United States
Environmental Protection
Agency

Office of Water
Office of Science and Technology
Washington, DC 20460

EPA-822-R-07-004
September 2007
www.epa.gov

Nutrient Criteria Technical Guidance Manual

Wetlands

DISCLAIMER

This manual provides technical guidance to States, Authorized Tribes, Territories and other authorized jurisdictions to establish water quality criteria and standards under the Clean Water Act (CWA), in order to protect aquatic life from acute and chronic effects of nutrient over-enrichment. Under the CWA, States and Authorized Tribes are directed to establish water quality criteria to protect designated uses. States and Authorized Tribes may use approaches for establishing water quality criteria that differ from those recommended in this guidance. This manual constitutes EPA's scientific recommendations regarding the development of numeric criteria reflecting ambient concentrations of nutrients that protect aquatic life. However, it does not substitute for the CWA or EPA's regulations; nor is it a regulation itself. Thus, it cannot impose legally binding requirements on EPA, States, Authorized Tribes, or the regulated community, and might not apply to a particular situation or circumstance. Further, States and Authorized Tribes may choose to develop different types of criteria for wetlands protection, including narrative criteria. EPA may change this guidance in the future.

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ACKNOWLEDGMENTS

The authors wish to acknowledge the efforts and input of several individuals. These include members of our EPA National Nutrient Team: Jim Carleton, Lisa Larimer, and Robert Cantilli; members of the EPA Wetlands Division: Kathy Hurld, Chris Faulkner and Donna Downing; members of the Office of Research and Development and their Office of Science Policy: Valerie Chan, Joseph Schubauer-Berigan, Charles Lane, John Morrice, Christine Weilhoefer, Tony Olsen, and Walt Nelson; the Office of General Counsel: Leslie Darman. We also want to thank Kristine Pintado (DEQ, Louisiana) for her contributions and her careful review and comments.

This document was peer reviewed by a panel of expert scientists. The peer review charge focused on evaluating the scientific validity of the processes and techniques for developing nutrient criteria described in the guidance. The peer review panel comprised Dr. Robert H. Kadlec, Dr. Lawrence Richards Pomeroy, Dr. Eliska Rejmankova and Dr. Li Zhang. Edits and suggestions made by the peer review panel were incorporated into the final version of the guidance.

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EXECUTIVE SUMMARY

The purpose of this document is to provide scientifically defensible guidance to assist States, Authorized Tribes, Territories, and other authorized jurisdictions—hereafter referred to as States—in assessing the nutrient status of their wetlands, and to provide technical assistance for developing numeric nutrient criteria for wetland systems in an eco-region. The development of nutrient criteria is part of an initiative by the U.S. Environmental Protection Agency (USEPA) to address the problem of cultural eutrophication, i.e., nutrient pollution caused by human activities (USEPA 1998a). Cultural eutrophication is not new; however, traditional efforts at nutrient control have been only moderately successful. Specifically, efforts to control nutrients in water bodies that have multiple nutrient sources (point and nonpoint sources) have been less effective in providing satisfactory, timely remedies for enrichment-related problems. Development and adoption of numeric criteria into water quality standards aids nitrogen and phosphorus pollution control efforts by providing clear numeric goals for water quality protection. Furthermore, numeric nutrient criteria provide specific water quality goals that will assist researchers in designing improved best management practices.

In 1998, the USEPA published a report entitled, *National Strategy for the Development of Regional Nutrient Criteria* (USEPA 1998a). This report outlines a framework for development of waterbody-specific technical guidance that can be used to assess nutrient status and develop region-specific numeric nutrient criteria. The document presented here is the wetland-specific technical guidance for developing numeric nutrient criteria. The Nutrient Criteria Technical Guidance Manuals for Rivers and Streams (USEPA, 2000b), Lakes and Reservoirs (USEPA, 2000a) and Estuarine and Coastal Marine Waters (USEPA, 2001) have been completed and are available at: http://www.epa.gov/waterscience/criteria/nutrient/guidance/index.html.

Section 303(c) of the Clean Water Act directs States to adopt water quality standards for waters that are "waters of the United States," including wetlands that are waters of the United States.[1] A water quality standard consists of three main elements: (1) one or more designated uses of each of the State's waters, such as recreation or propagation of fish; (2) criteria expressed as pollutant concentration levels or narrative statements representing a quality of water that supports a designated use; and, (3) an anti-degradation policy to protect existing uses and high quality waters.

[1] For further information regarding the scope of 'waters of the U.S.' in light of the U.S. Supreme Court's 2006 decision in *Rapanos v. United States*, see "Clean Water Act Jurisdiction Following the U.S. Supreme Court's Decision in Rapanos v. United States & Carabell v. United States," which was jointly issued by the U.S. Environmental Protection Agency and the Army Corps of Engineers and is available at: http://www.epa.gov/owow/wetlands/.

The information used in developing the technical approaches in this document came from references about studies of wetlands in a wide range of conditions, but not wetlands with a high degree of modification (e.g., wetlands that are considered "prior converted cropland" or artificial wetlands specifically engineered to protect or improve downstream water quality). This guidance is to assist States in developing numeric nutrient criteria for wetlands, should they choose to do so. States may choose to develop different types of criteria for wetlands protection, including site-specific or narrative criteria for wetlands protection, as long as they are scientifically defensible and protective of the designated uses (40 CFR § 131.11). This Guidance Manual includes chapters dealing with the following topics:

CLASSIFICATION OF WETLANDS

Classification strategies for nutrient criteria development include:

- physiographic regions
- hydrogeomorphic class
- water depth and duration
- vegetation type or zone

Choosing a specific classification scheme will likely depend on practical considerations, such as: whether a classification scheme is available in mapped digital form or can be readily derived from existing map layers; whether a hydrogeomorphic or other classification scheme has been refined for a particular region and wetland type; and, whether classification schemes are already in use for monitoring and assessment of other waterbody types in a State or region.

SAMPLING DESIGN

Three sampling designs for new wetland monitoring programs are described:

- probabilistic sampling
- targeted/tiered approach
- BACI (Before/After, Control/Impact)

These approaches are designed to obtain a significant amount of information for statistical analyses with relatively minimal effort. Sampling efforts should be designed to collect information that will answer management questions in a way that permits robust statistical analysis. In addition, site selection, characterization of reference sites or systems, and identification of appropriate index periods are all of particular concern when selecting an appropriate sampling design. Careful selection of sampling design will allow the best use of financial resources and result in the collection of high quality data for evaluation of the wetland resources of a State.

CANDIDATE VARIABLES FOR ESTABLISHING NUTRIENT CRITERIA

Candidate variables to use in determining nutrient condition of wetlands and to help identify appropriate nutrient criteria for wetlands consist of supporting variables, causal variables, and response variables. Supporting variables provide information useful in normalizing causal and response variables and categorizing wetlands. Causal variables are intended to characterize nutrient availability (or assimilation) in wetlands and could include nutrient loading rates and soil nutrient concentrations. Response variables are intended to characterize biotic response and could include community structure and composition of macrophytes and algae. Recommended variables for wetland nutrient criteria development described in this chapter are:

1. Causal variables – nutrient loading rates, land use, extractable and total soil nitrogen (N) and phosphorus (P), water column N and P;

2. Response variables – nutrient content of wetland vegetation (algal and/or higher plants), aboveground biomass and stem height, macrophyte, algal, and macroinvertebrate community structure and composition; and,

3. Supporting variables – hydrologic condition/balance, conductivity, soil pH, soil bulk density, soil organic matter content.

DATABASE DEVELOPMENT AND NEW DATA COLLECTION

A database of relevant water quality information can be an invaluable tool to States as they develop nutrient criteria. In some cases, existing data are available and can provide additional information that is specific to the region where criteria are to be set. However, little or no data are available for most regions or parameters, and creating a database of newly gathered data is strongly recommended. In the case of existing data, the data should be geolocated, and their suitability (type and quality and sufficient associated metadata) ascertained.

DATA ANALYSIS

Data analysis is critical to nutrient criteria development. Proper analysis and interpretation of data determine the scientific defensibility and effectiveness of the criteria. Therefore, it is important to evaluate short and long-term goals for wetlands of a given class within the region of concern. The purpose of this chapter is to explore methods for analyzing data that can be used to develop nutrient criteria consistent with these goals. Techniques discussed in this chapter include:

- Distribution-based approaches that examine distributions of primary and supporting variables (i.e., the percentile approach);

- Response-based approaches that develop relationships between measurements of nutrient exposure and ecological responses (i.e., tiered aquatic life uses);

- Partitioning effects of multiple stressors;
- Statistical techniques;
- Multi-metric indices; and,
- Linking nutrient availability to primary producer response.

CRITERIA DEVELOPMENT

Several methods can be used to develop numeric nutrient criteria for wetlands.They include, but are not limited to, criteria development methods that are detailed in this document:

- Comparing conditions in known reference systems for each established wetland type and class based on best professional judgment (BPJ) or identifying reference conditions using frequency distributions of empirical data and identifying criteria using percentile selections of data plotted as frequency distributions;
- Refining classification systems using models, and/or examining system biological attributes in comparison to known reference conditions to assess the relationships among nutrients, vegetation or algae, soil, and other variables and identifying criteria based on thresholds where those response relationships change; and,
- Using or modifying published nutrient and vegetation, algal, and soil relationships and values to identify appropriate criteria.

A weight of evidence approach with multiple attributes that combine one or more of the development approaches will generally produce criteria of greater scientific validity.

The purpose of this document is to provide guidance on developing numeric nutrient criteria in a scientifically valid manner, and is not intended to address the multiple, complex issues surrounding implementation of water quality criteria and standards. Implementation will be addressed in a different process and additional implementation assistance will also be provided through other technical assistance projects provided by EPA. For issues specific to constructed wetlands, States should refer to http://www.epa.gov/owow/wetlands/watersheds/cwetlands.html.

Chapter 1 Introduction

1.1 INTRODUCTION

PURPOSE

The purpose of this document is to provide technical guidance to assist States in assessing the nutrient status of their wetlands by considering water, vegetation, and soil conditions, and to provide technical assistance for developing regionally-based, scientifically defensible, numeric nutrient criteria for wetlands. In this document, the term "wetlands" or "wetland systems" refers to wetlands that are considered as "waters of the United States." However, States may, at their discretion, use this document to develop water quality criteria and standards for wetlands that are considered waters of the State.

EPA's development of recommended nutrient criteria is part of an initiative by the U.S. Environmental Protection Agency (USEPA) to address the problem of cultural eutrophication. In 1998, the EPA published a report entitled, *National Strategy for the Development of Regional Nutrient Criteria* (USEPA 1998a). The report outlines a framework for EPA's development of waterbody-specific technical guidance that can be used to assess nutrient status and develop region-specific numeric nutrient criteria. This document is the technical guidance for developing numeric nutrient criteria for wetlands. Approaches to nutrient criteria development are similar for freshwater and tidal wetlands, however, this document has a freshwater emphasis. EPA recognizes that wetlands are different from the other types of waters of the U.S. in that they frequently do not have standing or flowing water, and the soils and vegetation components are more dominant in these systems than in the other waterbody types (lakes, streams, estuaries). Additional, more specific information on sampling wetlands is available at: www.epa.gov/waterscience/criteria/nutrient/guidance/index.html.

BACKGROUND

Cultural eutrophication (human-caused inputs of excess nutrients in waterbodies) is one of the primary causal factors that impair surface waters in the U.S. (USEPA 1998a). Both point and nonpoint sources of nutrients contribute to impairment of water quality. Point source discharges of nutrients are relatively constant and are controlled by the National Pollutant Discharge Elimination System (NPDES) permitting program. Nonpoint source pollutant inputs have increased in recent decades, resulting in degraded water quality in many aquatic systems. Nonpoint sources of nutrients are most commonly intermittent and are usually linked to runoff, atmospheric deposition, seasonal agricultural activity, and other irregularly occurring events such as silvicultural activities. Control of nonpoint source pollutants typically focuses on land management activities and regulation of pollutants released to the atmosphere (Kronvang et al., 2005; Howarth et al., 2002; Carpenter et al., 1998).

The term eutrophication was coined in reference to lake systems. The use of the term for wetlands can be problematic due to the confounding nature of hydrodynamics, light, and the differences in the responses of algae and vegetation. Eutrophication in this document refers to human-caused inputs of excess nutrients and is not intended to indicate the same scale or responses to eutrophication found in lake systems and codified in the trophic state index for lakes (Carlson 1977). This manual is intended to provide guidance for identifying deviance from natural conditions with respect to cultural eutrophication in wetland systems. Hydrologic alteration and pollutants other than excess nutrients may amplify or reduce the effects of nutrient pollution, making specific responses to nutrient pollution difficult to quantify. EPA recognizes these issues, and presents recommendations for analyzing wetland systems with respect to nutrient condition for development of nutrient criteria in spite of these confounding factors.

Cultural eutrophication is not new; however, traditional efforts at nutrient control have been only moderately successful. Specifically, efforts to control nutrients in waterbodies that have multiple nutrient sources (point and nonpoint sources) have been less effective in providing satisfactory, timely remedies for enrichment-related problems (Azzellino et al., 2006; Merseburger et al., 2005; Carpenter et al., 1998). Development and adoption of numeric nutrient criteria into water quality standards aid State nutrient pollution control efforts by providing clear numeric goals for nutrient concentrations. Furthermore, numeric nutrient criteria provide specific water quality goals that will assist researchers in designing improved best management practices.

1.2 WATER QUALITY STANDARDS AND CRITERIA

States are responsible for setting water quality standards to protect the physical, biological, and chemical integrity of their waters. “Water quality standards (WQS) are provisions of State or Federal law which consist of a designated use or uses and water quality criteria for such waters to protect such uses.[2] Water quality standards are to protect public health or welfare, enhance the quality of the water, and serve the purposes of the Act (40 CFR 131.2 and 131.3(i))” (USEPA 1994). A water quality standard defines the goals for a wetland by: 1) designating its specific uses, 2) setting criteria to protect those uses, and, 3) establishing an antidegradation policy to protect existing water quality.

Designated uses are a State’s concise statements of its goals and expectations for each of the individual surface waters under its jurisdiction. With designated uses, States can work with their publics to identify a collective goal for their waters that they intend to strive for as they manage water quality. EPA encourages States to evaluate the attainability of these goals and expectations

[2] EPA published guidance on water quality standards for wetlands in 1990 (USEPA, 1990c). Examples of different state approaches for standards can be found at: http://www.epa.gov/owow/wetlands/initiatives/.

to ensure they have designated the appropriate uses. Generally, the effectiveness of designated uses in guiding water quality management programs is greater if they:

- Identify specific expectations based on as much data as possible to reduce ambiguity.
- Recognize and accommodate inherent natural differences among surface water types.
- Acknowledge certain human caused conditions that limit the potential to support uses.

Designated uses may involve a spectrum of expectations, depending on the type of wetland and associated hydropatterns, where the wetland is situated with respect to natural landscape features and human activity, and the historical and anticipated future functions that the wetland provides. Criteria to protect specific uses, in turn, should reflect these differing expectations where appropriate. The information used in developing the technical approaches in this document was drawn from references about studies of wetlands in a wide range of conditions, but not wetlands with a high degree of modification (e.g., wetlands that are considered "prior converted cropland" or artificial wetlands specifically engineered to protect or improve downstream water quality).

Water quality criteria may be expressed as numeric values or narrative statements. As of this writing, most of the Nation's waterbodies do not have numeric nutrient criteria, but instead rely on narrative criteria that describe the desired condition. Narrative criteria are descriptions of conditions necessary for a water to attain the designated uses. An example of a narrative criterion for nutrients is shown below:

> In no case shall nutrient concentrations of a body of water be altered so as to cause an imbalance in natural populations of aquatic flora or fauna.

Numeric criteria, on the other hand, are values assigned to measurable components of water quality to protect designated uses, such as the concentration of a specific constituent that is present in the water column. An example of a numeric criterion for specific waters is shown below:

> (4) Phosphorus Criterion.
> (a) The numeric phosphorus criterion for Class III waters shall be a long-term geometric mean of 10 ppb, but shall not be lower than the natural conditions of the Class II waters, and shall take into account spatial and temporal variability. Achievement of the criterion shall be determined by the methods in this subsection. Exceedences of the provisions of the subsection shall not be considered deviations from the criterion if they are attributable to the full range of natural spatial and temporal variability, statistical variability inherent in sampling and testing procedures, or higher natural background conditions.

In addition to narrative and numeric criteria, some States use numeric translator mechanisms—mechanisms that translate narrative (qualitative) standards into numeric (quantitative) values for use in evaluating water quality data. Translator mechanisms may be useful internally by the State

agency for water assessment and management and serve as an intermediate step between numeric and narrative criteria.

Numeric criteria provide distinct interpretations of acceptable and unacceptable conditions, form the foundation for measurement of environmental quality, and reduce ambiguity for management and enforcement decisions. The lack of numeric nutrient criteria in State water quality standards for most of the Nation's waterbodies makes it difficult to assess the condition of waters of the U.S. with respect to nutrients, and thus hampers the water quality manager's ability to protect designated uses and improve water quality. EPA encourages States to adopt numeric nutrient criteria into their water quality standards (USEPA 2007b).

Many States have adopted some form of nutrient criteria for surface waters related to maintaining natural conditions and avoiding nutrient enrichment. Most States with nutrient criteria in their water quality standards have broad narrative criteria for most waterbodies and may also have site-specific numeric criteria for certain waters of the State. Established criteria most commonly pertain to phosphorus (P) concentrations in lakes. Nitrogen (N) criteria, where they have been established, are usually protective of human health effects or relate to toxic effects of ammonia and nitrates. In general, levels of nitrate (10 ppm [mg/L] for drinking water) and ammonia high enough to be problematic for human health or toxic to aquatic life (1.24 mg N/L at pH = 8 and 25°C) will also cause problems of enhanced algal growth (USEPA 1986).

Numeric nutrient criteria can provide a variety of benefits and may be used in conjunction with State and Federal biological assessments, Nonpoint Source Programs, Watershed Implementation Plans, and in development of Total Maximum Daily Loads (TMDLs) to improve resource management and support watershed protection activities at local, State, and national levels. Information obtained from compiling existing data and conducting new surveys can provide water quality managers and the public a better perspective on the condition of State waters. The compiled information can be used to most effectively budget personnel and financial resources for the protection and restoration of State waters. In a similar manner, data collected in the criteria development and implementation process can be compared before, during, and after specific management actions. Analyses of these data can determine the response of the wetland and the effectiveness of management endeavors.

1.3 NUTRIENT ENRICHMENT PROBLEMS

Water quality can be affected when watersheds are modified by alterations in vegetation, sediment transport, fertilizer use, industrialization, urbanization, or conversion of native forests and grasslands to agriculture and silviculture (Turner and Rabalais 1991; Vitousek et al., 1997; Carpenter et al., 1998). Cultural eutrophication, one of the primary factors causing impairment of U.S. surface waters (USEPA 1998a), results from point and nonpoint sources of nutrient pollution. Nonpoint source pollutant inputs have increased in recent decades and have degraded

water quality in many aquatic systems (Carpenter et al., 1998). Control of nonpoint source pollutants focuses on land management activities and regulation of pollutants released to the atmosphere (Carpenter et al., 1998).

Nutrient enrichment frequently ranks as one of the top causes of water resource impairment. EPA reported to Congress that of the waterbodies surveyed and reported impaired, 20 percent of rivers and 50 percent of lakes were listed with nutrients as the primary cause of impairment (USEPA 2000c). Few States currently include wetland monitoring in their routine water quality monitoring programs (only eleven States reported attainment of designated uses for wetlands in the *National Water Quality Inventory 1998 Report to Congress* (USEPA 1998b) and only three States used monitoring data as a basis for determining attainment of water quality standards for wetlands); thus, the extent of nutrient enrichment and impairment of wetland systems is largely undocumented. Increased wetland monitoring by States will help define the extent of nutrient enrichment problems in wetland systems.

The best-documented case of cultural eutrophication in wetlands is the Everglades ecosystem. The Everglades ecosystem is a wetland mosaic that is composed primarily of oligotrophic freshwater marsh. Historically, the greater Everglades ecosystem included vast acreage of freshwater marsh, small stands of custard apple and some cattail south of Lake Okeechobee, and Big Cypress Swamp, which eventually drains into Florida Bay. Lake Okeechobee was diked to reduce flooding. The area directly south of Lake Okeechobee was then converted into agricultural lands for cattle grazing and row crop production. The cultivation and use of commercial fertilizers in the area now known as the “Everglades Agricultural Area” have resulted in release of nutrient-rich waters into the Everglades for more than thirty years. The effects of the nutrient-rich water, combined with coastal development and channeling to supply water to communities on the southern Florida coast, have significantly increased soil and water column phosphorus levels in naturally oligotrophic areas. In particular, nutrient enrichment of the freshwater marsh has resulted in an imbalance in the native vegetation. Cattail is now encroaching in areas that were historically primarily sawgrass; calcareous algal mats are being replaced by non-calcareous algae, changing the balance of native flora that is needed to support vast quantities of wildlife. Nutrient enriched water is also reaching Florida Bay, suffocating the native turtle grass as periphyton covers the blades (Davis and Ogden 1994; Everglades Interim Report 1999, 2003; Everglades Consolidated Report 2003). Current efforts to restore the Everglades are focusing on nutrient reduction and better hydrologic management (Everglades Consolidated Report 2003).

Monitoring to establish trends in nutrient levels and associated changes in biology has been infrequent for most wetland types as compared to studies in the Everglades or examination of other surface waters such as lakes. Noe et.al., (2001) have argued that phosphorus biogeochemistry and the extreme oligotrophy observed in the Everglades in the absence of anthropogenic inputs represents a unique case. Effects of cultural eutrophication, however, have been documented in a range of different wetland types. Existing studies are available to document potential impacts of anthropogenic nutrient additions to a wide variety of wetland

types, including bogs, fens, Great Lakes coastal emergent marshes, and cypress swamps. The evidence of nutrient effects in wetlands ranges from controlled experimental manipulations, to trend or empirical gradient analysis, to anecdotal observations. Consequences of cultural eutrophication have been observed at both community and ecosystem-level scales (Table 1). Changes in wetland vegetation composition resulting from cultural eutrophication of these systems have been demonstrated in bogs (Kadlec and Bevis 1990), fens (Guesewell et.al., 1998, Bollens and Ramseier 2001, Pauli et.al., 2002), meadows (Finlayson et.al., 1986), marshes (Bedford et.al., 1999) and cypress domes (Ewel 1976). Specific effects on higher trophic levels in marshes seem to depend on trophic structure (e.g., presence/absence of minnows, benthivores, and/or piscivores, Jude and Pappas 1992, Angeler et.al., 2003) and timing/frequency of nutrient additions (pulse vs. press; Gabor et.al., 1994, Murkin et.al., 1994, Hann and Goldsborough 1997, Sandilands et.al., 2000, Hann et.al., 2001, Zrum and Hann 2002).

Table 1. Observed consequences of cultural eutrophication in freshwater wetlands.[3]

Observed impact	References
Loss of submerged aquatic plants that have high light compensation points	Phillips et.al., 1978 Stephenson et.al., 1980 Galatowitsch and van der Valk 1996
Shifts in vascular plant species composition due to shifts in competitive advantage	Wentz 1976 Verhoeven et.al., 1988 Ehrenfeld and Schneider 1993 Gaudet and Keddy 1995 Koerselman and Verhoeven 1995
Increases in above-ground production	Barko 1983 Bayley et.al., 1985 Barko and Smart 1986 Vermeer 1986
Decreases in local or regional biodiversity	Mudroch and Capobianco 1979 Guntenspergen et al., 1980 Lougheed et.al., 2001 Balla and Davis 1995 VanGroenendael et.al., 1993 Bedford et.al., 1999
Increased competitive advantage of aggressive/invasive species (e.g., *Typha glauca, T. latifolia* and *Phalaris arundinacea)*	Woo and Zedler 2002 Svengsouk and Mitsch 2001 Green and Galatowitsch 2002 Maurer and Zedler 2002
Loss of nutrient retention capacity (e.g., carbon and	Nichols 1983

[3] Similar impacts in tidal and estuarine wetlands have been documented, but are not included in this table.

nitrogen storage, changes in plant litter decomposition)	Davis and van der Valk 1983 Rybczyk et.al., 1996
Major structural shifts between "clear water" macrophyte dominated systems to turbid phytoplankton dominated systems or metaphyton-dominated systems with reduced macrophyte coverage	McDougal et.al., 1997 Angeler et.al., 2003
Shifts in macroinvertebrate composition along a cultural eutrophication gradient	Chessman et.al., 2002

The cycling of nitrogen (N) and phosphorus (P) in aquatic systems should be considered when managing nutrient enrichment. The hydroperiod of wetland systems significantly affects nutrient transformations, availability, transport, and loss of gaseous forms to the atmosphere (Mitsch and Gosselink, 2000). Nutrients can be re-introduced into a wetland from the sediment, or by microbial transformation, potentially resulting in a long recovery period even after pollutant sources have been reduced. In open wetland systems, nutrients may also be rapidly transported downstream, uncoupling the effects of nutrient inputs from the nutrient source, and further complicating nutrient source control (Mitsch and Gosselink, 2000; Wetzel 2001). Recognizing relationships between nutrient input and wetland response is the first step in mitigating the effects of cultural eutrophication. When relationships are established, nutrient criteria can be developed to manage nutrient pollution and protect wetlands from eutrophication.

1.4 THE CRITERIA DEVELOPMENT PROCESS

The *National Strategy for the Development of Regional Nutrient Criteria* (USEPA 1998a) describes the principal elements of numeric nutrient criteria development. This document can be downloaded in PDF format at the Web site: http://www.epa.gov/waterscience/criteria/nutrient/strategy.html. The Strategy recognizes that a prescriptive, one-size-fits-all approach is not appropriate due to regional differences that exist and the scientific community's current technical understanding of the relationship between nutrients, algal and macrophyte growth, and other factors (e.g., flow, light, substrata). The approach chosen for criteria development therefore may be tailored to meet the specific needs of each State. The
EPA Strategy envisions a process by which State waters are initially monitored, reference conditions are established, individual waterbodies are compared to known reference waterbodies, and appropriate management measures are implemented. These measurements can be used to document change and monitor the progress of nutrient reduction activities and protection of water quality.

The National Nutrient Program represents an effort and approach to criteria development that, in conjunction with efforts made by State water quality managers, will ultimately result in a heightened understanding of nutrient-response relationships. As the proposed process is put into use to set criteria, program success will be gauged over time through evaluation of management and monitoring efforts. A more comprehensive knowledge-base pertaining to nutrient, and vegetation and/or algal relationships will be expanded as new information is gained and obstacles overcome, justifying potential refinements to the criteria development process.

The overarching goal of developing and adopting nutrient criteria is to protect and maintain the quality of our national waters. Protecting and maintaining water quality may include restoration of impaired systems, conservation of high quality waters, and protection of systems at high risk for future impairment. The specific goals of a State water quality program may be defined differently based on the needs of each State, but should, at a minimum, be established to protect the designated uses for the waterbodies within State lands. In addition, as numeric nutrient criteria are developed for the nation's waters, States should revisit their goals for water quality and revise their water quality standards as needed.

1.5 ROADMAP TO THE DOCUMENT

As set out in Figure 1.1, the process of developing numeric nutrient criteria begins with defining the goals of criteria development and water quality standards adoption. Those goals are pertinent to the classification of systems, the development of a monitoring program, and the application of numeric nutrient criteria to permit limits and water quality protection. These goals therefore should be determined with the intent of revising and adapting them as new information is obtained and the paths to achieving those goals are clarified. Defining the goals for criteria development is the first step in the process. The summaries below describe each chapter in this document. The document is written to provide recommendations for a stepwise procedure for criteria development. Some chapters contain information that is not needed by some readers; the descriptions below should serve as a guide to the most relevant information for each reader.

Chapter Two describes many of the functions of wetland systems and their role in the landscape with respect to nutrients. This chapter is intended to familiarize the reader with some basic scientific information about wetlands that will provide a better understanding of how nutrients move within a wetland and the importance of wetland systems in the landscape.

Chapter Three discusses wetland classification and presents the reader with options for classifying wetlands based on system characteristics. This chapter introduces the scientific rationale for classifying wetlands, reviews some common classification schemes, and discusses their role in establishing nutrient criteria for wetlands. The classification of these systems is important to identifying their nutrient status and their condition in relation to similar wetlands.

Chapter Four provides technical guidance on designing effective sampling programs for State wetland water quality monitoring programs. Most States should begin wetland monitoring programs to collect water quality and biological data in order to develop nutrient criteria protective of wetland systems. The best monitoring programs are designed to assess wetland condition with statistical rigor and maximize effective use of available resources. The sampling protocol selected, therefore, should be determined based on the goals of the monitoring program, and the resources available.

Chapter Five gives an overview of candidate variables that could be used to establish nutrient criteria for wetlands. Primary variables are expected to be most broadly useful in characterizing wetland conditions with respect to nutrients, and include nutrient loading rates, soil nutrient concentrations, and nutrient content of wetland vegetation. Supporting variables provide information useful for normalizing causal and response variables. The candidate variables suggested here are not the only parameters that can be used to determine wetland nutrient condition, but rather identify those variables that are thought to be most likely to identify the current nutrient condition and of the greatest utility in determining a change in nutrient status.

A database of relevant water quality information can be an invaluable tool to States as they develop nutrient criteria. If little or no data are available for most regions or parameters, it may be necessary for States to create a database of newly gathered data. Chapter Six provides the basic information on how to develop a database of nutrient information for wetlands, and supplies links to ongoing database development efforts at the State and national levels.

The purpose of Chapter Seven is to explore methods for analyzing data that can be used to develop nutrient criteria. The quality of the analysis and interpretation of data generally determines whether the criteria will be scientifically defensible and effective. This chapter describes recommended approaches to data analysis for developing numeric nutrient criteria for wetlands. Included are techniques to evaluate metrics, to examine or compare distributions of nutrient exposure or response variables, and to examine nutrient exposure-response relationships.

Chapter Eight describes the details of establishing scientifically defensible criteria in wetlands. Several approaches are presented that water quality managers can use to derive numeric criteria for wetland systems in their State waters. They include: (1) the use of the reference conditions concept to characterize natural or minimally impaired wetland systems with respect to causal and response variables; (2) applying predictive relationships to select nutrient concentrations that will protect wetland function; and, (3) developing criteria from established nutrient exposure-response relationships (as in the peer-reviewed, published literature). This chapter provides recommendations regarding how to determine the appropriate numeric criterion based on the data collected and analyzed.

The appendices include a glossary of terms and acronyms and case study examples of wetland nutrient enrichment and management.

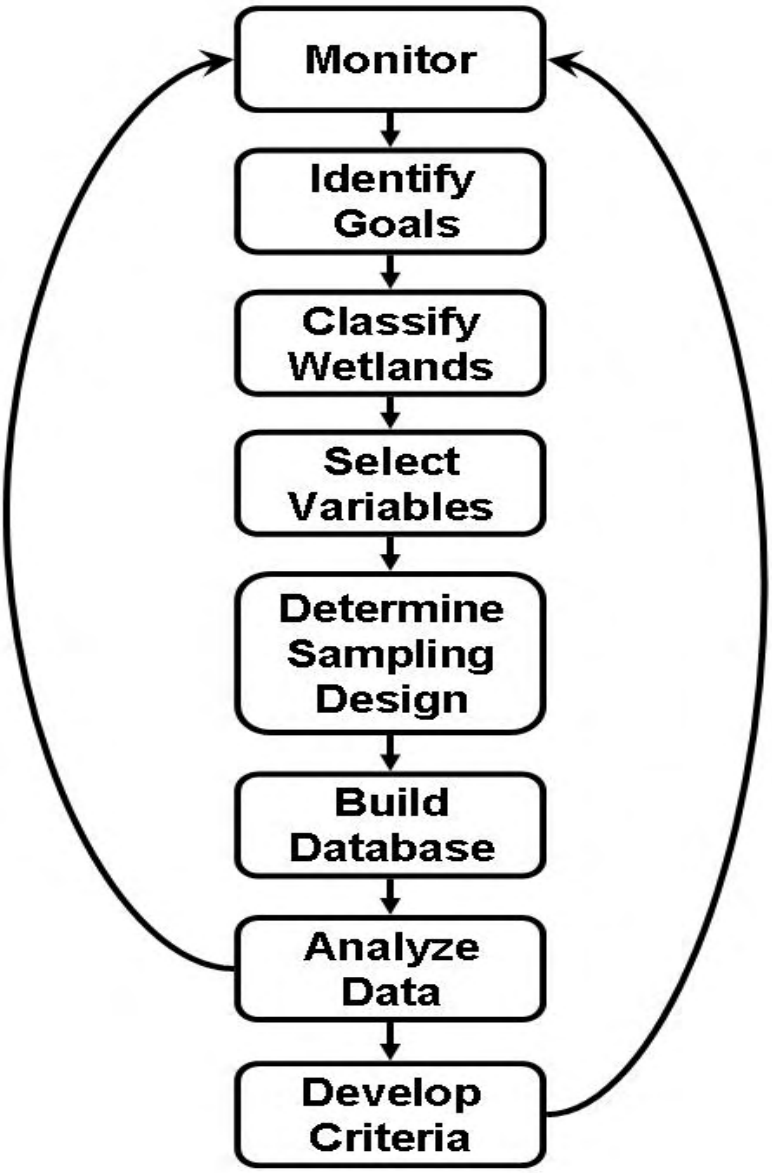

Figure 1.1 Flowchart identifying the steps of the recommended process to develop wetland nutrient criteria.

Chapter 2 Overview of Wetland Science

2.1 INTRODUCTION

Wetlands exist at the interface between terrestrial and aquatic environments. They serve as sources, sinks, and transformers of materials. (Figure 2.1) Wetlands serve as sites for transformation of nutrients such as nitrogen (N) and phosphorus (P). Dissolved inorganic forms of N and P are assimilated by microorganisms and vegetation and incorporated into organic compounds. Nitrate in surface- and ground-water is reduced to gaseous forms of N (NO, N_2O, N_2) by microorganisms, a process known as denitrification, and returned to the atmosphere. Phosphorus undergoes a variety of chemical reactions with iron (Fe), aluminum (Al), and calcium (Ca) that depend on the pH of the soil, availability of sorption sites, redox potential, and other factors. These biogeochemical reactions are important in evaluating the nutrient condition (oligotrophic, mesotrophic, eutrophic) of the wetland and its susceptibility to nutrient enrichment.

Wetlands also generally are sinks for sediment, and wetlands that are connected to adjacent aquatic ecosystems (e.g., rivers, estuaries) may trap more sediment as compared to wetlands that lack such connectivity (Fryirs et.al., 2007; Mitsch and Gosselink, 2000; Dunne and Leopold 1978). Wetlands also may be sources of organic carbon (C) (Bouchard 2007; Raymond and Bauer 2001) and nitrogen (N) (Mitsch and Gosselink 2000; Mulholland and Kuenzler 1979) to aquatic ecosystems. Production of plant biomass (leaves, wood, and roots) from riparian, alluvial, and floodplain forests and from fringe wetlands such as tidal marshes and mangroves provide organic matter to support heterotrophic foodwebs of streams, rivers, estuaries, and nearshore waters (Mitsch and Gosselink 2000; Day et.al., 1989).

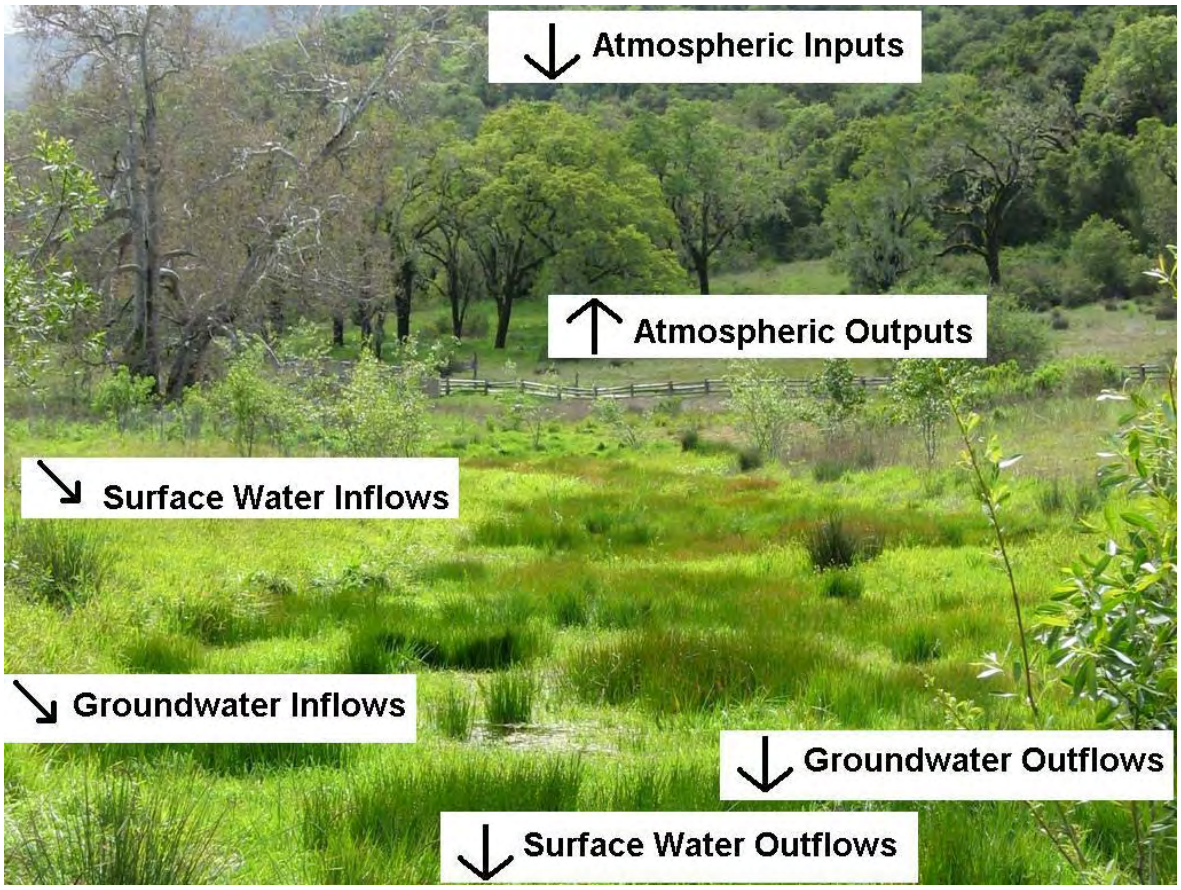

Figure 2.1. Schematic of nutrient transfer among potential system sources and sinks.

2.2 COMPONENTS OF WETLANDS

Wetlands are distinguished by three primary components: hydrology, soils, and vegetation. Wetland hydrology is the driving force that determines soil development, the assemblage of plants and animals that inhabit the site, and the type and intensity of biochemical processes. Wetland soils may be either organic or mineral, but share the characteristic that they are saturated or flooded at least some of the time during the growing season. Wetland vegetation consists of many species of algae, rooted plants that may be herbaceous and emergent, such as cattail (*Typha* sp.) and arrowhead (*Saggitaria* sp.), or submergent, such as pondweeds (*Potamogeton* sp.), or may be woody such as bald cypress (*Taxodium distichum*) and tupelo (*Nyssa aquatica*). Depending on the duration, depth, and frequency of inundation or saturation, wetland plants may be either obligate (i.e., species found almost exclusively in wetlands) or facultative (i.e., species found in wetlands but which also may be found in upland habitats). The discussion that follows provides an overview of wetland hydrology, soils, and vegetation, as well as aspects of biogeochemical cycling in these systems.

HYDROLOGY

Hydrology is characterized by water source, hydroperiod (depth, duration, and frequency of inundation or soil saturation), and hydrodynamics (direction and velocity of water movement). The hydrology of wetlands differs from that of terrestrial ecosystems in that wetlands are inundated or saturated long enough during the growing season to produce soils that are at least periodically deficient in oxygen. Wetlands differ from other aquatic ecosystems by their shallow depth of inundation that enables rooted vegetation to become established, in contrast to deep water aquatic ecosystems, where the depth and duration of inundation can be too great to support emergent vegetation. Anaerobic soils promote colonization by vegetation adapted to low concentrations of oxygen in the soil.

Wetlands primarily receive water from three sources: precipitation, surface flow, and groundwater (Figure 2.2). The relative proportion of these hydrologic inputs influences the plant communities that develop, the types of soils that form, and the predominant biogeochemical processes. Wetlands that receive mostly precipitation tend to be “closed” systems with little exchange of materials with adjacent terrestrial or aquatic ecosystems. Examples of precipitation-driven wetlands include “ombrotrophic” bogs and depressional wetlands such as cypress domes and vernal pools. Wetlands that receive water mostly from surface flow tend to be “open” systems with large exchanges of water and materials between the wetland and adjacent non-wetland ecosystems. Examples include floodplain forests and fringe wetlands such as lakeshore marshes, tidal marshes, and mangroves. Wetlands that receive primarily groundwater inputs tend to have more stable hydroperiods than precipitation- and surface water-driven wetlands, and, depending on the underlying bedrock or parent material, high concentrations of dissolved inorganic constituents such as calcium (Ca) and magnesium (Mg). Fen wetlands and seeps are examples of groundwater-fed wetlands.

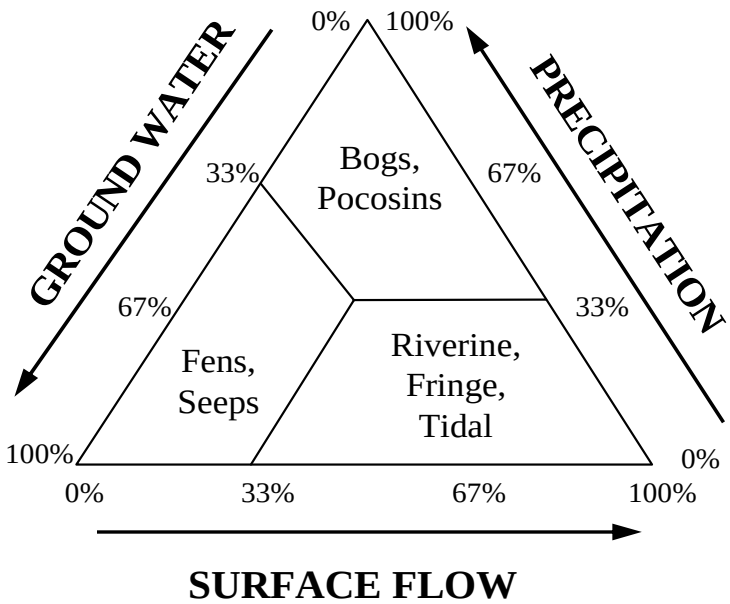

Figure 2. Relationship between water source and wetland vegetation. Modified from Brinson (1993).

Hydroperiod is highly variable depending on the type of wetland. Some wetlands that receive most of their water from precipitation (e.g., vernal pools) have very short duration hydroperiods. Wetlands that receive most of their water from surface flooding (e.g., floodplain swamps) often are flooded longer and to a greater depth than precipitation-driven wetlands. Fringe wetlands such as tidal marshes and mangroves are frequently flooded (up to twice daily) by astronomical

tides but the duration of inundation is relatively short. In groundwater-fed wetlands, hydroperiod is more stable and water levels are relatively constant as compared to precipitation- and surface water-driven wetlands, because groundwater provides a less variable input of water throughout the year.

Hydrodynamics is especially important in the exchange of materials between wetlands and adjacent terrestrial and aquatic ecosystems. In fact, the role of wetlands as sources, sinks, and transformers of material depends, in large part, on hydrodynamics. For example, many wetlands are characterized by lateral flow of surface- or ground-water. Flow of water can be unidirectional or bidirectional. An example of a wetland with unidirectional flow is a floodplain forest where surface water spills over the river bank, travels through the floodplain, and re-enters the river channel some distance downstream. In fringe wetlands such as lakeshore marshes, tidal marshes, and mangroves, flow is bidirectional as wind-driven or astronomical tides transport water into, then out of the wetland. These wetlands have the ability to intercept sediment and dissolved inorganic and organic materials from adjacent systems as water passes through them. In precipitation-driven wetlands, flow may occur more in the vertical direction as rainfall percolates through the wetland soils to underlying aquifers or nearby streams. Wetlands with lateral surface flow may be important in maintaining water quality of adjacent aquatic systems by trapping sediment and other pollutants. Surface flow wetlands also may be an important source of organic C to aquatic ecosystems as detritus, particulate C, and dissolved organic C are transported out of the wetland into rivers and streams down gradient or to adjacent lakes, estuaries, and nearshore waters.

SOILS

Wetland soils, also known as hydric soils, are defined as “soils that formed under conditions of saturation, flooding, or ponding long enough during the growing season to develop anaerobic conditions in the upper part” (NRCS 1998). Anaerobic conditions result because the rate of oxygen diffusion through water is approximately 10,000 times less than in air. Wetland soils may be composed mostly of mineral constituents (sand, silt, clay) or they may contain large amounts of organic matter. Because anaerobic conditions slow or inhibit decomposition of organic matter, wetland soils typically contain more organic matter than terrestrial soils of the same region or climatic conditions. Under conditions of near continuous inundation or saturation, organic soils (histosols) may develop. Histosols are characterized by high organic matter content, 20-30% (12-18% organic C depending on clay content) with a thickness of at least 40 cm (USDA 1999). Because of their high organic matter content, histosols possess physical and chemical properties that are very different from mineral wetland soils. For example, organic soils generally have lower bulk densities, higher porosity, greater water holding capacity, lower nutrient availability, and greater cation exchange capacity than many mineral soils.

Mineral wetland soils, in addition to containing greater amounts of sand, silt, and clay than histosols, are distinguished by changes in soil color that occur when elements such as Fe and manganese (Mn) are reduced by microorganisms under anaerobic conditions. Reduction of Fe leads to the development of grey or "gleyed" soil color as oxidized forms of Fe (ferric Fe, Fe^{3+}) are converted to reduced forms (ferrous Fe, Fe^{+2}). In sandy soils, development of a dark-colored, organic-rich surface layer is used to distinguish hydric soil from non-hydric (terrestrial) soil. An organic-rich surface layer, indicative of periodic inundation or saturation, is not sufficiently thick (<40 cm) to qualify as a histosol, which forms under near-continuous inundation.

Wetland soils serve as sites for many biogeochemical transformations. They also provide long and short term storage of nutrients for wetland plants. Wetland soils are typically anaerobic within a few millimeters of the soil-water interface. Water column oxygen concentrations are often depressed due to the slow rate of oxygen diffusion through water. However, even when water column oxygen concentrations are supported by advective currents, high rates of oxygen consumption lead to the formation of a very thin oxidized layer at the soil-water interface. Similar oxidized layers can also be found surrounding roots of wetland plants. Many wetland plants are known to transport oxygen into the root zone, thus creating aerobic zones in predominantly anaerobic soil. The presence of these aerobic (oxidizing) zones within the reducing environment in saturated soils allows for the occurrence of oxidative and reductive transformations to occur in close proximity to each other. For example, ammonia is oxidized to nitrate within the aerobic zone surrounding plant roots in a process called nitrification. Nitrate then readily diffuses into adjacent anaerobic soil, where it is reduced to molecular nitrogen via denitrification or may be reduced to ammonium in certain conditions through dissimilatory nitrate reduction (Mitsch and Gosselink 2000; Ruckauf et al., 2004; Reddy and Delaune, 2005). The anaerobic environment hosts the transformations of N, P, sulfur (S), Fe, Mn, and C. Most of these transformations are microbially mediated. The oxidized soil surface layer also is important to the transport and translocation of transformed constituents, providing a barrier to translocation of some reduced constituents. These transformations will be discussed in more detail below in *Biogeochemical Cycling.*

VEGETATION

Wetland plants consist of macrophytes and microphytes. Macrophytes include free-floating, submersed, floating-leaved, and rooted emergent plants. Microphytes are algae that may be free floating or attached to macrophyte stems and other surfaces. Plants require oxygen to meet respiration demands for growth, metabolism, and reproduction. In macrophytes, much (about 50%) of the respiration occurs below ground in the roots. Wetland macrophytes, however, live in periodically to continuously-inundated and saturated soils and, therefore, use specialized adaptations to grow in anaerobic soils (Cronk and Fennessy 2001). Adaptations consist of morphological/anatomical adaptations that result in anoxia avoidance, and metabolic adaptations that result in true tolerance to anoxia. Morphological/anatomic adaptations include shallow roots systems, aerenchyma, buttressed trunks, pneumatophores (e.g., black mangrove (*Avicennia*

germinans)), and lenticles on the stem. These adaptations facilitate oxygen transport from the shoots to the roots where most respiration occurs. Many wetland plants also possess metabolic adaptations, such as anaerobic pathways of respiration, that produce non-toxic metabolites such as malate to mitigate the adverse effects of oxygen deprivation, instead of toxic compounds like ethanol (Mendelssohn and Burdick 1988).

Species best adapted to anaerobic conditions are typically found in areas inundated for long periods, whereas species less tolerant of anaerobic conditions are found in areas where hydroperiod is shorter. For example, in southern forested wetlands, areas such as abandoned river channels (oxbows) are dominated by obligate species such as bald cypress (*Taxodium distichum*) and tupelo gum (*Nyssa aquatica*) (Wharton et.al., 1982). Areas inundated less frequently are dominated by hardwoods such as black gum (*Nyssa sylvatica*), green ash (*Fraxinus pennsylvanicus*), and red maple (*Acer rubrum*), and the highest, driest wetland areas are dominated by facultative species such as sweet gum (*Liquidambar styraciflua*) and sycamore (*Platanus occidentalis*) (Wharton et.al., 1982). Herbaceous-dominated wetlands also exhibit patterns of zonation controlled by hydroperiod (Mitsch and Gosselink 2000).

In estuarine wetlands such as salt- and brackish-water marshes and mangroves, salinity and sulfides also adversely affect growth and reproduction of vegetation (Webb and Mendelssohn 1996; Mitsch and Gosselink 2000). Inundation with seawater brings dissolved salts (NaCl) and sulfate. Salt creates an osmotic imbalance in vegetation, leading to desiccation of plant tissues. However, many plant species that live in estuarine wetlands possess adaptations to deal with salinity (Winchester et al., 1985; Whipple et al., 1981; Zheng et.al., 2004). These adaptations include salt exclusion at the root surface, salt secreting glands on leaves, schlerophyllous (thick, waxy) leaves, low transpiration rates, and other adaptations to reduce uptake of water and associated salt. Sulfate carried in by the tides undergoes sulfate reduction in anaerobic soils to produce hydrogen sulfide (H_2S) that, at high concentrations, is toxic to vegetation. At sub-lethal concentrations, H_2S inhibits nutrient uptake and impairs plant growth.

SOURCES OF NUTRIENTS

Point Sources

Point source discharges of nutrients to wetlands may come from municipal or industrial discharges, including stormwater runoff from municipalities or industries, or in some cases from large animal feeding operations. Nutrients from point source discharges may be controlled through the National Pollutant Discharge Elimination System (NPDES) permits, most of which are administered by States authorized to issue them. In general, point source discharges that are not stormwater related are fairly constant with respect to loadings.

Nonpoint Sources

Nonpoint sources of nutrients are commonly discontinuous and can be linked to seasonal agricultural activity or other irregularly occurring events such as silviculture, non-regulated construction, and storm events. Nonpoint nutrient pollution from agriculture is most commonly associated with row crop agriculture and livestock production that tend to be highly associated with rain events and seasonal land use activities. Nonpoint nutrient pollution from urban and suburban areas is most often associated with climatological events (rain, snow, and snowmelt), when pollutants are most likely to be transported to aquatic resources.

Urban and agricultural runoff is generally thought to be the largest source of nonpoint source pollution; however, growing evidence suggests that atmospheric deposition may have a significant influence on nutrient enrichment, particularly from nitrogen (Jaworski et.al., 1997). Gases released through fossil fuel combustion and agricultural practices are two major sources of atmospheric N that may be deposited in waterbodies (Carpenter et.al., 1998). Nitrogen and nitrogen compounds formed in the atmosphere return to the earth as acid rain or snow, gas, or dry particles. Atmospheric deposition, like other forms of pollution, may be determined at different scales of resolution. More information on national atmospheric deposition can be found at: http://www.arl.noaa.gov/research/programs/airmon.html and http://nadp.sws.uiuc.edu/. These national maps may provide the user with information about regional areas where atmospheric deposition, particularly of nitrogen, may be of concern. However, these maps are generally low resolution when considered at the local and site-specific scale and may not reflect areas of high local atmospheric deposition, such as local areas in a downwind plume from an animal feedlot operation.

Other nonpoint sources of nutrient pollution may include certain silviculture and mining operations; these activities generally constitute a smaller fraction of the national problem, but may be locally significant nutrient sources. Control of nonpoint source pollutants focuses on land management activities and regulation of pollutants released to the atmosphere (Carpenter et.al., 1998).

2.3 WETLAND NUTRIENT COMPONENTS

NUTRIENT BUDGETS

Wetland nutrient inputs mirror wetland hydrologic inputs (e.g., precipitation, surface water, and ground water), with additional loading associated with atmospheric dry deposition and nitrogen transformation (Figures 2.5 and 2.6). Total atmospheric deposition (wet and dry) may be the dominant input for precipitation-dominated wetlands, while surface- or ground-water inputs may dominate other wetland systems.

The total annual nutrient load (mg-nutrients/yr) into a wetland is the sum of the dissolved and particulate loads. The dissolved load (mg-nutrients/s) can be estimated by multiplying the

instantaneous inflow (L/s) by the nutrient concentration (mg-nutrients/L). EPA recommends calculating the annual load by the summation of this function over the year—greater loads may be found during periods of increased flow and EPA recommends monitoring during these intervals. Where continuous data are unavailable, average flows and concentrations may be used if a bias factor (Cohn et al., 1989) is included to account for unmeasured loads during high flows. Particulate loads (mg-nutrients/yr) can be estimated using the product of suspended and bedload inputs (kg-sediments/yr) and the mass concentrations (mg-nutrients/kg-sediment).

Surface-water nutrient inputs are associated with flows from influent streams, as well as diffuse sources from overland flow through the littoral zone. Ground-water inputs can also be concentrated at points (e.g., springs), or diffuse (such as seeps). The influence of allochthonous sources is likely to be greatest in those zones closest to the source.

Because wetlands generally tend to be low-velocity, depositional environments, they often sequester sediments and their associated nutrients. These sediment inputs generally accumulate at or near the point of entry into the wetland, forming deltas or levees near tributaries, or along the shoreline for littoral inputs. Coarser fractions (e.g., gravels and sands) tend to settle first; the finer fractions (silts, clays, and organic matter) tend to settle further from the inlet point. Particulate input from ground-water sources can usually be neglected, while particulate inputs from atmospheric sources may be important if local or regional sources are present.

Wetland nutrient outputs again mirror hydrologic outputs (e.g., surface- and ground-water), and loads are again estimated as the product of the flow and the concentration of nutrients in the flow. While evaporation losses from wetlands may be significant, there are no nutrient losses associated with this loss. Instead, loss of nutrients to the atmosphere may occur as a result of ammonia volatilization, as well as N_2O losses from incomplete denitrification. Because sediment outputs from wetlands may be minor, nutrient exports by this mechanism may not be important.

Nutrient accumulation in wetlands occurs when nutrient inputs exceed outputs. Net nutrient loads can be estimated as the difference between these inputs and outputs. It is important, therefore, to have some estimate of net accumulation by taking the difference between upstream and downstream loads. Sampling ground-water nutrient concentrations in wells located upstream and downstream of the wetland can provide some sense of net nutrient sequestration, while sampling wetland nutrient inflows and outflows is needed for determining the additional sequestration for this pathway.

BIOGEOCHEMICAL CYCLING

Biogeochemical cycling of nutrients in wetlands is governed by physical, chemical, and biological processes in the soil and water column. Biogeochemical cycling of nutrients is not unique to wetlands, but the aerobic and anaerobic interface generally found in saturated soils of wetlands creates unique conditions that allow both aerobic and anaerobic processes to operate

simultaneously. The hydrology and geomorphology of wetlands (Johnston et.al., 2001) influences biogeochemical processes and constituent transport and transformation within the systems (e.g., water-sediment exchange, plant uptake, and export of organic matter). Interrelationships among hydrology, biogeochemistry, and the response of wetland biota vary among wetland types (Mitsch and Gosselink, 2000; Reddy and Delaune, 2005).

Biogeochemical processes in the soil and water column are key drivers of several ecosystem functions associated with wetland values (e.g., water quality improvement through denitrification, long-term nutrient storage in the organic matter) (Figure 2.3). The hub for biogeochemistry is organic matter and its cycling in the soil and water column. Nutrients such as N, P, and S are primary components of soil organic matter, and cycling of these nutrients is always coupled to C cycling. Many processes occur within the carbon, nitrogen, phosphorus, and sulfur (C, N, P, or S) cycles; microbial communities mediate the rate and extent of these reactions in soil and the water column.

Aerobic-anaerobic interfaces are more common in wetlands than in upland landscapes and may occur at the soil-water interface, in the root zones of aquatic macrophytes, and at surfaces of detrital tissue and benthic periphyton mats. The juxtaposition of aerobic and anaerobic zones in wetlands supports a wide range of microbial populations and associated metabolic activities, with oxygen reduction occurring in the aerobic interface of the substrate, and reduction of alternate electron acceptors occurring in the anaerobic zone (D'Angelo and Reddy, 1994a or b). Under continuously saturated soil conditions, vertical layering of different metabolic activities can be present, with oxygen reduction occurring at and just below the soil-floodwater interface. Substantial aerobic decomposition of plant detritus occurs in the water column; however, the supply of oxygen may be insufficient to meet demands and drive certain microbial groups to utilize alternate electron acceptors (e.g., nitrate, oxidized forms of iron (Fe) and manganese (Mn), sulfate, and bicarbonate (HCO_3)).

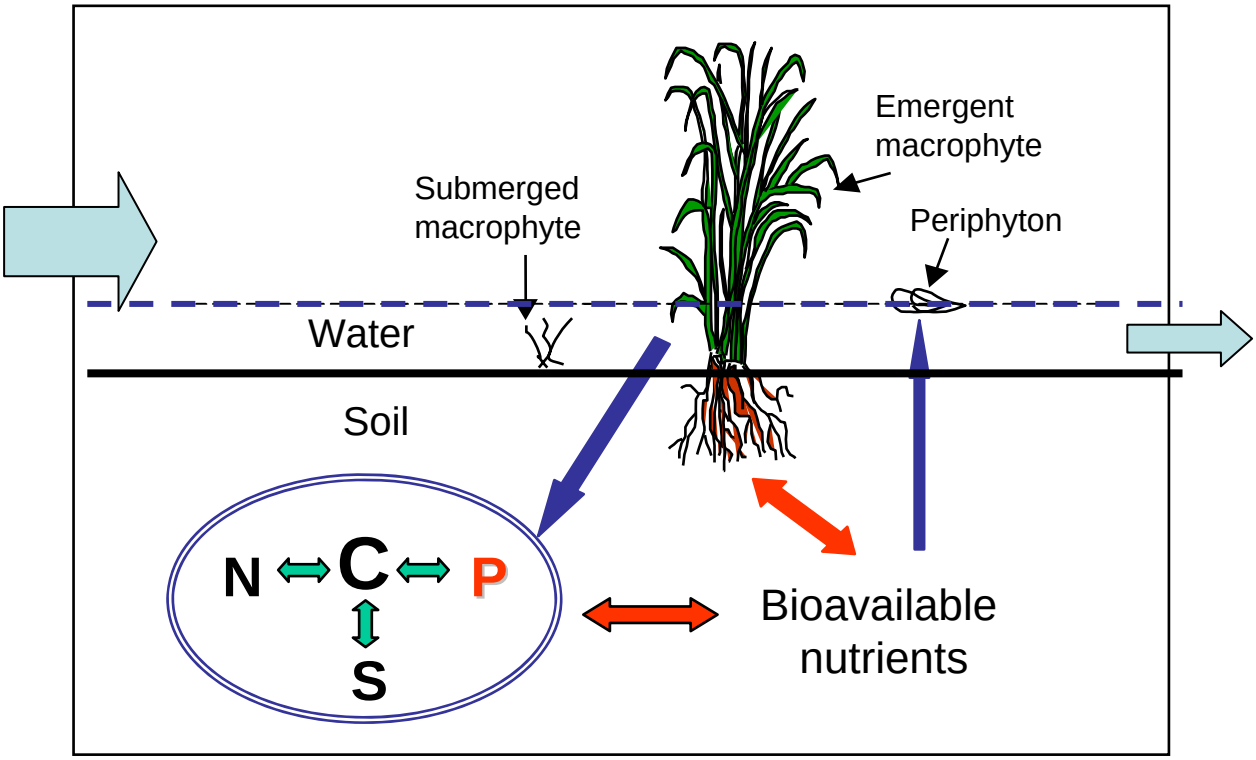

Figure 2.3 Schematic showing basic nutrient cycles in soil-water column of a wetland.

Soil drainage adds oxygen to the soil, while other inorganic electron acceptors may be added through hydraulic loading to the system. Draining wetland soil accelerates organic matter decomposition due to the introduction of oxygen deeper into the profile. In many wetlands, the influence of NO_3 and oxidized forms of Mn and Fe on organic matter decomposition is minimal. This is because the concentrations of these electron acceptors are usually low as a result of the fact that they have greater reduction potential than other alternate electron acceptors, so they generally are depleted rapidly from systems. Long-term sustainable microbial activity is then supported by electron acceptors of lower reduction potentials (sulfate and HCO_3). Methanogenesis is often viewed as the terminal step in anaerobic decomposition in freshwater wetlands, whereas sulfate reduction is viewed as the dominant process in coastal wetlands. However, both processes can function simultaneously in the same ecosystem and compete for available substrates (Capone and Kiene 1988).

A simple way to characterize wetlands for aerobic and anaerobic zones is to determine the oxidation-reduction potential or redox potential (Eh) of the soil-water column (Figure 2.4). Redox potential is expressed in units of millivolts (mV) and is measured using a voltmeter coupled to a platinum electrode and a reference electrode. Typically, wetland soils with Eh

values >300 mV are considered aerobic and typical of drained soil conditions, while soils with Eh values <300 mV are considered anaerobic and are devoid of molecular oxygen (Figure 2.4).

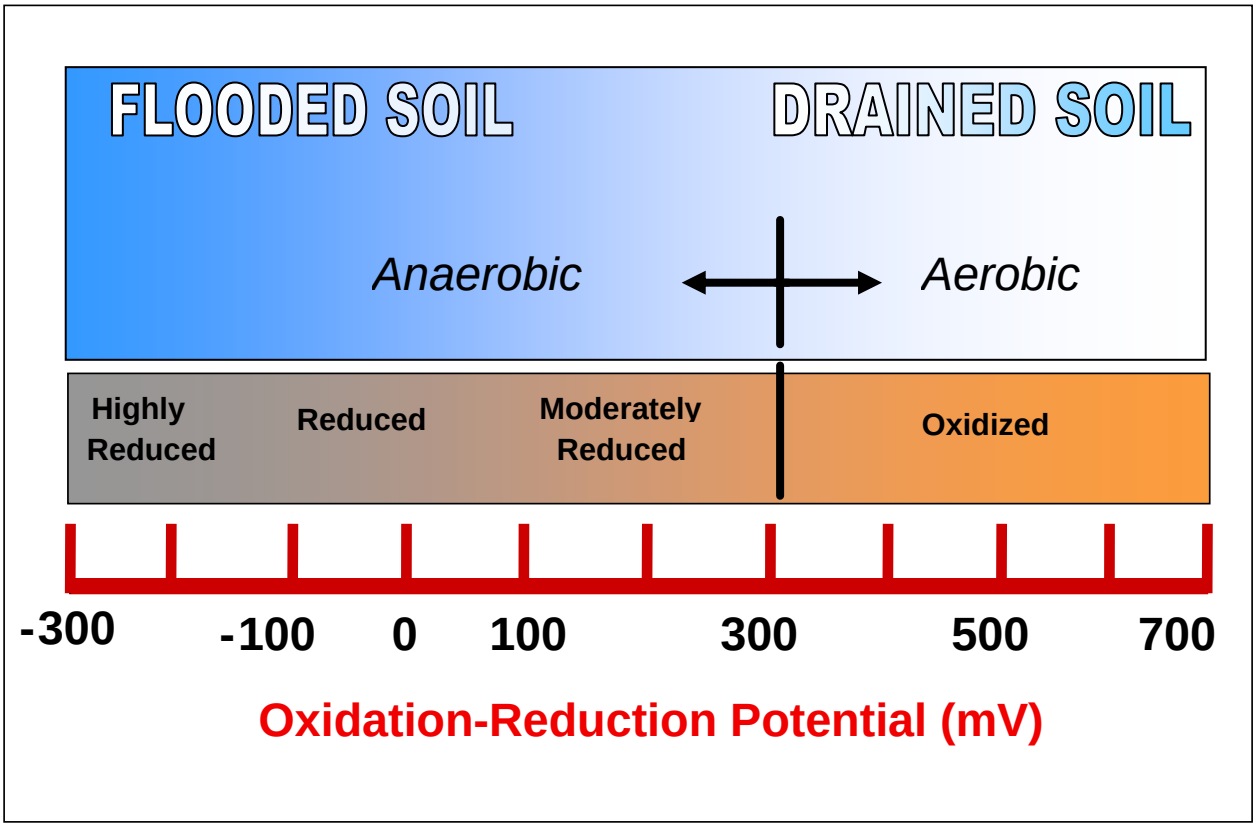

Figure 2.4. Range of redox potentials in wetland soils (Reddy and Delaune 2007).

Wetlands, as low-lying areas in the landscape, receive inputs from all hydrologically connected uplands. Many wetlands are open systems receiving inputs of carbon (C) and nutrients from upstream portions of the watershed that can include agricultural and urban areas.

Prolonged nutrient loading to wetlands can result in distinct gradients in water and soil. Mass loading and hydraulic retention time determine the degree and extent of nutrient enrichment. Continual nutrient loading to an oligotrophic wetland can result in a zone of high nutrient availability near the input, and low nutrient availability and possibly nutrient limiting conditions further from the input point. This enrichment effect can be seen in many freshwater wetlands, most notably in the sub-tropical Everglades where light is abundant and temperatures are high (Davis, 1991; Reddy et al., 1993; Craft and Richardson, 1993 a, b; DeBusk et al., 1994), and in some estuarine marshes (Morris and Bradley 1999). Between these two extremes, there can exist

a gradient in quality and quantity of organic matter, nutrient accumulation, microbial and macrobiotic communities, composition, and biogeochemical cycles.

Compared to terrestrial ecosystems, most wetlands show an accumulation of organic matter, and therefore wetlands function as global sinks for carbon. Accumulation of organic C in wetlands is primarily a result of the balance of C fixation through photosynthesis and losses through decomposition. Rates of photosynthesis in wetlands are typically higher than in other ecosystems, and rates of decomposition are typically lower due to anaerobic conditions, hence organic matter tends to accumulate. In addition to maintaining proper functioning of wetlands, organic matter storage also plays an important role in regulating other ecosystems and the biosphere. For example, organic matter contains substantial quantities of N, P, and S; therefore, accumulation of organic matter in wetlands decreases transport of these nutrients to downstream aquatic systems.

NITROGEN (N):

Nitrogen enters wetlands in organic and inorganic forms, with the relative proportion of each depending on the input source. Organic forms are present in dissolved and particulate fractions, while inorganic N (NH_4-N, NO_3-N and NO_2-N) is present in dissolved fractions (Figure 2.5) or bound to suspended sediments (NH_4-N). Particulate fractions are removed through settling and burial, while the removal of dissolved forms is regulated by various biogeochemical reactions functioning in the soil and water column. Relative rates of these processes are affected by physico-chemical and biological characteristics of plants, algae, and microorganisms.

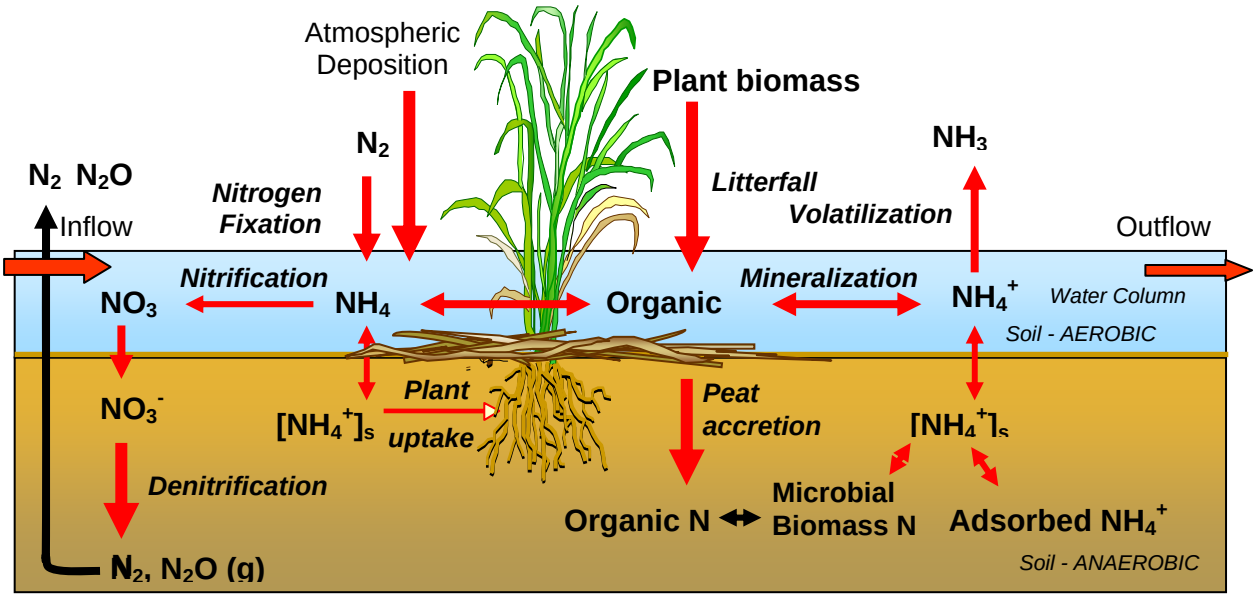

Figure 2.5. Schematic of the nitrogen cycle in wetlands.

Nitrogen reactions in wetlands effectively process inorganic N through nitrification and denitrification, ammonia volatilization, and plant uptake. These processes aid in lowering levels of inorganic N in the water column. A significant portion of dissolved organic N assimilated by plants is returned to the water column during breakdown of detrital tissue or soil organic matter, and the majority of this dissolved organic N is resistant to decomposition. Under these conditions, water leaving wetlands may contain elevated levels of N in organic form. Exchange of dissolved nitrogen species between soil and water column support several nitrogen reactions. For example, nitrification in the aerobic soil layer is supported by ammonium flux from the anaerobic soil layer. Similarly, denitrification in the anaerobic soil layer is supported by nitrate flux from the aerobic soil layer and water column. Relative rates of these reactions will, however, depend on the environmental conditions present in the soil and water column (Reddy and Delaune 2007).

PHOSPHORUS (P):

Phosphorus retention by wetlands is regulated by physical (sedimentation and entrainment), chemical (precipitation and flocculation), and biological mechanisms (uptake and release by vegetation, periphyton, and microorganisms). Phosphorus in the influent water is found in soluble and particulate fractions, with both fractions containing a certain proportion of inorganic and organic forms. Relative proportions of these pools depend on the input source. For example, municipal wastewater may contain a large proportion (>75%) as inorganic P in soluble forms, as compared to effluents from agricultural watersheds where a greater percentage of P loading may be in the particulate fraction.

Phosphorus forms that enter a wetland are grouped into: (i) dissolved inorganic P (DIP); (ii) dissolved organic P (DOP); (iii) particulate inorganic P (PIP); and, (iv) particulate organic P (POP) (Figure 2.6). The particulate and soluble organic fractions may be further separated into labile and refractory components. Dissolved inorganic P is generally bioavailable, whereas organic and particulate P forms generally must be transformed into inorganic forms before becoming bioavailable. Both biotic and abiotic mechanisms regulate relative pool sizes and transformations of P compounds within the water column and soil. Alterations in these fractions can occur during flow through wetlands and depend on the physical, chemical, and biological characteristics of the systems. Thus, both biotic and abiotic processes should be considered when evaluating P retention capacities of wetlands. Biotic processes include assimilation by vegetation, plankton, periphyton, and microorganisms. Abiotic processes include sedimentation, adsorption by soils, precipitation, and exchange processes between soil and the overlying water column (Reddy et.al., 1999, 2005; Reddy and Delaune, 2007). The processes affecting phosphorus exchange at the soil/sediment water interface include: (i) diffusion and advection due to wind-driven currents; (ii) diffusion and advection due to flow and bioturbation; (iii) processes within the water column (mineralization, sorption by particulate matter, and biotic uptake and release); (iv) diagenetic processes (mineralization, sorption, and precipitation dissolution) in

bottom sediments; (v) redox conditions (O_2 content) at the soil/sediment-water interface; and, (vi) phosphorus flux from water column to soil mediated by evapotranspiration by vegetation.

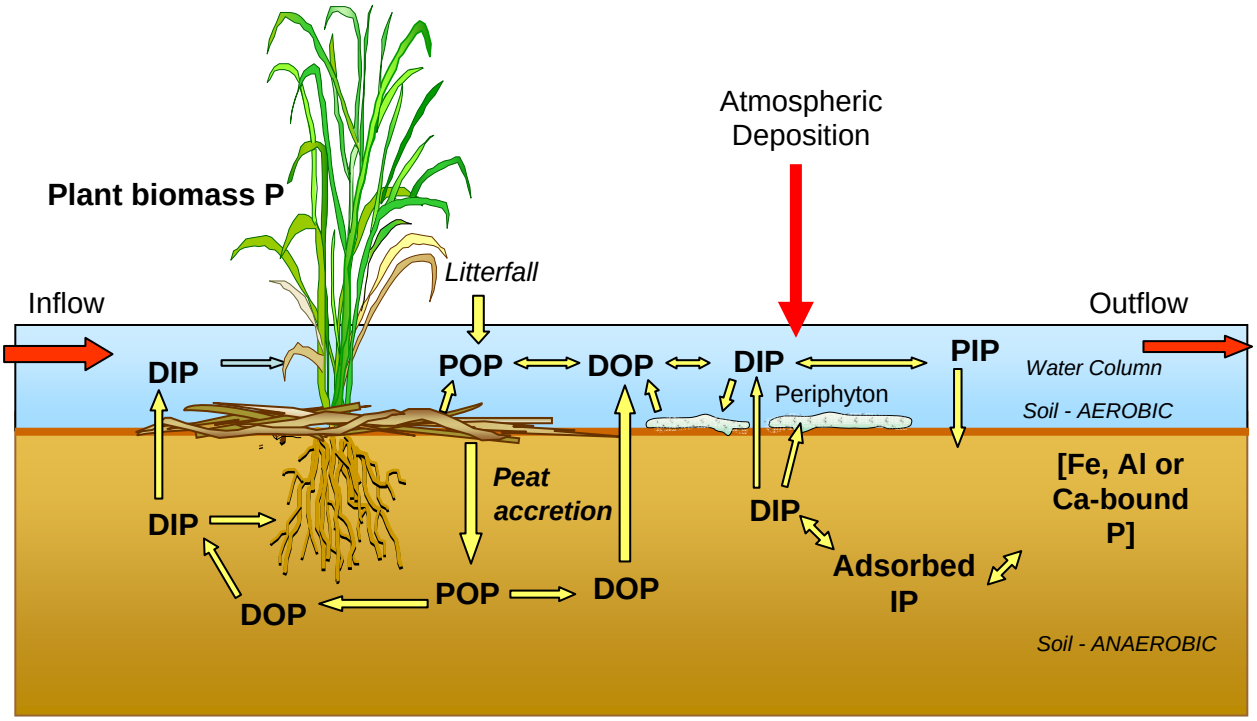

Figure 2.6. Schematic of the Phosphorous cycle in wetlands.

The key biogeochemical services provided by wetlands include nutrient transformation and removal by decreasing concentrations of nutrients and other contaminants, and sequestration of carbon and nutrients into stable pools (Kadlec and Knight 1996). The biogeochemical processes regulating water quality improvement are well established, and are made use of in treatment wetlands. Increased nutrient loading to oligotrophic wetlands results in increased primary productivity and nutrient enrichment. This resulting **eutrophication** can have both positive and negative impacts to the environment. Higher rates of primary productivity increase rates of organic matter accumulation, thus increasing **carbon sequestration**. However, eutrophication may lead to increased periodic and episodic export of DIP (Kadlec and Knight 1996; Reddy et.al., 1995, 1996, 2005; Reddy and Delaune 2007)).

Chapter 3 Classification of Wetlands

3.1 INTRODUCTION

Developing individual, site-specific nutrient criteria is not practical for every wetland. Instead, criteria for groups of similar wetlands in a region are needed. To this end, a means of grouping or classifying wetlands is required. This chapter introduces the scientific rationale for classifying wetlands, reviews some common classification schemes, and discusses their implications for establishing nutrient criteria for wetlands. Use of a common scheme across State boundaries should facilitate collaborative efforts in describing reference condition for biota or water quality and in developing assessment methods, indices of biotic integrity (IBI) (USEPA 1993b, http://www.epa.gov/emap/remap/index.html), nutrient-response relationships, and nutrient criteria for wetlands. This chapter describes a series of national classification systems that could be used to provide a common framework for development of nutrient criteria for wetlands, and suggests ways in which these classification schemes could be combined in a hierarchical fashion. Many existing classification schemes may be relevant and should be considered for use or modification, even if they were not originally derived for wetland nutrient criteria because: 1) they incorporate key factors that control nutrient inputs and cycling; 2) they have already been mapped; and, 3) they have been incorporated into sampling, assessment, and management strategies for wetland biology or for other surface water types, thus facilitating integration of monitoring strategies. Adoption of any classification scheme should be an iterative process, whereby initial results of biological or water quality sampling are used to test for actual differences in reference condition for nutrients or nutrient-response relationships among proposed wetland classes. Wetland classes that behave similarly can be combined, and apparent outliers in distributions of nutrient concentrations from reference sites or in nutrient-response relationships can be examined for additional sources of variability that may need to be considered. In addition, new classification schemes can be derived empirically through many multivariate statistical methods designed to determine factors that can discriminate among wetlands based on nutrient levels or nutrient-response relationships.

The overall goal of classification is to reduce variability within classes due to differences in natural condition related to factors such as geology, hydrology, and climate. This will minimize the number of classes for which reference conditions must be defined. For example, we would expect different conditions for water quality or biological community composition for wetland classes in organic soils (histosols), compared to wetlands in mineral soils. In assessing impacts to wetlands, comparing a wetland from within the same class would increase the precision of assessments, enable more sensitive detection of change, and reduce errors in characterizing the status of wetland condition.

REFERENCE CONCEPT

Reference conditions "describe the characteristics of waterbody segments least impaired by human activities and are used to define attainable biological or habitat conditions" (USEPA 1990, Stoddard et.al., 2006). At least two general approaches have been defined to establish reference condition—the site-specific and the regional (U.S. EPA 1990b, http://www.epa.gov/bioindicators/). The current approach to developing water quality criteria for nutrients also emphasizes the identification of expected ranges of nutrients by waterbody type and ecoregion for the least-impaired reference conditions (U.S. EPA 1998, http://www.epa.gov/waterscience/standards/nutrient.html).

Although different concepts of reference condition have been used in other programs (e.g., for evaluation of wetland mitigation projects (Smith et.al., 1995; http://el.erdc.usace.army.mil/wetlands/pdfs/wrpde9.pdf)), for the purposes of this document, the term "reference condition" refers to wetlands that are minimally or least impacted by human activities. Most, if not all, wetlands in the U.S. are affected to some extent by human activities such as acid precipitation, global climate change, or other atmospheric deposition of nitrogen and mercury, and changes in historic fire regime. "Minimally impacted" is therefore operationally defined by choosing sites with fewer stressors or fewer overall impacts as described by indicators of stressors, such as land-use or human activities within the watershed or buffer area surrounding a wetland and source inputs. Identifying reference wetlands in areas of high local or regional atmospheric deposition of nitrogen should also be carefully considered because indicators such as local land use activities may not be sufficient to indicate nutrient enrichment from dry or wet air deposition.

3.2 EXISTING WETLAND CLASSIFICATION SCHEMES

There are two different approaches for classification of aquatic resources. One is geographically-based, and the other is independent of geography but relies on environmental characteristics that determine aquatic ecosystem status and vulnerability at the region-, watershed-, or ecosystem-scale (Detenbeck et.al., 2000). Ecoregions (including "*nutrient ecoregions*") and Ecological Units represent geographically-based classification schemes that have been developed and applied nation-wide (Omernik 1987, Keys et.al., 1995). The goal of geographically-based classification schemes is to reduce variability in reference condition based on spatial co-variance in climate and geology, along with topography, vegetation, hydrology, and soils. Geographically-independent or environmentally-based classification schemes include those derived using watershed characteristics such as land-use and/or land-cover (Detenbeck et.al., 2000), hydro geomorphology (Brinson 1993), vegetation type (Grossman et.al., 1998), or some combination of these (Cowardin et.al., 1979). Both geographically- and environmentally-based schemes have been developed for wetland classification. These approaches can be applied individually or combined within a hierarchical framework (Detenbeck et.al., 2000).

GEOGRAPHICALLY-BASED CLASSIFICATION SCHEMES

Regional classification systems were first developed specifically for the United States by land management agencies. The U.S. Department of Agriculture (USDA) has described a hierarchical system of Land Resource Regions and Major Land Resource Areas based mainly on soil characteristics for agricultural management (USDA SCS 1981). Ecoregions were then refined for USDA and the U.S. Forest Service based on a hierarchical system in which each of several environmental variables such as climate, landform, and potential natural vegetation were applied to define different levels of classification (Bailey 1976). Subsequently, Omernik and colleagues developed a hierarchical, nationwide ecoregion system to classify streams using environmental features they expected would influence aquatic resources, as opposed to terrestrial resources (Hughes and Omernik 1981, Omernik et.al., 1982). The latter was based on an overlay of "component maps" for land use, potential natural vegetation, land-surface form, and soils, along with a subjective evaluation of the spatial congruence of these factors as compared to the hierarchical approach used by Bailey, which relied only on natural features (not land-use). Omernik has produced a national map of 84 ecoregions defined at a scale of 1:7,500,000 (Figure 3.1; Omernik 1987, http://water.usgs.gov/GIS/metadata/usgswrd/XML/ecoregion.xml). More detailed, regional maps have been prepared at a scale of 1:2,500,000 in which the most "typical" areas within each ecoregion are defined. Cowardin et.al., (1979) have suggested an amendment to Bailey's ecoregions to include coastal and estuarine waters (Figure 3.2a). In practice, Omernik's scheme has been more widely used for geographic classification of aquatic resources such as streams, but few examples to verify the appropriateness of this grouping to wetland nutrients are available.

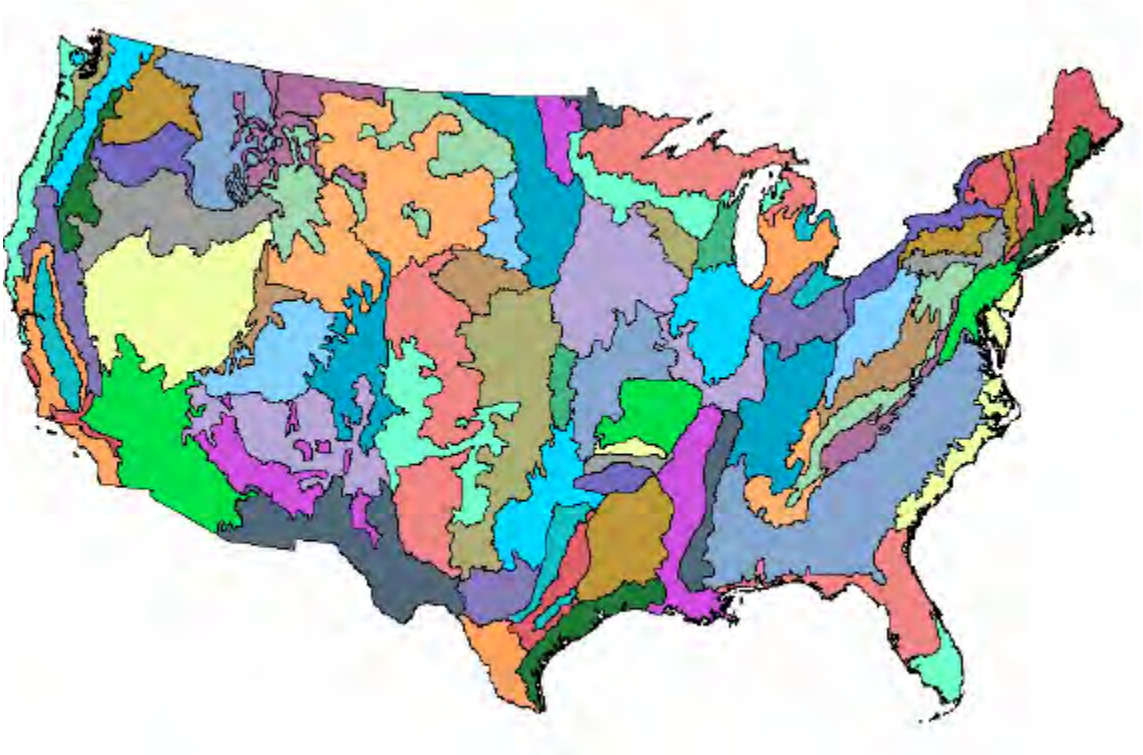

Figure 3.1 Map of Omernik aquatic ecoregions.

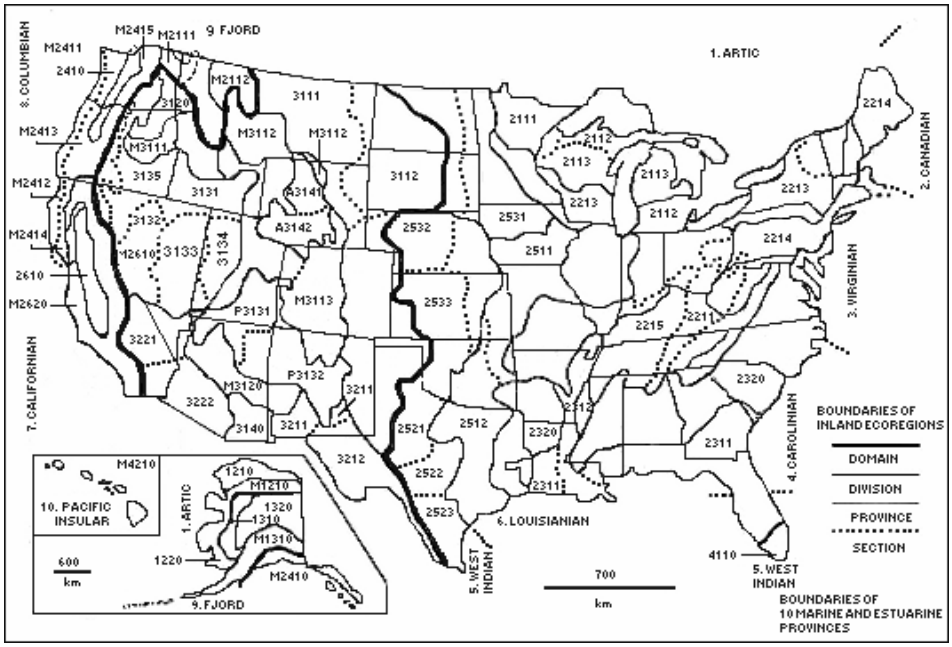

Figure 3.2a. Map of Bailey ecoregions with coastal and estuarine provinces (Cowardin et al., 1979).

Figure 3.2b. Legend

[a]Domains, Divisions, Provinces, and Sections used on Bailey's (1976) map and described in detail in Bailey (1978). Highland ecoregions are designated M mountain, P plateau, and A altiplano.

- 1000 Polar
  - 1200 Tundra
    - 1210 Arctic Tundra
    - 1220 Bering Tundra
  - M1210 Brooks Range
- 1300 Subarctic
  - 1310 Yukon Parkland
  - 1320 Yukon Forest
  - M1310 Alaska Range
- 2000 Humid Temperate
  - 2100 Warm Continental
    - 2110 Laurentian Mixed Forest
      - 2111 Spruce-Fir Forest
      - 2112 Northern Hardwoods-Fir Forest
      - 2113 Northern Hardwoods Forest
      - 2114 Northern Hardwoods-Spruce Forest
    - M2110 Columbia Forest
      - M2111 Douglas-fir Forest
      - M2112 Cedar-Hemlock-Douglas-fir Forest
  - 2200 Hot Continental
    - 2210 Eastern Deciduous Forest
      - 2211 Mixed Mesophytic Forest
      - 2212 Beech-Maple Forest
      - 2213 Maple-Basswood Forest + Oak Savanna
      - 2214 Appalachian Oak Forest
      - 2215 Oak-Hickory Forest
  - 2300 Subtropical
    - 2310 Outer Coastal Plain Forest
      - 2311 Beech-Sweetgum-Magnolia-Pine-Oak
      - 2312 Southern Floodplain Forest
    - 2320 Southeastern Mixed Forest
  - 2400 Marine
    - 2410 Willamette-Puget Forest
    - M2410 Pacific Forest (in conterminous U.S.)
      - M2411 Sitka Spruce-Cedar-Hemlock Forest
      - M2412 Redwood Forest
      - M2413 Cedar-Hemlock-Douglas-fir Forest
      - M2414 California Mixed Evergreen Forest
      - M2415 Silver fir-Douglas-fir Forest
      - M2410 Pacific Forest (in Alaska)
  - 2500 Prairie
    - 2510 Prairie Parkland
      - 2511 Oak-Hickory-Bluestem Parkland
      - 2512 Oak + Bluestem Parkland
    - 2520 Prairie Brushland
      - 2521 Mesquite-Buffalo Grass
      - 2522 Juniper-Oak-Mesquite
      - 2523 Mesquite-Acacia
    - 2530 Tall-Grass Prairie
      - 2531 Bluestem Prairie
      - 2532 Whestgrass-Bluestem-Needlegrass
      - 2533 Bluestem-Gamma Prairie
    - 2600 Mediterranean (Dry-summer Subtropical)
      - 2610 California Grassland
      - M2610 Sierran Forest
      - M2620 California Chaparral
- 3000 Dry 3100 Steppe
  - 3110 Great Plains-Shortgrass Prairie
    - 3111 Gramma-Needlegrass-Wheatgrass
    - 3112 Wheatgrass-Needlegrass
    - 3113 Grama-Buffalo Grass
  - M3110 Rocky Mountain Forest
  - M3111 Grand-fir-Douglas-fir Forest
    - M3112 Douglas-fir Forest
    - M3113 Ponderosa Pine-Douglas-fir Forest
  - 3120 Palouse Grassland
    - M3120 Upper Gila Mountains Forest
    - 3130 Intermountain Sagebrush
      - 3131 Sagebrush-Wheatgrass
      - 3132 Lahontan Saltbush-Greasewood
      - 3133 Great Basin Sagebrush
      - 3134 Bonneville Saltbush-Greasewood
      - 3135 Ponderosa Shrub Forest
    - P3130 Colorado Plateau
      - P3131 Juniper-Pinyon Woodland + Sagebrush Saltbush Mosaic
      - P3132 Grama-Galleta Steppe + Juniper-Pinyon Woodland Mosaic
  - 3140 Mexican Highland Shrub Steppe
  - A3140 Wyoming Basin
  - A3141 Wheatgrass-Needlegrass-Sagebrush
  - A3142 Sagebrush-Wheatgrass
    - 3200 Desert 3210 Chihuahuan Desert
      - 3211 Grama-Tobosa
      - 3212 Tarbush-Creosote Bush
    - 3220 American Desert
      - 3221 Creosote Bush
      - 3222 Creosote Bush-Bur Sage
- 4000 Humid Tropical
  - 4100 Savanna
  - 4110 Everglades
  - 4200 Rainforest
    - M4210 Hawaiian Islands

Finally, an attempt has been made to integrate approaches across Federal agencies to produce regional boundaries termed Ecological Units (Keys et.al., 1995). Information has been combined on climate, landform, geomorphology, geology, soils, hydrology, and potential vegetation to produce a nested series of boundaries for the eastern U.S. Different combinations of environmental parameters are emphasized at each hierarchical level of classification. This scheme was developed to explain variation in both terrestrial and aquatic systems, and is consistent with a more comprehensive strategy to classify lotic systems down to the level of stream reaches (Maxwell et.al., 1995). The mapped system for the eastern U.S. includes classification at the following levels:

domain (n=2) > divisions (n=5) > provinces (n=14) > sections (n=78) > subsections (n=xxx),

where Sections are roughly half the size of Omernik ecoregions (Figure 3.3). For lotic systems, additional spatial detail can be added by defining watersheds (at the level of land type associations), subwatersheds (at the level of land types), valley segments, stream reaches, and, finally, channel units (Maxwell et.al., 1995). In reality, not all watersheds nest neatly within subsections, and may cross-subsection boundaries.

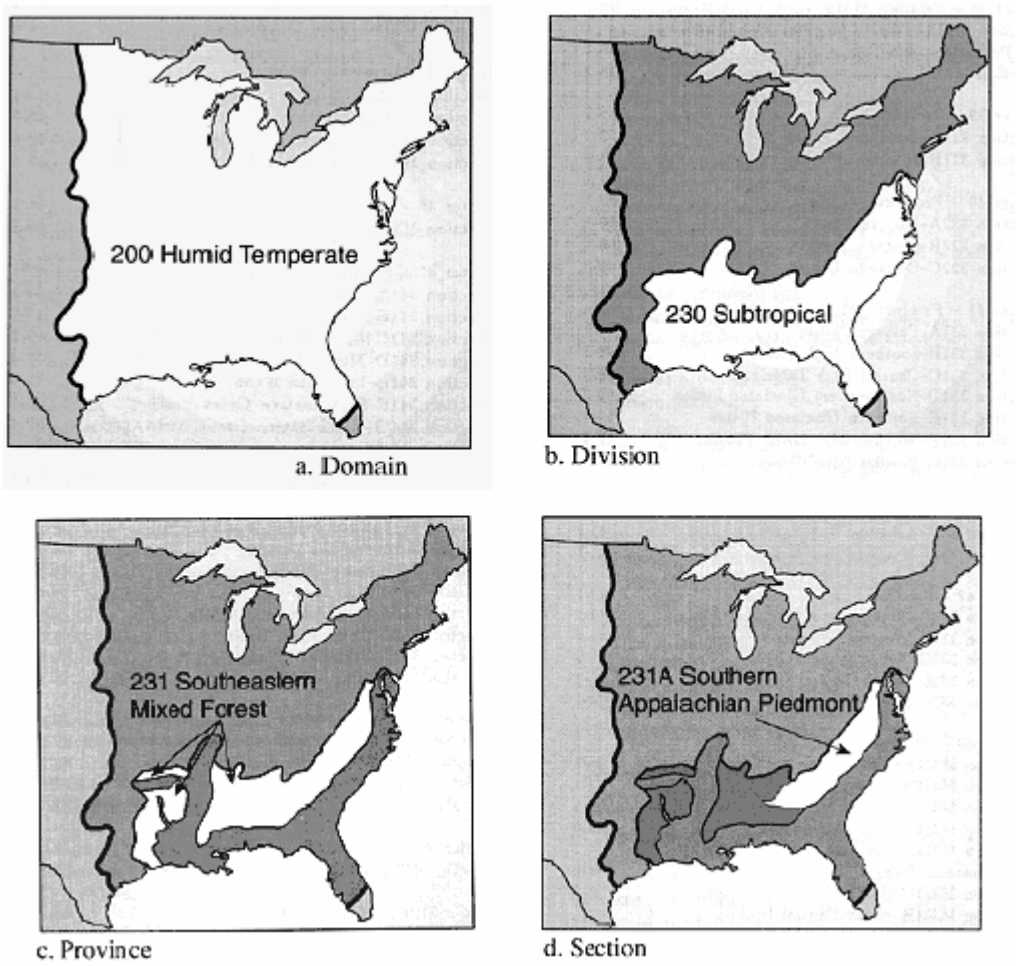

Figure 3.3 Examples of first four hierarchical levels of Ecological Units: domain, division, province, and section, from USEPA Environmental Atlas.

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Some States have chosen to refine the spatial resolution of Omernik's ecoregional boundaries for management of aquatic resources (e.g., Region 3 and Florida). For example, the State of Florida has defined subecoregions for streams based on analysis of macroinvertebrate data from 100

minimally-impacted sites. Efforts are currently underway to define ecoregions for Florida wetlands based on variables influencing the water budget and plant community composition (Dougherty et.al., 2000, Lane 2000).

ENVIRONMENTALLY-BASED CLASSIFICATION SYSTEMS

Wetland habitat types are described very simply but coarsely by Shaw and Fredine (1956, Circular 39), ranging from temporarily-flooded systems to ponds. A more refined hierarchical classification system is available based on vegetation associations; for example, the system developed by the Nature Conservancy for terrestrial vegetation includes some wetland types (Grossman et.al., 1998). Vegetation associations have also been used to classify Great Lakes coastal wetlands within coastal geomorphic type (Michigan Natural Features Inventory 1997).

COWARDIN CLASSIFICATION SYSTEM

The Cowardin classification system (Cowardin et.al., 1979) was developed for the U.S. Fish and Wildlife Service (FWS) as a basis for identifying, classifying, and mapping wetlands, special aquatic sites, and deepwater aquatic habitats. The Cowardin system combines a number of approaches incorporating landscape position, hydrologic regime, and habitat (vegetative) type (http://www.nwi.fws.gov) (Figure 3.4). Wetlands are categorized first by landscape position (tidal, riverine, lacustrine, and palustrine), then by cover type (e.g., open water, submerged aquatic bed, persistent emergent vegetation, shrub wetlands, and forested wetlands), and then by hydrologic regime (ranging from saturated or temporarily-flooded to permanently flooded). Modifiers can be added for different salinity or acidity classes, soil type (organic vs. mineral), or disturbance activities (impoundment, beaver activity). Thus, the Cowardin system includes a mixture of geographically-based factors, proximal forcing functions (hydrologic regime, acidity), anthropogenic disturbance regimes, and vegetative outcomes. In practice, the Cowardin system can be aggregated by combination of hydrogeomorphic (HGM) type and predominant vegetation cover if digital coverages are available (Ernst et.al.,1995).

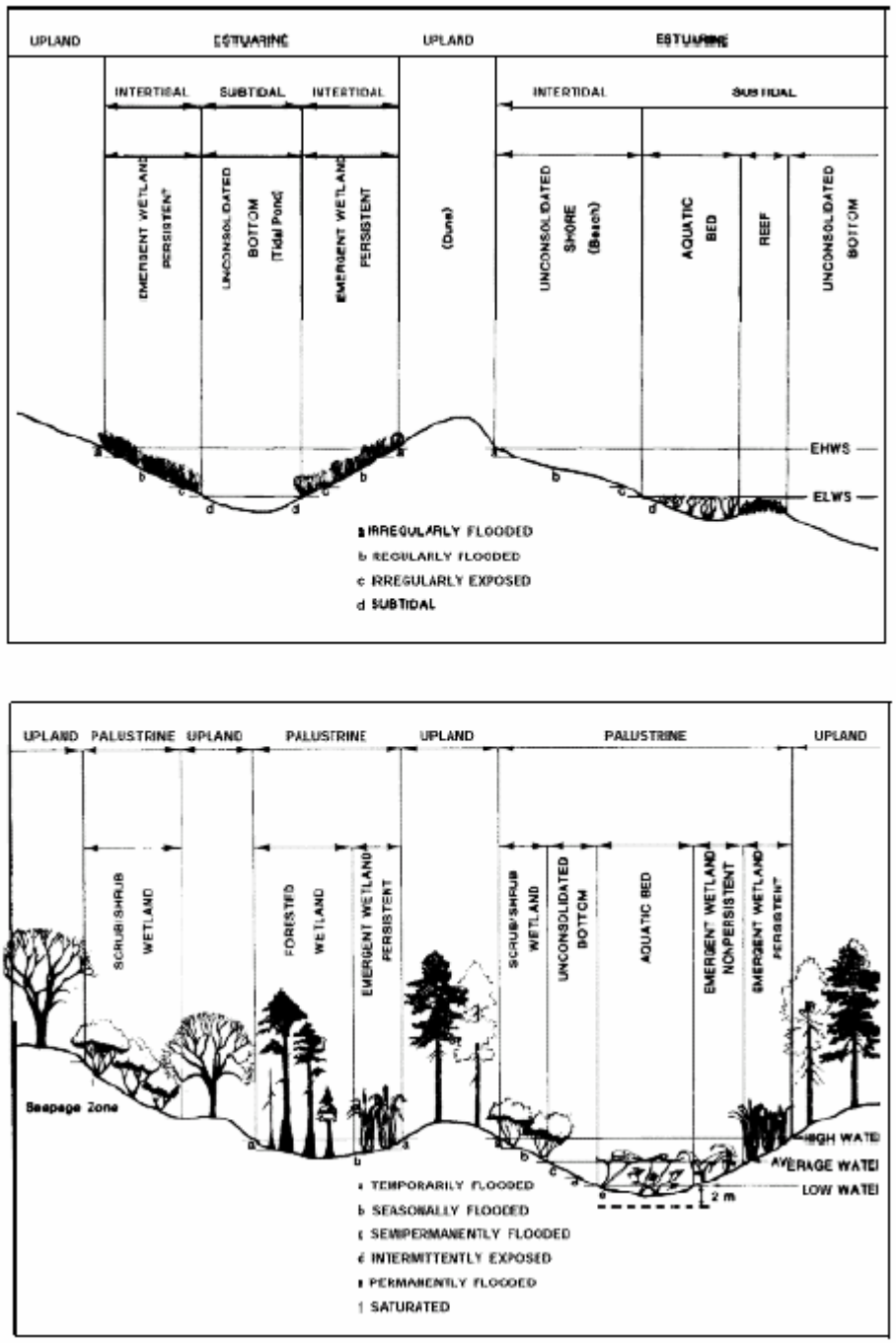


Figure 3.4. (Top) Cowardin hierarchy of habitat types for estuarine systems; (Bottom) Palustrine systems, from Cowardin et.al., 1979.

HYDROGEOMORPHIC CLASSIFICATION SYSTEM(S)

Brinson (1993) has defined a hydrogeomorphic classification system for wetlands based on geomorphic setting, dominant water source (Figure 3.5), and dominant hydrodynamics (Figure 3.6; http://www.wes.army.mil/el/wetlands). Seven classes have been described: depressional, lacustrine fringe, tidal fringe, slope, riverine, mineral soil flats, and organic soil flats (Smith et.al., 1995). Also see Hydrogeomorphic Classification in http://www.epa.gov/waterscience/criteria/wetlands/7Classification.pdf.

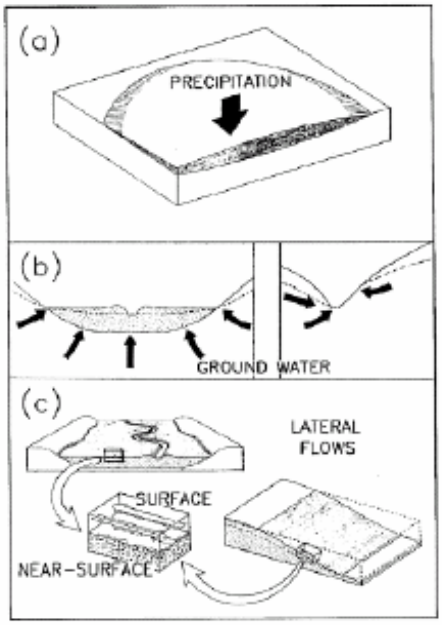


Figure 3.5. Dominant water sources to wetlands, from Brinson 1993.

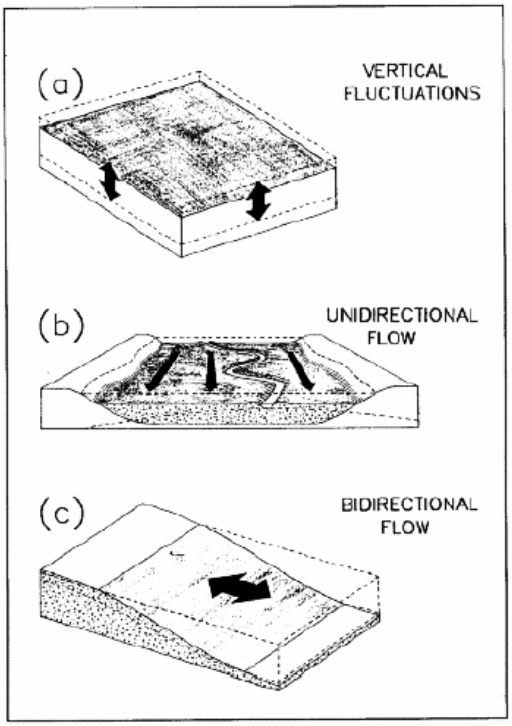


Figure 3.6. Dominant hydrodynamic regimes for wetlands based on flow pattern (Brinson 1993).

Depressional systems, as the name implies, are located in topographic depressions where surface water can accumulate. Depression wetlands can be further classified based on presence of inlets or outlets and primary water source as closed, open/groundwater, or open/surface water subclasses.

Lacustrine fringe wetlands are located along lake shores where the water elevation of the lake determines the water table of the adjacent wetland. Great Lakes coastal wetlands represent one important region of lacustrine fringe wetlands. These coastal systems are strongly influenced by coastal forming processes, and, as such, have been further classified by geomorphic type through various schemes (Jaworski and Raphael 1979, and others summarized in Michigan Natural Features Inventory 1997). These geomorphic coastal positions will further influence the predominant source of water and the degree and type of energy regime (riverine vs. seiche and wave activity). Tidal fringe wetlands occupy a similar position relative to marine coasts and estuaries, where water level is influenced by sea level. Tidal fringe wetlands can be broken down further based on salinity into euhaline vs. mixohaline subclasses. Slope wetlands occur on slopes where groundwater discharges to the land surface, but typically do not have the capacity for surface water storage (Figure 3.7). Riverine wetlands are found in floodplains and riparian zones associated with stream channels. Riverine systems can be broken down based on watershed position (and, thus, hydrologic regime) into tidal, lower perennial, upper perennial, and nonperennial subclasses. Mineral soil flats are in areas of low topographic relief (e.g.,

interfluves, relic lake bottoms, and large floodplain terraces) with precipitation as the main source of water. The topography of organic soil flats (e.g., peatlands), in contrast, is controlled by the vertical accretion of organic matter.

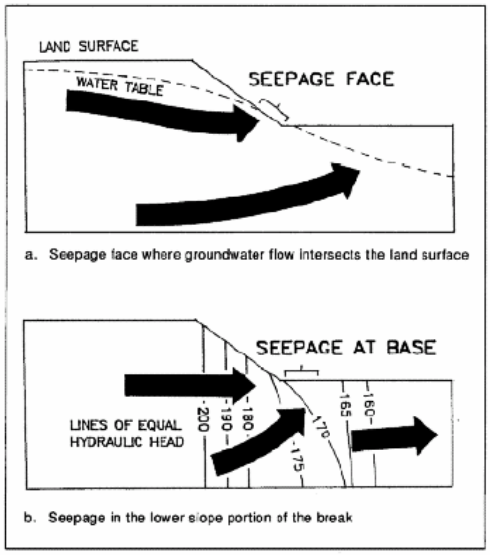


Figure 3.7. Interaction with break in slope with groundwater inputs to slope wetlands (Brinson 1993).

The HGM classification system is being further refined to the subclass level for different regions or States and classes (Cole et.al., 1997, http://www.wes.army.mil/el/wetlands). In addition to the classification factors described above, Clairain (2002) suggests using parameters such as the degree of connection between the wetland and other surface waters (depressional wetlands), salinity gradients (tidal), degree of slope or channel gradient (slope and riverine wetlands), position in the landscape (riverine, slope), and a scaling factor (stream order, watershed size or floodplain width for riverine subclasses). In some cases, existing regional schemes have been used as the basis for subclass definition (e.g., Stewart and Kantrud 1971, Golet and Larson 1974, Wharton et.al., 1982, Weakley and Schafale 1991, Keough et.al., 1999).

The HGM classification system has been applied primarily to assess wetland functions related to hydrology, biological productivity, biogeochemical cycling, and habitat (Smith et.al., 1995, http://www.wes.army.mil/el/wetlands/pdfs/wrpde9.pdf). The same environmental parameters that influence wetland functions also determine hydrologic characteristics and background water quality, which in turn drive wetland habitat structure and community composition and the timing of biotic events. Thus, the HGM classification system can serve as a basis for partitioning variability in reference trophic status and biological condition, as well as defining temporal strategies for sampling.

COMPARISON OF ENVIRONMENTALLY-BASED CLASSIFICATION SYSTEMS

If an integrated assessment of aquatic resources within a watershed or region is desired, it may be useful to consider intercomparability of classification schemes for wetlands, lakes, and riverine systems to promote cost-effective sampling and ease of interpretation. The HGM approach could integrate readily with a finer level of classification for lake type because lentic systems are separated out as lacustrine fringe or depressional wetlands based on lake or pond size and influence of water level on the adjacent wetland. Lacustrine classification systems for water quality have included geography (climate + bedrock characteristics, Gorham et.al., 1983) or hydrologic setting (Winter 1977, Eilers et.al., 1983) as factors for categorization. McKee et.al., (1992) suggest a modification of Cowardin's system for Great Lakes coastal wetlands incorporating landscape position (system), depth zone (littoral vs. limnetic subsystems), vegetative or substrate cover (class and subclass), and modifiers of ecoregions, water level regimes, fish community structure, geomorphic structure, and human modification. In contrast, the Michigan Natural Features Inventory (1997) categorizes Great Lakes coastal wetlands by Great Lake, then nine unique geomorphic types within lakes, then vegetative association.

For lotic systems, Brinson et.al., (1995) describes an approach to further classify riverine classes into subclasses based on watershed position and stream size/permanence. This strategy is consistent with current monitoring efforts to develop stream IBIs (Indices of Biotic Integrity), which typically use stream order as a surrogate for watershed size in explaining additional background variation in IBI scores (USEPA 1996). A more detailed classification of stream reach types, based on hydrogeomorphic character, is described by Rosgen (1996). This classification scheme has been predominantly applied to assessments of channel stability and restoration options, and not to development of criteria. Gephardt et.al., (1990) described a cross-walk between riparian and wetland classification and description procedures.

COMBINATIONS OF GEOGRAPHIC AND ENVIRONMENTALLY-BASED APPROACHES

It is possible to combine geographically-based classification with hydrogeomorphic and/or habitat-based approaches. For example, a scheme could be defined that nests Cowardin (Cowardin et.al., 1979) vegetative cover class within HGM class within ecoregion. Maxwell et.al., (1995) have defined a scheme for linking geographically-based units based on geoclimatic setting (domains => divisions => provinces => sections => subsections) to watersheds and subwatersheds, and thus to riverine systems composed of valley segments, stream reaches, and channel units, or to lacustrine systems composed of lakes, lake depth zones, and lake sites/habitat types.

Maxwell et.al., (1995) also define a series of fundamental hydrogeomorphic criteria for classifying wetlands based on Brinson (1993) and Winter (1992), including physiography (landscape position), water source, hydrodynamics, and climate. The first three of these are similar to the HGM classification system (see summary tables in Keys et.al., 1995). Finer scale variation in landforms is also discussed and may be of use in determining the dominance of different hydrogeomorphic classes of wetlands and associated surface waters (lakes and rivers). Characteristics and relative advantages and disadvantages of different classification systems are summarized in Table 3.2.

3.3 SOURCES OF INFORMATION FOR MAPPING WETLAND CLASSES

In order to select wetlands for sampling in a random- or random-stratified design (described in Chapter 4), it is important to have a record of wetland locations to choose from, preferably categorized by the classification system of interest. For some, but not all portions of the country, wetlands have been mapped from aerial photography through the National Wetlands Inventory (NWI) maintained by the U.S. Fish and Wildlife Service (http://www.fws.gov/nwi/; Dahl 2005). In other cases, individual States have developed inventories, or researchers have developed lists for specific types of wetlands within a given region, e.g., Great Lakes coastal wetlands (Herdendorf et.al., 1981). In order to sample these mapped wetland areas in a random fashion, it is important to have a list of each wetland that occurs within each class and its associated area. A geographic information system (GIS) allows one to automatically produce a list of all wetland polygons by type within a specified geographic region. Sources of digital information for mapping and/or classifying wetlands in a GIS are presented in the Land-Use Characterization for Nutrient and Sediment Risk Assessment Module (http://www.epa.gov/waterscience/criteria/wetlands/17LandUse.pdf).

Table 2. Comparison of landscape and wetland classification schemes.

Classification scheme	Scale	Hierarchical?	Levels of strata	Advantages	Disadvantages	Potential links with other schemes
Bailey's ecoregions	Nationwide	Yes	Domains Divisions Provinces Sections	Only natural attributes included Digital maps	Terrestrial basis Untested for wetlands No hydrology	Could form first strata for any of the schemes below ecological units
Omernik ecoregions	Nationwide	No	Ecoregions Subecoregions	Digital maps	Combines land-use with natural attributes Untested for most wetlands No hydrology	Could form first strata for any of the schemes below ecological units
Ecological units (Maxwell et.al.,1995)	Nationwide	Yes	Domain Divisions Provinces Sections Subsections	Digital maps	Greater number of strata and units than for ecoregions Untested for wetlands	Could form first strata for any of the schemes below ecological units Ties to classification schemes already defined within hydrogeomorphic types
US ACE Hydrogeomorphic Classes	Nationwide at class level; regionalized at subclass level	Yes - limited	Class Subclass	Specific for wetlands	Subclasses not comparable across different regions	Intermediate strata between geographic and habitat-scale
Rosgen channel types	Nationwide	Yes	Level I Level II	Captures differences in hydrologic regime for riverine wetlands	More focused on instream channel form than riparian characteristics Riverine only Not mapped	Intermediate strata between hydro-geomorphic type and habitat-scale

Table 2. Comparison of landscape and wetland classification schemes.

Classification scheme	Scale	Hierarchical?	Levels of strata	Advantages	Disadvantages	Potential links with other schemes
Anderson land-cover classes	Nationwide	Yes	Level I Level II Level III	Common basis for land-use/land-cover mapping	Not functionally based	Cross-walk w NWI system possible
Circular 39 classes	Nationwide	No	Class	Popular recognition	Mixture of criteria used to distinguish classes Not mapped	Strata below geographic but contains mixture of hydrogeomorphic type and habitat type
National Wetland Inventory	Nationwide	Yes	System Subsystem Class Subclass Hydrologic modifier Other modifiers	Digital maps available for much of nation (but smallest wetlands omitted)	Inconsistencies in mapping water quality modifiers Limited consideration of hydrogeomorphic type	Strata below geographic Hydrogeomorphic class could be improved by link w HGM system
Vegetation associations	International	Yes	System Formation class Formation subclass Formation group Formation subgroup Formation alliance Association	Consistency across terrestrial and aquatic systems	Not functionally based No digital maps Taxa specific	Could be used as lowest level within other schemes

In areas for which digital NWI maps do not yet exist, potential wetland areas can be mapped using GIS tools to predict relative wetness (e.g., Phillips 1990) or soil survey maps with hydric soil series can be used. It should be noted that in areas in which hydrology has been significantly altered (e.g., through ditching, tiling, or construction of urban stormwater systems), areas of potential wetlands could have been removed already. Similarly, although there are no current maps of wetlands by hydrogeomorphic class, these could be derived through GIS techniques using a combination of wetland coverages, hydrography (adjacency to large lakes and rivers), and digital elevation models to derive landforms (mineral and organic soil flats) and/or landscape position (slope and depressional wetlands).

3.4 DIFFERENCES IN NUTRIENT REFERENCE CONDITION OR SENSITIVITY TO NUTRIENTS AMONG WETLAND CLASSES

Very few studies to verify classification systems for wetland nutrient monitoring have been completed, although a number of monitoring strategies have been implemented based on pre-selected strata. Monitoring efforts to develop or assess biological criteria generally have used a combination of geographic region and hydrogeomorphic class or subclass (e.g., Cole et.al., 1997, Bennett 1999, Apfelbeck 1999, Michigan Natural Features Inventory 1997). Analysis of plant associations has been used to derive empirical classifications based on factors such as landscape position, water source, climate, bedrock, and sediment hydraulic conductivity (Weakley and Schafale 1991, Nicholson 1995, Halsey et.al., 1997, Michigan Natural Features Inventory 1997). Only one case of classification based on wetland macroinvertebrate composition was found. For Australian wetlands, wetland classes grouped by macroinvertebrate communities were distinguished by water chemistry extremes (low pH, high salinity), degree of nutrient enrichment, and water color (Growns et.al., 1992).

In some cases (e.g., northern peatlands) classification criteria derived on the basis of plant associations are less powerful in discriminating among nutrient regimes (e.g., Nicholson 1995); this may be particularly true where variation in vegetation type is related to differences in major ion chemistry and pH, rather than nutrients. The same is true in southern pocosins, where short and tall pocosins differ in seasonal hydrology but not soil chemistry. However, when contrasting pocosins and swamp forests, soil nutrients differed strongly (Bridgham and Richardson 1993). For some potential indicators of nutrient status such as vegetation nitrogen to phosphorus ratios, indicator thresholds will be consistent across species (Koerselman and Meuleman 1996), while response thresholds for other indicators of plant nutrient status vary across functional plant groupings with different life history strategies. These differences may indicate potential differences in sensitivity to excess nutrient loading (McJannet et.al., 1995). Thus, vegetation community types are not always a good predictor of background nutrient concentrations (reference condition) or sensitivity to nutrient loading.

Sensitivity to nutrient loading (as evidenced by differences in nutrient cycling and availability) may also be related to differences in hydroperiod among wetlands. Wetland mesocosms exposed to pulse discharges had higher nutrient loss from the water column than those exposed to continuous flow regimes (Busnardo et.al., 1992). Depending on the predominant mechanism for nutrient loss (e.g., plant uptake versus denitrification), nutrient-controlled primary production could be either stimulated or reduced. Mineralization rates of carbon, nitrogen, and phosphorus differ significantly among soils from northern Minnesota wetlands, related to an ombrotrophic to minerotrophic gradient (i.e., degree of groundwater influence), and aeration status (Bridgham et.al., 1998).

In general, very few definitive tests of alternative classification schemes for wetlands are available with respect to describing reference condition for either nutrient criteria or biocriteria. However, evidence from the literature suggests that in many cases both geographic factors (e.g., climate, geologic setting) and landscape setting (hydrogeomorphic type) are expected to affect water quality and biotic communities.

3.5 RECOMMENDATIONS

Classification strategies for nutrient criteria development should incorporate factors affecting background nutrient levels and wetland sensitivity to nutrient loading at several spatial scales.

- Classification of physiographic regions eliminates background variation in lithology and soil texture (affecting background nutrient levels and sorption capacity), in climate (affecting seasonality, productivity, decomposition, and peat formation), and in landforms, which determines the predominance of different hydrogeomorphic classes.

- Classification by hydrogeomorphic class reduces background variation in predominant water and nutrient sources, water depth and dynamics, hydraulic retention time, assimilative capacity, and interactions with other surface water types (Table 3).

- Classification by water depth and duration (which may or may not be incorporated into hydrogeomorphic classes) helps to explain variation in internal nutrient cycling, dissolved oxygen level and variation, and the ability of wetlands to support some higher trophic levels such as fish and amphibians.

- Classification by vegetation type or zone, whether to inform site selection or to determine sampling strata within a site, helps to explain background variation in predominant primary producer form (which will affect endpoint selection), as well as turnover and growth rates (which will affect rapidity of response to nutrient loadings).

In general, the choice of specific alternatives among the classification schemes listed above depends on their intrinsic value as well as practical considerations, e.g., whether a classification scheme is available in mapped digital form or can be readily derived from existing map layers, whether a hydrogeomorphic or other classification scheme has been refined for a particular region and wetland type, and whether classification schemes are already in use for monitoring and assessment of other waterbody types in a State or region. Revisiting classification decisions once data from a sufficient number of sites have been sampled may be useful to ensure the original classification was correct.

Table 3. Features of the major hydrogeomorphic classes of wetlands that may influence background nutrient concentrations, sensitivity to nutrient loading, nutrient storage forms and assimilative capacity, designated use and choice of endpoints.

HGM Class	Organic Flats	Mineral Flats	Depressional	Riverine	Fringe	Slope
Predominant Nutrient Source(S)	Atmospheric Deposition	Atmospheric Deposition, Groundwater	Runoff (Particulate and Dissolved), Surface and Groundwater	Runoff (Particulate), Overbank Flooding (Particulate, Dissolved)	Adjacent Lake, Possible Stream or Riverine Source, Groundwater	Groundwater
Landscape Position				Adjacent to Rivers	Adjacent to Lakes	Slope, Toe of Slope
Hydrologic Regime	Saturated, Little Standing Water	Saturated, Little Standing Water	Depth and Duration Varyfrom Saturated to Temporary to Seasonal to Semi-Permanent to Permanent Inundation	Depth, Duration Vary With River Flooding Regime	Standing Water In Emergent and Submerged Aquatic Zones, Short-Term Fluctuation Related to Seiche Activity, Long-Term to Wet-Dry Cycles	Saturated
Hydraulic Retention Time	Decades	Decades	Varies With Inflows/Outflows, Landscape Position	<Day to Few Days	< Day	< Day
Nutrient Assimilation Capacity	Low	High Sorption Capacity	High Sorption, Plant Uptake, (Limited) Sediment Storage	High Sorption, Sediment Trapping, Plant Uptake In Floodplain	Some Sediment Trapping Nutrient Transformer	High Sorption Capacity

Table 3 cont'd.

HGM Class	Organic Flats	Mineral Flats	Depressional	Riverine	Fringe	Slope
Predominant Vegetation Growth Form	Mosses Sedges	Sedges	Varies With Zone And Duration of Flooding: Wooded Grass/Sedge Emergents Submerged Aquatics*	Wooded, Emergent Vegetation Submerged Aquatics*	Varies With Zone: Grass/Sedge Emergents Submerged Aquatics*	Wooded Grasses Sedges
Top Trophic Level	Mammals Birds Amphibians Invertebrates	Mammals Birds Amphibians Invertebrates	Mammals Birds Mudminnows Amphibians Invertebrates	Fish Birds Mammals	Fish Birds Mammals	Mammals Birds Amphibians Invertebrates
Commercially-Important Fish/Wildlife			Waterfowl	Fish*	Waterfowl Fish*	
Recreational Use Likely			Yes	Yes	Yes	
Drinking Water Source Downstream			Possible	Likely	Possible	

Chapter 4 Sampling Design for Wetland Monitoring

4.1 INTRODUCTION

This chapter provides technical guidance on designing effective sampling programs for State wetland water quality monitoring programs. EPA recommends that States begin wetland monitoring programs to collect water quality and biological data in order to characterize the condition of existing wetlands as they develop nutrient criteria that will protect their wetlands. The best monitoring programs are designed to assess wetland conditions with statistical rigor while maximizing available resources.

At the broadest level, monitoring data should:

1. Detect and characterize the condition of existing wetlands.

2. Describe whether wetland conditions are improving, degrading, or staying the same.

3. Define seasonal patterns, impairments, and deviations in status of wetland conditions.

Water quality monitoring programs should collect a sufficient number of samples over time and space to identify changes in system condition or estimate average conditions with statistical rigor. Three approaches to study design for assessing water quality and biological and ecological condition, and identifying degradation in wetlands are described in this chapter. Specific issues to consider in designing monitoring programs for wetland systems are also discussed in this chapter. The study designs presented here can be tailored to fit the goals of specific monitoring programs.

The three approaches described below (Section 4.3) (probabilistic sampling, targeted/tiered, and Before/After-Control/Impact [BACI]), present study designs that allow one to obtain a significant amount of information with relatively minimal effort. Probabilistic sampling begins with a large-scale, random monitoring design that is reduced as the wetland system conditions are characterized. This approach is used to find the average condition of each wetland class in a specific region. Probabilistic sampling design is frequently used for new large-scale monitoring programs at the State and Federal level (e.g., Environmental Monitoring and Assessment Program (EMAP), Regional Environmental Monitoring and Assessment Program (REMAP), State programs [e.g., Maine, Montana, Wisconsin]). The tiered or targeted approach to monitoring begins with coarse screening and proceeds to more detailed monitoring protocols as impaired and high-risk systems are identified and targeted for further investigation. Targeted sampling design provides a triage approach to more thoroughly assess condition and diagnose stressors in wetland systems in need of restoration, protection, and intensive management. Several State pilot projects use this method or a modification of this method for wetland

assessment (e.g., Florida, Ohio, Oregon, and Minnesota). The synoptic approach described in Kentula et.al., (1993) uses a modified targeted sampling design. The BACI design and its modifications are frequently used to assess the success of restoration efforts or other management experiments. BACI design allows for comparisons in similar systems over time to determine the rate of change in relation to the management activity, e.g., to assess the success of a wetland hydrologic restoration. The BACI design, in particular, is included to assist States in evaluating ongoing management actions, and may provide less statistical rigor if adopted as a general monitoring program design. This design, however, is of considerable value in assessing restoration success and has been included at the request of States with ongoing wetland restoration. Detenbeck et.al., (1996) used BACI design for monitoring water quality of wetlands in the Minneapolis/St. Paul, Minnesota metro area.

Monitoring programs should be designed to describe what the current conditions are and to answer under what conditions impairment may occur. A well-designed monitoring program can contribute to determining those conditions.

Sampling design is dependent on the management question being asked. Sampling efforts should be designed to collect information that will answer the management question. For example, probabilistic sampling might be good for ambient (synoptic) monitoring programs, BACI for evaluating management actions such as restoration, and targeted sampling/stratified and random sampling for developing index of biotic integrity (IBIs) or nutrient criteria thresholds. In practice, some State programs likely will need to use a combination of approaches.

4.2 CONSIDERATIONS FOR SAMPLING DESIGN

DESCRIBING THE MANAGEMENT QUESTION

Clearly defining the question being asked (identifying the hypothesis) encourages the use of appropriate statistical analyses, reduces the occurrence of Type I (false positive) errors, and increases the efficient use of management resources (Suter 1993; Leibowitz et al., 1992; Kentula et al., 1993). Beginning a study or monitoring program with carefully defined questions and objectives helps to identify the statistical analyses most appropriate for the study and reduces the chance that statistical assumptions will be violated. Management resources are optimized because resources are directed at monitoring that which is most likely to answer management questions. In addition, defining the specific hypotheses to be tested, carefully selecting reference sites, and identifying the most useful sampling interval can help reduce the uncertainty associated with the results of any sampling design and further conserve management resources (Kentula et al., 1993). Protecting or improving the quality of a wetland system often depends on the ability of the monitoring program to identify cause-response relationships, for example, the relationship of nutrient concentration (causal variable) to nutrient content of vegetation or vegetation biomass (response variable). Cause-response relationships can be identified using

large sample sizes and systems that span the gradient (low to high) of wetland quality. All ranges of response should be observed along the causal gradient from minimally disturbed to high levels of human disturbance.

Monitoring efforts often are prioritized to best utilize limited resources. For example, the Oregon case study chose not to monitor depressional wetlands due to funding constraints. They further tested the degree of independence of selected sites (and thus the need to monitor all of those sites) using cluster analysis and other statistical tests (http://www.epa.gov/owow/wetlands/bawwg/case/or.html). Frequency of monitoring should be determined by the management question being asked and the intensity of monitoring necessary to collect enough information to answer the question. In addition, monitoring should identify the watershed level activities that are likely to result in ecological degradation of wetland systems (Suter et al., 1993).

SITE SELECTION

Site selection is one of many important tasks in developing a monitoring program (Kentula et.al., 1993). Site selection for a monitoring program is based on the need to sample a sufficiently large number of wetlands to establish the range of wetland quality in a specific regional setting. Wetland monitoring frequently includes an analysis of both watershed/landscape characteristics and wetland specific characteristics (Kentula et al., 1993; Leibowitz et al., 1992). Therefore, wetland sampling sites should be selected based on land use in the region so that watersheds range from minimally impaired with few expected stressors to high levels of development (e.g., agriculture, forestry, or urban) with multiple expected stressors (see the Land-Use Characterization for Nutrient and Sediment Risk Assessment, Wetland module #17). There is often a lag in time between the causal stress and the response in the wetland system. This time lag between stress and response and the duration of this lag depends on many factors, including the type of stressor, climate, and system hydrology; these factors should be considered when selecting sites to establish the range of wetland quality within a region.

LANDSCAPE CHARACTERIZATION

The synoptic approach described in Liebowitz et.al., (1992) provides a method of rapid assessment of wetlands at the regional and watershed levels that can help identify the range of wetland quality within a region. Liebowitz et.al., (1992) recommend an initial assessment for site selection based on current knowledge of watershed and landscape level features; modification of such an assessment can be made as more data are collected. Assessing watershed characteristics through aerial photography and the use of geographical information systems (GIS) linked to natural resource and land-use databases can aid in identifying reference and degraded systems (see the Land-Use Characterization for Nutrient and Sediment Risk Assessment, Wetland module #17); Johnston et al., 1988, 1990; Gwin et al., 1999; Palik et al., 2000; Brown and Vivas 2004). Some examples of watershed characteristics which can be evaluated using GIS and aerial

photography include land use, land cover (including riparian vegetation), soils, bedrock, hydrography, and infrastructure (e.g., roads or railroads). Changes in point sources can be monitored through the NPDES permit program (USEPA 2000). Changes in nonpoint sources can be evaluated through the identification and tracking of wetland loss and/or degradation, increased residential development, urbanization, increased tree harvesting, shifts to more intensive agriculture with greater fertilizer use or increases in livestock numbers, and other land use changes. Local planning agencies should be informed of the risk of increased anthropogenic stress and encouraged to guide development accordingly.

IDENTIFYING AND CHARACTERIZING REFERENCE WETLANDS

The term "reference" in this document refers to those systems that are least impaired by anthropogenic effects. The use of the term reference is confusing because of the different meanings that are currently in use in different classification methods, particularly its use in hydrogeomorphic [HGM] wetland classification. A discussion of the term reference and its multiple meanings is provided in Chapter 3.

Watersheds with little or no development that receive minimal anthropogenic inputs could potentially contain wetlands that may serve as minimally impaired reference sites. Watersheds with a high percentage of the drainage basin occupied by urban areas, agricultural land, and altered hydrology are likely to contain wetlands that are impaired or could potentially be considered "at risk" for developing problems. Wetland loss in the landscape also should be considered when assessing watershed characteristics for reference wetland identification. Biodiversity can become impoverished due to wetland fragmentation or decreases in regional wetland density even in the absence of site-specific land-use activities. Reference wetlands may be more difficult to locate if fragmentation of wetland habitats is significant and may no longer represent the biodiversity of minimally disturbed wetlands in the region. The continued high rate of wetland loss in most States dictates that multiple reference sites be selected to ensure some consistency in reference sites for multiple year sampling programs (Liebowitz et al., 1992; Kentula et al., 1993). Once the watershed level has been considered, a more site-specific investigation can be initiated to better assess wetland condition.

The ideal reference site will have similar soils, vegetation, hydrologic regime, and landscape setting to other wetlands in the region (Adamus 1992; Liebowitz et al., 1992; Kentula et al., 1993; Detenbeck et al., 1996). Classification of wetlands, as discussed in Chapter 3, may aid in identifying appropriate reference wetlands for specific regions and wetland types. Wetland classification should be supplemented with information on wetland hydroperiod to assure that the selected reference wetlands are truly representative of wetlands in the region, class, or subclass of interest. Reference wetlands may not be available for all wetland classes. In that case, data from systems that are as close as possible to the assumed unimpaired state of wetlands in the wetland class of interest should be sought from States within the same geologic province. Development of a conceptual reference may be important if appropriate reference sites cannot be

found in the local region or geologic province. Techniques for defining a conceptual reference are discussed at some length in Harris et.al., (1995), Trexler (1995), and Toth et.al., (1995).

Reference wetlands should be selected based on low levels of human alteration in their watersheds (Liebowitz et.al., 1992; Kentula et.al., 1993; USEPA 2000). Selecting reference wetlands usually involves assessment of land-use within watersheds and visits to individual wetland systems to ground-truth expected land-use and check for unsuspected impacts. Ground-truthing visits to reference wetlands are crucial for identification of ecological impairment that may not be apparent from land-use and local habitat conditions. Again, sufficient sample size is important to characterize the range of conditions that can be expected in the least impacted systems of the region (Detenbeck et.al., 1996). Reference wetlands should be identified for each ecoregion or geological province in the State lands and then characterized with respect to ecological integrity. A minimum of three low impact reference systems is recommended for each wetland class for statistical analyses. However, power analysis can be performed to determine the degree of replication necessary to detect an impact to the systems being investigated (Detenbeck et.al., 1996; Urquhart et.al., 1998). Highest priority should be given to identifying reference systems for those wetland types considered to be at the greatest risk from anthropogenic stress.

WHEN TO SAMPLE

Sampling may be targeted to the periods when effects are most likely to be detected – the index period. The appropriate index period should be defined by what the investigator is trying to investigate and what taxonomic assemblage or parameters are being used for that investigation (Barbour et.al., 1999). For example, increased nutrient concentrations and sedimentation from non-point sources may occur following periods of high runoff during spring and fall, while point sources of nutrient pollutants may cause plankton blooms and/or increased water and soil nutrient concentrations in wetland pools during times of low rainfall. Hence, different index periods may be needed to detect effects from point source and nonpoint source nutrients, respectively. Each taxonomic assemblage studied also should have an appropriate index period—usually in the growing season (see assemblage methods in the Maine case study: http://www.epa.gov/waterscience/criteria/wetlands/index.html).

The index period window may be early in the growing season for amphibians and algae. Other assemblages, such as vegetation and birds, may benefit from a different sampling window for the index period; see the assemblage specific modules for recommendations. Once wetland condition has been characterized, one-time annual sampling during the appropriate index period may be adequate for multiple year monitoring of indicators of nutrient status, designated use, and biotic integrity. However, criteria and ecological indicator development may benefit from more frequent sampling to define conditions that relate to the stressor or perturbor of interest (Karr and Chu 1999; Stevenson 1996; Stevenson 1997). Regardless of the frequency of sampling, selection

of index periods and critical review of the data gathered and analyzed should be done to scientifically validate the site characterization and index periods for data collection.

Ideally, water quality monitoring programs produce long-term data sets compiled over multiple years to capture the natural, seasonal, and year-to-year variations in biological communities and constituent concentrations (e.g., Tate 1990; Dodds et.al., 1997; McCormick et.al., 1999; Craft 2001; Craft et al., 2003; Zheng et al., 2004). Multiple-year data sets can be analyzed with statistical rigor to identify the effects of seasonality and variable hydrology. Once the pattern of natural variation has been described, the data can be analyzed to determine the ecological state of the wetland. Long-term data sets have also been important in influencing management decisions about wetlands, most notably in the Everglades, where long-term data sets have induced Federal, State, and Authorized Tribal actions for conservation and restoration of the largest wetland system in the U.S. (see Davis and Ogden 1994; Everglades Interim Report, South Florida Water Management District [SFWMD, 1999]; Everglades Consolidated Report [SFWMD, 2000, 2001]; 1994 Everglades Forever Act, Florida Statute § 373.4592).

In spite of the documented value of long-term data sets, there is a tendency to intensively study a wetland for one year before and one year after treatment. A more cost-effective approach may be to measure only the indices most directly related to the stressor of interest (i.e., those parameters or indicators that provide the best information to answer the specific management question), but to double or triple the monitoring period. Multiple years (two or more) of data are often needed to identify the effects of years with extreme climatic or hydrologic conditions. Comparisons over time between reference and at risk or degraded systems can help describe biological response and annual patterns in the presence of changing climatic conditions. Multi-year data sets also can help describe regional trends. Flooding or drought may significantly affect wetland biological communities and the concentrations of water column and soil constituents. Effects of uncommon climatic events can be characterized to discern the overall effect of management actions (e.g., nutrient reduction, water diversion) if several years of data are available to identify the long-term trends.

At the very minimum, two years of data before and after specific management actions, but preferably three or more each, are recommended to evaluate the cost-effectiveness of management actions with some degree of certainty (USEPA 2000). If funds are limited, restricting sampling frequency and/or numbers of indices analyzed should be considered to preserve a longer-term data set. Reducing sampling frequency or numbers of parameters measured will allow for effectiveness of management approaches to be assessed against the high annual variability that is common in most wetland systems. Wetlands with high hydrological variation from year to year may benefit from more years of sampling both before and after specific management activities to identify the effects of the natural hydrologic variability (Kadlec and Knight 1996).

CHARACTERIZING PRECISION OF ESTIMATES

Estimates of cause-response relationships, nutrient and biological conditions in reference systems, and wetland conditions in a region are based on sampling; hence, precision should be assessed. Precision is defined as the "measure of the degree of agreement among the replicate analyses of a sample, usually expressed as the standard deviation" (APHA 1999). Determining precision of measurements for one-time assessments from single samples in a wetland is often important. The variation associated with one-time assessments from single samples can be determined by re-sampling a specific number of wetlands during the survey. Measurement variation among replicate samples then can be used to establish the expected variation for one-time assessment of single samples. Re-sampling does not establish the precision of the assessment process, but rather identifies the precision of an individual measurement (Kentula et.al., 1993).

Re-sampling frequency is often conducted for one wetland site in every block of 10 sites. However, investigators should adhere to the objectives of re-sampling (often considered an essential element of QA/QC) to establish an assessment of the variation in a one-time/sample assessment. Often, more than one in 10 samples should be replicated in monitoring programs to provide a reliable estimate of measurement precision (Barbour et.al., 1999). The reader should understand that this is a very brief description of the concerns about precision, and that any monitoring program or study involving monitoring should include consultation with a professional statistician before the program begins and regularly during the course of the monitoring program to assure statistical rigor.

4.3 SAMPLING PROTOCOL

APPROACHES TO SAMPLING DESIGN

The following sections discuss three different approaches to sampling design, probabilistic, targeted, and BACI. These approaches have advantages and disadvantages that under different circumstances warrant the choice of one approach over the other (Table 4). The decision as to the best approach for sample design in a new monitoring program should be made by the water quality resource manager or management team after careful consideration of the different approaches. For example, justification of a dose-response relationship is confounded by lack of randomization and replication and should be considered in choosing a sampling design for a monitoring program.

PROBABILISTIC SAMPLING DESIGN FOR ASSESSING CONDITION

Probabilistic sampling – a sampling process wherein randomness is requisite (Hayek 1994) – can be used to characterize the status of water quality conditions and biotic integrity in a region's

wetland system. This type of sampling design is used to describe the average conditions of a wetland population, identify the variability among sampled wetlands, and help determine the range of wetland system conditions in a region. Data collected from a probabilistic random sample design generally will be characteristic of the dominant class or type of wetland in the region, but rare wetlands may be under-represented or absent from the probabilistically sampled wetlands. Additional sampling sites may need to be added to precisely characterize the complete range of wetland conditions and types in the region.

Probabilistic designs are often modified by stratification (such as classification). Stratified random sampling is a type of probabilistic sampling where a target population is divided into relatively homogenous groups or classes (strata) prior to sampling based on factors that influence variability in that population (Hayek 1994). Stratification by wetland size and class or types ensures more complete information about different types of wetlands within a region. Sample statistics from random selection alone would be most characteristic of the dominant wetland type in a region if the population of wetlands is not stratified.

Many State 305(b) and watershed monitoring programs utilize stratified random sampling designs, and we will further discuss this type of probabilistic sampling. Pilot projects in Maine, Montana, and Wisconsin all use stratified random sampling design. Details of these monitoring designs can be found in the Case Studies module #14 and on the Web at http://www.epa.gov/waterscience/criteria/wetlands/index.html.

Stratification is based on identifying wetland systems in a region (or watershed) and then selecting an appropriate sample of systems from the defined population. The determination of an appropriate sample population usually is dependent on the management questions being asked. A sample population of isolated depressional wetlands could be identified as a single stratum, but investigations of these wetlands would not provide any information on riparian wetlands in the same region. If the goal of the monitoring program is to identify wetland condition for all wetland classes within a region, then a sample population of wetlands should be randomly selected from all wetlands within each class. In practice, most State programs stratify random populations by size, wetland class (see Chapter 3), and landscape characteristics or location (see http://www.epa.gov/waterscience/criteria/wetlands/index.html, module #14).

Once the wetlands for each stratum have been identified, the list of wetlands can be used to select a spatially-balanced stratified random sample. Spatial-balance will ensure spatial coverage over the assessment region, usually increase the types of wetlands sampled (assuming classes of wetlands vary spatially), and reduce spatial autocorrelation among the sampled wetlands. For example, EMAP implements spatially-balanced samples using Generalized Random Tessellation Stratified (GRTS) designs applied to GIS coverages of wetlands within the assessment region. GRTS using a hierarchical grid randomization process to ensure the sites are spatially distributed (Paulsen et.al., 1991; Stevens and Olsen 2004). Estimates of ecological conditions from these kinds of modified probabilistic sampling designs can be used to characterize the water quality

conditions and biological integrity of wetland systems in a region, and over time, to distinguish trends in ecological condition within a region. (See http://www.epa.gov/owow/wetlands/bawwg/case/mtdev.html and http://www.epa.gov/owow/wetlands/bawwg/case/fl1.html).

TARGETED DESIGN

A targeted approach to sampling design may be more appropriate when resources are limited (Stern 2004). The example of targeted sampling described here involves defining a gradient of impairment. Once the gradient has been defined and systems have been placed in categories of impairment, investigators focus the greatest efforts on identifying and characterizing wetland systems or sites likely to be impacted by anthropogenic stressors, and on relatively undisturbed wetland systems or sites (see Identifying and Characterizing Reference Systems, Chapter 3), that can serve as regional, sub-regional, or watershed examples of natural biological integrity. Florida Department of Environmental Protection (FL DEP) uses a targeted sampling design for developing thresholds of impairment with macroinvertebrates (http://www.epa.gov/owow/wetlands/bawwg/case/fl2.html). Choosing sampling stations that best allow comparison of ecological integrity at reference wetland sites of known condition can conserve financial resources. A sampling design that tests specific hypotheses (e.g., the FL DEP study tested the effect of elevated water column phosphorus on macroinvertebrate species richness) generally can be analyzed with statistical rigor and can conserve resources by answering specific questions. Furthermore, identification of systems with problems and reference conditions eliminates the need for selecting a random sample of the population for monitoring.

Targeted sampling assumes some knowledge of the systems sampled (Stern 2004; Kentula et.al., 1993). Systems based on independent variables with evidence of degradation are compared to reference systems that are similar in their physical structure (i.e., in the same class of wetlands). Wetland systems should be viewed along a continuum from reference to degraded. An impaired or degraded wetland is a system in which anthropogenic impacts exceed acceptable levels or interfere with beneficial uses. Comparison of the monitoring data to that collected from reference wetlands will allow characterization of the sampled systems. Wetlands identified as “at risk” should be evaluated through a sampling program to characterize the degree of degradation. Once characterized, the wetlands should be placed in one of the following categories:

1. Degraded wetlands—wetlands in which the level of anthropogenic perturbance interferes with designated uses.

2. High-risk wetlands—wetlands where anthropogenic stress is high but does not significantly impair designated uses. In high-risk systems, impairment is prevented by one or a few factors that could be changed by human actions, though characteristics of ecological integrity are already marginal.

3. Low-risk wetlands—wetlands where many factors prevent impairment, stressors are maintained below problem levels, and/or no development is contemplated that would change these conditions.

4. Reference wetlands—wetlands where the ecological characteristics most closely represent the pristine or minimally impaired condition.

Once wetland systems have been classified based on their physical structure (see Chapter 3) and placed into the above categories, specific wetlands need to be selected for monitoring. At this point, randomness is introduced; wetlands should be randomly selected within each class and risk category for monitoring. An excellent example of categorizing wetlands in this manner is given in the Ohio Environmental Protection Agency's (OH EPA) case study, available at: http://www.epa.gov/owow/wetlands/bawwg/case/oh1.html. They used the Ohio Rapid Assessment Method to categorize wetlands by degree of impairment. The Minnesota Pollution Control Agency (MPCA) also used a targeted design for monitoring wetlands (http://www.epa.gov/owow/wetlands/bawwg/case/mn1.html). They used the best professional judgment of local resource managers to identify reference sites and those with known impairment from identified stressors (agriculture and stormwater runoff).

Targeted sampling design involves monitoring identified degraded systems and comparable reference systems most intensively. Low risk systems are monitored less frequently (after initial identification) unless changes in the watershed indicate an increased risk of degradation.

Activities surrounding impaired wetland systems may be used to help identify which actions negatively affect wetlands, and therefore may initiate more intensive monitoring of at-risk wetlands. Monitoring should focus on factors likely to identify ecological degradation and anthropogenic stress and on any actions that might alter those factors. State water quality agencies should encourage adoption of local watershed protection plans to minimize ecological degradation of natural wetland systems. Development plans in the watershed should be evaluated to identify potential future stressors. Ecological degradation often gradually increases due to many growing sources of anthropogenic stress. Hence, frequent monitoring may be warranted for high-risk wetlands if sufficient resources remain after meeting the needs of degraded wetlands. Whenever development plans appear likely to alter factors that maintain ecological integrity in a high-risk wetland (e.g., vegetated buffer zones), monitoring should be initiated at a higher sampling frequency in order to enhance the understanding of baseline conditions (USEPA 2000).

BEFORE/AFTER, CONTROL/IMPACT (BACI) DESIGN

An ideal before/after impact survey has several features: 1) the type of impact, time of impact, and place of occurrence should be known in advance; 2) the impact should not have occurred

yet; and, 3) control areas should be available (Green 1979). The first feature allows the surveys to be efficiently planned to account for the probable change in the environment. The second feature allows a baseline study to be established and extended as needed. The last feature allows the surveyor to distinguish between temporal effects unrelated to the impact and changes related to the impact. In practice however, advance knowledge of specific impacts is rare, and the ideal impact survey is rarely conducted. BACI designs modified to monitor impacts during or after their occurrence still can provide information, but there is an increase in the uncertainty associated with the results and the likelihood of finding a statistically significant change due to the impact is less probable. In addition, other aspects of survey design are dependent on the study objectives, e.g., the sampling interval, the length of time the survey is conducted (i.e., sampling for acute versus chronic effects), and the statistical analyses appropriate for analyzing the data (Suter 1993).

The best interval for sampling is determined by the objectives of the study (Kentula et.al., 1993). If the objective is to detect changes in trends (e.g., regular monitoring for detection of changes in water quality or biotic integrity), regularly spaced intervals are preferred because the analysis is easier. On the other hand, if the objective is to assess differences before and after impact, then samples at random time points are advantageous. Random sample intervals reduce the likelihood that cyclic differences unforeseen by the sampler will influence the size of the difference before and after the impact. For example, surveys taken every summer for a number of years before and after a clear-cut may show little difference in system quality; however, differences may exist that can only be detected in the winter and therefore may go undetected if sampling occurs only during summer.

The simplest impact survey design involves taking a single survey before and after the impact event (Green 1979). This type of design has the obvious pitfall that there may be no relationship between the observed event and the changes in the response variable—the change may be entirely coincidental. This pitfall is addressed in BACI design by comparing before and after impact data to data collected from a similar control system nearby. Data are collected before and after a potential disturbance in two areas (treatment and a control), with measurements on biological and environmental variables in all combinations of time and area (Green 1979). We will use a clear-cut adjacent to a wetland as an example to illustrate the BACI design. The sampling design is developed to identify the effects of clear-cutting on adjacent wetland systems. In the simplest BACI design, two wetlands would be sampled. One wetland would be adjacent to the clear-cut (the treatment wetland); the second wetland would be adjacent to a control site that is not clear-cut. The control site should have characteristics (soil, vegetation, structure, functions) similar to the treatment wetland and is exposed to climate and weather similar to the first wetland. Both wetlands are sampled at the same time points before the clear-cut occurs and at the same time point after the clear-cut takes place. This design is technically known as an area-by-time factorial design. Evidence of an impact is found by comparing the control site samples (before and after) with the treatment site before and after samples. Area-by-time factorial design allows for both natural wetland-to-wetland variation and coincidental time

effects. If there is no effect of the clear-cut, then change in system quality between the two time points should be the same. If there is an effect of the clear-cut, the change in system quality between the two time points should be different.

CONSIDERATIONS FOR BACI DESIGN

There are some potential problems with BACI design. First, because the control and impact sites are not randomly assigned, observed differences between sites may be related solely to some other factor that differs between the two sites. One could argue that it is unfair to ascribe the effect to the impact (Hurlbert 1984; Underwood 1991). However, as pointed out by Stewart-Oaten et.al., (1986), the survey is concerned about a particular impact in a particular place, not in the average of the impact when replicated in many different locations. Consequently, it may be possible to detect a difference between these two specific sites. Even so, if there are no randomized replicate treatments, the results of the study cannot be generalized to similar events at different wetlands. In any case, the likelihood that the differences between sites are due to factors other than the impact can be reduced by monitoring several control sites (Underwood 1991) because multiple control sites provide some information about potential effects of other factors.

The second and more serious concern with the simple Before-After design with a single sampling point before and after the impact is that it fails to recognize that there may be natural fluctuations in the characteristic of interest that are unrelated to any impact (Hurlbert 1984; Stewart-Oaten 1986). Single samples before and after impact would be sufficient to detect the effects of the impact if there were no natural fluctuations over time. However, if the population also has natural fluctuations over and above the long-term average, then it is impossible to distinguish between cases where there is no effect from cases where there is an impact. Consequently, measured differences in system quality may be artifacts of the sampling dates and natural fluctuations may obscure differences or lead one to believe differences are present when they are not.

The simple BACI design was extended by Stewart-Oaten et.al., (1986) by pairing surveys at several selected time points before and after the impact to help resolve the issue of psuedoreplication (Hulbert 1984). This modification of the BACI design is referred to as BACI-PS (Before-After, Control-Impact Paired Series design). The selected sites are measured at the same time points. The rationale behind this paired design is that repeated sampling before the impact gives an indication of the pattern of differences of potential change between the two sites. BACI-PS study design provides information both on the mean difference in the wetland system quality before and after impact and on the natural variability of the system quality measurements. The resource manager has detected an effect if the changes in the mean difference are large relative to natural variability. Considerations for sampling at either random or regularly spaced intervals also apply here. Replication of samples should also be included if resources allow in order to improve certainty of analytical results.

Violation of the BACI assumptions may invalidate conclusions drawn from the data. Enough data should be collected before the impact to identify the trends in the communities of each sampling site if the BACI assumptions are to be met. Clearly defining the objectives of the study and identifying a statistically testable model of the relationships the investigator is studying can help resolve these issues (Suter 1993).

The designs described above are suitable for detecting longer-term chronic effects in the mean level of the variable of interest. However, the impact may have an acute effect (i.e., effects only last for a short while) or may change the variability in response (e.g., seasonal changes become more pronounced) in some cases. The sampling schedule can be modified so that it occurs at two temporal scales (enhanced BACI-PS design) that encompass both acute and chronic effects (Underwood 1991). The modified temporal design introduces randomization by randomly choosing sampling occasions in two periods (Before and After) in the control or impacted sites. The two temporal scales (sampling periods vs. sampling occasions) allow the detection of a change in mean and of a change in variability after impact. For example, groups of surveys could be conducted every year with five surveys one week apart randomly located within each group. The analysis of such a design is presented in Underwood (1991). Again, multiple control sites should be used to counter the argument that detected differences are specific to the sampled site. The September 2000 issue of the *Journal of Agricultural, Biological, and Environmental Statistics* discusses many of the advantages and disadvantages of the BACI design and provides several examples of appropriate statistical analyses for evaluation of BACI studies.

4.4 SUMMARY

State monitoring programs should be designed to assess wetland condition with statistical rigor while maximizing available management resources. The three approaches described in this module—probabilistic sampling, targeted/tiered approach, and BACI (Before/After, Control/Impact)—present study designs that allow one to obtain a significant amount of information for statistical analyses. The sampling design selected for a monitoring program should depend on the management question being asked. Sampling efforts should be designed to collect information that will answer management questions in a way that will allow robust statistical analysis. In addition, site selection, characterization of reference sites or systems, and identification of appropriate index periods are all of particular concern when selecting an appropriate sampling design. Careful selection of sampling design will allow the best use of financial resources and will result in the collection of high quality data for evaluation of the wetland resources of a State. Examples of different sampling designs currently in use for State wetland monitoring are described in the Case Study module #14 on the Web site: http://www.epa.gov/waterscience/criteria/wetlands/index.html. Well-designed monitoring programs tend to produce data that managers can use in nutrient criteria development, such as in developing reference networks or utilizing distribution-based approaches.

Table 4. Comparison of Probabilistic, Targeted, and BACI Sampling Designs

Probabilistic	Targeted	BACI
Random selection of wetland systems from entire population within a region.	Targeted selection of wetlands based on problematic (wetland systems known to have problems) and reference wetlands.	Selection of wetlands based on a known impact.
This design requires minimal prior knowledge of wetlands within the sample population for stratification.	This design requires prior knowledge of wetlands within the sample population.	This design requires knowledge of a specific impact to be analyzed.
This design may use more resources (time and money) to randomly sample wetland classes because more wetlands may need to be sampled.	This design utilizes fewer resources because only targeted systems are sampled.	This design may use fewer resources because only wetlands with known impacts and associated control systems are sampled.
System characterization for a class of wetlands is more statistically robust.	System characterization for a class of wetlands is less statistically robust, although characterization of a targeted wetland may be statistically robust.	Characterization of the investigated systems is statistically robust.
Rare wetlands may be under-represented or absent from the sampled wetlands.	This design may miss important wetland systems if they are not selected for the targeted investigation.	The information gained in this type of investigation is not transferable to wetland systems not included in the study.
This design is potentially best for regional characterization of wetland classes, especially if water quality conditions are not known.	This design is potentially best for site-specific and watershed-specific criteria development when water quality conditions for the wetland of interest are known.	This design is potentially best for monitoring restoration or creation of wetlands and systems that have specific known stressors.

Chapter 5 Candidate Variables for Establishing Nutrient Criteria

5.1 OVERVIEW OF CANDIDATE VARIABLES

This chapter provides an overview of candidate variables that could be used to establish nutrient criteria for wetlands. A more detailed discussion of sampling methods and laboratory analysis with useful references can be found in the Methods for Evaluating Wetland Condition module series for sampling wetlands[4, 5] at:
http://www.epa.gov/waterscience/criteria/wetlands/index.html.

A good place to start with selecting candidate variables is by developing a conceptual model of how human activities affect nutrients and wetlands. These conceptual models may vary from complex to very simple models, such as relating nitrogen concentrations in sediments and plant biomass or species composition. Conceptual models establish the detail and scope of the project and the most important variables to select. In addition, they define the cause-effect relationships that should be documented to determine whether a problem occurs and what is causing the problem.

In general, for the purposes of numeric nutrient criteria development, it is helpful to develop an understanding of the relationships among human activities, nutrients and habitat alterations, and attributes of ecosystem structure and function to establish a simple causal pathway among three basic elements in a conceptual model. These three basic groups of variables are important to distinguish because we use them differently in environmental management (Stevenson et.al., 2004a). A fourth group of variables is important in order to account for variation in expected condition of wetlands due to natural variation in landscape setting.

The overview of candidate variables in this chapter follows the outline provided in the conceptual model in Figure 5.1. Historically, variables in conceptual models have been grouped many ways with a variety of group names (Paulsen et.al., 1991; Stevenson 1998; Stevenson 2004a, b). In this document, three groups and group names are used to emphasize cause-effect relationships, simplify their presentation and discussion for a diversity of audiences, and maintain some continuity between their use in the past and their use here. The three groups are: supporting variables, causal variables, and response variables.

Supporting variables provide information useful in normalizing causal and response variables and categorizing wetlands. (These are in addition to characteristics used to define wetland

[4] EPA is developing and revising additional modules as a part of the Methods for Evaluating Wetland Conditions Module series; *Biogeochemical Indicators, Wetland Hydrology*, and *Nutrient Loading Estimation*.
[5] The references for these modules can be found in the Supplementary References following the References section.

classes as described in Chapter 3.) **Causal variables characterize pollution or habitat alterations.** Causal variables are intended to characterize nutrient availability in wetlands and could include nutrient loading rates and soil nutrient concentrations. **Response variables are direct measures or indicators of ecological properties.** Response variables are intended to characterize biotic response and could include community structure and composition of vegetation and algae. The actual grouping of variables is much less important than understanding relationships among variables.

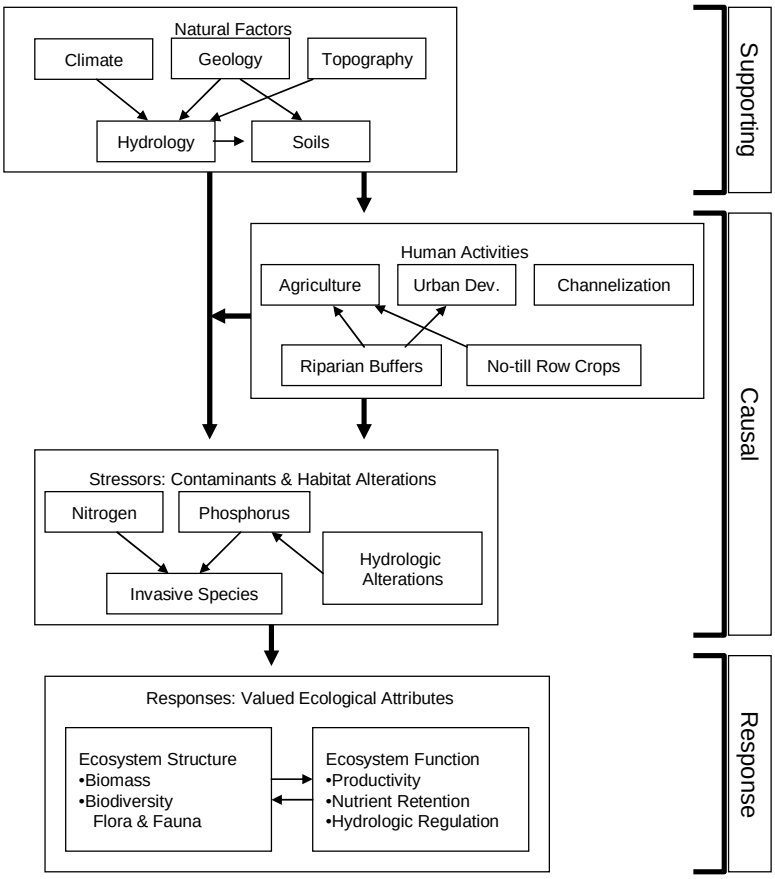
It is important to recognize the complex temporal and spatial structure of wetlands when measuring or interpreting causal and response variables with respect to nutrient condition. The complex interaction of climate, geomorphology, soils, and internal interactions has led to a diverse array of wetland types ranging from infrequently flooded, isolated depressional wetlands such as seasonal prairie potholes and playa lakes, to very large, complex systems such as the Everglades and the Okefenokee Swamp. In addition, most wetlands are complex temporal and spatial mosaics of habitats with distinct structural and functional characteristics illustrated most visibly by patterns in vegetation structure.

Horizontal zonation is a common feature of wetland ecosystems, and in most wetlands, relatively distinct bands of vegetation develop in relation to water depth. Bottomland hardwood forests and prairie pothole wetlands provide excellent illustrations of zonation in two very divergent wetland types. However, vegetation zones are not static. Seasonal and long-term changes in vegetation structure are a common characteristic of most wetland ecosystems. Wetlands may exhibit dramatic shifts in vegetation patterns in response to changes in hydrology, with entire wetlands shifting between predominantly emergent vegetation to completely open water within only a year or two. Such temporal patterns in fact are important features of many wetlands and should be considered in interpreting any causal or response variable. For example, seasonal cycles are an essential feature of floodplain forests, which are typically flooded during high spring flows but dry by mid to late summer. Longer-term cycles are similarly essential features of prairie pothole wetlands, which exhibit striking shifts in vegetation in response to water level fluctuations over periods of a few years in smaller wetlands to decades in larger, more permanent wetlands (van der Valk 2000). Vegetation patterns can significantly affect the physical and chemical characteristics of sediments and overlying waters and are likely to control major aspects of wetland biogeochemistry and trophic dynamics (Rose and Crumpton 1996).

The complex temporal and spatial structure of wetlands should influence the selection of variables to measure and methods for measuring them. Most wetlands are characterized by extremely variable hydrologic and nutrient loading rates and close coupling of soil and water column processes. As a result, estimates of nutrient loading may prove more useful than direct measurements of water column nutrient concentrations as causal variables for establishing the nutrient condition of wetlands. In addition, soil nutrients that integrate a wetland's variable nutrient history over a period of years may provide the most useful metric against which to evaluate wetland response.

Fig 5.1

This conceptual model illustrates the causal pathway between human activities and valued ecological attributes. It includes the role of nutrients in a broader context that includes natural variation among wetlands. The relationship between different approaches of grouping variables is illustrated to emphasize the importance of cause-effect relationships. Here, natural factors and human activities regulate the physical, chemical and biological attributes of wetlands. Some wetland attributes are more valued than others and provide the endpoints of assessment and management. Some physical, chemical, and biological attributes are stressors, i.e. contaminants and habitat alterations caused by human activities that negatively affect valued ecological attributes. The overview of variables in Chapter 5 is organized in three sections: supporting, causal, and response variables. Supporting variables are natural landscape-level factors that classify expected condition of wetlands. Causal factors "cause" effects in response variables.



5.2 SUPPORTING VARIABLES

Supporting variables are not intended to characterize nutrient availability or biotic response but, rather, to provide information that can be useful in normalizing causal and response variables. Below is a brief overview of supporting variables that might be useful for categorizing wetlands and for normalizing and interpreting causal and response variables.

CONDUCTIVITY

Conductivity (also called electrical conductance or specific conductance) is an indirect measure of total dissolved solids. This is due to the ability of water to conduct an electrical current when there are dissolved ions in solution—water with higher concentrations of dissolved inorganic compounds have higher conductivity. Conductivity is commonly measured *in situ* using a handheld probe and conductivity meter (APHA 1999) or using automated conductivity loggers. Because the conductivity changes with temperature, the raw measurement should be adjusted to

a reference temperature of 25°C. A multiplier of 0.7 is commonly applied to estimate the total dissolved solids concentration (mg/L) in fresh water when the conductivity is measured in units of microSiemens per centimeter (μS/cm), although this multiplier varies with the types of dissolved ions and should be adjusted for local chemical conditions.

Conductivity is a useful tool for characterizing wetland inputs and interpreting nutrient condition because of its sensitivity to changes in these inputs. Rainfall tends to have lower conductivity than surface water, with ground water often having higher values due to the longer residence time of water in the subsurface. Coastal and marine waters—as well as water in terminal lakes and wetlands—have even higher conductivity due to the influence of salinity. Municipal and industrial discharges often have higher conductivity than their intake waters due to the addition of soluble wastes. Wetland hydrologic inputs can be identified by comparing the measured input conductivity with the conductivity of potential local sources.

SOIL pH

Soil pH can be important for categorizing wetland soils and interpreting soil nutrient variables. The pH of wetland soils and water varies over a wide range of values. Many ombrotrophic organic wetland soils (histosols) such as bogs and non-limestone based wetlands are often acidic, and mineral wetland soils are frequently neutral or alkaline. Flooding a soil results in consumption of electrons and protons. In general, flooding acidic soils results in an increase in pH, and flooding alkaline soils decreases pH (Mitsch and Gosselink 2000). The increase in pH of low pH (acidic) wetland soils is largely due to the reduction of iron and manganese oxides. However, the initial decrease in pH of alkaline wetland soils is due to rapid decomposition of soil organic matter and accumulation of CO_2. The decrease in pH that generally occurs when alkaline soils are flooded results from the buildup of CO_2 and carbonic acid. In addition, the pH of alkaline soils is highly sensitive to changes in the partial pressure of CO_2. Carbonates of iron and manganese also can buffer the pH of soil to neutrality. Soil pH determinations should be made on wet soil samples. Once the soils are air-dried, oxidation of various reduced compounds results in a decrease in pH and the values may not represent ambient conditions.

Soil pH is measured using commercially available combination electrodes on soil slurries. If air dry or moist soil is used, a 1:1 soil to water ratio should be used. For details on methodology, the reader is referred to Thomas (1996).

Soil pH can explain the availability and retention capacity of phosphorus. For example, phosphorus bioavailability is highest at soil pH near neutral conditions. For mineral soils, phosphorus adsorption capacity has been directly linked to extractable iron and aluminum. For details, the reader is referred to Supplementary References.

SOIL BULK DENSITY

Soil bulk density is the mass of dry solids per unit volume of soil, which includes the volume of solids plus air- and water-filled pore space. Bulk density is a useful parameter for expressing the concentration of nutrients on a volume basis, rather than mass basis. For example, concentration of nutrients in organic wetland soils can be high when expressed on a mass basis (mg/kg or µg/g of dry soil), as compared to mineral wetland soils. However, the difference in concentration may not be as high when expressed on a volume (cm^3) basis, which is calculated as the product of bulk density and nutrient concentration per gram of soil. Expressing soil nutrient concentrations on a volume basis is especially relevant to uptake by vegetation since plant roots explore a specific volume, not mass, of soil. Expressing nutrients on a volume basis also helps in calculating total nutrient storage in a defined soil layer.

Bulk density is measured by collecting an intact soil core of known volume at specific depths in the soil (Blake and Hartge, 1986). Cores are oven-dried at 70^oC and weighed. Bulk density is calculated as follows:

Bulk density (dry) (g/cm^3) = mass dry weight (grams)/volume (cm^3)

Bulk densities of wetland organic soils range from 0.1 to 0.5 g/cm^3, whereas bulk densities of mineral wetland soils range from 0.5 to 1.5 g/cm^3. Soil bulk densities are directly related to soil organic matter content, as bulk densities decrease with increases in soil organic matter content.

SOIL ORGANIC MATTER CONTENT

Soil organic matter can be important for categorizing wetland soils and interpreting soil nutrient variables. Wetland soils often are characterized by the accumulation of organic matter because rates of primary production often exceed rates of decomposition. Some wetlands accumulate thick layers of organic matter that, over time, form peat soil. Organic matter provides nutrient storage and supply, increases the cation exchange capacity of soils, enhances adsorption or deactivation of organic chemicals and trace metals, and improves overall soil structure, which results in improved air and water movement. A number of methods are now routinely used to estimate soil organic matter content expressed as total organic carbon or loss on ignition (APHA, 1999; Nelson and Sommers, 1996).

Soil organic matter content represents the soil organic carbon content of soils. Typically, soil organic matter content is approximately 1.7 to 1.8 times that of total organic carbon. The carbon to nitrogen and carbon to phosphorus ratios of soils can provide an indication of nutrient availability in soils.

HYDROLOGIC CONDITION

Wetland hydrologic condition is important for characterizing wetlands and for normalizing many causal and response variables. Hydrologic conditions can directly affect the chemical and physical processes governing nutrient and suspended solids dynamics within wetlands (Mitsch and Gosselink, 2000). Detailed, site-specific hydrologic information available is best, but at a minimum, some estimate of water level fluctuation should be made. A defining characteristic of wetlands is oxygen deficiency in the soil caused by flooding or soil saturation. These conditions influence vegetation dynamics through differential growth and survival of plant species and also exert significant control over biogeochemical processes involved in carbon flow and nutrient cycling within wetlands. Spatial and temporal patterns in hydrology can create complex patterns in soil and water column oxygen availability, including alternating aerobic and anaerobic conditions in wetland soils, with obvious implications for plant response and biogeochemical process dynamics. Water levels in wetlands can be determined using a staff gauge when surface water is present. A staff gauge measures the depth of surface flooding relative to a reference point such as the soil surface. Other methods to assess past water levels when standing water is not present include moss collars, staining, and cypress knee heights. While surface flooding may be rare or absent in a wetland, high water tables may still cause soil saturation in the rooting zone. In wetlands where soils are saturated, water level can be measured with a small diameter perforated tube installed in the soil to a specified depth (Amoozegar and Warrick 1986). Automated water level recorders using floats, capacitance probes, or pressure transducers are suitable for measuring water levels both above- and below-ground. The reader is referred to the Supplementary References for details.

5.3 CAUSAL VARIABLES

Causal variables are intended to characterize nutrient availability in wetlands. Most wetlands are characterized by extremely variable nutrient loading rates and close coupling of soil and water column processes. As a result, estimates of nutrient loading and measurements of soil nutrients may prove more useful than direct measurements of water column nutrient concentrations as causal variables for establishing the nutrient condition of wetlands. Nutrient loading history and soil nutrient measures can integrate a wetland's variable nutrient history over a period of years and may provide especially useful metrics against which to evaluate nutrient condition. Wetlands exhibit a high degree of spatial heterogeneity in chemical composition of soil layers, and areas impacted by nutrients may exhibit more variability than unimpacted areas of the same wetland. Thus, sampling protocols should capture this spatial variability. Developing nutrient criteria and monitoring the success of nutrient management programs involves important considerations for sampling designed to capture spatial and temporal patterns.

Below is a brief overview of the use of nutrient loading and soil and water column nutrient measures for estimating nutrient condition of wetlands. Please refer to Supplementary

References for a list of references on both nutrient load estimation and biogeochemical indicators, with a focus on soil and water column nutrient measures.

NUTRIENT LOADING

External nutrient loads to wetlands are determined primarily by surface and subsurface transport from the contributing landscape, and vary significantly as a function of weather and landscape characteristics such as soils, topography, and land use. Most wetlands are characterized by extremely variable hydrologic and nutrient loading rates, which present considerable obstacles to obtaining adequate direct measurement of nutrient inputs. Adequate measurement of loads may require automated samplers capable of providing flow-weighted samples when loading rates are highly variable. In many cases, nonpoint source loads simply may not be adequately sampled. The more detailed the loading measurements the better, but it is not reasonable to expect adequate direct measurement of loads for most wetlands. In the absence of sufficient, direct measurements, it may be possible to estimate nutrient loading using an appropriate loading model or at least to provide a relative ranking of wetlands based on expected nutrient load. One advantage of loading models is that nutrient loading can be integrated over the appropriate time scale for characterizing wetland nutrient condition and, in some cases, historical loading patterns can be reconstructed. Loading models also can provide hydrologic loading rates to calculate critical supporting variables such as hydroperiod and residence times.

Loading function models are based on empirical or semi-empirical relationships that provide estimates of pollutant loads on the basis of long-term measurements of flow and contaminant concentration. Generally, loading function models contain procedures for estimating pollutant load based on empirical relationships between landscape physiographic characteristics and phenomena that control pollutant export. McElroy et.al., (1976) and Mills (1985) described loading functions employed in screening models developed by the USEPA to facilitate estimation of nutrient loads from point and nonpoint sources. The models contain simple empirical expressions that relate the magnitude of nonpoint pollutant load to readily available or measurable input parameters such as soils, land use and cover, land management practices, and topography. Preston and Brakebill (1999) described a spatial regression model that relates the water quality conditions within a watershed to sources of nutrients and to those factors that influence transport of the nutrients. The regression model, Spatially-Referenced Regressions on Watersheds (SPARROW), involves a statistical technique that utilizes spatially referenced information and data to provide estimates of nutrient load (Smith et al., 1997; Smith et al., 2003; http://water.usgs.gov/nawqa/sparrow/).

In general, the SPARROW methodology was designed to provide statistically based relationships between stream water quality and anthropogenic factors such as contaminant sources within the contributing watersheds, land surface characteristics that influence the delivery of pollutants to the stream, and in-stream contaminant losses via chemical and biological process pathways. The Generalized Watershed Loading Functions (GWLF) model

(Haith and Shoemaker, 1987; Haith et al., 1992) uses daily time steps, and to some extent, both can be used to examine seasonal variability and the response to landscape characteristics of specific watersheds. The GWLF model was developed to evaluate the point and nonpoint loading of nitrogen and phosphorus in urban and rural watersheds. The model enhances assessment of effectiveness of certain land use management practices and makes extensive use of readily available watershed data. The GWLF also provides an analytical tool to identify and rank critical areas of a watershed and evaluate alternative land management programs.

Process-oriented simulation models attempt to explicitly represent biological, chemical, and physical processes controlling hydrology and pollutant transport. These models are at least partly mechanistic in nature and are built from equations that contain directly definable, observable parameters. Examples of process-oriented simulation models that have been used to predict watershed hydrology and water quality include the Agricultural Nonpoint Source model (AGNPS), the Hydrologic Simulation Program-Fortran (HSPF), and the Soil and Water Assessment Tool (SWAT). AGNPS (Young et. al., 1987) is a distributed parameter, event-based and continuous simulation model that predicts the behavior of runoff, sediment, nutrients, and pesticide transport from watersheds that have agriculture as the primary land use. Because of its simplicity and ease of use, AGNPS is probably one of the most widely used hydrologic and water quality models of watershed assessment. HSPF (Johansen et al., 1984; Bicknell et al., 1993; Donigian et al., 1995a) is a lumped parameter, continuous simulation model developed during the mid-1970s to predict watershed hydrology and water quality for both conventional and toxic organic pollutants. HSPF is one of the most comprehensive models available for simulating nonpoint source nutrient loading. The capability, strengths, and weaknesses of HSPF have been demonstrated by its application to many urban and rural watersheds and basins (e.g., Donigian et al., 1990; Moore et al., 1992; and Ball et al., 1993). SWAT (Arnold et al., 1995) is a lumped parameter, continuous simulation model developed by USDA-Agricultural Research Services that provides long-term simulation of impact of land management practices on water, sediment, and agricultural chemical yields in large complex watersheds. Because of its lumped parameter nature, coupled with its extensive climatic, soil, and management databases, the SWAT model is one of the most widely used hydrologic and water quality models for large watersheds and basins, and the model has found widespread application in many modeling studies that involve systemic evaluation of impact of agricultural management on water quality.

These loading models address only gross, external nutrient inputs. It is important to consider the overall mass balance for the receiving wetland in developing measures of nutrient loading against which to evaluate wetland nutrient condition. This requires some estimate of nutrient export, storage, and transformation. In the absence of sufficient, direct measurements from which to calculate nutrient mass balance, it may be possible to estimate nutrient mass balances using an appropriate wetland model. Strictly empirical, regression models can be used to estimate nutrient retention and export in wetlands but these regressions are of little value outside the data domain in which they are developed. When developed for a diverse set of systems, the scatter in these regressions can be quite large. In contrast to strictly empirical regressions, mass

balance models incorporate principles of mass conservation. These models integrate external loading to the wetland, nutrient transformation and retention within the wetland, and nutrient export from the wetland. Mass balance models allow time varying hydrologic and nutrient inputs and can provide estimates of spatial nutrient distribution within the wetland. The most difficult problem is developing removal rate equations which adequately represent nutrient transformation and retention across the range of conditions for which estimates are needed.

LAND USE

Identifying land uses in regions surrounding wetlands is important for characterizing reference condition, identifying reference wetlands, and providing indicators of nutrient loading rates for criteria development. Most simply, the percentage of natural area or the percentage of agricultural and urban lands can be used to characterize land uses around wetlands. More detailed quantitative data can be gathered from GIS analysis, which provides higher resolution identification of land use types such as pastures, row crops, and confined animal feeding operations for agriculture. Ideally these characterizations should be done for the entire sourceshed, including both air and water, in the regions around wetlands. Air-sheds should incorporate potential atmospheric sources of nutrients, and watersheds should incorporate potential aquatic sources. However, in practice, land use around wetlands is typically used for defining reference wetlands and also in most nutrient loading models to characterize groundwater and surface water sources. Land use in buffer zones, one kilometer zones around wetlands and wetland watersheds (delineated by elevation), has been used to characterize human activities that could be affecting wetlands (Brooks et.al., 2004).

EXTRACTABLE SOIL NITROGEN AND PHOSPHORUS

Ammonium is the dominant form of inorganic N in wetland soils, and unlike total soil N (Craft et.al., 1995, Chiang et.al., 2000), soil extractable NH_4-N increases in response to N loadings. Enrichment leads to enhanced cycling of N between wetland biota (Valiela and Teal 1974, Broome et.al., 1975, Chalmers 1979, Shaver et.al., 1998), greater activity of denitrifying bacteria (Johnston 1991, Groffman 1994, White and Reddy 1999), and accelerated organic matter and N accumulation in soil (Reddy et.al., 1993, Craft and Richardson 1998). In most cases, extractable soil N should be measured in the surface soil where roots and biological activity are concentrated.

Extractable N is measured by extraction of inorganic (NH_4-N) N with 2 M KCl (Mulvaney 1996). Ten to twenty grams of field moist soil is equilibrated with 100 ml of 2 M KCl for one hour on a reciprocating shaker, followed by filtration through Whatman No. 42 filter paper. Ammonium-N in soil extracts is determined colorimetrically using the phenate or salicylate method (APHA 1999, Method 350.2, USEPA, 1993a).

Extractable P is often a reliable indicator of the P enrichment of soils, and in wetlands, extractable P is strongly correlated with surface water P concentration and P enrichment from external sources (Reddy et.al., 1995, 1998). Selected methods used to extract P are described below (Kuo 1996). Many soil testing laboratories perform these analyses on a routine basis. Historically, these methods have been used to determine nutrient needs of agronomic crops, but the methods have been used more recently to estimate P impacts in upland and wetland soils (Sharpley et.al., 1992; Nair et.al., 1995; Reddy et.al., 1995, 1998).

The Mehlich I method is typically used in the Southeast and Mid-Atlantic regions on mineral soils with pH of < 7.0 (Kuo 1996). The extractant consists of dilute concentrations of strong acids. Many plant nutrients such as P, K, Ca, Mg, Fe, Zn, and Cu extracted with Mehlich I methods have been calibrated for production of crops in agricultural ecosystems. This solvent extracts some Fe and Al- bound P, and some Ca-bound P. Soil (dry) to extractant ratio is set at 1:4, for mineral soils, while wider ratios are used for organic soils. Soil solutions are equilibrated for a period of five minutes on a mechanical shaker and then filtered through a Whatman No. 42 filter. Filtered solutions are analyzed for P and other nutrients using standard methods (Method 365.1, USEPA, 1993a).

The Bray P-1 method has been widely used as an index of available P in soils (Kuo 1996). The combination of dilute concentration of strong acid (HCl at 0.025 M) and ammonium fluoride (NH_4F at 0.03 M) is designed to easily remove acid extractable soluble P forms such as Ca-bound P, and some Fe and Al-bound P. Soil (dry) to extractant ratio is set at 1:7 for mineral soils with wider ratios used for highly organic soils, then shaken for five minutes and filtered through a Whatman No. 42 filter. Filtered solutions are analyzed for P and other nutrients using the same methods used for the Mehlich I extraction (Method 365.1, USEPA 1993a).

Bicarbonate Extractable P is a suitable method for calcareous soils. Soil P is extracted from the soil with 0.5 M $NaHCO_3$ at a nearly constant pH of 8.5 (Kuo 1996). In calcareous, alkaline, or neutral soils containing Ca-bound P, this extractant decreases the concentration of Ca in solution by causing precipitation of Ca as $CaCO_3$. As a result, P concentration in soil solution increases. Soil (dry) to extraction ratio is set at 1:20 for mineral soils and 1:100 for highly organic soils. Soil solutions are equilibrated for a period of 30 minutes on a shaker , filtered through a Whatman No. 42 filter paper, and analyzed for P using standard methods (Method 365.1, USEPA, 1993a).

TOTAL SOIL NITROGEN AND PHOSPHORUS

Nutrient enrichment leads to enrichment of total soil P (Craft and Richardson 1993, Reddy et.al., 1993, Bridgham et al., 2001). In contrast, soil total N usually does not increase in response to nutrient enrichment (Craft et.al., 1995, Chiang et.al., 2000). Rather, enrichment leads to enhanced cycling of N between wetland biota that is reflected in greater N uptake and net primary production (NPP) of wetland vegetation (Valiela and Teal 1974, Broome et.al., 1975,

Chalmers 1979, Shaver et.al., 1998), greater activity of denitrifying bacteria (Johnston 1991, Groffman 1994, White and Reddy 1999), and accelerated organic matter and N accumulation in soil (Reddy et.al., 1993, Craft and Richardson 1998). In most cases, total N and P should be measured in at least the surface soil where most roots and biological activity are concentrated.

Since ammonium N is the dominant form of inorganic nitrogen in saturated wetland soils with very little nitrate (NO_3) present, total Kjeldahl nitrogen (TKN) can generally be taken as a measure of total N in such soils. The difference between TKN and ammonium N provides information on soil organic N. The soil organic carbon to soil organic nitrogen ratio can provide an indication of the soil's capacity to mineralize organic N and provide ammonium N to vegetation. TKN in soils is determined by converting organic forms of N to NH_4-N by digestion with concentrated H_2SO_4 at temperatures of 300-350 ^{o}C (Bremner 1996). The NH_4-N in digested samples is analyzed using colorimetric (e.g., phenate, salicylate) methods (APHA 1999, Mulvaney 1996).

Total P in soils is determined by oxidation of organic forms of P and acid (nitric-perchloric acid) dissolution of minerals at temperatures of <300°C (Kuo 1996). Digested solutions are analyzed for P using colorimetric methods (e.g., ascorbic acid-molybdate) (APHA 1999, Kuo 1996). Many laboratories may not have access to perchloric acid fume-hoods. Alternatively, soil total phosphorus can be determined using the ashing method (Anderson, 1976). Results obtained from this method are reliable and comparable to total phosphorus measurements made using perchloric acid digestion method.

WATER COLUMN NITROGEN AND PHOSPHOROUS

Nutrient inputs to wetlands are highly variable across space and time, hence, single measurements of water column N and P represent only a "snap-shot" of nutrient condition and may or may not reflect the long-term pattern of nutrient inputs that alter biogeochemical cycles and affect wetland biota. The best use of water column N and P concentrations for nutrient criteria development will be based on frequent monitoring of nutrient concentrations over time (e.g., weekly or monthly measurements). Of course, in wetlands that are seldom flooded, measurements of water column N and P may not be practical or even relevant for assessing impacts. Whenever water samples are obtained, it is important that the water depth is recorded because nutrient concentration is related to water depth. In the case of tidal estuarine or freshwater wetlands, it is also important to record flow and the point in the tidal cycle that the samples were collected.

Methodologies to monitor N in surface waters are well developed for other ecosystems and can be readily adopted for wetlands. The most commonly monitored N species are total Kjeldahl nitrogen (TKN), ammonium N, and nitrate plus nitrite N (APHA 1999). The TKN analysis includes both organic and ammonium N, but does not include nitrate plus nitrite N. Organic N is determined as the difference between TKN and NH_4-N. Forms of N in surface water are

measured by standard methods, including phenol-hypochlorite for ammonium N, cadmium reduction of nitrate to nitrite for nitrate N, and Kjeldahl digestion of total N to ammonium for analysis of total N (APHA 1999). Dissolved organic N is primarily used by heterotrophic microbes, whereas plants and various microorganisms take up inorganic forms of N (ammonium N and nitrate N) to support metabolism and new growth.

Methodologies to monitor P in surface waters are well developed for aquatic ecosystems and can be readily adopted for wetlands (APHA 1999). The most commonly measured forms of P in surface water are total P, dissolved inorganic P (i.e., PO_4-P), and total dissolved P. To trace the transport and transformations of P in wetlands, it might be useful to distinguish four forms of P: (i) dissolved inorganic P (DIP, also referred to as dissolved reactive P (DRP) or soluble reactive phosphorous (SRP)); (ii) dissolved organic P (DOP); (iii) particulate inorganic P (PIP); and, (iv) particulate organic P (POP). Dissolved inorganic P (PO_4-P) is considered bioavailable (e.g., available for uptake and use by microorganisms, algae, and vegetation), whereas organic and particulate P forms generally must be transformed into inorganic forms before being considered bioavailable. In P limited wetlands, a significant fraction of DOP can be hydrolyzed by phosphatases and utilized by bacteria, algae, and macrophytes.

5.4 RESPONSE VARIABLES

Biotic measures that can integrate a wetland's variable nutrient history over a period of months to years may provide the most useful measures of wetland response to nutrient enrichment. Microorganisms, algae, and macrophytes respond to nutrient enrichment by: (1) increasing the concentration of nutrients (P, N) in their tissues; (2) increasing growth and biomass production; and, (3) shifts in species composition. The biotic response to nutrient enrichment generally occurs in a sequential manner as nutrient uptake occurs first, followed by increased biomass production, followed by a shift in species composition as some species disappear and other species replace them. Macroinvertebrates respond to nutrient enrichment indirectly as a result of changes in food sources, habitat structure, and dissolved oxygen. Because of their short life cycle, microorganisms and algae respond more quickly to nutrient enrichment than macrophytes. However, biotic measures that can integrate a wetland's variable nutrient history over a period of months to years may provide the most useful measures of wetland response.

Below is a brief overview of the use of macrophytes, algae, and macroinvertebrates to assess nutrient condition of wetlands. Please refer to the relevant modules in the EPA series "Methods for Evaluating Wetland Condition" for details on using vegetation (http://www.epa.gov/waterscience/criteria/wetlands/16Indicators.pdf;

http://www.epa.gov/waterscience/criteria/wetlands/10Vegetation.pdf), algae; (http://www.epa.gov/waterscience/criteria/wetlands/11Algae.pdf); and, macroinvertebrates (http://www.epa.gov/waterscience/criteria/wetlands/9Invertebrate.pdf) to assess wetland condition, including nutrients.

MACROPHYTE NITROGEN AND PHOSPHORUS

Wetland macrophytes respond to nutrient enrichment by increasing uptake and storage of N and P (Verhoeven and Schmitz 1991, Shaver et.al., 1998, Chiang et.al., 2000). In wetlands where P is the primary limiting nutrient, the P content of vegetation increases almost immediately (within a few months) in response to nutrient enrichment (Craft et.al., 1995). Increased P uptake by plants is known as "luxury uptake" because P is stored in vacuoles and used later (Davis 1991). Like P, leaf tissue N may increase in response to N enrichment (Brinson et.al., 1984, Shaver et.al., 1998). However, most N is directly used to support new plant growth so that luxury uptake of N is not usually observed (Verhoeven and Schmitz 1991). Tidal marsh grasses, however, do appear to store nitrogen in both living and dead tissues that can be accessed by living plant tissue. A discussion of conservation and translocation of N in saltwater tidal marshes can be found in Hopkinson and Schubauer (1980) and Thomas and Christian (2001).

Nutrient content of macrophyte tissue holds promise as a means to assess nutrient enrichment of wetlands. However, several caveats should be kept in mind when using this diagnostic tool (Gerloff 1969, Gerloff and Krombholz 1966, EPA 2002c).

1. The most appropriate plant parts to sample and analyze should be determined. It is generally recognized that the plant or plant parts should be of the same physiological age.

2. Samples from the same species should be collected and analyzed. Different species assimilate and concentrate nutrients to different levels.

3. Tissue nutrient concentrations vary with (leaf) position, plant part, and age. It is important to sample and analyze leaves from the same position and age (e.g., third leaf from the terminal bud on the plant) to ensure comparability of results from sampling of different wetlands.

4. Tissue P may be a more reliable indicator of nutrient condition than N. This is because N is used to increase production of aboveground biomass, whereas excess P is stored via luxury uptake.

Another promising macrophyte-based tool is the measurement of nutrient resorption of N and P prior to leaf senescence and dieback. Nutrient resorption is an important strategy used by macrophytes to conserve nutrients (Hopkinson and Schubauer 1984; Shaver and Melillo 1984). In nutrient-poor environments, macrophytes resorb N and P from green leaves prior to

senescence, leading to low concentrations of N and P in senesced leaves. In nutrient-rich environments, resorption becomes less important so that senesced leaves retain much of the N and P that was present when the leaves were green.

Nitrogen and phosphorus should be measured in green leaves of the same approximate age collected from the dominant wetland plant species. Samples also should be collected throughout the wetland to account for spatial variability. If an environmental gradient is known or suspected to exist within the wetland, then sites along this gradient should be sampled separately. At each sampling location, approximately five green leaves are collected from each of the dominant plant species. Leaves are collected from the middle portion of the stem, avoiding very young leaves at the top of the stem and very old leaves at the bottom of the stem. At each location, leaf samples by species are combined for analysis, oven-dried at $70^{o}C$, and ground.

Nitrogen is measured by dry combustion using a CHN analyzer. Phosphorus is measured colorimetrically after digestion in strong acid (H_2SO_4-H_2O_2) (Allen et.al., 1986). Many land-grant universities, State agricultural testing laboratories, and environmental consulting laboratories perform these analyses. Contact your local U.S. Department of Agriculture office or land-grant agricultural extension office for information on laboratories that perform plant tissue nutrient analyses.

Please see the EPA module #16, *Vegetation-based Indicators of Wetland Nutrient Enrichment* (http://www.epa.gov/waterscience/criteria/wetlands/16Indicators.pdf) for a detailed description of indicators derived from N and P content of macrophytes.

ABOVEGROUND BIOMASS AND STEM HEIGHT

Wetland macrophytes also respond to nutrient enrichment by increased net primary production (NPP) and growth if other factors such as light are not limiting growth (Chiang et.al.,2000). Net primary production is the amount of carbon fixed during photosynthesis that is incorporated into new leaves, stems, and roots. Most techniques to measure NPP focus on aboveground biomass and discount root production because it is difficult to measure, even though root production may account for 50% of NPP. The simplest way to measure aboveground biomass is by harvesting all of the standing material (biomass) at the end of the growing season (Broome et al., 1986). The harvest method is useful for measuring NPP of herbaceous emergent vegetation, especially in temperate climates where there is a distinct growing season. If root production desired, it can be determined by sequentially harvesting roots at monthly intervals during the year (Valiela et. al, 1976).

Enhanced NPP often is reflected by increased height and, sometimes, stem density of herbaceous emergent vegetation (Broome et. al., 1983). Because increased stem density may reflect other factors like vigorous clonal growth, it is not recommended as an indicator of nutrient enrichment.

Aboveground biomass of herbaceous vegetation may be determined by end-of-season harvest of aboveground plant material in small 0.25 m^2 quadrats stratified by macrophyte species or inundation zone (Broome et.al., 1986). Stem height of individuals of dominant species is measured in each plot. Height of the five to 10 tallest stems in each plot has been shown to be a reliable indicator of NPP (Broome et.al., 1986) that saves time as compared to height measurements of all stems in the plot. Aboveground biomass is clipped at the end of the growing season, in late summer or fall. Clipped material is separated into live (biomass) versus dead material, then dried at 70°C to a constant weight. For stem height and biomass sampling, five to 10 plots per vegetation zone are collected. In forested sites, biomass production is defined as the sum of the leaf and fruit fall and aboveground wood production (Newbould, 1967). Please see the EPA module *Vegetation-based Indicators of Wetland Nutrient Enrichment* (http://www.epa.gov/waterscience/criteria/wetlands/16Indicators.pdf) for a detailed description of sampling aboveground biomass in wetlands.

ALGAL NITROGEN & PHOSPHORUS

In some cases, measurements of algal N and P can provide a useful complement to vegetation and soil nutrient analyses that integrate nutrient history over a period of months in the case of vegetation (Craft et.al., 1995), to years in the case of soils (Craft and Richardson 1998, Chiang et.al., 2000). Nutrient concentrations in algae can integrate variation in water column N and P bioavailability over a time scale of weeks, potentially providing an indication of the recent nutrient status of a wetland (Fong et al., 1990; Stevenson et.al., 2001). Caution is warranted for this method because it is not useful in all wetlands; for example, in wetlands where surface inundation occurs intermittently or for short periods of time, where the water surface is severely shaded as in some forested wetlands, or under other circumstances where unrelated environmental factors exert primary control over algal growth.

Algae should be sampled by collecting grab samples from different locations in the wetland to account for spatial variability in the wetland. If an environmental gradient is known or suspected (i.e., decreasing canopy or impacted land uses) or exists within the wetland as a result of specific source discharges, then sites along this gradient should be sampled separately. Comparisons among wetlands or locations within a wetland should be done on a habitat-specific basis (e.g., phytoplankton vs. periphyton). Samples are processed in the same manner as wetland plants to determine N and P content. Nitrogen is determined using a CHN analyzer, whereas P is measured colorimetrically after acid digestion.

Please see the EPA module *Using Algae to Assess Environmental Conditions in Wetlands* (http://www.epa.gov/waterscience/criteria/wetlands/11Algae.pdf) for a detailed description of indicators derived from to N and P content of algae.

MACROPHYTE COMMUNITY STRUCTURE AND COMPOSITION

The composition of the plant community and the changes that result from human activities can be used as sensitive indicators of the biological integrity of wetland ecosystems. In particular, aggressive, fast-growing species such as cattail (*Typha* spp.), giant reed (*Phragmites communis*), reed canarygrass (*Phalaris arundincea*), and other clonal species invade and may eventually come to dominate the macrophyte community. Data collection methods and analyses for using macrophyte community structure and composition as an indicator of nutrient enrichment and ecosystem integrity for wetlands are described in *Vegetation-based Indicators of Wetland Nutrient Enrichment* (http://www.epa.gov/waterscience/criteria/wetlands/16Indicators.pdf) and *Using Vegetation to Assess Environmental Conditions in Wetlands* (http://www.epa.gov/waterscience/criteria/wetlands/10Vegetation.pdf), respectively.

ALGAL COMMUNITY STRUCTURE AND COMPOSITION

Algae can be used as a valuable indicator of biological and ecological condition of wetlands. Structural and functional attributes of algae can be measured including diversity, biomass, chemical composition, productivity, and other metabolic functions. Species composition of algae, particularly of the diatoms, is commonly used as an indicator of biological integrity and physical and chemical conditions of wetlands. Discussions of sampling, data analyses, and interpretation are included in *Using Algae to Assess Environmental Conditions in Wetlands* (http://www.epa.gov/waterscience/criteria/wetlands/11Algae.pdf).

INVERTEBRATE COMMUNITY STRUCTURE AND COMPOSITION

Aquatic invertebrates can be used to assess the biological and ecological condition of wetlands. The approach for developing an Index of Biological Integrity (IBI) for wetlands based on aquatic invertebrates is described in *Developing an Invertebrate Index of Biological Integrity for Wetlands* (http://www.epa.gov/waterscience/criteria/wetlands/9Invertebrate.pdf).

5.5 SUMMARY

Candidate variables to use in determining nutrient condition of wetlands and to help identify appropriate nutrient criteria for wetlands consist of supporting variables, causal variables, and response variables. Supporting variables provide information useful in normalizing causal and response variables and categorizing wetlands. Causal variables are intended to characterize nutrient availability (or assimilation) in wetlands and could include nutrient loading rates and soil nutrient concentrations. Response variables are intended to characterize biotic response and could include community structure and composition of macrophytes and algae.

The complex temporal and spatial structure of wetlands will influence the selection of variables to measure and methods for measuring them. The information contained in this chapter is a brief summary of suggested analyses that can be used to determine wetland condition with respect to

nutrient status. The authors recognize that the candidate variables and analytical methods described here will generally be the most useful for identifying wetland nutrient condition, while other methods and analyses may be more appropriate in certain systems.

Chapter 6 Database Development and New Data Collection

6.1 INTRODUCTION

A database of relevant water quality information can be an invaluable tool to States as they develop nutrient criteria. In some cases, existing data are available and can provide additional information that is specific to the region where criteria are to be set. However, little or no data are available for most regions or parameters and creating a database of newly gathered data is strongly recommended. In the case of existing data, the data should be located and their suitability (type and quality and sufficient associated metadata) ascertained. It is also important to determine how the data were collected to ensure that future monitoring efforts are compatible with earlier approaches.

Databases operate much like spreadsheet applications but have greater capabilities. Databases store and manage large quantities of data and allow viewing and exporting of data sorted in a variety of ways, while spreadsheets analyze and graphically display small quantities of data. Databases can be used to organize existing information, store newly gathered monitoring data, and manipulate data for water quality criteria development. Databases can sort data for export into statistical analyses programs, spreadsheets, and graphics programs. This chapter will discuss the role of databases in nutrient criteria development and provide a brief review of existing sources of nutrient-related water quality information for wetlands.

6.2 DATABASES AND DATABASE MANAGEMENT

A database is a collection of information related to a particular subject or purpose. Databases are arranged so that individual values are kept separate, yet can be linked to other values based on some common denominator (such as association of time or location). Geographic Information Systems (GIS) are geo-referenced relational databases that have a geographical component (i.e., spatial platform) in the user interface. Spatial platforms associated with a database allow geographical display of sets of sorted data. GIS platforms such as ArcView™, ArcInfo™, and MapInfo™ are frequently used to integrate spatial data with monitoring data for watershed analysis. Data stored in simple tables, relational databases, or geo-reference databases can also be located, retrieved, and manipulated using queries. A query allows the user to find and retrieve only the data that meets user-specified conditions. Queries can also be used to update or delete multiple records simultaneously and to perform built-in or custom calculations of data. Data in tables can be analyzed and printed in specific layouts using reports. Data can be analyzed or presented in a specific way in print by creating a report. The most effective use of these tools requires a certain amount of training, expertise, and software support, especially when using geo-referenced data.

To facilitate data storage, manipulation, and calculations, it is highly recommended that historical and present-day data be transferred to a relational database (i.e., Access™). Relational databases store data in tables as sets of rows and columns and are powerful tools for data manipulation and initial data reduction. They allow selection of data by specific, multiple criteria and definition and redefinition of linkages among data components. Data queries can also be exported to GIS, provided that the data is related to some geo-referenced coordinate system.

POTENTIAL DATA SOURCES

EPA Water Quality Data

STORET
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.

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 Web site: 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. Data from the EMAP program can be downloaded directly from the EMAP Web site (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 Web site.

U.S. Geological Surve) (USGS) 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 databases is through the USGS State representative. Every State office can be reached through the USGS home page at: http://www.usgs.gov.

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 over the past 30 years. The HBN consists 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.

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 Web site for more details (http://water.usgs.gov/nrp/webb/about.html).

US Department of Agriculture (USDA)
Agricultural Research Service (ARS)

The USDA ARS houses the Natural Resources and Sustainable Agricultural Systems Scientific Directory (http://hydrolab.arsusda.gov/arssci.html), 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 Web site (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 Web site (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 (NSF) funds projects for the Long Term Ecological Research (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.

U.S. Army Corps of Engineers (COE)

The U.S. Army Corps of Engineers (COE) is responsible for many federal wetland jurisdiction issues. Although a specific network of water quality monitoring data does not exist, specific studies on wetlands by the COE may provide suitable data. The COE focuses more on water quantity issues than on water quality issues. As a result, much of the wetland system data collected by the COE does not include nutrient data. 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 can be found at http://www.usace.army.mil/public.html.

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

The Bureau of Reclamation of the U.S. Department of the Interior manages many irrigation and water supply reservoirs in the West, some of which may have wetland applicable 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 if a flow component of criteria development is chosen. These data can be gathered on a site-specific basis from the BuRec Web site: http://www.usbr.gov.

State Monitoring Programs

Some States may have wetland water quality data as part of a research study, use attainability analysis (UAA), or to assess mitigation or nutrient related impacts. Most of this data is collected by State natural resources or environmental protection agencies, or by regional water management authorities. Data collected by State water quality monitoring programs can be used

for nutrient criteria development and may provide pertinent data sources, although they may be regionally limited. These data should be available from the agencies responsible for monitoring.

Volunteer Monitoring Programs

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 or local governments to address problem areas. The EPA supports volunteer monitoring and local involvement in protecting our water resources.

Academic and Literature Sources

Most of the data available on water and soil quality in wetlands is the result of research studies conducted by academic institutions. Much of the research conducted by the academic community, however, was not conducted for the purpose of spatial or long-term biogeochemical characterization of the nation's wetlands; instead, water quality information was often collected to characterize the environmental conditions under which a particular study or experiment was conducted. Infrequently, spatial studies of limited extent or duration were conducted. Data collected from these sources, therefore, may not be sufficiently representative of the population of wetlands within an ecoregion. However, this limited data may be the only information available and therefore could be useful for identifying reference conditions or determining where to begin a more comprehensive survey to support development of nutrient criteria. Academic research data is available from researchers and the scientific literature.

6.3 QUALITY OF HISTORICAL AND COLLECTED DATA

The value of older historical data 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. Supporting documentation should be examined to determine the consistency of sampling and analytical protocols. The suitability of data in large, heterogeneous data repositories for establishing nutrient criteria are described below. These same factors need to be taken into account when developing a new database such that future investigators will have sufficient information necessary to evaluate the quality of the database.

LOCATION

Geo-referenced data is extremely valuable in that it allows for aggregating and summarizing data according to any GIS coverage desired, whether the data was historically related to a particular coverage theme or not. However, many studies conducted prior to the availability and accuracy of hand held Global Positioning System (GPS) units relied on narrative and less definitive descriptions of location such as proximity to transportation corridor, county, or nearest municipal center. This can make comparison of data, depending upon desired spatial resolution, difficult. Knowledge of the rationale and methods of site selection from the original investigators may supply valuable information for determining whether inclusion of the site or study in the database is appropriate based on potential bias relative to overall wetland data sources. STORET and USGS data associated with the National Hydrography Dataset (NHD) are geo-referenced with latitude, longitude, and Reach File 3 (RF3) codes (http://nhd.usgs.gov/). In addition, STORET often contains a site description to supplement location information. Metadata of this type, when known, is frequently stored within large long-term databases.

VARIABLES AND ANALYTICAL METHODS

Each separate analytical method yields a unique variable. For example, five ways of measuring TP result in five unique variables. Data generated using different analytical methods should not be combined in data analyses because methods differ in accuracy, precision, and detection limits. Data generated from one method may be too limited, making it important to select the most frequently used analytical methods in the database. Data that were generated using the same analytical methods may not always be obvious because of synonymous names or analytical methods. Consistency in taxonomic conventions and indicator measurements is likewise important for biological variables and multimetric indices comparisons. Review of recorded data and analytical methods by knowledgeable personnel is important to ensure that there are no problems with data sets developed from a particular database.

LABORATORY QUALITY CONTROL (QC)

Data generated by agencies or laboratories with known quality control/quality assurance protocols are most reliable. Laboratory QC data (blanks, spikes, replicates, known standards) are infrequently reported in larger data repositories. Records of general laboratory quality control protocols and specific quality control procedures associated with specific data sets 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.

Water column nutrient data can be reported in different units, e.g., ppm, mg/L, mmoles. Reporting of nutrient data from other strata such as soils, litter, and vegetation can further

expand the list of reporting units (e.g., mg/kg, g/kg, %, mg/cm^3). In many instances, conversion of units is possible; however, in other instances unit conversion is not possible or is lacking support information for conversion. Consistency in reporting units and the need to provide conversion tables cannot be overemphasized.

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 7.

INDEX PERIOD

An index period—the time period most appropriate for sampling—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 wetland characteristics for the region, the quality and quantity of data available, and estimates of temporal variability (if available). Consideration of the data available relative to longer-term oscillations in environmental conditions (e.g., dry years, wet years) should also be taken into account such that the data is representative and appropriate. Additional information and considerations for establishing an index period are discussed in Chapter 7.

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 wetland classes of interest. The investigator should determine if all wetlands or a subset of the wetlands in the database are representative of the population of wetlands to be characterized. If a sufficient sample of representative wetlands cannot be found, then a new survey is strongly recommended.

6.4 COLLECTING NEW DATA

New data should be collected when no data presently exist or the data available are not suitable, and 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 in the database and collecting spatially representative regional monitoring data. In many cases, this may mean starting from scratch because no data presently exists or the data available are not suitable. Data gathered under new monitoring programs should be imported into databases or spreadsheets and, if comparable, merged with existing data for criteria development. It is best to archive the data with as much data-unique information (meta-data) as possible. It is always possible to aggregate at a later time, but impossible to separate lumped data without having the parameter needed to partition the data set. Redundancy may also be a problem but can more easily be avoided when common variables or parameters are kept in each database (i.e., dates may be very important). The limitations and qualifications of each data set should be known and data 'tagged', if possible, before combining them. The following five factors should be considered when collecting new data and before combining new data with existing data sets: representativeness, completeness, comparability, accuracy, and precision.

REPRESENTATIVENESS

Sampling program design (when, where, and how you sample) should produce samples that are *representative* or typical of the regional area being described and the classes of wetlands present. Sampling designs for developing nutrient criteria are addressed in Chapter 4. Databases populated by data from the literature or historical studies will not likely provide sufficient spatial or class representation of a region. Data interpretation should recognize these gaps and be limited until gaps are filled using additional survey information.

COMPLETENESS

A QA/QC plan should describe how to complete the data set in order to answer 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. Precautions to ensure completeness may include collecting extra samples, having back-up equipment in the field, 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 should 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 and examined for compatibility with other monitoring programs prior to initiation of a survey. Comparability should be assessed for field sample collection, sample preservation, sample preparation and analysis, and among laboratories used for sample analyses.

ACCURACY

To assess the accuracy of field instruments and analytical equipment, a standard (a sample with a known value) should 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, 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 vegetation, soils, and sediments. Standards and methods of calibration are typically included with turbidity meters, pH meters DO meters, and DO testing kits. The U.S. EPA, USGS, and some private companies provide reference standards or QC samples for nutrients.

VARIABILITY

The variability in field measurements and analytical methods should be demonstrated and documented to identify the source and magnitude of variability when possible. EPA QA/QC guidance provides an explanation and protocols for measuring sampling variability (USEPA 1998c).

DATA REDUCTION

For data reduction, it is important to have a clear idea of the analysis that will be performed and a clear definition of the sample unit for analysis. For example, a sample unit might be defined as “a wetland during July- August.” For each variable measured, a mean value would then be estimated for each wetland during the July-August index period on record. Analyses are then conducted on the observations (estimated means) for each sample unit, not with the raw data. Steps recommended for reducing the data include:

1. Selecting the long-term time period for analysis;

2. Selecting an index period;

3. Selecting relevant variables of interest;

4. Identifying the quality of analytical methods;

5. Identifying the quality of the data recorded; and,

6. Estimating values for analysis (mean, median, minimum, maximum) based on the reduction selected.

6.5 QUALITY ASSURANCE / QUALITY CONTROL (QA/QC)

The validity and usefulness of data depend on the care with which they were collected, analyzed, and documented. EPA provides guidance on data quality assurance (QA) and quality control (QC) (USEPA 1998c) 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.

1. Who will use the data?
2. What the project's goals/objectives/questions or issues are?
3. What decision(s) will be made from the information obtained?
4. How, when, and where project information will be acquired or generated?
5. What possible problems may arise and what actions can be taken to mitigate their impact on the project?
6. What type, quantity, and quality of data are specified?
7. How "good" those data have to be to support the decision to be made?
8. How the data will be analyzed, assessed, and reported?

Chapter 7 Data Analysis

7.1 INTRODUCTION

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

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

7.2 FACTORS AFFECTING ANALYSIS APPROACH

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

Wetland hydrogeomorphic type http://el.erdc.usace.army.mil/wrap/wrap.html may determine the sensitivity of wetlands to nutrient inputs, as well as the interaction of nutrients with other driving factors in producing an ecological response. Hydrogeomorphic types differ in landscape position, predominant water source, and hydrologic exchanges with adjacent water bodies (Brinson 1993). These factors, in turn, influence water residence time, hydrologic regime, and disturbance regime. In general, isolated depressional wetlands will have greater residence times than fringe wetlands, which, in turn, will have greater residence times than riverine wetlands. Systems with long residence times are likely to behave more like lakes than flow-through systems and may show a greater response to cumulative loadings. Thus, nutrient loading rates or indicators thereof are likely to be a more sensitive predictor of ecological effects for depressional wetlands, while nutrient water column or sediment concentrations are likely to be a more sensitive predictor of responses for riverine wetlands. Water column concentrations will influence the response of algal communities, while macrophytes derive nutrients from both the

water column and sediments. Fringe wetlands are likely to be influenced both by concentration of nutrients in the adjacent lake or estuary as well as the accumulation of nutrients within these systems from groundwater inflow and, in some cases, riverine inputs. The relative influence of these two sources will depend on the exchange rate with the adjacent lake, e.g., through seiche activity (Keough et al., 1999; Trebitz et al., 2002). In practice, it is difficult to measure loadings from multiple sources including groundwater and exchange with adjacent water bodies. If sediment concentrations are shown to be a good indicator of recent loading rates, then sediment concentrations might be the best predictor to use across systems.

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

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

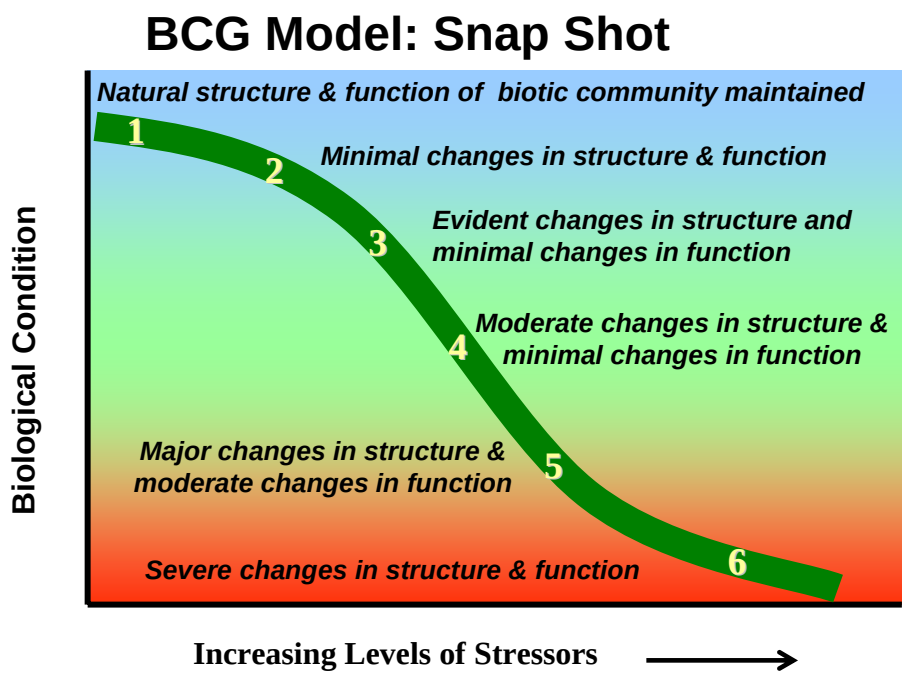


Figure 7.1. Biological condition gradient model describing biotic community condition as levels of stressors increase.

7.3 DISTRIBUTION-BASED APPROACHES

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

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

Distributions of nutrient exposure metrics or response variables can be developed to represent either an entire population of wetlands or only a subset of those considered to be minimally

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

7.4 RESPONSE-BASED APPROACHES

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

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

Designated uses such as contact recreation and drinking water may not be applicable to wetlands, hence, it may not be readily apparent what the relative significance of changes in different primary producers is for organisms at higher trophic levels. Wetland food webs have traditionally been considered to be detritus-based (Odum and de la Cruz 1967; Mann 1972, 1988). However, more recent research on wetland food webs utilizing stable isotope analysis

have identified the importance of phytoplankton, periphyton, or benthic algae as the base of the food chain for higher trophic levels (Fry 1984, Kitting et al., 1984, Sullivan and Moncreiff 1990, Hamilton et al., 1992, Newell et al., 1995, Keough et al., 1996); in these cases, it would be particularly important to monitor shifts in algal producers.

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

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

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

from macrophyte pollen or diatom frustrules, which will be particularly valuable in the absence of sites representing reference condition (Cooper et al., 1999, Jensen et. al., 1999).

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

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

7.5 PARTITIONING EFFECTS AMONG MULTIPLE STRESSORS

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

among multiple factors can be used, such as partial CCA or partial redundancy analysis (Cooper et al., 1999, Jensen et al., 1999).

7.6 STATISTICAL TECHNIQUES

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

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

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

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

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

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

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

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

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

MULTIMETRIC INDICIES

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

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

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

Another approach for characterizing biotic integrity of assemblages as a function of trophic status is to calculate the deviation in species composition or growth forms at assessed sites from composition in the reference condition. Similarity or dissimilarity indices can be used for the determining the differences in biotic integrity of a wetland in comparison to the reference condition. Multivariate similarity or dissimilarity indices need to be calculated for multivariate attributes such as taxonomic composition (Stevenson 1984; Raschke 1993) as defined by relative

abundance of different growth forms or species, or species presence/absence. One standard form of these indices is percent community similarity (PS_c, Whittaker 1952):

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

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

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

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

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

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

7.7 LINKING NUTRIENT AVAILABILITY TO PRIMARY PRODUCER RESPONSE

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

DEFINING THE LIMITING NUTRIENT

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

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

Limitation may be detected by other means, such as alkaline-phosphatase activity, to determine if phosphorus is limiting. Alkaline phosphatase is an extracellular enzyme excreted by some algal species and from roots in some macrophytes in response to P limitation. This enzyme hydrolyzes phosphate ester bonds, releasing orthophosphate (PO_4) from organic phosphorus compounds (Mullholland et.al., 1991). Therefore, the concentration of alkaline phosphatase in

the water can be used to assess the degree of P limitation. Alkaline phosphatase activity, monitored over time in a wetland, can be used to assess the influence of P loads on the growth limitation of algae (Richardson and Qian 1999).

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

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

Chapter 8 Criteria Development

8.1 INTRODUCTION

This chapter describes recommendations for setting scientifically defensible criteria for nutrients in wetlands by using data that address causal and biotic response variables. Causal variables (external nutrient loading, soil extractable P, soil extractable N, total soil N and P, and water column N and P), and biotic response variables (vegetation N and P, biomass, species composition, and algal N and P) and the supporting variables (hydrologic condition, conductivity, soil pH, soil bulk density, particle size distribution, and soil organic matter), as described in Chapter 5 provide an overview of environmental conditions and nutrient status of the wetland; these parameters are considered critical to nutrient assessment in wetlands. Several recommended approaches that water quality managers can use to derive numeric criteria in combination with other biological response variables are presented. These recommended approaches can be used alone, in combination, or may be modified for use by State water quality managers to derive criteria for wetlands that are scientifically defensible and protective of the designated use. Criteria developed from multiple lines of evidence using combined approaches will provide the greatest scientific defensibility. Recommended approaches for numeric nutrient criteria development presented here include:

- the use of reference conditions to characterize natural or minimally impaired wetland systems with respect to causal and exposure indicator variables;
- applying predictive relationships to select nutrient concentrations that will protect wetland structure and/or function; and,
- developing criteria from established nutrient exposure-response relationships (as in the peer-reviewed published literature).

The first approach is based on the assumption that maintaining nutrient levels within the range of values measured for reference systems will maintain the biological integrity of wetlands. This presumes that a sufficient number of reference systems can be identified. The second two approaches are response-based; hence, the level of nutrients associated with biological impairment should be used to identify criteria. Ideally, both kinds of information (background variability and exposure-response relationships) will be available for criteria development. Recommendations are also presented for deriving criteria based on the potential for effects to downstream receiving waters (i.e., the lake, reservoir, stream, or estuary influenced by wetlands). States should consider relating these measures to metrics of ecological integrity and periodically assessing measures to verify assumptions made in criteria development. The chapter concludes with a recommended process for evaluating proposed criteria, suggestions of how to

interpret and apply criteria, considerations for sampling for comparison to criteria, potential modifications to established criteria, and adoption of criteria into water quality standards.

The RTAG is composed of State and Regional specialists who will help the Agency and States establish nutrient criteria for adoption into their water quality standards. Expert evaluations are important throughout the criteria development process. The data upon which criteria are based and the analyses performed to arrive at criteria should be assessed for veracity and applicability.

8.2 METHODS FOR DEVELOPING NUTRIENT CRITERIA

The following discussions focus on three general methods that can be used in developing nutrient criteria. First, identification of reference or control systems for each established wetland type and class should be based on either best professional judgment (BPJ) or percentile selections of data plotted as frequency distributions. The second method uses refinement of classification systems, models, and/or examination of system biological attributes to assess the relationships among nutrients, vegetation or algae, soil, and other variables. Finally, the third method identifies published nutrient and vegetation, algal, and soil relationships and values that may be used (or modified for use) as criteria. **A weight of evidence approach with multiple attributes that combines one or more of these three approaches should produce criteria of greater scientific validity.**

USING REFERENCE CONDITION TO ESTABLISH CRITERIA

One approach to consider in setting criteria is the concept of reference condition. This approach involves using relatively undisturbed wetlands as reference systems to serve as examples for the natural or least disturbed ecological conditions of a region. These approaches are most useful for estimating reference conditions appropriate to the specific designated use for a class of wetlands. Three recommended ways of using reference condition to establish criteria are:

1. Characterize reference systems for each class within a region using best professional judgment and use these reference conditions to define criteria.

2. Identify the 75th to 95th percentile of the frequency distribution for a class of reference wetlands as defined in Chapter 3 and use this percentile to define the criteria.

3. Calculate a 5th to 25th percentile of the frequency distribution of the general population of a class of wetlands and use the selected percentile to define the criteria.

Defining the nutrient condition of wetlands within classes will allow the manager to identify protective criteria and determine which systems may benefit from management action. Criteria that are identified using reference condition approaches may require comparisons to similar systems in other States that share the ecoregion so that reference condition and developed

criteria can be validated. Furthermore, the 95th percentile of the reference population and the 5th percentile of the general population are best used to define the criteria when there is great confidence that the group of reference waters truly reflects reference conditions as opposed, for example, to best available condition.

Reference wetlands should be identified for each class of wetland within a State or ecoregion and then characterized with respect to external nutrient loading, water column N and P, biotic response variables (macrophytes, algae, soils) and supporting environmental conditions. Wetlands classified as reference quality should be verified by comparing the data from the reference systems to general population data for each wetland class. Reference systems should be minimally disturbed and should have biotic response values that reflect this condition.

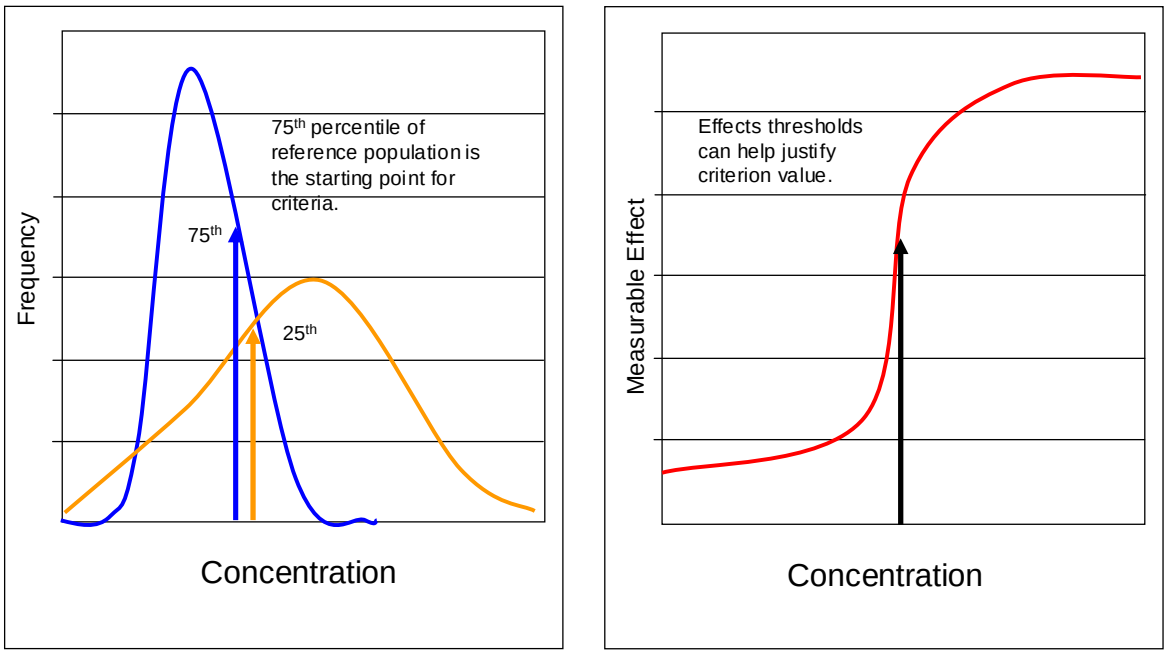
Conditions at reference sites may be characterized using either of two frequency distribution approaches (see 2 and 3 above). In both approaches, an optimal reference condition value is selected from the distribution of an available set of wetland data for a given wetland class. This approach may be of limited value at this time because few States currently collect wetland monitoring data. However, as more wetlands are monitored and more data become available, this approach may become more viable.

In the first frequency distribution approach, a percentile (75th – 95th is recommended) is selected from the distribution of causal and biotic response variables of reference systems selected *a priori* based on very specific criteria (i.e., highest quality or least impacted wetlands for that wetland class within a region). The values for variables at the selected quartile may be used as the basis for nutrient criteria. The selection of a specific percentile as the basis for the criterion should be determined by the uses designated for that water.

If reference wetlands of a given class are rare within a given region or if inadequate information is available to assign wetlands with historic nutrient data as “reference” versus “impacted” wetlands, another approach may be appropriate. The second frequency distribution approach involves selecting a percentile of: (1) all wetland data in the class (reference and non-reference); or, (2) a random sample distribution of all wetland data within a particular class. Due to the random selection process, a lower percentile should be selected because the sample distribution is expected to contain some degraded systems. This option is most useful in regions where the number of legitimate “natural” reference wetlands is usually very small, such as in highly developed land use areas (e.g., the agricultural lands of the Midwest and the urbanized east or west coasts). EPA’s recommendation in this case is the 5th to 25th percentile depending upon the number of “natural” reference systems available. If almost all systems are impaired to some extent, then a lower percentile, generally the 5th percentile, is recommended for selection of reference wetlands.

Both the 75th percentile for the subset of reference systems and the 5th to 25th percentile from a representative random sample distribution are only recommendations. The actual distribution of

the observations should be the major determinant of the threshold point chosen. For example, a bi-modal distribution of sediment or water-column nutrients might indicate a natural breakpoint between reference and enriched systems. To illustrate, Figure 8.1 shows both options and illustrates the presumption that these two alternative methods should approach a common reference condition along a continuum of data points. In this illustration, the 75th percentile of the reference data distribution produces an extractable soil P reference condition that corresponds to the 25th percentile of the random sample distribution.



Nutrient data from reference waters (blue) or from all waters (gold) similar physical characteristics.

Paired nutrient and effects data from waters with similar physical characteristics.

Figure 8.1 Use of frequency distributions of nutrient concentration for establishing criteria (left graphic), and use of effects thresholds with nutrient concentration for establishing criteria (right graphic).

The choice of a distribution cut-off to define the upper range of reference wetland nutrient levels is analogous to defining an acceptable level of Type I error, the frequency for rejecting wetlands as members of the "unimpacted" class when in fact they are part of the reference wetland

population (a false designation of impairment). If a distribution cut-off of 25% is chosen, the rate of falsely designating wetlands as impaired will be higher than if a distribution cutoff of 5% is chosen; however, the frequency of committing Type II errors (failing to identify anthropogenically-enriched wetlands) will be lower. As described in Chapter 7, there is a trade-off between Type I and Type II errors. When additional information is available, it may be possible to justify a range of values that are representative of least-impaired wetlands that would reduce Type I errors on a system by system basis.

State water quality managers also may consider analyzing wetlands data based on designated use classifications. Using this approach, frequency distributions for specific designated uses, as opposed to frequency distributions of reference or general populations, could be examined and criteria proposed based on maintenance of high quality systems that are representative of each designated use. For example, one criterion could be derived that protects superior quality wetland habitat (SWLH), and a second criterion could be identified that maintains good quality wetland habitat (function maintained but some loss of sensitive species (Figure 8.2); see Office of Water tiered aquatic life use training module: (http://www.epa.gov/waterscience/biocriteria/modules/wet101-05-alus-monitoring.pdf). This recommended approach is designated as the Tiered Aquatic Life Use (TALU) and is being developed by the EPA Office of Water in a more detailed publication. Using this approach, a criterion range is created and a greater number of wetland systems will likely be considered protective of the designated use. In this case, emphasis may be shifted from managing wetland systems based on a central tendency,toward more pristine systems associated with Tiers I and II. This approach also will aid in prioritizing systems for protection and restoration. Subsequent management efforts using this approach should focus on improving wetland conditions so that, over time, plots of wetland data shift to the left (i.e., improved nutrient condition) of their initial position.
p.p

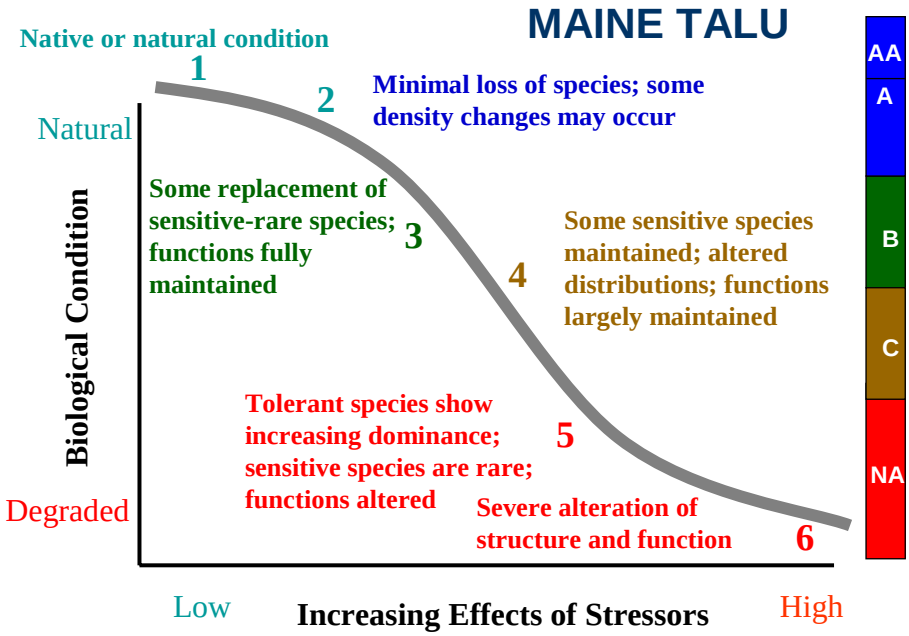


Figure 8.2. Tiered Aquatic Life Use model used in Maine.

APPLYING PREDICTIVE RELATIONSHIPS

Two fundamental reasons are commonly considered for using biological attributes in developing nutrient criteria. The concepts basically promote the use of biotic responses or biocriteria to nutrient enrichment, i.e., both rationales support evaluation of physical and chemical conditions in conjunction with biological parameters when establishing water quality criteria. The first reason is that the primary goal of environmental assessment and management is to protect and restore ecosystem services and ecological attributes, which are often closely related to biological features and functions in ecosystems. Therefore, it is the effects of nutrients on the living components of ecosystems that should become the critical determinant of nutrient criteria, rather than
the actual nutrient concentrations. The second reason for using biocriteria is that attributes of biological assemblages usually vary less in space and time than most physical and chemical characteristics measured in environmental assessments. Thus, fewer mistakes in assessment may occur if biocriteria are employed in addition to physical and chemical criteria. In those environments where biological attributes change fairly rapidly, such as in Louisiana's coastal wetland environment where salinity can vary dramatically in response to wet versus drought years, other techniques will need to be developed. Information on some other techniques can be found at: Louisiana State University's School of the Coast and Environment

[http://www.wetlandbiogeochemistry.lsu.edu/] and also in interagency efforts through the Los Angeles Department of Natural Resources) to assess coastal area ecology. [http://data.lca.gov/Ivan6/app/app_c_ch9.pdf]

Multimetric indices are a special form of indicators of biological condition in which several metrics are used to summarize and communicate in a single number the state of a complex ecological system. Multimetric indices for macroinvertebrates and fish are used successfully to establish biocriteria for aquatic systems in many States, and several States are developing multimetric indices for wetlands (see http://www.epa.gov/owow Web site).

Another recommended approach is to identify threshold or non-linear biotic responses to nutrient enrichment. Some biological attributes respond linearly with increasing nutrient concentrations, whereas some attributes change in a non-linear manner. Non-linear changes in metrics indicate thresholds along environmental gradients where small changes in environmental conditions cause relatively great changes in a biological attribute. In an example from the Everglades, a specific level of P concentration and loadings was associated with a dramatic shift in algal composition and loss of the calcareous algal mats typical of this system (Figure 8.3). Overall, metrics or indices that change linearly (typically higher-level community attributes such as diversity or a multimetric index) provide better variables for establishing biocriteria because they respond to environmental change along the entire gradient of human disturbance. However, metrics that change in a non-linear manner along environmental gradients are valuable for determining where along the environmental gradient the physical and chemical criteria should be set and, correspondingly, how to interpret other biotic response variables of interest (Stevenson et.al., 2004a).

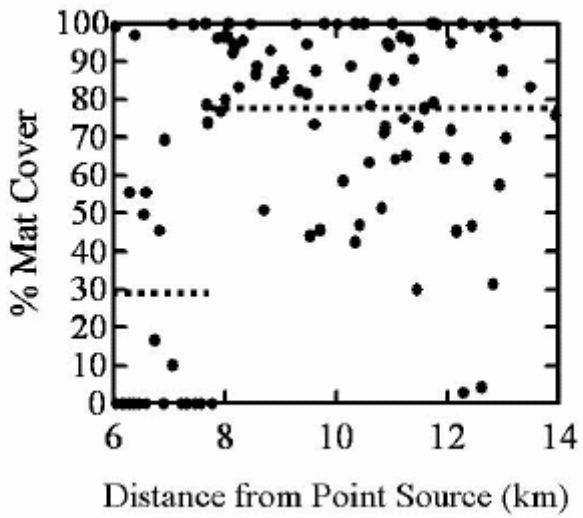


Figure 8.3. Percent calcareous algal mat cover in relation to distance from the P source showing the loss of the calcareous algal mat in those sites closer to the source (Stevenson et.al., 2002).

USING DATA PUBLISHED IN THE LITERATURE

Values from the published literature may be used to develop nutrient criteria if a strong rationale is presented that demonstrates the suitability of these data to the wetland of interest (i.e., the system of interest should share the same characteristics with the systems used to derive the published values). Published data, if there is enough of it, could be used to develop criteria for: (1) reference condition; (2) predictive (cause and effect) relationships between nutrients and biotic response variables; (3) tiered criteria; or, (4) criteria that exhibit a threshold response to nutrients. However, published data from similar wetlands should not substitute for collection and analysis of data from the wetland or wetlands of interest.

CONSIDERATIONS FOR DOWNSTREAM RECEIVING WATERS

More stringent nutrient criteria may be appropriate for wetlands that drain into lentic or standing waters. For example, it is proposed that 35 µg/L TP concentration and a mean concentration of 8 µg/L chlorophyll *a* constitute the dividing line between eutrophic and mesotrophic lakes (OECD 1982). Natural nutrient concentrations in some wetlands may be higher than downstream lakes. In addition, assimilative capacity for nutrients without changes in valued attributes may also be higher in wetlands than lakes. Nutrient criteria for wetlands draining into lakes may need to be lower than typically would be set if only effects on wetlands were considered. This is because EPA's regulations require States to take into consideration the water quality standards of downstream waters when designating uses of a water body and adopting appropriate criteria to protect those uses. (See 40 CFR 131.10(b).) Therefore, when adopting nutrient criteria for wetlands draining into lakes, States should take into account the protection of the downstream waters of receiving lakes in addition to wetlands.

8.3 EVALUATION OF PROPOSED CRITERIA

Following criteria derivation, an expert assessment of the proposed criteria and their applicability to all wetlands within the class of interest is encouraged. Criteria should be verified in many cases by comparing criteria values for a wetland class within an ecoregion across State boundaries. In fact, development of interstate criteria should be an integral part of a State's water quality standards program. In addition, prior to recommending any proposed criterion, it is recommended that States take into consideration the water quality standards of downstream waters to ensure that their water quality standards provide for attainment and maintenance of the water quality standards of downstream waters. (see 40 CFR 131.10(b)). Load estimating models,

such as those recommended by EPA (USEPA 1999), can assist in this determination (see External Nutrient Loading in Chapter 5.3). Water quality managers responsible for downstream receiving waters also should be consulted.

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APPENDIX A. ACRONYM LIST AND GLOSSARY

ACRONYMS

ACOE/ACE/COE - Army Corps of Engineers
AGNPS - Agricultural Nonpoint Source Pollution model
ARS - Agricultural Research Service
BACI - Before/After, Control/Impact
BMP - Best Management Practice
BuRec - Bureau of Reclamation
CCC - Commodity Credit Corporation
CENR - Committee for the Environment and Natural Resources
CGP - Construction General Permit
CHN - Carbon-Hydrogen-Nitrogen
CPGL - Conservation of Private Grazing Land
CPP - Continuing Planning Process
CREP - Conservation Reserve Enhancement Program
CRP - Conservation Reserve Program
CSO - Combined Sewer Overflow
CWA - Clean Water Act
CZARA - Coastal Zone Act Reauthorization Amendment
DIP - Dissolved inorganic phosphorus
DO - Dissolved oxygen
DOP - Dissolved organic phosphorus
DRP - Dissolved reactive phosphorus
ECARP - Environmental Conservation Acreage Reserve Program
EDAS - Ecological Data Application System
Eh - Redox potential
EMAP - Environmental Monitoring and Assessment Program
EQIP - Environmental Quality Incentive Program
FDEP - Florida Department of Environmental Protection
FIP - Forestry Incentive Program
GIS - Geographic Information System
GPS - Geospatial Positioning System
GWLF - Generalized Watershed Loading Function
HEL - Highly erodible land
HGM - Hydrogeomorphic approach

HSPF - Hydrologic Simulation Program - Fortran
MPCA - Minnesota Pollution Control Agency
NAAQS - National Ambient Air Quality Standard
NASQAN - National Stream Quality Assessment Network
NAWQA - National Water Quality Assessment
NIS - Network Information System
NIST - National Institute of Standards and Technology
NOAA - National Oceanic and Atmospheric Administration
NPDES - National Pollution Discharge Elimination System
NPP - Net primary production
NRCS - Natural Resources Conservation Service
NSF - National Science Foundation
NWI - National Wetlands Inventory
OH EPA - Ohio EPA
ONRW - Outstanding Natural Resource Waters
PCB - Polychlorinated biphenyls
PCS - Permit Compliance System
PIP - Particulate inorganic phosphorus
POP - Particulate organic phosphorus
PSA - Particle size analysis
QA/QC - Quality Assurance/Quality Control
QC - Quality Control
REMAP - Regional Environmental Monitoring and Assessment Program
RF3 - Reach File 3
SCS - Soil Conservation Service
SPARROW - Spatially Referenced Regressions on Watersheds
SRP - Soluble reactive phosphorus
STORET - Storage and Retrieval System
SWAT - Soil and Water Assessment Tool
TKN - Total Kjeldahl Nitrogen
TMDL - Total Maximum Daily Load
TP - Total Phosphorus
TWINSPAN -
USDA - United States Department of Agriculture
USEPA - United States Environmental Protection Agency
USFWS - United States Fish and Wildlife Service
USGS - United States Geological Survey
WEBB - Water, Energy, and Biogeochemical Budgets
WHIP - Wildlife Habitat Incentive Program
WLA - Wasteload Allocation
WQBEL - Water Quality Based Effluent Limit
WQS - Water Quality Standard
WRP - Wetlands Reserve Program

GLOSSARY

biocriteria
(biological criteria) Narrative or numeric expressions that describe the desired biological condition of aquatic communities inhabiting particular types of waterbodies and serve as an index of aquatic community health. (USEPA 1994).

cluster analysis
An exploratory multivariate statistical technique that groups similar entities in an hierarchical structure.

criteria
Elements of State water quality standards, expressed as constituent concentrations, levels, or narrative statements, representing a quality of water that supports a particular use. When criteria are met, water quality will generally protect the designated use (40 CFR 131.3(b)).

designated use(s)
Uses defined in water quality standards for each waterbody or segment whether or not the use is being attained (USEPA 1994).

detritus
Unconsolidated sediments comprised of both inorganic and dead and decaying particulate organic matter inhabited by decomposer microorganisms (Wetzel 1983).

ecological unit
Mapped units that are delineated based on similarity in climate, landform, geomorphology, geology, soils, hydrology, potential vegetation, and water.

ecoregion
A region defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, and other ecologically relevant variables.

emergent vegetation
"Erect, rooted herbaceous angiosperms that may be temporarily to permanently flooded at the base but do not tolerate prolonged inundation of the entire plant; e.g., bulrushes (Scirpus spp.), saltmarsh cordgrass" (Cowardin et.al., 1979).

eutrophic
Abundant in nutrients and having high rates of productivity frequently resulting in oxygen depletion below the surface layer (Wetzel 1983).

eutrophication
The increase of nutrients in [waterbodies] either naturally or artificially by pollution (Goldman and Horne 1983).

GIS (Geographical Information Systems)
A computerized information system that can input, store, manipulate, analyze, and display geographically referenced data to support decision-making processes. (NDWP Water Words Dictionary)

HGM, hydrogeomorphic

Land form characterized by a specific origin, geomorphic setting, water source, and hydrodynamic (NDWP Water Words Dictionary)

index of biotic integrity (IBI)
An integrative expression of the biological condition that is composed of multiple metrics. Similar to economic indexes used for expressing the condition of the economy.

interfluve
An area of relatively unchannelized upland between adjacent streams flowing in approximately the same direction.

lacustrine
"Includes wetlands and deepwater habitats with all of the following characteristics: (1) situated in a topographic depression or a dammed river channel; (2) lacking trees, persistent emergents, emergent mosses or lichens with greater than 30% areal coverage; and, (3) total area exceeds 8 ha (20 acres). Similar wetland and deepwater habitats totaling less than 8 ha are also included in the Lacustrine System if an active wave-formed or bedrock shoreline feature makes up all or part of the boundary, or if the water depth in the deepest part of the basin exceeds 2 m (6.6 feet) at low water...may be tidal or nontidal, but ocean-derived salinity is always less than 0.5%" (Cowardin et.al., 1979).

lentic
Relatively still-water environment (Goldman and Horne 1983).

limnetic
The open water of a body of fresh water.

littoral
Region along the shore of a non-flowing body of water.

lotic
Running-water environment (Goldman and Horne 1983).

Macrophyte
(Also known as SAV-Submerged Aquatic Vegetation) Larger aquatic plants, as distinct from the microscopic plants, including aquatic mosses, liverworts, angiosperms, ferns, and larger algae as well as vascular plants; no precise taxonomic meaning (Goldman and Horne 1983).

µg/L
micrograms per liter, 10^{-6} grams per liter

mg/L
milligrams per liter, 10^{-3} grams per liter

mineral soil flats
Level wetland landform with predominantly mineral soils

minerotrophic
Receiving water inputs from groundwater, and thus higher in salt content (major ions) and pH than ombrotrophic systems.

mixohaline
Water with salinity of 0.5 to 30%, due to ocean salts.

M
Molarity, moles of an element as concentration

multivariate
Type of statistics that relates one or more independent (explanatory) variables with multiple dependent (response) variables.

nutrient ecoregion
Level II ecoregions defined by Omernik according to expected similarity in attributes affecting nutrient supply (http://www.epa.gov/OST/standards/ecomap.html).

oligotrophic
Trophic status of a waterbody characterized by a small supply of nutrients (low nutrient release from sediments), low production of organic matter, low rates of decomposition, oxidizing hypolimnetic condition (high DO) (Wetzel 1983).

palustrine
"Nontidal wetlands dominated by trees, shrubs, persistent emergents, emergent mosses orlichens, and all such wetlands that occur in tidal areas where salinity due to ocean-derived salts is below 0.5%. It also includes wetlands lacking such vegetation, but with all of the following four characteristics: (1) area less than 8 ha (20 acres); (2) active wave-formed or bedrock shoreline features lacking; (3) water depth in the deepest part of basin less than 2 m at low water; and, (4) salinity due to ocean-derived salts less than 0.5%" (Cowardin et.al., 1979).

peatland
"A type of wetland in which organic matter is produced faster than it is decomposed, resulting in the accumulation of partially decomposed vegetative material called Peat. In some mires peat never accumulates to the point where plants lose contact with water moving through mineral soil. Such mires, dominated by grasslike sedges, are called Fens. In other mires peat becomes so thick that the surface vegetation is insulated from mineral soil. These plants depend on precipitation for both water and nutrients. Such mires, dominated by acid forming sphagnum moss, are called Bogs." (NDWP Water Words Dictionary)

periphyton
Associated aquatic organisms attached or clinging to stems and leaves of rooted plants or other surfaces projecting above the bottom of a waterbody (USEPA 1994).

pocosin
Evergreen shrub bog, found on Atlantic coastal plain.

riverine wetland
A hydrogeomorphic class of wetlands found in floodplains and riparian zones associated with stream or river channels.

slope wetland
A wetland typically formed at a break in slope where groundwater discharges to the surface. Typically there is no standing water.

trophic status
Degree of nutrient enrichment of a waterbody.

waters of the U.S.
Waters of the United States is defined at 40 CFR § 230.3(s)) as including:
a. All waters that are currently used, were used in the past, or may be susceptible to use in interstate or foreign commerce, including all waters that are subject to the ebb and flow of the tide;
b. All interstate waters, including interstate wetlands; and,
c. All other waters such as interstate lakes, rivers, streams (including intermittent streams), mudflats, sandflats, wetlands, sloughs, prairie potholes, wet meadows, playa lakes, or natural ponds the use, degradation, or destruction of which would affect or could affect interstate or foreign commerce including any such waters:

1 That are or could be used by interstate or foreign travelers for recreational or other purposes;
2 From which fish or shellfish are or could be taken and sold in interstate or foreign commerce; or,
3 That are used or could be used for industrial purposes by industries in interstate commerce;

d. All impoundments of waters otherwise defined as waters of the United States under this definition;
e. Tributaries of waters identified in paragraphs (a) through (d) of this definition;
f. The territorial sea; and,
g. Wetlands adjacent to waters (other than waters that are themselves wetlands) identified in paragraphs (a) through (f) of this definition.
For further information regarding the scope of 'waters of the U.S.' in light of the U.S. Supreme Court's 2006 decision in Rapanos v. United States, see "Clean Water Act Jurisdiction Following the U.S. Supreme Court's Decision in Rapanos v. United States & Carabell v. United States," which was jointly issued by the U.S. Environmental Protection Agency and the Army Corps of Engineers and is available at: http://www.epa.gov/owow/wetlands/.

wetland(s)
Those areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions [EPA, 40 CFR§ 230.3 (t)/USACE,33 CFR § 328.3 (b)].

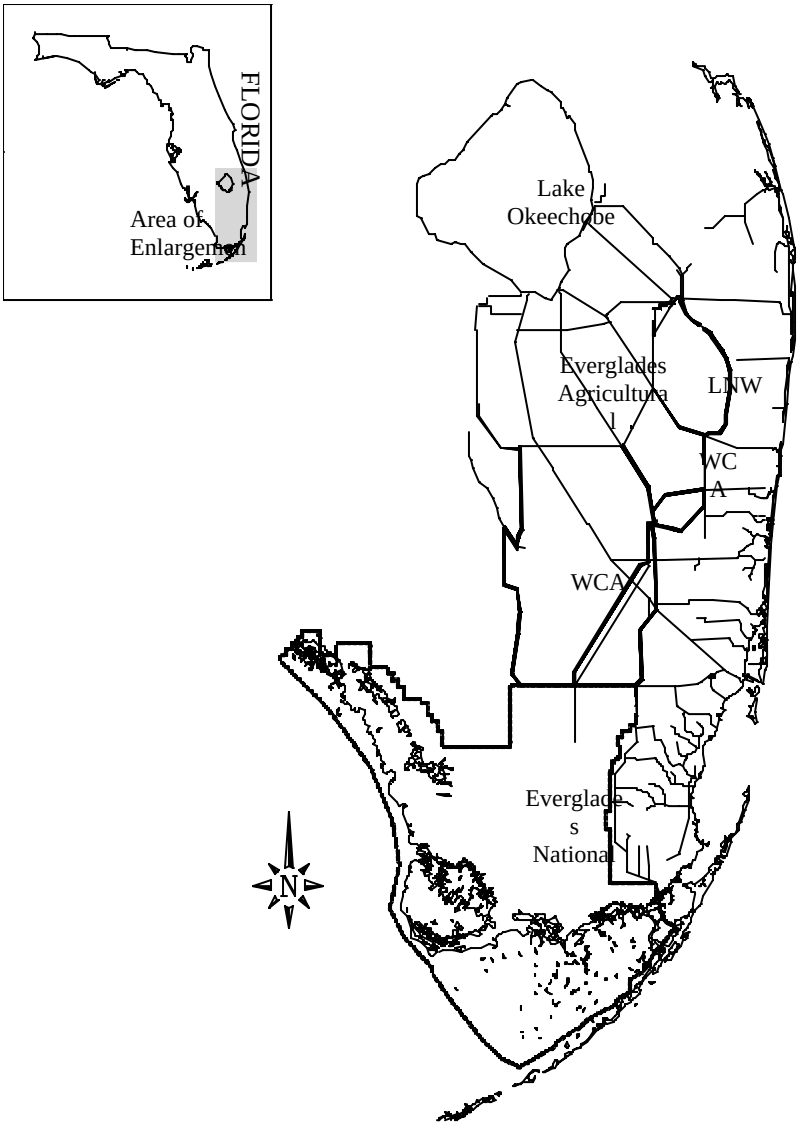
APPENDIX B. CASE STUDY: DERIVING A PHOSPHORUS CRITERION FOR THE FLORIDA EVERGLADES

INTRODUCTION

The Everglades (Figure B1.1) is the largest subtropical wetland in North America and is widely recognized for its unique ecological character. It has been affected for more than a century by rapid population growth in south Florida. Roughly half of the ecosystem has been drained and converted to agricultural and urban uses. Among other changes, the conversion of 500,000 acres of the northern Everglades to agriculture (the Everglades Agricultural Area or EAA) and the subsequent diking of the southern rim of Lake Okeechobee eliminated the normal seasonal flow of water southward from Lake Okeechobee. Furthermore, the construction of a complex network of internal canals and levees disrupted the natural sheetflow of water through the system and created a series of impounded wetlands known as "Water Conservation Areas" or WCAs. This conversion from a hydrologically open to a highly managed wetland occurred gradually, beginning with the excavation of four major canals during the 1900-1910 period and culminating with the construction of the Central and South Florida Flood Control Project (CSFFCP) during the 1950s and 60s (Light and Dineen 1994).

The remnant Everglades is managed for multiple and often conflicting uses including water supply, flood control, and the hydrologic needs of the natural ecosystem. Water management operations have altered the quantity, quality, timing, and delivery of flows to the Everglades relative to the pre-disturbance system; some parts of the system have been damaged by overdrainage, excessive flooding in other areas has stressed native vegetation communities. Changes to the seasonal pattern of flooding and drying have influenced many ecological processes, including changes in the dominant micro- and macro-phytic vegetation, declines in critical species, and the nesting success of wading bird populations that rely on drawdowns during a narrow window of time to concentrate fish prey. Canal inputs containing runoff from agricultural and urban lands contribute roughly 50% of flows to the managed system and have increased loads of nutrients and contaminants. In particular, phosphorus (P) has been identified as a key limiting nutrient in the Everglades and increased inputs of this nutrient have been identified as a significant factor affecting ecological processes and communities.

The primary source of P to the pre-disturbance Everglades was rainfall, although seasonal flows from Lake Okeechobee likely contributed significant P to the northern fringe of the wetland. Prior to the implementation of P control efforts in the late 1990s, canal flows were estimated to contribute more than half of the P load to the managed Everglades (SFWMD 1992). Discharge from the EAA is the main source of water to the Everglades, with approximately 500,000 acres of farmland draining southward via SFWMD canals, and is the major source of anthropogenic P. Significant inputs also come from Lake Okeechobee, a naturally mesotrophic lake that has also been enriched by agricultural runoff. Several other agricultural and urban catchments contribute



Appendix B1. Figure B1.1. Major hydrologic units of the remnant Florida Everglades (shaded region) including (from north to south) the A.R.M. Loxahacthee National Wildlife Refuge (LNWR), Water Conservation Area (WCA) 2A, WCA 3A, and Everglades National Park. Shaded lines represent the regional canal and levee system that conveys water southward from Lake Okeechobee and the Everglades Agricultural Area to the Everglades and urban areas along the coast.

smaller amounts of P via canal discharges into various parts of the Everglades. However, in general, canal P concentrations and loads (and associated wetland concentrations) decline from north to south.

The history of P enrichment and associated ecological impacts is not well documented but probably occurred at a limited scale for much of the last century. Early reports by the South Florida Water Management District (e.g., Gleason et.al., 1975, Swift and Nicholas 1987) showed an expansion of cattail and changes in the periphyton community in portions of the northern Everglades receiving EAA runoff. The severity and extent of P impacts were more fully recognized by 1988 when the Federal Government sued the State of Florida for allowing P-enriched discharges and associated impacts to occur in the Everglades. Settlement of this lawsuit eventually resulted in the enactment of the Everglades Forever Act by the Florida Legislature in 1994, which required the Florida Department of Environmental Protection (FDEP) to derive a numeric water quality criterion for P that would "prevent ecological imbalances in natural populations of flora or fauna" in the Everglades. These legal and legislative events provided the basis for numerous research and monitoring efforts designed to better understand the effects of P enrichment and to determine levels of enrichment that produced undesirable ecosystem changes.

Research and monitoring were initiated by the State of Florida (the Florida Department of Environmental Protection and the South Florida Water Management District) and other university research groups (e.g., Duke University, Florida International University, University of Florida) to better understand ecological responses to anthropogenic P inputs and to identify a P concentration or range of concentrations that result in unacceptable degradation of the Everglades ecosystem. This case study reviews research and monitoring conducted by the State to derive a P criterion for the Everglades. This criterion was proposed by the FDEP in 2001 and approved in 2003. This process is divided into three parts:

1. Define the reference (i.e., historical) conditions for P and the oligotrophic ecology of the Everglades;

2. Determine the types of ecological impacts caused by P enrichment; and,

3. Identify wetland P concentrations that produce these impacts,and determine a criterion that will protect the resource from those impacts.

DEFINING THE REFERENCE CONDITION

Several sources of information were used to characterize reference conditions across the Everglades. Sampling in minimally impacted locations (i.e., reference sites) believed to best reflect historical conditions provided the quantitative basis for establishing reference conditions with respect to P concentrations and associated ecological conditions. Where possible, this

characterization was augmented by historical evidence. Written accounts of surveys conducted during the 1800s and early 1900s provided useful qualitative data on past ecological conditions. Early scientific literature contained substantial information on large-scale vegetation patterns (e.g., Davis 1943, Loveless 1959). Paleoecological assessments, including the dating and analysis of soil cores with respect to nutrient content and preserved materials such as pollen provided, further information (e.g., Cooper and Goman 2001, Willard et.al., 2001).

Predisturbance Everglades exhibited significant spatial and temporal variation, and, while its conversion to a smaller, more managed wetland resulted in the loss of some of this heterogeneity, the legacy of past variations in hydrology, chemistry, and biology remain in many areas. Legislation mandating the development of a P criterion stipulated that natural variation in P concentrations and ecological conditions within the remnant ecosystem be considered. This required that sampling efforts encompass the expected range of background variability in the remnant ecosystem. To ensure that spatial variation in P conditions were considered, sampling was conducted in all four major hydrologic units: The Loxahatchee National Wildlife Refuge (LNWR), WCA-2A, WCA-3A, and Everglades National Park (see Figure B1.1).

Water Column Phosphorus

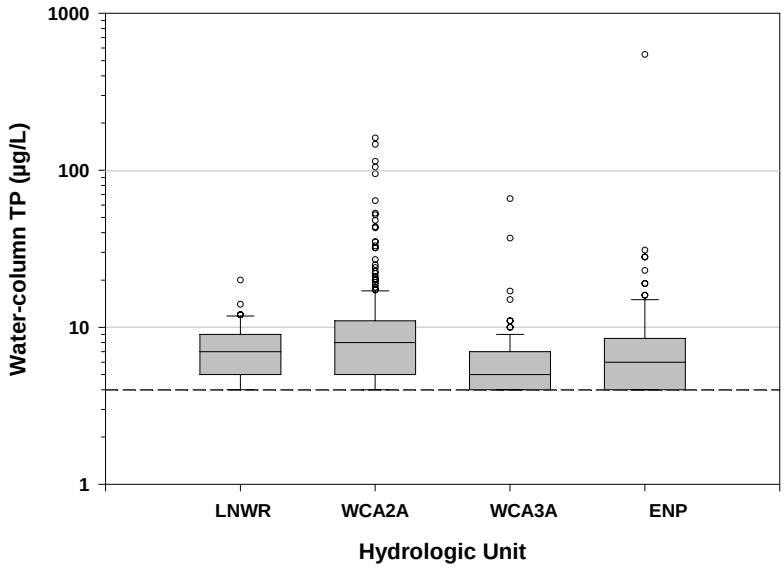
Nutrient inputs to the Everglades were historically derived primarily from atmospheric deposition (rainfall and dry fallout), which is typically low in P. Historical loading rates have been estimated from annual atmospheric P inputs in south Florida and reconstructions of P accumulation in Everglades soils and probably averaged less than 0.1 g P m^{-2} y^{-1} (SFWMD 1992). Atmospheric inputs of P were augmented by inflows from Lake Okeechobee, which was connected by surface-water flows to the northern Everglades during periods of high water (Parker et.al., 1955). While inflows from this historically eutrophic lake were undoubtedly enriched in P compared with the Everglades, the influence of these inputs were likely limited to wetlands along the lake's southern fringe (Snyder and Davidson 1994) as is demonstrated by the limited extent of pond apple and other vegetation that require more nutrients for growth than the sawgrass (*Cladium jamaicense)* that dominates most of the Everglades.

Interior areas of the Everglades generally retain the oligotrophic characteristics of the predrainage ecosystem and, thus, provide the best contemporary information on historical P concentrations. Water chemistry data were available for several interior locations that had been sampled by the State for many years. Median water-column TP concentrations at these stations ranged between 4 and 10 µg L^{-1}, with lowest concentrations occurring in southern areas that have been least affected by anthropogenic P loads (Figure B1.2). Phosphorus concentrations >10 µg L^{-1} were measured periodically at many of these sites. Isolated high P concentrations at reference stations were attributed to P released as a result of oxidation of exposed soils, increased fire frequency during droughts, and difficulties in collecting water samples that are not contaminated by flocculent wetland sediments when water depths are low. Data from reference sites may represent an upper estimate of historical TP concentrations in the Everglades since

several stations are located in areas that either have been overdrained, a condition which promotes soil oxidation and P release, or so heavily exposed to canal inflows (e.g., WCA 2A) that some P inputs have likely intruded even into interior areas. However, in the absence of reliable historical data these values were deemed as best available for defining reference condition.

Soil Phosphorus

Extensive soil mapping projects across interior portions of the central and northern Everglades indicate a reference range for soil TP in the surface 0-10 cm of soil of between 200 and 500 mg kg^{-1} on a mass basis (DeBusk et.al., 1994, Reddy et.al., 1994a, Newman et.al., 1997, Richardson et.al., 1997a, Newman et.al., 1998). Fewer data are available from ENP, but available evidence indicates background concentrations of < 400 mg kg^{-1} (Doren et.al., 1997). Soil P content also varies volumetrically as a function of changing soil bulk density. The typical bulk density of flooded Everglades peat soils is approximately 0.08 g cm^{-3}, whereas soils that have been subjected to extended dry out and oxidation can have bulk densities greater than 0.2 g cm^{-3} (Newman et.al., 1998). Increases in volumetric nutrient concentrations resulting from increased bulk density can have a stimulatory effect on plant growth even in the absence of external P inputs (see Chapter 2). Following correction for the varying bulk densities in the peat soils of the Everglades, a historical TP concentration of <40 µg cm^{-3} may be applicable for most regions (DeBusk et.al., 1994, Reddy et.al., 1994a, Newman et.al., 1997, Newman et.al., 1998, Reddy et.al., 1998). In the LNWR, most of the interior area has soil TP < 20 µg TP cm^{-3} (Newman et.al., 1997).



Appendix B1. Figure B1.2. Box plots showing surface-water P concentrations at long-term monitoring stations in each major hydrologic unit that illustrate the minimally impacted (i.e., reference) condition of the Everglades with respect to P. The top, mid-line, and bottom of each box represents the 75th, 50th (median), and 25th percentile of data, respectively; the error bars represent the 90th and 10th percentiles; open circles are data outside the 90th percentile; the dashed line is the analytical limit for TP (4 µg L^{-1}).

REFERENCE ECOLOGICAL CONDITIONS

The Everglades is perhaps the most intensively studied wetland in the world and, therefore, the ecological attributes that defined the predisturbance structure and function of this ecosystem are well understood compared with most wetlands. Clearly, not all of the valued ecological attributes of this or any other wetland are affected directly by P enrichment. Thus, in order to define the reference condition of the ecosystem with respect to the role of P, this assessment focused on those processes and communities that are most sensitive to P enrichment. Based on available information and preliminary scoping studies, five ecological features were selected as biotic response variables. These features included three indicators of ecosystem structure, one indicator of ecosystem function, and one indicator of landscape change. Structural indicators included the periphyton community, dominant macrophyte populations, and the benthic macroinvertebrate community. Diel fluctuations in water column DO provided an important indicator of shifts in

aquatic metabolism. The landscape indicator of change was the loss of open-water slough-wet prairie habitats—areas of high natural diversity and productivity.

Periphyton

Aquatic vegetation and other submerged surfaces in the oligotrophic Everglades interior are covered with periphyton, a community of algae, bacteria and other microorganisms. Periphyton accounts for a significant portion of primary productivity in sloughs and wet prairies (Wood and Maynard 1974, Browder et.al., 1982, McCormick et.al., 1998), and floating and attached periphyton mats provide an important habitat and food source for invertebrates and small fish (Browder et.al., 1994, Rader 1994). These mats store large amounts of P (approaching 1 kg TP m^{-2} in some locations) and, thus, may play a critical role in maintaining low P concentrations in reference areas (McCormick et.al., 1998, McCormick and Scinto 1999). Periphyton biomass and productivity peak towards the end of the wet season (August through October) and reach a minimum during the colder months of the dry season (January through March). Periphyton biomass in open-water habitats can exceed 1 kg m^{-2} during the wet season (Wood and Maynard 1974, Browder et.al., 1982, McCormick et.al., 1998) when floating mats can become so dense as to cover the entire water surface. Aerobic conditions in slough-wet prairie habitats is maintained by the high productivity of this community and the capacity of dense algal mats to trap oxygen released during photosynthesis (McCormick and Laing 2003).

Two types of periphyton communities occur in reference areas of the Everglades. Mineral-rich waters, such as those found WCA 2A and Taylor Slough (ENP), support a periphyton assemblage dominated by a few species of calcium-precipitating cyanobacteria and diatoms, while the soft-water interior of LNWR contain a characteristic assemblage of desmid green algae and diatoms. Waters across much of the southern Everglades (WCA-3A and portions of ENP) tend to be intermediate with respect to mineral content and contain some taxa from both assemblages.

The chemical composition of periphyton in the oligotrophic Everglades is indicative of severe P limitation. Periphyton samples from reference areas of major hydrologic units within the Everglades are characterized by an extremely low P content (generally <0.05%) and extremely high N:P ratios (generally >60:1 w:w). This observational evidence for P limitation is supported by experimental fertilization studies that have shown that: 1) periphyton responds more strongly to P enrichment than to enrichment with other commonly limiting nutrients such as nitrogen (Scheidt et.al., 1989, Vymazal et.al., 1994); and, 2) periphyton changes in response to experimental P enrichment mimic those that occur along field nutrient gradients (McCormick and O'Dell 1996). Thus, it is well-established that periphyton is strongly P-limited in reference areas of the Everglades.

Dissolved Oxygen

Interior Everglades habitats exhibit characteristic diel fluctuations in water-column dissolved oxygen (DO), although aerobic conditions are generally maintained throughout much or all of the diel cycle (Belanger et.al., 1989, McCormick et.al., 1997, McCormick and Laing 2003). High daytime concentrations in open-water habitats (i.e., sloughs, wet prairies) are a product of photosynthesis by periphyton and other submerged vegetation. These habitats may serve as oxygen sources for adjacent sawgrass stands, where submerged productivity is low (Belanger et.al., 1989). Oxygen concentrations decline rapidly during the night due to periphyton and sediment microbial respiration and generally fall below the 5 mg L^{-1} standard for Class III Florida waters (Criterion 17-302.560(21), F.A.C.). However, these diurnal excursions are characteristic of reference areas throughout the Everglades (McCormick et.al., 1997) and are not considered a violation of the Class III standard (Nearhoof 1992). In fact, a site specific criterion for DO has been adopted by the State; a copy of the technical support document (Weaver 2004) can be found at:
http://www.dep.state.fl.us/water/wqssp/everglades/docs/DOTechSupportDOC2004.pdf.

Vegetation

The vegetation communities characteristic of the pristine Everglades are dominated by species adapted to low P, seasonal patterns of wetting and drying, and periodic natural disturbances such as fire, drought, and occasional freezes (Duever et.al., 1994, Davis 1943, Steward and Ornes 1983, Parker 1974). Major aquatic vegetation habitats in oligotrophic areas include sawgrass wetlands, wet prairies, and sloughs (Loveless 1959, Gunderson 1994). The spatial arrangement of these habitats is dynamic and controlled by environmental factors such as fire, water depth, nutrient availability, and local topography (Loveless 1959).

Sawgrass (*Cladium jamaicense*) is the dominant macrophyte in the Everglades, and stands of this species compromise approximately 65 to 70% of the total vegetation cover of the Everglades (Loveless 1959). Wet prairies include a collection of low-stature, graminoid communities occurring on both peat and marl soils (Gunderson 1994). Dominant macrophyte taxa in these habitats include *Rhynchospora*, *Panicum*, and *Eleocharis* (Loveless 1959, Craighead 1971). Sloughs are deeper water habitats that remain wet most or all of the year and are characterized by floating macrophytes such as fragrant white water lily (*Nymphaea odorata*), floating hearts (*Nymphoides Aquaticum*), and spatterdock (*Nuphar advena*) (Loveless 1959, Gunderson 1994). Submerged aquatic plants, primarily bladderworts (*Utricularia foliosa* and *U. purpurea* in particular), also can be abundant in these habitats and, in the case of *U. purpurea*, provide a substrate for the formation of dense periphyton mats.

Several studies have concluded that macrophyte communities in the Everglades are P-limited. Sawgrass is adapted to the low-P conditions indicative of the pristine Everglades (Steward and Ornes 1975b, Steward and Ornes 1983). During field and greenhouse manipulations, sawgrass responded to P enrichment either by increasing the rate of growth or P uptake (Steward and Ornes 1975a, Steward and Ornes 1983, Craft et.al., 1995, Miao et.al., 1997, Daoust and Childers 1999). Furthermore, additions of N alone had no effect on sawgrass or cattail growth under low-P conditions (Steward and Ornes 1983, Craft et.al., 1995). Recent experimental evidence in the Everglades National Park (Daoust and Childers 1999) has shown that other native vegetation associations such as wet prairie communities are also limited by P.

Historically, cattail (*Typha* spp.) was one of several minor macrophyte species native to the Everglades (Davis 1943, Loveless 1959). In particular, cattail is believed to have been associated largely with areas of disturbance such as alligator holes and recent burns (Davis 1994). Analyses of Everglades peat deposits reveal no evidence of cattail peat, although the presence of cattail pollen indicates its presence historically in some areas (Gleason and Stone 1994, Davis et.al., 1994, Bartow et.al., 1996). Findings such as these confirm the historical presence of cattail in the Everglades but provide no evidence for the existence of dense cattail stands covering large areas (Wood and Tanner 1990) as now occurs in the northern Everglades. In contrast, sawgrass and water lily peats have been major freshwater Everglades soils for approximately 4,000 years (McDowell et.al., 1969).

Macroinvertebrates

Aquatic invertebrates (e.g., insects, snails, and crayfish) represent a key intermediate position in energy flow through the Everglades food web as these taxa are the direct consumers of primary production and, in turn, are consumed by vertebrate predators. Invertebrates occupy several functional niches within the Everglades food web; however, most taxa are direct consumers of periphyton and/or plant detritus (e.g., Rader and Richardson 1994, McCormick et.al., 2004). Rader (1994) sampled both periphyton and macrophyte habitats in this same area and, based on the proportional abundance of different functional groups, suggested that grazer (periphyton) and detrital (plant) pathways contributed equally to energy flow in low-nutrient areas of the Everglades.

The macroinvertebrate fauna of the Everglades is fairly diverse (approximately 200 taxa identified) and is dominated by Diptera (49 taxa), Coleoptera (48 taxa), Gastropoda (17 taxa), Odonata (14 taxa), and Oligochaeta (11 taxa) (Rader 1999). Most studies have focused on a few conspicuous species (e.g., crayfish and apple snails) considered to be of special importance to vertebrate predators, and relatively little is known about the distribution and environmental tolerances of most taxa. An assemblage of benthic microinvertebrates (meiofauna) dominated by Copepoda and Cladocera is also present in the Everglades (Loftus et.al., 1986), but even less is known about the distribution and ecology of these organisms.

Invertebrates are not distributed evenly among Everglades habitats but, instead, tend to be concentrated in periphyton-rich habitats such as sloughs. In an early study, Reark (1961) noted that invertebrate densities in ENP were higher in periphyton habitats compared with sawgrass stands. Rader (1994) reported similar findings in the northern Everglades and found mean annual invertebrate densities to be more than six-fold higher in sloughs than in sawgrass stands. Invertebrate assemblages in sloughs were more species-rich and contained considerably higher densities of most dominant invertebrate groups. Functionally, slough invertebrate assemblages contained similar densities of periphyton grazers and detritivores compared with a detritivore-dominated assemblage in sawgrass stands. Higher invertebrate densities in sloughs were attributed primarily to abundant growths of periphyton and submerged vegetation, which provide oxygen and a source of high-quality food.

QUANTIFYING P IMPACTS

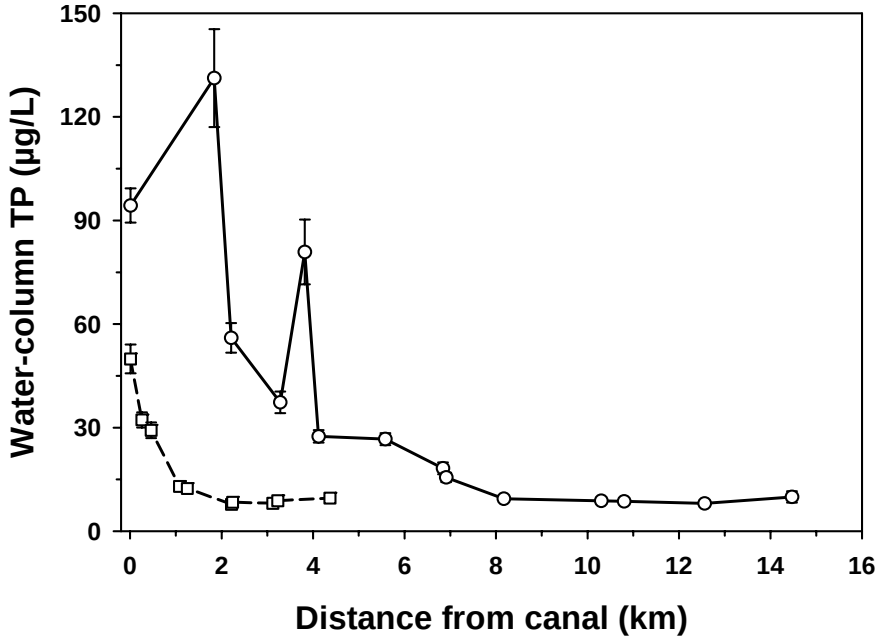
A targeted design (see Chapter 4) was used to quantify changes in key ecological attributes in response to P enrichment. Discharges of canal waters through fixed water-control structures are the primary source of anthropogenic P for the Everglades and produce P gradients that extend several kilometers into the wetland in several locations. These gradients have existed for several decades and provided the clearest example of the long-term ecological impacts associated with P enrichment. Monitoring was conducted along gradients in different parts of the Everglades to assess ecological responses to P enrichment. Fixed sampling stations were located along the full extent of each gradient to document ecological conditions associated with increasing levels of P enrichment. Intensive monitoring was performed along gradients in two northern Everglades wetlands, WCA 2A and the LNWR. WCA 2A is a mineral-rich, slightly basic peatland and contains the most pronounced and well studied P gradient in the Everglades, whereas LNWR is a soft-water, slightly acidic peatland. These two wetlands represent the most extreme natural water chemistry conditions in the Everglades and support distinct periphyton assemblages and macrophyte populations while sharing dominant species such as sawgrass and water lily. Less intensive sampling along gradients in other parts of the Everglades (WCA 3A and ENP) to confirm that P relationships were consistent across the wetland.

Chemical and biological conditions were measured at each sampling station along the two intensively sampled gradients. Repeated sampling, sometimes over several years, was performed to ensure that temporal variation in each metric was considered in the final data analysis. Monthly surface-water sampling and less frequent soil sampling were performed to quantify P gradients in each area. Diel DO regimes, periphyton, and benthic macroinvertebrates were sampled quarterly when surface water was present. Macrophyte sampling included ground-based methods to document shifts in species composition and remote sensing to determine changes in landscape patterns. The hydrology of each site was characterized to determine whether P gradients were confounded with hydrologic gradients, which can also exert a strong influence on ecological patterns.

Numerous field experiments have been conducted to quantify ecological responses to P enrichment and to better understand how interactions between P enrichment and other factors such as hydrology may affect these responses. The design of these experiments varied in complexity with respect to size and dosing regime depending on the specific objective of each study and has included enclosed fertilizer plots (e.g., Craft et.al., 1995), semi-permeable mesocosms receiving periodic P additions to achieve fixed loading rates in the form of periodic additions (e.g., McCormick and O'Dell 1996), flumes receiving semi-continuous enrichment at a fixed rate (Pan et.al., 2000), and flumes receiving flow-adjusted dosing to achieve constant inflow concentrations (Childers et.al., 2002). These experiments were useful in establishing the causal nature of responses to P enrichment documented along the P gradients described above.

GRADIENT P CONCENTRATIONS

Strong gradients in P concentrations were documented downstream of canal discharges into most Everglades wetlands (Figure B1.3). Inflow TP concentrations in from 1996-1999 have averaged as high as 100 µg L^{-1} as compared with reference and pre-disturbance concentrations $\leq$ 10 µg L^{-1}. The degree and spatial extent of P enrichment varies among areas depending on the source and magnitude of inflows. The most extensive enrichment has occurred in the northern Everglades near EAA inflows, while southern areas (e.g., ENP) have been relatively less affected. The most extensive enrichment has occurred in WCA-2A, which, unlike other areas, receives most of its water from canal discharges. Soil TP was strongly correlated with surface-water concentrations and exceeded 1500 mg kg^{-1} at the most enriched locations as compared with concentrations < 500 mg kg^{-1} in reference areas. In general, this enrichment effect is limited to the surface 30 cm of soil depth (Reddy et.al., 1998).



Appendix B1. Figure B1.3. Mean water-column TP concentrations (1996-1999) at long-term monitoring stations downstream of canal discharges in two northern Everglades wetlands, WCA 2A (circles connected by solid line) and LNWR (squares connected by dashed line). Error bars are ± 1 SE.

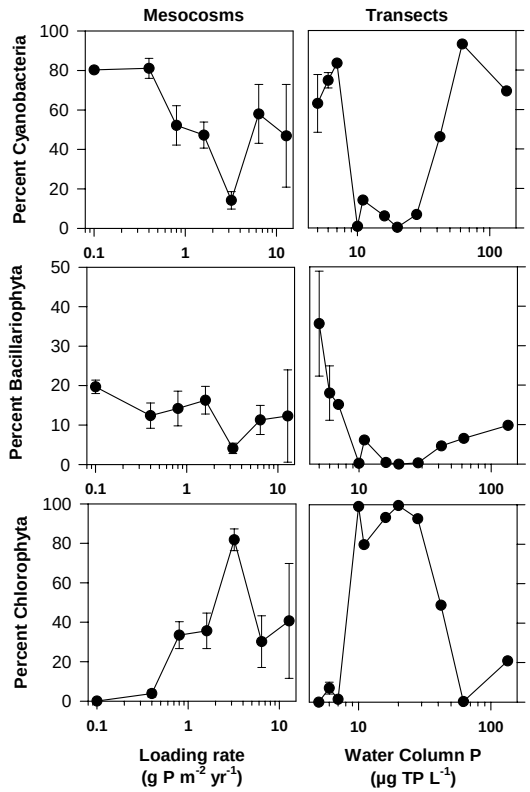
ECOLOGICAL RESPONSES TO P ENRICHMENT

Periphyton

Periphyton responses to P enrichment include changes in productivity, biomass, and species composition. Periphyton rapidly accumulates P from the water (McCormick et.al., 2001, Noe et.al., 2003), and, thus, a strong relationship between P concentrations in the water and periphyton is maintained along the P gradients (Grimshaw et.al., 1993, McCormick et.al., 1996). In fact, increases in periphyton P may provide one of the earliest signals of P enrichment (e.g., Gaiser et.al., 2004). Rapid increases in periphyton photosynthetic activity and growth rates occur in response to P enrichment (e.g., Swift and Nicholas 1987, McCormick et.al., 1996, McCormick et.al., 2001). All of these responses are consistent with the P-limited nature of Everglades periphyton.

Paradoxically, these physiological responses are associated with sharply lower periphyton biomass in P-enriched areas due to the loss of the abundant community of calcareous

cyanobacteria and diatoms that is indicative of mineral-rich reference areas. This community is replaced by a eutrophic community of filamentous cyanobacteria, filamentous green algae, and diatoms in areas having even slightly elevated P concentrations. For example, McCormick and O'Dell (1996) found that the calcareous assemblage that existed at low water-column P concentrations (TP = 5 to 7 µg L^{-1}) was replaced by a filamentous green algal assemblage at moderately elevated concentrations (TP = 10 to 28 µg L^{-1}) and by eutrophic cyanobacteria and diatoms species at even higher concentrations (TP = 42 to 134 µg L^{-1}). These results are representative of those documented by other investigators (e.g., Swift and Nicholas 1987, Pan et.al., 2000). Taxonomic changes in response to controlled P enrichment in field experiments have been shown to be similar to those documented along field enrichment gradients (Figure B1.4), thereby providing causal evidence that changes in the periphyton assemblage were largely a product of P enrichment (McCormick and O'Dell 1996, Pan et.al., 2000).



Appendix B1. Figure B1.4. Changes in percent biomass (as biovolume) of major algal groups in field enclosures dosed weekly with different P loads (left panel) and along a P enrichment gradient downstream of canal discharges (right panel) in WCA 2A. From McCormick and O'Dell (1996).

Community metabolism and dissolved oxygen concentrations

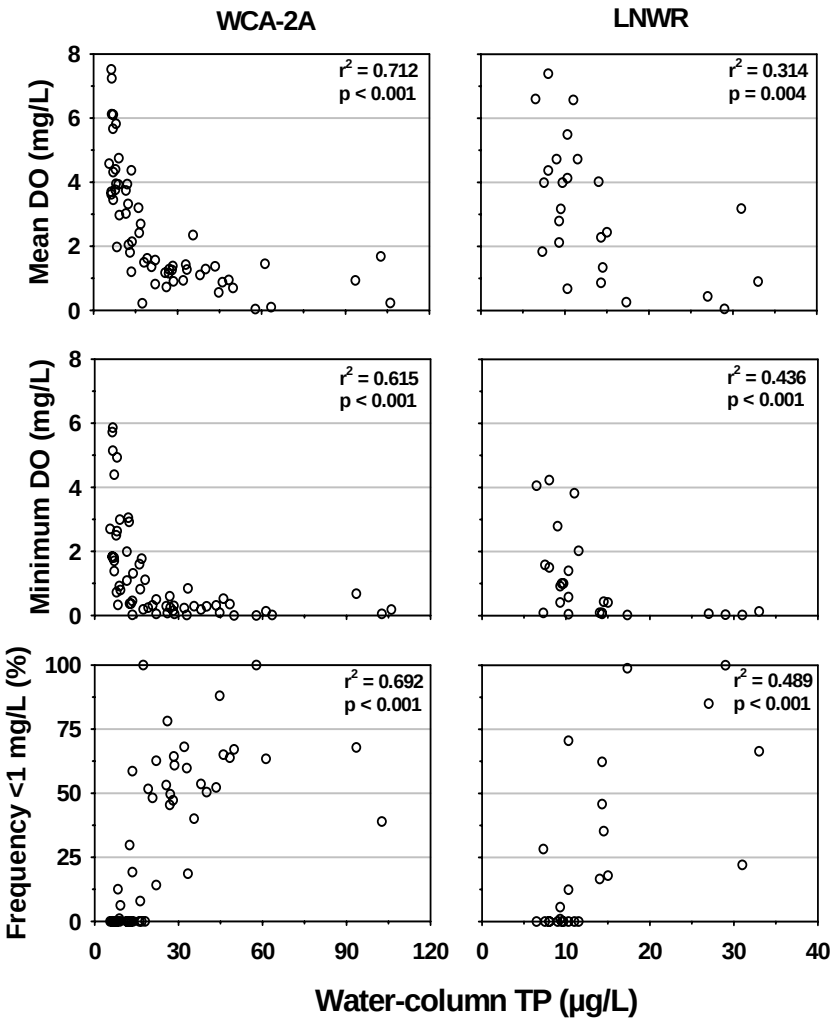
Phosphorus enrichment causes a shift in the balance between autotrophy and heterotrophy in the water column as a result of contrasting effects on periphyton productivity and microbial respiration. Rates of aquatic primary productivity (P) and respiration (R) are approximately balanced (P:R ratio = 1) across the diel cycle in minimally impacted sloughs throughout the Everglades (Belanger et al., 1989; McCormick et al., 1997). In contrast, respiration rates exceed productivity by a considerable margin (P:R ratio << 1) at enriched locations. This change is related primarily to a large reduction in areal periphyton productivity as a result of shading by dense stands of cattail (*Typha domingensis*) that form a nearly continuous cover in the most enriched areas (McCormick and Laing, 2003). Increased cattail production also stimulates microbial respiration (e.g., sediment oxygen demand) (e.g., Belanger et.al., 1989) due to an increase in the quantity and decomposability of macrophyte litter.

The shift towards dominance of heterotrophic processes with P enrichment, in turn, affects dissolved oxygen (DO) concentrations in enriched areas. For example, DO concentrations at an enriched site in WCA 2A rarely exceeded 2 mg L^{-1} compared with concentrations as high as 12 mg L^{-1} at reference locations (McCormick et al., 1997). Depressed water-column DO concentrations have subsequently been documented in enriched areas of WCA 2A and the LNWR (Figure B1.5) and confirmed in experimental P-enrichment studies (McCormick and Laing 2003). Declines in DO along field P gradients were steepest within a range of water-column TP concentrations roughly between 10 and 30 µg L^{-1}. Lower DO in enriched areas are associated with other changes including an increase in anaerobic microbial processes and a shift in invertebrate species composition toward species tolerant of low DO, described later in this study.

Macrophytes

Nutrient enrichment initially stimulates the growth of existing vegetation as evidenced by increased plant P content, photosynthesis, and biomass production, as it does for periphyton. Persistent enrichment eventually produces a shift in vegetation composition toward species better adapted to rapid growth and expansion under conditions of high P availability. Two major shifts in Everglades plant communities have been documented along P gradients, including: 1) the replacement of sawgrass stands by cattail; and, 2) the replacement of slough-wet prairie habitat by cattail.

Sawgrass populations in the Everglades have life-history characteristics indicative of plants adapted to low-nutrient environments (Davis 1989, Davis 1994, Miao and Sklar 1998). Sawgrass responses to P enrichment include an increase in tissue P, plant biomass, P storage, annual leaf production and turnover rates, and seed production (e.g., Davis 1989, Craft and Richardson 1997, Miao and Sklar 1998). Cattail is characterized by a high growth rate, a short life cycle, high reproductive output, and other traits that confer a competitive advantage under enriched conditions (Davis 1989, Davis 1994, Goslee and Richardson 1997, Miao and Sklar 1998).



Appendix B1. Figure B1.5. Relationship between water-column DO metrics and TP concentration at several stations and time intervals along P gradients downstream of canal discharges into two northern Everglades wetlands (see Figure 1 for map). Total P concentrations are mean values for all samples (n = 3 to 6) collected during the three-month period preceding DO measurements, which were typically collected over 3-4 diel cycles using dataloggers. Correlation coefficients are Spearman rank coefficients based on all data in the plot. Adapted from McCormick and Laing (2003).

Measurements and controlled enrichment experiments have shown that cattail growth rates exceed those of sawgrass under enriched conditions (Davis 1989, Newman et.al., 1996, Miao and DeBusk 1999). The replacement of sawgrass by cattail in P enriched areas may be facilitated by disturbances such as flooding or severe fires that weaken or kill sawgrass plants and create openings. Consequently, sawgrass distributional patterns were not as clearly related to P gradients as were other ecological indicators of enrichment.

Sloughs and wet prairies appear to be particularly sensitive to replacement by cattail under P-enriched conditions, possibly due to the sparser vegetation cover in these habitats. The process of slough enrichment and replacement by cattail as shown in satellite imagery is supported by ground-based sampling methods (McCormick et.al., 1999) that documented changes in slough vegetation and encroachment of these habitats by cattail in areas where soil TP concentrations averaged between 400 and 600 mg kg^{-1} and water-column TP in recent years averaged > 10 µg L^{-1}. *Eleocharis* declined in response to increased soil P, and *Nymphaea* was stimulated by enrichment and was dominant in slightly enriched sloughs. Increased occurrence of cattail in sloughs was associated with a decline in *Nymphaea*, probably as a result of increased shading of the water surface. These findings are consistent with those of Vaithiyanathan et.al., (1995) who documented a decline in slough habitats along this same nutrient gradient and the loss of sensitive taxa such as *Eleocharis* at locations where soil TP exceeded 700 mg kg^{-1}. As discussed by McCormick et.al., (2002), loss of these open-water areas is a sensitive landscape indicator of P enrichment (Figure B1.6).

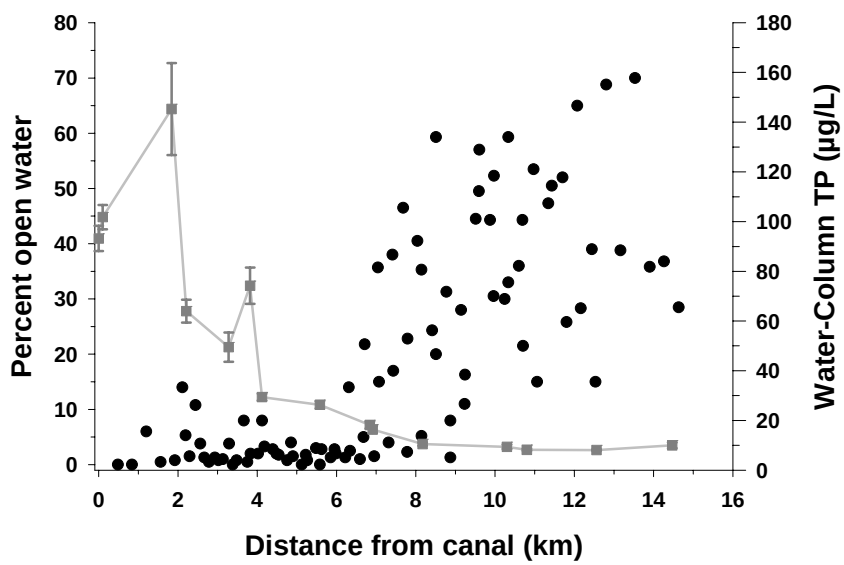


Figure B1.6. Changes in the percentage of open-water (i.e., sloughs, wet prairies, or other opening caused by natural disturbance or airboats) cover at 94 locations along a P enrichment gradient in WCA 2A as determined using aerial

photography. Gray line shows the mean (± 1 SE) water-column TP concentration (1996-1999) at 15 long-term monitoring stations along the gradient.

Benthic Macroinvertebrates

Macroinvertebrates are the most widely used biological indicator of water quality impacts, and several changes that occur in this community along P enrichment gradients in the Everglades are similar to those documented in response to eutrophication in other aquatic ecosystems. Several studies have documented an overall increase in macroinvertebrate abundance with increasing P enrichment (Rader and Richardson 1994, Trexler and Turner et.al., 1999, McCormick et.al., 2004). However, differences in sampling methodology have apparently produced conflicting results with respect to changes in species richness and diversity. For example, Rader and Richardson (1994) documented an increase in both macroinvertebrate species richness and diversity with P enrichment in open-water (i.e., low emergent macrophyte cover) habitats and concluded that enrichment had not impacted this community. McCormick et.al., (2004), however, using a landscape approach that involved habitat-weighted sampling, found little change in either species richness or diversity in response to enrichment. This latter study accounted for the decline in the cover of habitats such as sloughs and wet prairies, which contain the most diverse and abundant macroinvertebrate communities (Rader 1994). McCormick et.al., (2004) also documented a pronounced shift in community composition with increasing P enrichment as taxa characteristic of the oligotrophic interior of the wetland are replaced by common pollution-tolerant taxa of oligochaetes and chironomids. These changes were indicative of habitat degradation as determined using biotic indices derived by the Florida DEP to assess stream condition based on macroinvertebrate composition (results available at http://www.epa.gov/owow/wetlands/bawwg/case/fl2.html).

As for many other P-induced biological changes, the greatest change in the macroinvertebrate community occurred in response to relatively small increases in P concentration. Along field enrichment gradients, community shifts were associated with increases in water-column TP above approximately 10 ug L^{-1} (McCormick et.al., 2004). Similarly, Qian et.al., (2004) documented several shifts in community structure and function in response to long-term experimental dosing at average concentrations of approximately 10-15 ug L^{-1}.

ESTABLISHING A P CRITERION

The FDEP was charged with reviewing and analyzing available P and ecological data collected throughout the Everglades to establish a numeric P criterion. A brief summary of this process is provided here, and more detailed information can be found in Payne et al., (2000, 2001a,b; available at http://www.dep.state.fl.us/water/wqssp/everglades/pctsd.htm; and, 2002, 2003, available at http://www.sfwmd.gov/sfer/previous_ecr.html).

The narrative nutrient standard for Class III Florida waters such as the Everglades states that “in no case shall nutrient concentrations of a body of water be altered so as to cause an imbalance in

natural populations of aquatic flora or fauna." The FDEP approach to detecting violations of this standard with respect to surface-water P concentrations in the Everglades was to test for statistically significant departures in ecological conditions from those at reference sites (i.e., interior sampling locations with background P concentrations). Biological and chemical data collected along anthropogenic P gradients throughout the Everglades were analyzed to determine P concentrations associated with such departures. Results showed that sampling sites with average (geometric mean) surface-water TP concentrations significantly greater than 10 ppb consistently exhibited significant departures in ecological condition from that of reference sites. A key finding supporting this concentration as the standard was the fact that multiple changes in each of the major indicator groups—periphyton, dissolved oxygen, macrophytes, and macroinvertebrates—all occurred at or near this same concentration (e.g., Payne et.al., 2001).

Data from field and laboratory experiments conducted by various research groups provided valuable supporting information for understanding responses to P enrichment. While such experiments were not used directly to derive the P criterion, they established cause-effect relationships between P enrichment and ecological change that supported correlative relationships documented along field P gradients. For example, McCormick and O'Dell (1996) and Pan et.al., (2000) showed that major shifts in periphyton species composition documented along field P gradients matched those elicited by controlled P dosing in field enrichment experiments. McCormick and Laing (2003) confirmed that controlled P enrichment produced declines in water-column DO similar to those measured along the gradients. Macroinvertebrate community changes were documented experimentally, Qian et.al., (2004).

While the criterion established a surface-water concentration of 10 ug L^{-1} TP as protective of native flora and fauna, the methodology used to measure compliance with the criterion needed to normalize background fluctuations in concentration. Additional analyses of P data collected over several years at reference sites was used to set both a longer-term average concentration and a shorter-term maximum concentration for each site. Based on these analyses, the FDEP concluded that annual maximum concentrations at a given sampling location should not exceed 15 ug L^{-1} TP over the long-term, while five-year average concentrations should not exceed 10 ug L^{-1} TP. These limits would be applied to reference areas to ensure no further degradation and to areas already impacted by P enrichment to gauge the rate and extent of recovery in response to a suite of P control measures, including agricultural BMPs and the construction of treatment wetlands to remove P from surface runoff prior to being discharged into the Everglades. Additional information on Florida's progress in assessing and implementing the adopted standard can be found on the South Florida Water Management District Web site: www.sfwmd.gov.

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