compiled in a format that is easily imported into database and spreadsheets. Ideally, data will be compiled in the Nutrients Database described above. Potential data sources for river and stream nutrient data that will be useful for developing criteria are discussed below. These data sources contain extensive water quality data, however, data collection should not be limited to these sources. Collection of scientifically sound water quality data from any reliable source is encouraged.

POTENTIAL DATA SOURCES

Potential sources of data include water quality monitoring data from Federal, State, Tribal and local water quality agencies; university studies; and volunteer monitoring information. The data sources described in this section do not encompass the full extent of available data sources. Many State/Tribal, and Federal programs that are regional or site-specific are excellent data sources, but are not included in this discussion.

EPA Water Quality Data

EPA has many programs of national scope that focus on collection and analysis of water quality data. The following presents information on several of the databases and national programs that may be useful to water quality managers as they compile data for criteria development.

STORET

STOrage and RETrieval system (STORET) is EPA's national database for water quality and biological data. EPA's original STORET System, operated continuously since the 1960s, was historically the largest repository of water quality data in the nation. This legacy mainframe-based system will cease to exist in the year 2000. In its place, EPA will support two independent, web-accessible databases. The older database, called the STORET Legacy Data Center (LDC) is the repository of all data held in EPA's original STORET System as of the end of 1998. The newer, modernized database, simply called STORET, is designed as a replacement for the original STORET System. It is the repository for more current data, and offers major improvements in database content and quality control documentation.

Interested parties may view both databases on the World-Wide-Web, where the capability will exist to produce printed reports and download data files. Queries for data via the web will be designed for use by the general public and will require no special training or software. The web site will be announced in the first quarter of FY2000.

STORET (the new STORET system) is a compendium of data supplied by Federal, State, and local organizations which evaluates environmental conditions in the field. The data in STORET is organized by both geographic location and data ownership. Every field study site is identified by at least one latitude/longitude and, where appropriate, also by State/Province, County, drainage basin, and stream reach. Monitoring activities recorded include field measurements, habitat assessments, water and sediment samples, and biological population surveys. Records cover the complete spectrum of physical properties, concentrations of substances, and abundance and distribution of species observed during biological monitoring. STORET is designed for maximum compatibility with commercial software, including Geographic Information Systems such as the ESRI ArcView package, and statistical packages such as PC SAS. STORET download files import easily into all standard spread sheet packages.

Further information about STORET may be obtained by e-mailing STORET@epa.gov, or telephoning toll-free at 1-800-424-9067.

National Surface Water Survey (NSWS)
EPA's National Surface Water Survey consists of two parts: the National Lake Survey and the National Stream Survey. The purpose of the National Lake Survey is to quantify, with known statistical confidence, the current status, extent, and chemical and biological characteristics of lakes in regions of the United States that are potentially sensitive to acidic deposition. The purpose of the National Stream Survey (NSS) is to determine the percentage, extent, and location of streams in the United States that are presently acidic or have low-acid neutralizing capacity and may, therefore, be susceptible to future acidification, as well as to identify streams that represent important classes in each region for possible use in more intensive studies or long-term monitoring. The NSS provides an overview of stream water quality chemical characterisitics in regions of the United States that are expected, on the basis of previous alkalinity data, to contain predominantly low-acid neutralizing capacity waters (EPA website http://www.epa.gov/ceisweb1/ceishome/ceisdocs/usguide/prog(56).htm).

Environmental Monitoring and Assessment Program (EMAP)
The Environmental Monitoring and Assessment Program is an EPA research program designed to develop the tools necessary to monitor and assess the status and trends of national ecological resources (see EMAP Research Strategy on the EMAP website: www.epa.gov/emap). EMAP's goal is to develop the scientific understanding for translating environmental monitoring data from multiple spatial and temporal scales into assessments of ecological condition and forecasts of future risks to the sustainability of the Nation's natural resources. EMAP's research supports the National Environmental Monitoring Initiative of the Committee on Environment and Natural Resources (CENR) (www.epa.gov/emap/). Data from the EMAP program can be downloaded directly from the EMAP website (www.epa.gov/emap/). The EMAP Data Directory contains information on available data sets including data and metadata (language that describes the nature and content of data). Current status of the data directory as well as composite data and metadata files are available on this website.

Clean Lakes Program (CLP)
The EPA Clean Lakes Program was initiated to assess water quality in impaired public lakes and reservoirs and to restore these systems where appropriate. CLP included a monitoring and assessment component to identify the efforts needed to restore water quality. Lakes in this program were selected because they were perceived to have water quality impairment. Major tributaries into lakes and reservoirs included in this program were sampled on a regular basis. EPA encouraged States in its May 1996 section 319 nonpoint source guidance to use section 319 funds to fund eligible activities that might have been funded in previous years in the CLP under Section 314. Data from this program may be useful for positioning river and stream systems on a nutrient gradient continuum, but are unlikely to provide data for reference stream reaches. Information about EPA's CLP can be found at the website: http://www.epa.gov/owowwtr1/lakes/lakes.old.html.

Ecological Data Application System (EDAS)
EDAS is EPA's program-specific counterpart to STORET. EDAS was developed by EPA's Office of Water to manipulate data obtained from biological monitoring and assessment and to assist States/Tribes in developing biocriteria. It contains built-in data reduction and recalculation queries that are used in

biological assessment. The EDAS database is designed to enable the user to easily manage, aggregate, integrate, and analyze data to make informed decisions regarding the condition of a water resource. Biological assessment and monitoring programs require aggregation of raw biological data (lists and enumeration of taxa in a sample) into informative indicators. EDAS is designed to facilitate data analysis, particularly the calculation of biological metrics and indexes. Pre-designed queries that calculate a wide selection of biological metrics are included with EDAS. Future versions of EDAS will include the capability to upload data to, and download data from, the distributed version of modernized STORET. EDAS is not a final data warehouse, but is a program or project-specific customized data application for manipulating and processing data to meet user requirements. The EDAS application is currently under development; more information will be available at a later date through the EPA website.

USGS (U.S. Geological Survey) Water Data

The USGS has national and distributed databases on water quantity and quality for waterbodies across the nation. Much of the data for rivers and streams are available through the National Water Information System (NWIS). These data are organized by state, Hydrologic Unit Codes (HUCs), latitude and longitude, and other descriptive attributes. Most water quality chemical analyses are associated with an instantaneous streamflow at the time of sampling and can be linked to continuous streamflow to compute constituent loads or yields. The most convenient method of accessing the local data bases is through the USGS State representative. Every State office can be reached through the USGS home page on the Internet at URL http://www.usgs.gov/wrd002.html.

HBN and NASQAN

USGS data from several national water quality programs covering large regions offer highly controlled and consistently collected data that may be particularly useful for nutrient criteria analysis. Two programs, the Hydrologic Benchmark Network (HBN) and the National Stream Quality Accounting Network (NASQAN) include routine monitoring of rivers and streams during the past 30 years. The HBN consisted of 63 relatively small, minimally disturbed watersheds. HBN data were collected to investigate naturally-induced changes in streamflow and water quality and the effects of airborne substances on water quality. The NASQAN program consists of 618 larger, more culturally influenced watersheds. NASQAN data provides information for tracking water-quality conditions in major U.S. rivers and streams. The watersheds in both networks include a diverse set of climatic, physiographic, and cultural characteristics. Data from the networks have been used to describe geographic variations in water-quality concentrations, quantify water-quality trends, estimate rates of chemical flux from watersheds, and investigate relations of water quality to the natural environment and anthropogenic contaminant sources. Since 1995, the NASQAN Program has focused on monitoring the water quality of four of the Nation's largest river systems—the Mississippi (including the Missouri and Ohio), the Columbia, the Colorado, and the Rio Grande. NASQAN currently operates a network of 40 stations in which the concentration of a broad range of chemicals—including pesticides and trace elements—and stream discharge are measured.

Alexander and others (1996) assembled much of the historical water-quality and streamflow data collected by the NASQAN and HBN on two CD-ROMs, including supporting documentation and quality assurance information (see Internet URL http://wwwrvares.er.usgs.gov/wqn96/). These data are collectively referred to as Water-Quality Networks (WQN). The CD-ROMs are designed to allow users to efficiently browse text files and retrieve data for subsequent use in user-supplied software including spreadsheet, statistical analysis, or geographic information systems. The data may be extracted from one of the CD-ROMs (the "DOS disc") using the supplied DOS-based software, and output in a variety of formats. This software allows the user to search, retrieve, and output data according to user-specified requirements. Alternatively, the ASCII form of the WQN data may be accessed on a second CD-ROM (the "ASCII disc") from user-supplied software including a Web browser, spreadsheet, or word processor.

A comprehensive review of sources, concentrations, and loads of nutrients in the Mississippi River Basin was completed by USGS under the Committee of Environmental Natural Resources. The review focused on analyzing issues related to the Gulf of Mexico hypoxia. Much of Topic 3, Flux and sources of nutrients in the Mississippi-Atchafalaya River Basin, includes data and analysis that could be useful for the development of nutrient criteria in large river systems, such as the Mississippi River. Results of this effort, which was led by the National Oceanic and Atmospheric Administration, have been published and can also be found at the Internet site http://www.nos.noaa.gov/products/pubs_hypox.html.

NAWQA

The USGS National Water-Quality Assessment (NAWQA) Program is building a third national database of stream quality information from data collected and analyzed for more than 50 river basins and aquifer systems, called Study Units, across the Nation. NAWQA studies are based on a complex sampling design that targets specific land uses and hydrologic conditions in addition to assessing the most important aquifers and large streams and rivers in each area studied. Gilliom and others (1995) describe the NAWQA sampling design in detail. A comprehensive data screening, computer retrieval, and review of existing data on nutrients in streams was completed for each of the first 20 Study Units (Mueller et al. 1995). A major component of the sampling design for streams is to target specific watersheds influenced primarily by a single dominant land use (agricultural or urban) that is important in a particular area of the United States. Some of the watersheds were selected as undeveloped areas relative to the rest of the Study Unit to use in comparative analysis of land-use effects on water quality. Water-quality data collection during 1992-1996 include analyses of eight nutrient species from about 8,500 samples of streams and rivers in the first 20 Study Units. A data set used for national synthesis of water quality has been compiled and can be viewed and downloaded via the Internet URL http://wwwrvares.er.usgs.gov/nawqa/nutrient.html. Mueller and others (1997) describe quality control of the NAWQA stream data and Mueller (1998) provides a rigorous assessment of the quality of these data.

WEBB

The Water, Energy, and Biogeochemical Budgets (WEBB) program was developed by USGS to study water, energy, and biogeochemical processes in a variety of climatic/regional scenarios. Five ecologically diverse watersheds, each with an established data history, were chosen. This program may prove to be a rich data source for ecoregions in which the five watersheds are located. Many publications on the WEBB project are available. See the USGS website for more details (http://water.usgs.gov/nrp/webb/about.html).

USDA

Agricultural Research Service (ARS)

ARS houses Natural Resources and Sustainable Agricultural Systems, which has seven national programs to examine the effect of agriculture on the environment. The program on Water Quality and

Management addresses the role of agriculture in nonpoint source pollution through research on Agricultural Watershed Management and Landscape Features, Irrigation and Drainage Management Systems, and Water Quality Protection and Management Systems. Research is conducted across the country and several models and databases have been developed. Information on research and program contacts is listed on the website (http://www.nps.ars.usda.gov/programs/nrsas.htm).

Forest Service

The Forest Service has designated research sites across the country, many of which are Long Term Ecological Research (LTER) sites. Many of the data from these experiments are available in the USFS databases located on the website (http://www.fs.fed.us/research/). Most of the data are forest-related, but may be of use for determining land uses and questions on silviculture runoff.

National Science Foundation (NSF)

The National Science Foundation funds projects for the LTER Network. The Network is a collaboration of over 1,100 researchers investigating a wide range of ecological topics at 24 different sites nationwide. The LTER research programs are not only an extremely rich data source, but also a source of data available to anyone through the Network Information System (NIS), the NSF data source for LTER sites. Data sets from sites are highly comparable due to standardization of methods and equipment. Data can be accessed from the website http://www.lternet.edu/research/data/nis/.

U.S. Army Corps of Engineers (COE)

The U.S. Army Corps of Engineers is responsible for more than 750 reservoirs. Many have extensive monitoring data that could contribute to the development of nutrient criteria for tributaries to those reservoirs. The COE focuses more on water quantity issues than on water quality issues. As a result, much of the river and stream system data collected by the COE does not include nutrient or algal constituents. Nonetheless, the COE does have a large water sampling network and supports USGS and EPA monitoring efforts in many programs. A list of the water quality programs that the COE actively participates in was compiled in 1997. This information can be found at the website: http://cw71.cw-wc.usace.army.mil/wqinfo/wq98sem/ANNWQMGT.HTM.

U.S. Department of the Interior, Bureau of Reclamation (BuRec)

The Bureau of Reclamation manages many irrigation and water supply reservoirs in the West, some of which may have operational data available. These data focus on water supply information and limited water quality data. However, real time flow data are collected for rivers supplying water to BuRec, which may be useful for the flow component of criteria development. These data can be gathered on a site-specific basis from the BuRec website: www.usbr.gov. Extensive remote sensing data are available from the website: http://wais.rsgis.do.usbr.gov/html/rsgig_wq.html.

State/Tribal Monitoring Programs

Most states monitor some subset of stream and river systems within their borders for algal and nutrient variables. Data collected by State/Tribal water quality monitoring programs can be used for nutrient criteria development. These data should be available from the agencies responsible for monitoring.

Volunteer Monitoring Programs

Many States have volunteer water quality monitoring programs. Some programs are state-sponsored, while others are independent organizations such as Adopt-A-Stream. Citizens in many areas donate their time, money, or experience to aid State, Tribal, and local governments in collecting water quality data. Volunteers analyze water samples for DO (dissolved oxygen), nutrients, pH, temperature, and a host of other water constituents; evaluate the health of stream habitats and aquatic biological communities; note stream-side conditions and land uses that may affect water quality; catalogue and collect beach debris; and restore degraded habitats.

State and local agencies may use volunteer data to screen for water quality problems, establish trends in waters that would otherwise be unmonitored, and make planning decisions. Volunteers benefit from learning more about their local water resources and identifying what conditions or activities might contribute to pollution problems. As a result, volunteers frequently work with clubs, environmental groups, and State/Tribal or local governments to address problem areas.

The EPA supports volunteer monitoring and local involvement in protecting our water resources. EPA support takes many forms including: sponsoring national and regional conferences to encourage information exchange among volunteer groups, government agencies, businesses, and educators; publishing sampling methods manuals for volunteers; producing a nationwide directory of volunteer programs; and providing technical assistance (primarily on quality control and lab methods) and Regional coordination through the ten EPA Regional offices. In addition, grants to States/Tribes that can be used to support volunteer monitoring in lakes and for nonpoint source pollution control are managed by the EPA Regions (http://www.epa.gov/OWOW/monitoring/volunteer/epavm.html).

Adopt-A-Stream

The Adopt-A-Stream Foundation (AASF) is a non-profit organization that works to increase public awareness and involvement in water quality issues, stream enhancement, and environmental education. Their two main areas of focus are Environmental Education and Habitat Restoration. AASF seeks to protect streams through volunteer work, encouraging school and community groups, sports clubs, civic organization, and individuals to become “Streamkeepers.” “Adoption” of a stream requires that volunteers provide long-term care of the stream and establish stream monitoring, restoration, and community-wide environmental education activities. AASF provides education materials, classes, and tools for monitoring. Data collected through the volunteer monitoring associated with Adopt-A-Stream is usually site-specific, focusing on a single stream. However, if volunteers have been properly trained, the data collected may be useful in helping identify streams at risk for nutrient problems. The AASF website contains additional information on this organization and data they may be able to provide (http://www.streamkeeper.org/).

American Heritage Rivers

The American Heritage Rivers Initiative is a program launched by President Clinton to help communities restore their local waters and waterfront areas. Participation is voluntary and must be initiated by the community. To date, fourteen rivers have been designated on the basis of historical, economic, and environmental considerations. One goal of the program is to develop additional information that can be used by communities to improve any river system. Through the American Heritage Rivers website (http://www.epa.gov/OWOW/heritage/rivers.html), valuable information about our nation's rivers is easily available to everyone. Information organized geographically on flood events, population change, road networks, condition of water resources, and partnerships already at work in the area is available. Additionally, customized maps and environmental and educational assessment models will be made available through this initiative.

Electric Utilities
Many electric utilities own reservoirs for hydroelectric power generation, and are required to monitor the reservoirs' water quality. The largest of these, the Tennessee Valley Authority (TVA), has extensive chemical and biological monitoring data from most of its reservoirs from the early 1980s to the present. Data collected in conjunction with hydroelectric reservoirs must be gathered from the facility owners or managers.

Drinking Water Facilities
Many local drinking water facilities are supplied from river systems. These facilities continuously monitor some water quality parameters at the intake pipe. Nutrients are infrequently monitored by most of these facilities, but supplemental data, i.e., turbidity, pH, and flow are usually measured. These data may not provide the necessary parameters for deriving criteria, but may be very useful in combination with State/Tribal water quality monitoring data to develop criteria. Data from these facilities should be accessed locally for the waterbody of concern.

Academic and Literature Sources
Many research studies are conducted by academic institutions that may provide data useful for developing nutrient criteria. Much of the research conducted by the academic community concentrates on unimpaired or minimally impaired systems. While data collected from these sources may not be directly representative of the population of stream systems within an ecoregion, they could be useful for identifying reference conditions. Academic research also tends to be site-specific and span a limited number of years, although data for some systems may span 20 years or more. Academic research data should be available from researchers and the scientific literature.

QUALITY OF HISTORICAL DATA

The value of older historical data sets is a recurrent problem because data quality is often unknown. Knowledge of data quality is also problematic for long-term data repositories such as STORET and long-term State databases, where objectives, methods, and investigators may have changed many times over the years. The most reliable data tend to be those collected by a single agency, using the same protocol, for a limited number of years. Supporting documentation should be examined to determine the consistency of sampling and analysis protocols. Investigators must determine the acceptability of data contained in large, heterogeneous data repositories. Considerations and requirements for acceptance of these data are described below.

Location
STORET and USGS data are geo-referenced with latitude, longitude, and Reach File 3 (RF3) codes. Geo-reference data can be used to select specific locations, or specific USGS Hydrologic Units. In addition, STORET often contains a site description. Knowledge of the rationale and methods of site

selection from the original investigators may supply valuable information. Metadata of this type, when known, is frequently stored within large long-term databases.

Variables and Analytical Methods
Thousands of variables are recorded in database records. Each separate analytical method yields a unique variable. For example, five ways of measuring TP results in five unique variables. We do not recommend mixing analytical methods in data analyses because methods differ in accuracy, precision, and detection limits. Data analyses should concentrate on a single analytical method for each parameter of interest. Selection of a particular "best" method may result in too few observations, in which case it may be more fruitful to select the most frequently used analytical method in the database. Data may have been recorded using analytical methods under separate synonymous names, or analytical methods incorrectly entered when data were first added to the database. Review of recorded data and analytical methods recorded by knowledgeable personnel is necessary to correct these problems.

Laboratory Quality Control (QC)
Laboratory QC data (blanks, spikes, replicates, known standards, etc.) are infrequently reported in larger data repositories. Records of general laboratory quality control protocols and specific quality control procedures associated with specific datasets are valuable in evaluating data quality. However, premature elimination of lower quality data can be counterproductive, because the increase in variance caused by analytical laboratory error may be negligible compared to natural variability or sampling error, especially for nutrients and related water quality parameters. However, data of uncertain and undocumented quality should not be accepted.

Data Collecting Agencies
Selecting data from particular agencies with known, consistent sampling and analytical methods and known quality will reduce variability due to unknown quality problems. Requesting data review for quality assurance from the collecting agency will reduce uncertainty about data quality.

Time Period
Long-term records are critically important for establishing trends. Determining if trends exist in the time series database is also important for characterizing reference conditions for nutrient criteria. Length of time series data needed for analyzing nutrient data trends is discussed in Chapter 4.

Index Period
An index period for estimating average concentrations can be established if nutrient and water quality variables were measured through seasonal cycles. The index period may be the entire year or the summer growing season. The best index period is determined by considering stream characteristics for the region, the quality and quantity of data available, and estimates of temporal variability (if available). Additional information and considerations for establishing an index period are discussed in Chapter 4.

Representativeness
Data may have been collected for specific purposes. Data collected for toxicity analyses, effluent limit determinations, or other pollution problems may not be useful for developing nutrient criteria. Further, data collected for specific purposes may not be representative of the region or stream classes of interest. The investigator must determine if stream systems or a subset of the stream systems in the database are

representative of the population of stream systems to be characterized. If a sufficient sample of representative systems cannot be found, then a new survey will be necessary (see Chapter 4).

Gather New Data

New data should be gathered following the sampling design protocols discussed in Chapter 4. New data collection activities for developing nutrient criteria should focus on filling in gaps the database and collecting regional monitoring data. Data gathered under new monitoring programs should be imported into databases or spreadsheets and merged with existing data for criteria development.

Data Reduction

Data reduction requires a clear idea of the analysis that will be performed and a clear definition of the sample unit for the analysis. For example, a sample unit might be defined as "a watershed during July-August". For each variable measured, a mean value would then be estimated for each watershed in each July-August index period on record. Analyses are then done with the observations (estimated means) for each sample unit, not with the raw data. Steps in reducing the data include:

- Selecting the long-term time period for analysis;
- Selecting an index period;
- Selecting relevant chemical species;
- Identifying the quality of analytical methods;
- Identifying the quality of the data recorded; and
- Estimating values for analysis (mean, median, minimum, maximum) based on the reduction selected.

QUALITY ASSURANCE/QUALITY CONTROL

The validity and usefulness of data depend on the care with which they were collected, analyzed and documented. The EPA provides guidance on data quality assurance (QA) and quality control (USEPA 1998b) to assure the quality of data. Factors that should be addressed in a QA/QC plan are elaborated below. The QA/QC plan should state specific goals for each factor and should describe the methods and protocols used to achieve the goals. The five factors discussed below are: representativeness, completeness, comparability, accuracy and precision.

Representativeness

Sampling program design (when, where, how you sample) should produce samples that are *representative* or typical of the environment being described. Sampling designs for developing nutrient criteria are addressed in Chapter 4.

Completeness

Data sets are often incomplete because of spilled samples, faulty equipment, and/or lost field notebooks. A QA/QC plan should describe how complete the data set must be in order to answer the questions posed (with a statistical test of given power and confidence) and the precautions being taken to ensure that completeness. Data collection procedures should document the extent to which these conditions have been met. Incomplete data sets may not invalidate the collected data, but may reduce the rigor of statistical analyses. Therefore, precautions should be taken to ensure data completeness. Precautions to ensure completeness may include collecting extra samples, having back-up equipment in the field, installing alarms on freezers, copying field notebooks after each trip, and/or maintaining duplicate sets of data in two locations.

Comparability

In order to compare data collected under different sampling programs or by different agencies, sampling protocols and analytical methods must demonstrate comparable data. The most efficient way to produce comparable data is to use sampling designs and analytical methods that are widely used and accepted.

Accuracy

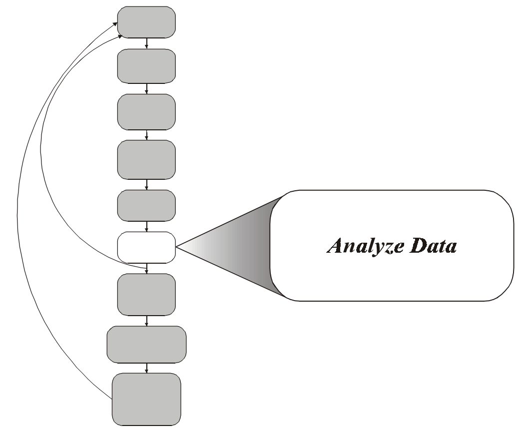
To assess the accuracy of field instruments and analytical equipment, a standard (a sample with a known value) must be analyzed and the measurement error or bias determined. Internal standards should periodically be checked with external standards provided by acknowledged sources. At Federal, State, Tribal, and local government levels, the National Institute of Standards and Technology (NIST) provides advisory and research services to all agencies by developing, producing, and distributing standard reference materials. For calibration sevices and standards see: http://ts.nist.gov/ts/htdocs/230/233/calibration/home.html.

Standards and methods of calibration are typically included with turbidity meters, pH meters DO meters, and DO testing kits. The USGS, the EPA and some private companies provide reference standards or QC samples for nutrients. Reference standards for chlorophyll are also available from the EPA and some private companies, although chlorophyll standards are time and temperature sensitive because they degrade over time.

Variability

Natural variability rather than imprecision in the method used, is usually the greatest source of error in the constituent measured. The variability in field measurements and analytical methods should be demonstrated and documented to identify the source of variability when possible. EPA QA/QC guidance provides an explanation and protocols for measuring sampling variability (USEPA 1998b). Methods for creating a chlorophyll standard to determine if the spectrophotometer is measuring chlorophyll consistently from one year to the next or from the beginning to the end of an analytical run are described in Wetzel and Likens (1991). In addition, a large number of replicates for each sample time and site must be collected because the largest source of variation is likely to be natural (i.e., in the samples).

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:

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

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

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

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

where seasonal mean and maximum benthic chlorophyll are in mg/m^2 and TN and TP are in $\mu g/L$. 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.

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

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

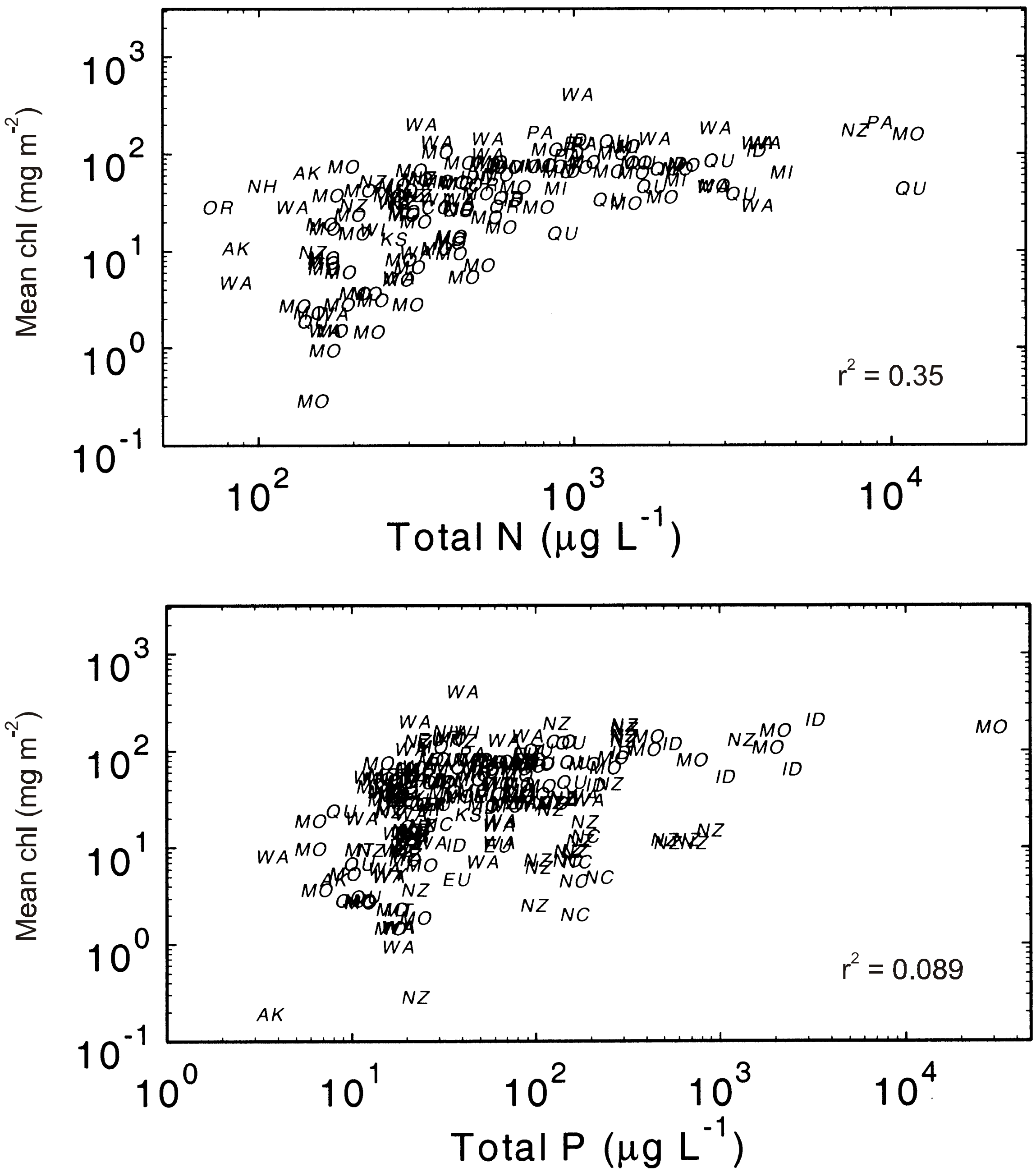


Figure 7. Relationships of log-transformed mean chlorophyll *a* as a function of TN and TP.

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:

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

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.

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

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:

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

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