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Nutrient Criteria
Technical Guidance Manual

Wetlands

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

Chapter 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.

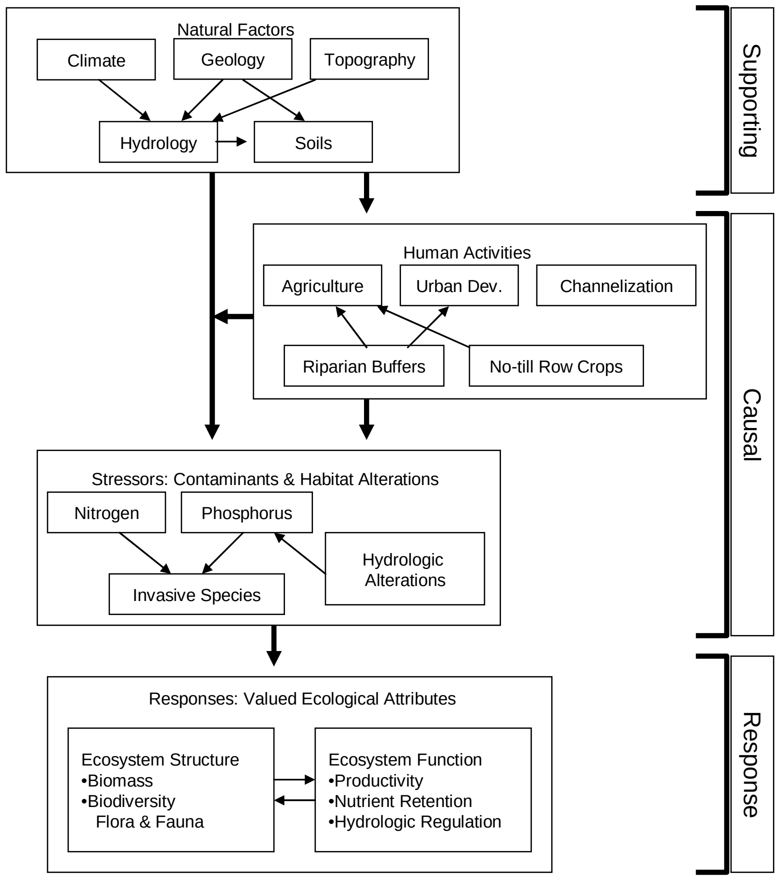
It is important to recognize the complex temporal and spatial structure of wetlands when measuring or interpreting causal and response variables with respect to nutrient condition. The complex interaction of climate, geomorphology, soils, and internal interactions has led to a diverse array of wetland 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^{o}C 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 ^oC (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 $<300^oC$ (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^{o}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.