg/L. Mixing and light attenuation from non-algal particulate matter, which are typical in deep, slow-moving rivers, may further limit light availability for photosynthesis.

A conceptual distribution of algal biomass in the euphotic zone over a range of water detention times was suggested by Rickert et al. (1977) (see Welch 1992). For example, the lower Duwamish River, Washington estuary typically contained around 2 μg/L chl *a* during summer, even though it was heavily enriched with secondary treated sewage effluent. However, when the water detention time increased and mixing decreased as a combined result of minimum range tidal conditions and low river flow in August, chl *a* reached a maximum of 70 μg/L (Welch 1992).

Algal biomass data in fast-flowing, gravel/cobble bed streams and deep, slow-moving, turbid rivers must be interpreted in light of the physical constraints that determine the potential for nutrient utilization (see Chapter 2). Relatively low biomass can be observed in highly enriched waters, if physical (light, temperature, current) or grazing constraints are severe. Relatively high algal biomass can occur with low enrichment if physical constraints approach the optima for algal growth. However, chl *a* concentrations near the maximum values cited above will not occur without nutrient enrichment.

TOTAL SUSPENDED SOLIDS, TRANSPARENCY, AND TURBIDITY

Total suspended solids (particulate matter suspended in the water column) attenuate light and reduce transparency, whether the source is algae, algal detritus or inorganic sediment. Streams may also have high concentrations of light-absorbing dissolved compounds (e.g., blackwater streams). The concentration of total suspended solids can be determined directly or as an effect on light transmission or scattering. Quantitative relationships have been developed for individual and/or groups of waters to predict transparency from particulate matter and/or chl *a* (Reckhow and Chapra 1983; Welch 1992). However, relationships of chl *a* and transparency (as an effect of nutrients) are not prevalent in fast-moving streams systems; most likely because of interference from time- and flow-variable inorganics and large diameter suspended solids. Total suspended solids may increase due to algae and detritus sloughed from large algal mats, but caution should be exercised in interpreting these data. During high flow, the concentration of suspended solids (and water clarity) will likely be more strongly influenced by inputs of inorganic sediment or channel erosion in streams, especially in urbanized and agricultural watersheds.

Turbidity, as NTUs (Nephelometric Turbidity Units), measures suspended matter in the water column whether of organic (i.e., chl *a*) or inorganic origin. Turbidity correlated with rain-event sampling may help identify non-point source loadings. Although turbidity is not commonly used as an index of eutrophication in either lakes or streams, it nonetheless should increase in streams with increasing algal biomass due to nutrient enrichment.

Periphyton are directly affected by suspended solids (as turbidity) due to light attenuation. Quinn et al. (1992) found that waters with turbidity measurements that range between 7-23 NTUs have reduced abundance and diversity of benthic invertebrates. They attributed the reduction in benthic invertebrates to turbidity, largely because of its adverse effect on periphyton production as an invertebrate food source (Quinn et al. 1992). In Illinois, the turbidity of agricultural streams (NTU 10-19) had more effect on periphyton accrual than did nutrient enrichment (Munn et al. 1989). Total suspended solids ranging from about 22 to 30 mg/L increased the loss rate of periphyton (mixture of filamentous blue-green and diatoms) tenfold, although increased velocity with and without solids caused more loss (Horner et al. 1990).

The vertical water column in relatively clear-water, gravel/cobble bed streams/rivers is usually insufficient to determine Secchi disk depth. However, the white Secchi disk routinely used in lakes and reservoirs to determine transparency is appropriate for slow-moving streams and rivers (Welch 1992). Transparency, as influenced by low concentrations of particulate matter in shallow, fast-flowing streams systems, can also be determined with a black disk (Davies-Colley 1988). The path length for transparency is measured horizontally in such shallow streams, as opposed to vertically in lakes, reservoirs and deep rivers/estuaries. As periphyton biomass increases, particulate matter sloughed and/or eroded from the substratum also increases, reducing transparency.

FLOW AND VELOCITY

The rate of discharge or flow in a stream system can be separated into two primary components, baseflow and storm or direct runoff. Baseflow comprises the regular groundwater inputs to a stream. This water typically reaches the stream through longer flow paths than direct runoff and sustains streamflow during rainless periods. Direct runoff is hillslope or overland flow runoff that reaches a stream channel during or shortly after a precipitation event. Both components of flow are reflected in a hydrograph (a graph of the rate of discharge plotted against time) of the stream segment. Runoff processes (including stream discharge and groundwater recharge), seasonal variation of flow, and methods to calculate average stream velocity, the annual probability hydrograph and flow duration curves are discussed at length in Dunne and Leopold (1978).

The flow of a river or stream affects the concentration and distribution of nutrients. Generally, point source concentrations are higher during low flow conditions due to reduced water volumes; in contrast, nutrients from non-point sources may be more highly concentrated during high flow conditions due to increased flow paths through the upper soil horizons and overland flow. There is also a rough correlation of total dissolved solids concentration with climate and hydrology. Streams in arid regions tend to have high concentrations of total dissolved solids (though the total annual solute transport is low because of low runoff), whereas in humid regions, concentrations tend to be lower with higher total annual solute transport (Dunne and Leopold 1978). However, the complexity of the interactions of nutrient concentration and flow make it important to examine both point sources and non-point sources of nutrients and wet weather (high flow) and dry weather (low flow) stream conditions to verify nutrient sources and concentrations in multiple flow conditions (Dunne and Leopold 1978).

Brandywine Creek, Pennsylvania, provides an example of how stream flow can affect nutrient concentrations in a stream system (Dunne and Leopold 1978). The Brandywine Creek watershed drains portions of the Piedmont plateau and Atlantic coastal plain into the Delaware River. The watershed land

use is a mix of urban, agricultural and suburban uses, and includes both point and non-point pollution sources. Brandywine Creek was sampled during periods of storm runoff and dry-weather flow for P and stream discharge. Point discharges of P were diluted as stream discharge increased following storm events. As storm runoff occurred, concentrations of P increased dramatically at sampling sites not dominated by point discharges. At sites not dominated by point discharges, runoff from forested and cultivated hillslopes washed large amounts of P into the Brandywine Creek in both solid organic form and sorbed to soil particles.

Hydrologic variability is an important consideration in the development of nutrient and algal criteria for all streams; nonetheless, there is often a higher degree of variability for specific types of regional stream systems. In particular, the spatial and temporal heterogeneity found in arid regions, the stark contrast between wet and dry, can be dramatic (see Desert Streams Case Study, Appendix A). When viewing desert catchments from above, the observer is often presented with a dry landscape of high relief bisected by the string of glistening beads that is the spatially intermittent stream. The dry arroyos or quiet, disconnected pools and short reaches of wetted stream that characterize desert streams during dry periods are in complete contrast to the raging torrents that they can become at flood stage. This hydrologic variability and the unique chemical and biological characteristics of arid lands aquatic ecosystems make the use of broad generalizations to explain nutrient regimes difficult.

In arid landscapes, stream ecosystems are dynamically linked with the surrounding upland ecosystem. In addition, surface discharge regimes may vary from completely dry, to flows as much as three to five orders of magnitude greater than mean annual flow, all within a period of hours or days. In comparison to streams in more mesic regions, the coefficient of variation of annual flow is 467% greater in arid lands streams (Davies et al. 1994). The aquatic ecosystems structured by these chaotic flow regimes (Thoms and Sheldon 1996) may require different techniques for nutrient criteria development than those used in more homogeneous environments.

Drying disturbance, or more specifically the contraction and fragmentation of a stream ecosystem, is a critical component of the hydrologic regime of desert streams. Drying occurs as a spatially or temporally intermittent stream recedes after a wet period. In streams where the dry period and extent may be greater than the wet, drying is likely to be an important determinant of biological pattern and process (Stanley et al. 1997; Stanley and Boulton 1995).

In order to properly characterize the nutrient regime of a stream ecosystem, the flow of water, surface and subsurface, flood or base flow, wet or dry must be considered at ecologically significant temporal and spatial scales. It is also important that the manager address this hydrologic regime at the scale of the question to be answered. If a stream is dry for 75% of the average year, or for 75% of its length, is it correct to characterize it from surface water data alone? If 50% of the entire annual load of a limiting nutrient passes through a stream ecosystem in three discrete storm events, what is the effect of that nutrient on the stream ecosystem itself? What is the effect to downstream ecosystems? Due to the spatial and temporal variability of flow patterns, the characterization of desert stream nutrient dynamics is an intricate undertaking. However, stream complexities will only be understood through appropriate assessment and evaluation.

3.3 SECONDARY RESPONSE VARIABLES

The following sections describe additional variables that may be useful in criteria development. These variables comprise chemical, physical, and biological parameters, some of which exhibit heightened response to nutrient enrichment.

SENSITIVE RESPONSE VARIABLES

The variables discussed below that are apt to be most sensitive to nutrient enrichment, via increased algal productivity and biomass are: 1) DO and pH, 2) benthic community metabolism, and 3) autotrophic index. These variables should vary directly with algal productivity and detect relatively small changes in nutrient condition. While other variables such as total suspended solids, macroinvertebrate indices, dissolved organic matter, and secondary production may be directly affected by algal productivity and biomass, they may also be strongly dependent on other natural factors and/or sources/types of pollutants.

Dissolved Oxygen and pH
Periphyton algal biomass above nuisance levels often produces large diurnal fluctuations in DO and pH. Photosynthesis/respiration by dense periphyton mats commonly causes water quality violations (Anderson et al. 1994; Watson et al. 1990; Wong and Clark 1976). These water quality impairments occur in stream systems as a result of nutrient-produced excessive algal biomass in fast-flowing, gravel/cobble bed streams as well as sluggish stream systems. Excessive macrophyte biomass can produce similar swings in DO and pH (Wong and Clark 1979; Wong et al. 1979).

The extent of diurnal swings in DO and pH will depend on several factors, such as turbulence (which affects reaeration), light, temperature, buffering capacity, and the amount and health of algal and/or macrophyte biomass. Sluggish streams and rivers may show a greater range in DO and pH per unit biomass compared to faster streams due to less turbulence and associated atmospheric exchange of CO_2 and O_2 (Odum 1956; Welch 1992). Light limitation may also be a common feature of algae in enriched streams, and therefore, light is likely an important control on diurnal DO and pH swings (Jasper and Bothwell 1986; Boston and Hill 1991; Hill 1996). Higher temperatures tend to enhance algal growth in many streams and may increase photosynthesis and respiration in many systems resulting in greater variation in diurnal DO and pH values. Streams with low buffering capacity will show greater diurnal swings in pH. Furthermore, biomass-specific metabolic rate (especially respiration–see photosynthesis/respiration discussion) tends to be greater in fast-flowing waters because periphytic growth is stimulated by velocity. The influence of the above factors on DO concentration and pH value reduce the specificity and potentially reduce the reliability of these variables to indicate response from nutrient enrichment. Therefore, direct measures of algal biomass, such as chl *a*, are preferred response variables.

Aquatic animals are affected most by maximum pH and minimum DO, rather than by the daily means for these variables (Welch 1992). Hence, monitoring for water quality should include pre-dawn hours to observe the diurnal minimum DO and afternoon hours for maximum pH. Routine grab samples in monitoring programs usually do not include such strict protocols. It may be possible to estimate minimum DO from equilibrated average and maximum DO (Slack 1971) which occurs during mid-day to afternoon, along with maximum pH.

Metabolism
Photosynthetic rate, or primary productivity, is often considered a more sensitive variable of response to nutrients than algal biomass. Biomass is a net result of gains (productivity) minus losses (algae lost due to death, scour, etc.) (see discussion in Stevenson 1996). Productivity is essentially growth, and therefore is a more direct measure of nutrient effects. Productivity can be determined for whole stream reaches by monitoring diurnal DO concentrations (see methods section, Appendix B) or alternatively, productivity and respiration may be measured using light/dark chambers. Whole-stream metabolism measurements are integrative over all components of the stream system and eliminate artifacts of enclosure that commonly confound results in chamber experiments. Marzolf et al. (1994, 1998) detail the methods for measuring whole-stream metabolism. Productivity and respiration in light/dark chambers may vary on an hourly and daily basis with temperature, light, and nutrients; short-term measurements must be corrected for those factors (Welch et al. 1992). The necessity of normalizing measurements and the greater analytical difficulty of productivity, has made algal biomass the preferred variable to indicate nutrient effects on periphyton and phytoplankton as evidenced by the generally established trophic state criteria for lakes and reservoirs (Welch 1992), and proposed for streams/rivers (Dodds et al. 1998). The rate at which maximum biomass is attained is dependent mostly on nutrient availability, minus losses to grazing and scouring, or washout in the case of phytoplankton. While integrated daily productivity is usually directly related to biomass as chl *a* (Boston and Hill 1991), there can be considerable variability in the relationship due to the variables discussed above, as shown by the ratio of productivity to biomass as chl *a* (Figure 6). The ratio of productivity to biomass as chl *a* is an index of growth rate. If there is no variability in productivity:biomass, the relationship will be constant and will not vary on a day-to-day basis.

Gross photosynthesis/respiration ratios (P/R ratios) can be useful indicators of trophic characteristics. P/R ratios have long been recognized to indicate the relative autotrophic (P/R >1) or heterotrophic (P/R <1) character of streams and rivers. Measurement of P/R and interpretation of results is dependent on the scale at which the measurements are made, and the point in the annual cycle when the measurements are taken. For example, low-order streams that flow through forested watersheds tend to be heterotrophic with photosynthesis limited by light due to shading; mid-order streams and rivers flowing through areas with minimal riparian vegetation, or largely unshaded due to width, are usually autotrophic (unless organic waste inputs are significant); high order rivers tend to return to a heterotrophic character due to light limitation brought on by increased depth and turbidity (Vannote et al. 1980; Bott et al. 1985). Furthermore, the P/R ratio for a short-term measurement (24-72 hours) in the spring may indicate an autotrophic stream, while on an annual basis the stream is heterotrophic (Hall and Moll 1975; Wetzel 1975; Wetzel and Ward 1992).

There are problems with interpreting P/R ratios, however. Photosynthesis/respiration ratios can vary seasonally and could actually reflect a temporary heterotrophic condition during a period of low periphyton biomass, due to scouring or low light, while otherwise it would be autotrophic. Decreased velocity can also decrease stream/river P/R, because mat thickness of periphytic diatoms can increase while the depth of active photosynthesis remains relatively constant (Biggs and Hickey 1994). Thus, photosynthesis is limited by light attenuation in the mat, but respiration is stimulated by movement of organic materials to heterotrophic organisms in the mat.

Gross Primary Productivity
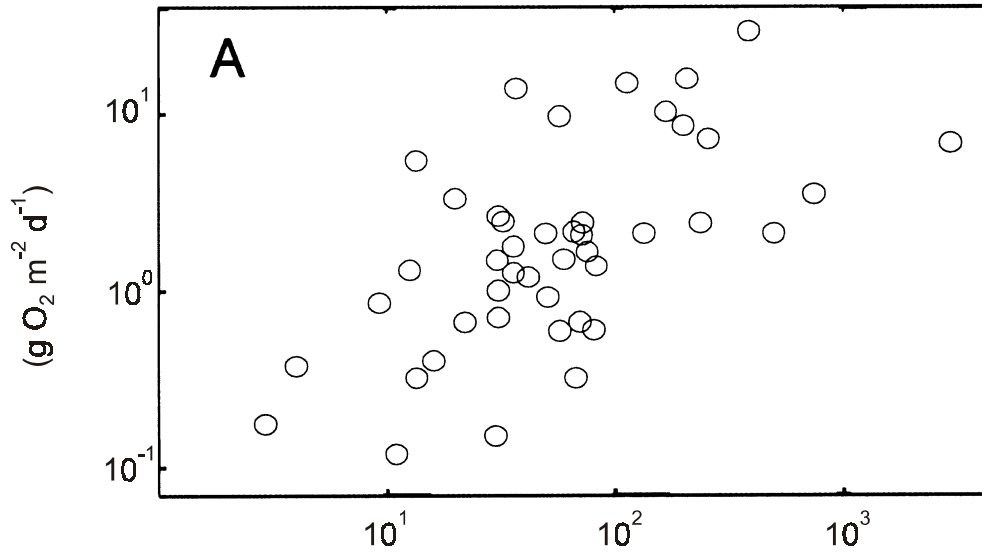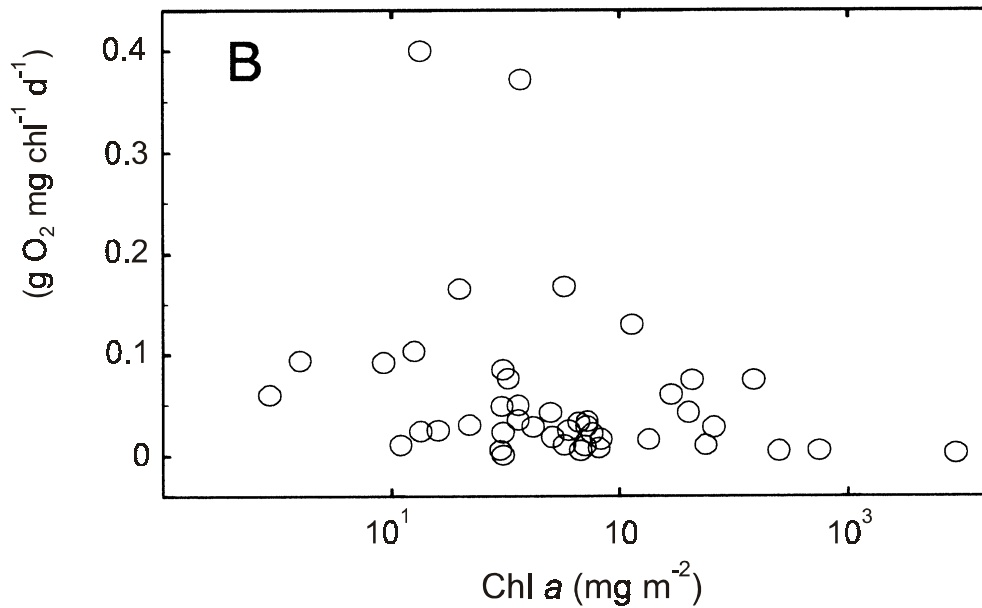

Assimilation Number

Figure 6. Integrated daily productivity related to biomass as chlorophyll *a* (data compiled by Dodds from published literature; many of the data from Bott et al. 1985).

Autotrophic Index
The ratio of AFDM to chl *a* is termed the autotrophic index for periphyton and is used to distinguish the relative response of inorganic (N and P) and organic (BOD) enrichment. Periphyton growing in surface water that is relatively free of organic matter contain approximately one to two percent chl *a* by weight. Surface water that is high in particulate organic matter may support large populations of bacteria, fungi and other non-chlorophyll bearing microorganisms, and have a larger ratio of AFDM to chl *a*. Increased ratios indicate that heterotrophs utlizing organic substances comprise a larger percentage of AFDM than autotrophic periphyton that rely largely on inorganic nutrients to increase biomass (Weber 1973). Ratios of AFDM/chl *a* can vary over three orders of magnitude, with values >400 indicating organically polluted conditions (Collins and Weber 1978). Ratios of AFDM/chl *a* around 250 are more typical for streams enriched with inorganic nutrients that are likely to have existing or potential eutrophication problems (Watson and Gestring 1996; Biggs 1996). The autotrophic index should be used with caution, because non-living organic detrital material may artificially inflate the ratio.

Interpretation of Sensitive Response Variables
High algal productivity can cause supersaturated DO and high pH during the day, P/R ratios >1, and unusually low autotrophic indices. Unfortunately, broad predictive relationships do not exist between nutrient concentration and algal/macrophyte biomass, DO, or pH. However, relationships could be developed for individual streams and rivers. Nevertheless, without inclusion of other factors that affect DO and pH (such as exchange with the atmosphere for specific stream systems), a biomass limit to prevent low DO (e.g., <5 mg/L) cannot be determined from any existing relationship, such as the chl *a* - TP relationships discussed earlier (Lohman et al. 1992; Dodds et al. 1997). As concentrations of nutrients and algae increase, diel fluctuations in DO and pH also increase (see Dissolved Oxygen and pH discussion above). However, established relationships observed in lakes and reservoirs, such as TP loading and hypolimnetic DO deficit (Welch 1992), do not exist for streams and rivers.

OTHER SECONDARY RESPONSE VARIABLES

Additional chemical, physical, and biological attributes may be useful when evaluating nutrient and algal relationships. Descriptions for several potential useful variables are provided below.

Chemical Waterbody Characteristics

Conductivity
Specific conductance (typically measured as conductivity) has also been used as an indicator of nutrient enrichment (Biggs and Price 1987; Biggs 1996). Conductance reflects the concentrations of macro-ions, so nutrients dissolved from bedrock are assumed to increase proportionately with increases in total ions. Conductance at low flow was found to increase proportionately with urbanization in 23 western Washington streams and was hypothesized to be a loose surrogate for soluble nutrient supply during summer when residual soluble nutrient concentration was low due to algal demand (May et al. 1997). However, conductance may be a poor indicator of nutrient availability in calcareous regions or those with high concentrations of dissolved salts that are not typically limiting nutrients.

Dissolved Organic Carbon
DOC is an important energy source that drives the heterotrophic community and can alter a river's response to algal growth problems. DOC can originate as allochthonous inputs naturally from the

watershed through decomposition of terrestrial primary production, or from cultural waste production. The heterotrophic community will dominate the periphyton in gravel/cobble bed streams and rivers that have high inputs of labile DOC.

Inflow and in-stream DOC should be related to the autotrophic index, as discussed previously. Streams and rivers enriched with DOC will have high autotrophic indices, and may be more prone to low oxygen events that can be exacerbated by excessive periphyton biomass. High rates of autochthonous DOC production` is usually a result of inorganic nutrient enrichment. Such eutrophication-caused DOC production can be an important source of decomposition by-products (e.g., tri-halomethane precursors and other sources of taste and odor problems) which is a concern for drinking water supplies.

Physical Waterbody Characteristics

Temperature
Algal metabolic rate, at a given biomass and growth phase (relative cell health), is controlled by temperature (DeNicola 1996), water movement, nutrients and light. In general, the response to enrichment will be faster at higher than lower temperature; e.g., twice as fast at 20°C as at 10°C (McIntire and Phinney 1965; Welch 1992). However, the maximum biomass will depend on nutrient availability; temperature will determine only the rate at which the maximum is reached (Welch 1992).

Temperature, as it interacts with light and nutrients, will determine which taxa dominate the algal biomass. The various algal taxa have individual thermal optima. In general blue-greens have higher optima than greens which have higher optima than diatoms (Rodhe 1948; Cairns 1956; Hutchinson 1967). For example, the nuisance filamentous green, *Cladophora*, apparently has an optimum around 18°C and its growth stops at 25°C (Storr and Sweeney 1971). As a result of differing thermal optima, seasonal succession of taxa is often observed, with diatom dominance during spring low temperature and greens and blue-greens dominating in summer. However, nutrients often override temperature effects, with diatoms dominating the periphyton throughout the spring-summer period at low nutrient concentrations and greens (and/or blue-greens) dominating for the whole period at high nutrient concentrations (Welch 1992).

Biological Attributes

Algal Biomass as Ash-Free Dry Mass
Algal biomass or standing crop is often expressed as AFDM. However, the weight of particulate detritus in fresh water frequently exceeds that of the algae. No reasonable method currently exists to separate algae from detrital material in the water. Therefore, chl *a* is usually the primary biomass indicator because it is specific to algae, while AFDM can include other living or non-living organic matter (Darley 1982; Wetzel 1975).

Algal Biomass - % Cover of Bottom by Nuisance Algae
Extent of periphyton coverage of a stream bed can be an important indicator of algal biomass problems. As enrichment increases, the fraction of periphyton biomass composed of filamentous greens increases, as does the percent of stream bed covered with algae (Welch et al. 1988; Lohman et al. 1992; Biggs 1996). However, there may be an uncoupling between percent cover and total biomass depending on the thickness of the algal mat, e.g. , a system might have 100% algal cover, but if the algal growth was very

thin (e.g., "sheets" of *Oscillatoria* filaments), the total biomass could be far less than a system with 50% cover of *Cladophora*. Nevertheless, estimates of percent cover are often a useful indicator of the intensity of algal proliferation in gravel/cobble-bed streams, and as an index of aesthetic appeal. The occurrence of floating blue-green algae scums in slow-moving rivers, lakes, and reservoirs is likewise an aesthetic nuisance, but there has been no attempt to quantify scum intensity/surface-cover similarly to periphyton in fast-flowing streams, largely due to the variable, diurnal nature of floating blue-green scums.

Pigment Ratios

Two pigment ratios are commonly used in periphyton assessments. One is the chl *a*:AFDM ratio, which is a modified version of the autotrophic index (Weber 1973; Stevenson 1996; Stevenson and Bahls 1999) and indicates the relative importance of autotrophy versus heterotrophy in streams. Values of the autotrophic index increase when algae (chl *a*) become a greater proportion of benthic biomass. The second is the chl *a*:phaeophytin ratio, which is an indicator of periphyton health. Phaeophytin is a degradation product of chlorophyll. Relatively low values of phaeophytin, thus relatively high values of the chl *a*:phaeophytin index, indicate periphyton is actively growing.

Chemical Composition of Algae (N:P Stoichiometry)

Phosphorus and N concentrations in periphyton increase with nutrient concentrations and trophic status of streams (Humphrey and Stevenson 1992; Biggs 1995). Periphyton can be analyzed for P and N content, as well as chl *a* or AFDM. Then P and N concentrations in periphyton can be expressed as a fraction of algal biomass as indicated by chl *a* or AFDM (μg P/μg chl *a* or μg P/mg AFDM). This metric can be another valuable complement to assessments of P and N availability, especially when P and N concentrations are variable in the stream.

Nutrient ratios in periphyton may provide a line of evidence to indicate whether N or P is limiting algal growth. The range of ambient or cellular N:P ratios has been used as to define the transition between N and P limitation for benthic algae (Schanz and Juon 1983). If ambient N:P ratios are greater than 20:1, then P can be assumed to be in limiting supply. If the ambient N:P ratio is less than 10:1, then N can be assumed to be in limiting supply. The distinction of the limiting nutrient when ambient N:P ratios are between 10 and 20 to 1 is not precise. Nutrient enrichment studies have supported these transition ratios in broad terms (e.g., Grimm and Fisher 1986a; Peterson et al. 1993). However, the accuracy of ambient nutrient ratio analysis decreases when greater amounts of detritus occur in periphyton samples. In streams, N:P ratios of periphyton can be different than N:P ratios in the water column (Humphrey and Stevenson 1992). Periphyton N:P ratios may better indicate relative nutrient availability to the periphyton than ratios based on water column nutrient concentrations. In addition, ambient ratios may not reflect the cellular ratio relevant to physiological growth processes when nutrients are abundant. Cellular nutrient ratios are a more direct measurement of nutrient limitation (Borchardt 1996). Even so, nutrient ratios only suggest limitation–bioassays are required to establish cause and effect relationships.

Phosphatase Activity

Alkaline phosphatase is an enzyme excreted by algae in response to P limitation. Alkaline phosphatase hydrolyzes phosphate ester bonds, releasing PO_4 from organic P compounds (Steinman and Mulholland 1996). Concentration of alkaline phosphatase in the water column can be used to evaluate P limitation. Alkaline phosphatase activity (APA), 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). Artificial stream channel

experiments by Klotz (1992) support the hypothesis that stream N:P ratio is the important factor in determining periphyton APA. In this study, APA varied seasonally, and shading of the stream channel resulted in lower APA. Results from studies of cultured algae appear to indicate that phosphatase levels above 0.003 mmol (micromoles) mg chl a^{-1} h^{-1} indicate moderate P deficiency, and phosphatase levels above 0.005 mmol mg chl a^{-1} h^{-1} indicate severe P deficiency (Steinman and Mulholland 1996).

Algal Species Composition
Assessment of algal species composition can indicate that nutrient related problems exist or that conditions are right for such problems to develop (Kelly and Whitton 1995; Pan et al. 1996). Since algae are often the problem associated with nutrient contamination, assessments of algal species composition can show whether nuisance algae are present or whether biotic integrity of this target community has changed. Assessment of algal species composition is more time consuming than simpler measurements of water chemistry or chl a measurement, however algal species composition may provide more reliable indicators of trophic status in streams and rivers than one-time sampling and assessment of water chemistry and benthic algal biomass (Stevenson, unpublished data). Assessment of algal species composition is an element of periphyton programs in all States that monitor periphyton. One of the reasons for relying on species composition is periphyton biomass is so variable spatially and temporally, and challenging to measure accurately. In addition, species composition is highly informative, especially when linked to the ecology of a species in relation to the environment, i.e., the autecological information about the species (Stevenson and Bahls 1999).

Many attributes of algal species composition can be used as metrics or indicators of nutrient conditions, trophic status, and biotic integrity (Stevenson and Bahls 1999). Indicators of nutrient status based on algal taxa fall in three categories: diversity, deviations in species composition from reference conditions, and weighted-average autecological indices. Diversity is comprised of two components: 1) the variety of species (species richness), and 2) the relative abundance of species (eveness). Shannon diversity (a measure of diversity which combines the components of diversity [Pielou 1975]) usually decreases with increasing trophic status because evenness decreases. Weighted-average autecological indices based on pollution tolerance, or more specifically, nutrient requirements can be used to infer nutrient status or trophic conditions in a habitat (Steinberg and Scheifele 1988; Schiefele and Schreiner 1991; Van Dam et al. 1994; Kelly and Whitton 1995; Pan et al. 1996). Dissimilarity in species composition between test and reference sites can be used to determine whether water quality is similar in test and reference sites. A more complete review of metrics and how algae can be used in environmental assessment of rivers and streams can be found in McCormick and Cairns (1994), Stevenson and Pan (1999) or Stevenson and Bahls (1999).

Grazers and Secondary Production
Dense populations of algae-consuming grazers may lead to negligible algal biomass in spite of high levels of nutrients (Steinman 1996). The existence of a "trophic cascade" (control of algal biomass by community composition of grazers and their predators) has been demonstrated for some streams (e.g., Power 1990). Grazer biomass was related more strongly with P concentration in 12 Quebec streams than was periphytic algal biomass, which was considered controlled by grazing in spite of TP concentrations ranging from 5 to 60 μg/L (Bourassa and Cattaneo 1998). The potential for manipulations of foodwebs to control eutrophication certainly warrants more investigation, but there is not currently enough information on trophic cascades in streams to allow for use of foodweb dynamics as a management option. Managers still should realize the potential control of algal biomass by grazers, but also be aware

that populations of grazers may fluctuate seasonally or unpredictably, and fail to control biomass at times. Consideration of grazer populations may at least explain why some stream systems with high nutrients have low algal biomass.

Phytoplankton losses in slow-moving rivers due to filter-feeding grazers can also be significant. Bivalve communities can filter large volumes of water on a daily basis (as much as 10-100% of the water column, depending on population density) (Strayer et al. 1999). The amount of particulate matter grazed from this filtration may exceed losses to pelagic filter-feeders or downstream advection. Significant losses of pelagic phytoplankton have been observed in large rivers. Strayer et al. (1999) describe a zebra mussel invasion of the Hudson River ecosystem that drastically reduced phytoplankton (and zooplankton) biomass by 80-90%, as well as a 50% reduction in phytoplankton biomass in a reach of the Potomac River following colonization by the bivalve *Corbicula fluminea*. Ecosystem response to severe biomass reduction by filter-feeding grazers is often characterized by an increase in dissolved nutrients like SRP, reduced turbidity, and proliferation of macrophytes. Inherent qualities of the waterbody (e.g., mixing, sediment stability, and light attenuation) are a factor in determining whether phytoplankton biomass is permanently reduced, regardless of increases in nutrient concentration, or temporarily reduced and then replenished with a shift in dominant phytoplankton species (Caraco et al. 1997).

Production and biomass of consumers is expected to be greater in streams/rivers enriched with N and P. At some point, however, productivity and biomass will cease to increase at all or the rate of increase per unit nutrient will be greatly reduced. One feature of highly enriched lakes and reservoirs is the switch to grazer-resistant filamentous/colonial blue-green algae, which reduces the efficiency of nutrient utilization and energy conversion to higher trophic levels (Welch 1992). Although not well documented, the same phenomenon may be expected in enriched streams and rivers resulting in increased biomass and percent coverage of filamentous green algae. On the other hand, low-level enrichment of oligotrophic streams and rivers may result in pronounced increases in benthic invertebrates and fishes in addition to increased algal biomass. For example, continuous enrichment of the P-limited Keogh River and Grilse Creek on Vancouver Island, British Columbia, led to substantial increases in secondary producers, but did not produce nuisance biomass levels of periphyton (Perrin et al. 1987; Slaney and Ward 1993). Enrichment of the Keogh River and Grilse Creek with 5-10 and 5 μg/L SRP, respectively, produced maximum periphyton biomass (chl *a*) levels of 100-150 and 50-100 mg/m^2. Consequently, benthic invertebrate biomass increased from 2-7 fold and fish size 1.4-2 fold. Phosphorus fertilization (10 μg/L) of a tundra river led to increased fish and algae production, but negligible increases in invertebrate production (Peterson et al. 1993). In some cases, enrichment of oligotrophic waters may result in increased grazer biomass with little or no change in periphyton biomass (Biggs and Lowe 1994).

Even if nuisance levels of periphyton are produced, secondary production will probably be higher than in unenriched waters in spite of reduced efficiency of conversion. Enrichment of Berry Creek, Oregon, with sucrose (1-4 mg/L) produced large, nuisance mats of filamentous bacteria, but benthic invertebrate biomass increased 4.5 fold and fish (cutthroat trout) increased 6.3 fold with enrichment (Warren et al. 1964). Although adverse effects of periphytic mats and water quality were apparently not evaluated, fish growth obviously prospered from the large biomass of chironomids that consumed the filamentous bacteria.

Secondary production can clearly respond to enrichment and the response may be more efficient and beneficial in oligotrophic than eutrophic streams systems. A transition region in enrichment from

beneficial to detrimental effects has not been defined to the extent that it has for lakes and reservoirs (Welch 1992), but probably exists for different physical types of streams and rivers. Two recent studies have provided independent estimates of target streamwater nutrient concentrations that should be maintained in order to assure acceptable water quality needed for fish growth (Smith et al. 1999). McGarrigle (1993) concluded that maintaining a mean annual SRP concentration <47 mg m^{-3} was necessary to prevent the nuisance growth of attached algae and to preserve water quality suitable for salmonid fishes in Irish rivers. Similarly, Miltner and Rankin (1998) observed deleterious effects of eutrophication on fish communities in low order Ohio streams when total inorganic nitrogen (TIN) and SRP concentrations exceeded 610 mg m^{-3} and 60 mg m^{-3}, respectively.

Invertebrate and fish biomass are considered very useful variables, albeit more demanding to measure than other indices discussed above. Measuring such variables could prove useful because: 1) both may respond to enrichment, 2) fish are of direct economic and recreational importance, and 3) case studies are needed to develop guidelines for regions of enrichment that represent a transition between beneficial and detrimental effects of enrichment.

Macrophytes
Macrophyte is a general term of no taxonomic significance that is applied to many species of aquatic vegetation. Aquatic plants (macrophytes) can be classified into four groups: emergent, floating-leaved, submersed, and freely floating and are large enough to be observed by the naked eye. Aquatic macrophytes represent a taxonomically diverse group of aquatic plants and include flowering vascular plants, mosses, ferns, and macroalgae (USEPA 1973; Wetzel 1975).   Macrophytes are found in most waterbodies and play an important role in the aquatic community providing food for other aquatic organisms, processing nutrients or toxic elements in the water column, and aiding in the stabilization of river/stream sediments (Davis 1985).

The four categories of macrophytes are defined by their connection or anchor to the waterbody substrate: free-floating, emergent (rooted but breaking the water surface), floating leaf anchored, and immersed floating mat anchored (USEPA 1973). The type of growth form plays an important role in the effects of eutrophication on macroscopic plant communties in rivers and streams. For example, the large surface area provided by the thin narrow leaves of *Potamogeton pectinatus* (sago pondweed) allow this species to persist in flowing water with high turbidity (Hynes 1969; Goldman and Horne 1983). Emergent macrophytes grow on the banks of rivers and streams in depths of water less than a meter and are typically rooted in the sediment, have their basal portions submersed in water and have their upper structural biomass growing in the air. Most emergent macrophytes are perennials (living for more than one year). Common emergent macrophytes include plants such as reeds (*Phragmites* spp.), bulrushes (*Scirpus* spp.), cattails (*Typha* spp.), and wild rice (*Zizania* spp.). Floating-leaved macrophytes are rooted to the river bottom with leaves that float on the surface of the water such as waterlilies (*Nymphaea* spp.) and spatterdock (*Nuphar* spp). Submersed macrophytes are a diverse group that grow completely under the water and include mosses (*Fontinalis* spp.), muskgrasses (*Chara* spp.), stoneworts (*Nitella* spp.) and numerous native vascular plants such as various pondweeds (*Potamogeton* spp.), tape-grass (*Vallisneria* spp.), and exotic species including hydrilla and Eurasian watermilfoil. Free-floating macrophytes typically float on or just under the water surface with their roots suspended in the water column. These unattached macrophytes range in size from small duckweeds (*Lemna* spp.) and water fern (*Salvinia* spp.) to larger surface floating plants such as water hyacinth (*Eichhornia crassipes*). Free-floating species are entirely dependent on the water for their nutrient supply. The distribution and

abundance of free-floating macrophytes in streams is affected by current velocity and wind. Thus, they are most frequently found in backwaters and embayments (Goldman and Horne 1983).

The most important environmental factors affecting the abundance and distribution of aquatic macrophytes in rivers are light availability (Spence 1975; Chambers and Kalff 1985; Canfield et al. 1985), nutrients and water chemistry (Hutchinson 1975; Beal 1977; Kadono 1982; Hoyer et al. 1996), substratum characteristics (sediment texture, nutrient content) (Pearsall 1920; Barko et al. 1986; Nichols 1992), and current velocity. Aquatic plants require light for growth, thus light availability is often considered the single most crucial environmental factor regulating the maximum depth of plant growth (Pearsall 1920; Spence 1975; Chambers and Kalff 1985). Light availability is directly linked to water clarity; as water depth increases or water clarity decreases, both the amount and spectral quality of light for photosynthesis decreases (Canfield et al. 1985; Chambers and Kalff 1985). Light availability in rivers is controlled by riparian canopy cover and water clarity, which can be due to both organic and inorganic suspended particles (Vannote et al. 1980). Thus, shaded, turbid, and deep rivers will have fewer aquatic macrophytes.

There are few reports of nutrient-related growth limitation for aquatic plants; nutrients supplied from sediments combined with those in solution are usually adequate to meet nutritional demands of rooted aquatic plants, even in oligotrophic systems (Barko et al. 1986). There are exceptions, however. Barko et al. (1991) showed that interstitial ammonia limited the growth of hydrilla in the Potomac estuary. Nutrient enrichment of nutrient poor waters will increase plant production if no other factors constrain growth. However, the effects of enrichment for macrophytes are confounded by competition with planktonic and epiphytic algae that may reduce underwater light penetration of submerged macrophytes and negate any direct effects of nutrient enrichment (Chambers et al. 1999). Bottom sediments act as the primary nutrient source for macrophytes, and for the most part, water column nutrients must be incorporated into the sediments before they become available for uptake by macrophytes (Chambers et al. 1999).

The physical aspects of sediment texture and as an anchoring point for aquatic plants are also important to the success of macrophytes in stream systems. Some bottom types (e.g., rocks or cobble) are so hard that plant roots cannot penetrate them and fast flowing gravel/cobble bottom stream systems rarely contain enough sediment to support rooted macrophytes. Other sediments are too soft or unstable to anchor rooted macrophytes well enough to endure changes in velocity. In addition, extremely coarse-textured sediment (sand) can be nutritionally poor and therefore require accumulation of organic matter from plant growth or erosion to provide suitable substrate for macrophyte growth (Goldman and Horne 1983).

Macrophytes affect the water quality and human uses of water, other resident organisms, and nutrient cycling. In turn, the above factors influence the growth and abundance of the macrophyte community. To obtain the desired biological integrity of an aquatic community, macrophytes should be present and healthy. However, excess natural or cultural enrichment may yield an overabundance or nuisance growth of macrophytes (USEPA 1973). Macrophytes can inhibit phytoplankton growth by competing for nutrients and sunlight, and by limiting light penetration and therefore photosynthetic processes below the surface (Wetzel 1975). Macrophytes affect the DO and carbon dioxide (CO_2) concentrations, alkalinity, pH, and nutrient supply of a water body through primary production and respiration. Overgrowth of macrophytes in rivers and streams may decrease sediment transport by lowering the flow velocity.

Current velocity, sediment type, and light availability to a large extent determine the plant types that occur in rivers (Hynes 1969; Goldman and Horne 1983; Chambers et al. 1999).

Macrophytes can be an important index of biological health in a waterbody. Their abundance or shortage may be an indicator of excess or deficient nutrient supply. By monitoring macrophytes over a long period of time (along with other parameters), relationships may be developed between macrophyte productivity and nutrients, nutrient cycling, eutrophication, sediment, and other biota (USEPA 1973). Depending on natural nutrient conditions or waterbody trophic state, N or P may be the limiting nutrient in algal/macrophyte biomass accumulation (USEPA 1973; Smart 1990). Phosphorus in particular, but also N and other nutrients, may be taken up by submerged macrophytes from sediment, uncoupling macrophyte growth from water column nutrient concentrations (Welch 1992). Hence, water column measurements of total N and P (or soluble N and P) are usually not indicative of macrophyte growth potential. However, macrophyte growth has been shown to be responsive to sediment pore-water ammonia content. As noted in the Bow River case study (see Appendix A), macrophytes declined in the Bow River following N removal from point source wastewater plants. This decline was hypothesized to have resulted from reductions in sediment N.

<u>Macroinvertebrate Multi-Metric Indices</u>
Indices employing macroinvertebrates as indicators of nutrient pollution have great potential because they are the most reliable and frequently used organisms to indicate the quality of water. Macroinvertebrates are 1) highly sensitive to changes in water quality and disturbance, 2) relatively immobile, long-lived and easy to sample, and 3) an important food supply for fish and therefore economically important. While the productivity and biomass of macroinvertebrates, as secondary producers, readily respond to enrichment as noted above, the individual taxa also respond. Some macroinvertebrates are particularly sensitive to nutrient enrichment, but local metrics of macroinvertebrates must be developed to reliably use macroinvertebrates as indicators of nutrient enrichment. The peer-reviewed stream ecology literature describing nutrient and macroinvertebrate interactions is extensive. Wallace and Webster (1996) provide a review of the literature. Specific methods for sampling macroinvertebrates and developing metrics for different stressors are described in Barbour et al. (1999). Further discussion of macroinvertebrate multi-metric index development can be found in Resh and Rosenberg (1984) and Resh et al. (1996). This type of metric development could be used to derive macroinvertebrate indices of nutrient enrichment in wadeable streams and rivers. In addition, Norton et al. (2000) describes procedures to use biological assessments, including multi-metric indices, for identifying nutrient stress on both macroinvertebrates and fish.