VIRGINIA WATER RESOURCES RESEARCH CENTER

A LITERATURE REVIEW FOR USE IN NUTRIENT CRITERIA DEVELOPMENT FOR FRESHWATER STREAMS AND RIVERS IN VIRGINIA

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A LITERATURE REVIEW FOR USE IN NUTRIENT CRITERIA DEVELOPMENT FOR FRESHWATER STREAMS AND RIVERS IN VIRGINIA

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ACRONYMS AND ABBREVIATIONS

AAC: Academic Advisory Committee
ac: acre
AFDM: ash free dry mass
ag: agricultural
ANOVA: analysis of variance
BMP: best management practices
Chl-a: chlorophyll-a
CO_2: carbon dioxide
DEQ: Department of Environmental Quality
DIN: dissolved inorganic nitrogen
DNA: deoxyribonucleic acid
D.O.: dissolved oxygen
EPA: U.S. Environmental Protection Agency
EPT: Ephemeroptera (mayflies), Plecoptera (stoneflies) and Tricoptera (caddisflies)
FTU: Formazin Turbidity Units (Calibration based on formazin primary standards. Does not specify how the instrument measures the sample [angle to the incident light])
g/m^2: grams per square meter
ha: hectare
HPLC: high pressure liquid chromatography
HPO_4^{2-}: monohydrogen phosphate ion
$H_2PO_4^-$: dihydrogen phosphate ion
IBI: Index of Biotic Integrity
ICI: Invertebrate Community Index
KDHE: Kansas Department of Health and Environment
KDOW: Kentucky Division of Water
kg/d: kilograms per day
km^2: square kilometers
L: liter
m: meter
μg/L: micrograms per liter (1 μg/L = 0.001 mg/L)
μS/cm: microSiemens per centimeter
mg/L: milligrams per liter (1 mg/L = 1,000 μg/L or 1,000 mg/m^3)
mg/m^2: milligrams per square meter
mg/m^3: milligrams per cubic meter (1 mg/m^3 = 0.001 mg/L)
m/sec: meters per second
n: number of observations in a sample (sample size)
N: nitrogen
N_c: cellular nitrogen
NAWQA: National Water-Quality Assessment
NH_3: ammonia
NH_4^+: ammonium ion
NO_2^-: nitrite ion
NO_3^-: nitrate ion

NPDES: National Pollutant Discharge Elimination System
NTU: Nephelometric Turbidity Units (Calibration based on formazin primary standards. Measures scattered light from the sample at a 90-degree angle from the incident light.)
O-P: ortho-phosphate
p: observed significance level
P: phosphorus
$\mathbf{P_c}$: cellular phosphorus
PIBI: Periphyton Index of Biological Integrity
$\mathbf{PO_4^{3-}}$: phosphate ion (ortho-phosphate ion)
QHEI: Qualitative Habitat Evaluation Index
r: coefficient of correlation for a sample
$\mathbf{r^2}$ or $\mathbf{R^2}$: coefficient of determination for a sample
RMSE: root mean square error
RTAG: Regional Technical Assistance Groups
s: sample standard deviation
SE: standard error
SIN: soluble inorganic nitrogen
SRP: soluble reactive phosphorus
TIN: total inorganic nitrogen
TKN: total Kjeldahl nitrogen
TMDL: total maximum daily load
TN: total nitrogen
TP: total phosphorous
Turb: turbidity
U.S. EPA: United States Environmental Protection Agency
USGS: United States Geological Survey
WWTF: wastewater treatment facilities

ABSTRACT

To protect the designated uses of streams and rivers, the U.S. Environmental Protection Agency (U.S. EPA) is directing states and authorized tribes to develop numeric criteria for nutrients. As a part of the efforts in Virginia, the Virginia Department of Environmental Quality requested specifically that the Academic Advisory Committee conduct a comprehensive literature search on the following topics:

- Investigate methods for defining undesirable (nuisance) levels of periphytic algae in wadeable streams, and what such studies have concluded as undesirable (nuisance) levels.
- Investigate a corresponding approach to planktonic algae in non-wadeable streams.

The purpose of this review is to provide background information for the interested public, the Academic Advisory Committee, and the Virginia Department of Environmental Quality to facilitate discussions concerning nutrient criteria development in Virginia. The review aims to identify approaches that could help Virginia in its task of developing nutrient-related numeric criteria for non-tidal freshwater streams and rivers and highlights criteria values defined in other areas through application of such methods.

Section I contains background information pertaining to nutrient criteria development, nitrogen, phosphorus, and primary production. Section II provides information about and examples of research or criteria proposed in other regions that pertain to the approaches recommended by the U.S. EPA for establishing numeric nutrient criteria. These approaches include: (1) the reference approach, (2) the predictive relationship approach, and (3) the published nutrient criteria and threshold approach. Section II also includes information about the development of numeric criteria to protect downstream waters. Section III of the review provides an alternative approach to developing numeric nutrient criteria proposed by the Kansas Department of Health and Environment but not yet reviewed by the U.S. EPA. This alternative approach emphasizes nutrient reductions by adhering to a developed nutrient export budget.

This document does not provide information on many issues that need to be discussed before Virginia sets numeric nutrient criteria. The literature review, therefore, should not be used to select numeric values for non-tidal freshwater streams and rivers in Virginia simply based on the research or criteria of others. Additional work is needed to address issues not discussed in this review.

SECTION I -- BACKGROUND INFORMATION

INTRODUCTION

The U.S. Environmental Protection Agency (U.S. EPA) has identified nutrients as a major reason for impaired water quality in the nation's streams and rivers (U.S. EPA 2000a). The U.S. EPA is therefore directing states and authorized tribes to develop numeric criteria for nutrients to protect the designated uses of streams and rivers. The purpose of this mandate by U.S. EPA to develop nutrient criteria is to address cultural eutrophication (waters enriched with nutrients because of human activities).

To assist water quality managers in developing numeric criteria, U.S. EPA developed a technical guidance, *Nutrient Criteria Technical Guidance Manual: Rivers and Streams* (U.S. EPA 2000a). In the guidance document, the agency has outlined three main approaches:

- the use of reference reaches,
- the application of predictive relationships (e.g., models), and
- the utilization of published nutrient thresholds or recommended algal limits.

The agency recommends that states and authorized tribes use these approaches or a combination of these approaches. It also directs states and authorized tribes to consider downstream effects when developing nutrient-related numeric criteria. In addition, U.S. EPA has developed a set of guidance criteria for two causal variables (total nitrogen [TN] and total phosphorus [TP]) and two early indicator response variables (chlorophyll-a [Chl-a] and turbidity) (U.S. EPA 2000a). States and authorized tribes can use U.S. EPA's guidance criteria in developing criteria applicable to their own streams and rivers.

U.S. EPA directs that the developed criteria must be scientifically defensible and must protect the designated uses of the streams and rivers. In Virginia, numeric criteria are needed to meet water quality standards that require protection of a "balanced, indigenous population of aquatic life" (which includes algal populations) (9 VAC 25-260-10) and require the control of substances that "nourish undesirable or nuisance aquatic plant life" (9 VAC 25-260-20).

U.S. EPA proposes the following goal for states to aim towards when developing nutrient criteria: Maintain an acceptable level of primary production in freshwater streams and rivers, even when other resources besides nutrients are not limiting (biomass accumulation not limited by light, flow, grazing invertebrates, etc.) (U.S. EPA 2000a). Reckhow et al. (2005) suggest that states should develop criteria that are easily measured, good predictors of the designated use attainment, and consider societal values that balance environmental protection and cost. Reckhow et al. (2005, p. 2918) caution: "…natural variability and criterion-use prediction uncertainty will almost certainly result in some risk of nonattainment…. Furthermore, the selection of the acceptable probability [of nonattainment] is a value judgment best left to policy makers and should not be 'hard-wired' into the criteria level analysis."

Section I of this literature review covers general information about nitrogen, phosphorus, and primary production, and the effects of excess nutrients and primary production in streams and rivers. Section II of the review is organized according to the three main approaches suggested by U.S. EPA and includes possible downstream effects. Section III provides a summary of an

alternative approach proposed by the Kansas Department of Health and Environment. Throughout the document the term "streams" is used to refer collectively to both non-tidal, freshwater streams and non-tidal, freshwater rivers.

PURPOSE

The purpose of this review is to provide background information for the interested public, the Academic Advisory Committee (AAC), and the Virginia Department of Environmental Quality (DEQ) as discussions begin concerning nutrient criteria development in Virginia. The review aims to identify approaches that could help Virginia in its task of developing nutrient-related numeric criteria for non-tidal, freshwater streams and rivers and highlights criteria values defined in other areas through application of those methods.

Virginia DEQ requested that the AAC conduct a comprehensive literature search on the following topics:

- Investigate methods for defining undesirable (nuisance) levels of periphytic algae in wadeable streams, and what such studies have concluded as undesirable (nuisance) levels.
- Investigate a corresponding approach to planktonic algae in non-wadeable streams.

This literature review should not be used to select numeric values for freshwater streams in Virginia simply based on the research or criteria of others. Furthermore, this document does not provide an exhaustive review of the literature on the topic of nutrients and freshwater streams and rivers. Instead, it focuses on research that addresses the question: "How much is too much?"

While some publications cite thresholds levels for streams and rivers that are based on data from lakes (e.g., lake data are provided in Novotny and Olem 1994, Ryding and Rast 1989, Wetzel 2001), few lake-derived values are included in this review. When included, they are identified as coming from lake water samples.

Further work is needed to address issues not discussed in this review. For example, because selected nutrient criteria need to relate to the uses of particular stream types, an assignment of uses for the freshwater streams in Virginia is needed prior to setting nutrient criteria. Furthermore, a discussion is needed on the purpose of the criteria; e.g., "Will the criteria be used simply for screening purposes to look for possible problems?" Also, the state needs to decide whether or not it will follow the approach taken by Ohio in categorizing streams according to their anticipated attainability level (e.g., streams in forested regions are expected to be able to attain higher levels of water quality than are streams in urban settings).

NITROGEN

Nitrogen (N) is an essential nutrient for living organisms. It is primarily used to form proteins, which are the building blocks of all living matter. Proteins provide structural support, act as enzymes, regulate cell activity, etc. Nitrogen is also an important component of chlorophyll, the green pigment that makes photosynthesis possible.

Nitrogen in streams may come from natural sources, such as the decomposition of plants and animals, waste products from aquatic life within the streams, urine and feces of wildlife, or (in generally small amounts) mineral dissolution of rocks. Much of the nitrogen that enters streams is often of direct human origin (such as discharges from sewage treatment plants or leachate from septic systems) or is related to human activities (such as wastes from poultry and livestock facilities, runoff of fertilizers, or nitrous oxides from fuel combustion). Nitrogen can be transported to streams through atmospheric deposition, runoff, or groundwater.

Most algae and other primary producers (organisms able to photosynthesize) are able to utilize inorganic forms of nitrogen: nitrates (NO_3^-), nitrites (NO_2^-), ammonia (NH_3), and ammonium ions (NH_4^+). Various forms of organic nitrogen (nitrogen that is bound to carbon-based molecules) may also be available to algae. For example, urea ($[NH_2]_2CO$), a soluble organic compound containing nitrogen that is excreted in urine and applied to land as fertilizer, easily degrades into inorganic forms of nitrogen. Likewise, organic nitrogen found in plant and animal tissues can become available for use by primary producers if converted by bacteria into inorganic forms of nitrogen.

Nitrogen in water samples can be measured in several different forms, for example:

- nitrate-N = (NO_3-N)
- nitrite-N = (NO_2-N)
- total ammonia-N = ammonia-N (NH_3-N) + ammonium-N (NH_4-N)
- dissolved inorganic nitrogen (DIN; filtered samples), soluble inorganic nitrogen (SIN; filtered samples), or total inorganic nitrogen (TIN; unfiltered samples) = nitrate-N + nitrite-N + total ammonia-N
- total Kjeldahl nitrogen (TKN) = total ammonia-N + total organic nitrogen
- total nitrogen (TN) = nitrate-N + nitrite-N + total ammonia-N + total organic nitrogen

Nitrogen values are typically measured in units of µg/L, mg/L, or mg/m^3. To make comparisons between different studies easier, values within this review are reported as mg/L.

PHOSPHORUS

Phosphorus (P) is an essential nutrient for living organisms. For instance, phosphorus is found in DNA (the genetic material of living organisms), used to form cell membranes, and is utilized at the cell level (as ATP, adenosine tri-phosphate) to generate energy.

Phosphorus enters streams from a number of different sources, e.g., point-source discharges, terrestrial runoff, feces from waterfowl, decaying organisms, and streambed rocks containing phosphorus. Some sources of phosphorus to streams are natural, such as waste products from

aquatic organisms and wildlife, and decaying tissues of plants and animals. Other natural sources of phosphorus in streams include dissolved minerals containing phosphorus and atmospheric deposition of particulate-bound phosphorus (e.g., phosphorus attached to wind blown soils). Sources of phosphorus in streams that result from human activities often include industrial and municipal effluents and surface runoff from lands affected by fertilizer, poultry litter, and/or livestock waste. Human activities that increase soil erosion may also contribute phosphorus to streams as particulate-bound phosphorus.

In the aquatic ecosystem, phosphorus can be found in the water column, within the bodies of aquatic organisms, or attached to particles (such as in sediment) in the water. Primary producers are able to directly incorporate inorganic forms of phosphates. Primary producers may also be able to indirectly obtain phosphorus from various organic compounds (phosphorus bound to carbon-based molecules, as in excrement and plant and animal matter). For example, organic phosphorus incorporated in plant and animal tissues may be made available for use by primary producers through bacterial conversion into soluble inorganic phosphates. Likewise, particulate-bound phosphates (phosphates bound to particles) can be used by primary producers if the phosphorus disassociates from its particle to become soluble in the water column.

Because phosphorus tends to bind to mineral particles, water quality laboratories often filter samples through a 0.45-micron filter. The phosphorus that passes through the filter is called *filterable phosphorus*. Filterable phosphorus is often referred to as "soluble" or "dissolved" phosphorus even though the filtrate may contain both dissolved and colloidal (tiny particles in suspension) forms of phosphorus. The part of the sample that cannot pass through the 0.45-micron filter is referred to as *particulate phosphorus*. These particulate samples may contain mineral materials as well as organic components such as bacteria, algae, zooplankton, particles of plant material, etc.

The phosphorus parameters reported in the literature and reviewed for this document include:

- soluble reactive phosphorus (SRP), filterable reactive phosphorus (FRP), or ortho-phosphate (O-P) (mainly filterable dihydrogen phosphates [H_2PO_4-P], monohydrogen phosphates [HPO_4-P], and ortho-phosphates [PO_4-P]), and
- total phosphorus (TP: all the organic and inorganic, filterable and particulate phosphorus forms).

Phosphorus values are generally measured in units of µg/L, mg/L, or mg/m^3. In this review, phosphorus values were converted to mg/L to make comparisons between studies easier.

PRIMARY PRODUCTION

In general terms, primary production is the amount of organic matter made from inorganic materials through the process of photosynthesis. Primary producers need essential nutrients — nitrogen, phosphorus, magnesium, calcium, iron, zinc, etc. — in sufficient amounts in order to live and grow. Of the essential nutrients, nitrogen and phosphorus are most likely to be limiting in aquatic environments. Nitrogen and phosphorus are considered to be "causal parameters" because in excessive amounts, they may cause proliferation of primary producers: periphyton, phytoplankton and/or macrophytes.

Periphyton refers to a community of organisms usually dominated by algae but also including bacteria, fungi, protozoa, and other microbes. The primary types of algae that make up periphyton include diatoms, green algae, red algae, chrysophytes, and xanthophytes. Periphyton assemblages, also known as benthic algae, grow on stable surfaces, such as rocks, woody debris, and vascular plants. Periphyton accumulation tends to occur along the streambed of shallow waters.

Phytoplankton are suspended in the water column and are made up of photosynthesizing organisms such as algae and cyanobacteria (formerly called blue-green algae). Phytoplankton may exist as single cells, filaments, or colonies of cells. Most phytoplankton have limited mobility so are carried downstream with the flow of the water (or settle to the streambed). Phytoplankton tend to be the dominant algal community in deeper waters, where the amount of sunlight reaching the streambed is inadequate for the growth of periphyton.

Macrophytes are plants large enough to be seen with the naked eye. They generally have roots, stems, and leaves. However, mosses are macrophytes that lack these tissues. Macrophytes may be rooted in the sediment or free-floating.

Algae and other primary producers serve as the basis for aquatic food chains. Some filamentous algae and macrophytes also provide habitat for other organisms. In nutrient-enriched streams (eutrophic conditions), however, these primary producers are often found at high levels and can interfere with the uses of a stream. For example, nuisance levels of *Cladophora* (green algae) have been observed in many streams with high phosphorus conditions (e.g., Wong and Clark 1976, Wharfe et al. 1984, Watson and Gestring 1996, Biggs et al. 1998a, Chételat et al. 1999, Ponader and Charles 2003). In low-order streams (small, headwater streams), nuisance algae are often filamentous algae (periphyton). In large streams (4th order or larger), blooms of phytoplankton may occur when certain types of microscopic phytoplankton grow quickly in water, forming visible patches. Nuisance phytoplankton blooms are frequently caused by diatoms or cyanobacteria.

Chlorophyll-a

The biomass of periphyton and phytoplankton are often estimated by measuring the amount of Chl-a, the predominant green pigment used in photosynthesis. Chl-a is considered an appropriate surrogate measurement for primary production because researchers have consistently found strong correlations between Chl-a values and algal biomass. Chl-a can be determined from a sample of periphyton collected from rocks (or other surfaces) in the stream or from a sample of phytoplankton collected from the water column. To determine the amount of Chl-a, the chlorophyll is extracted from the cells using a solvent such as acetone. The Chl-a value is then measured by such means as spectrophotometry, fluorometry, or high pressure liquid chromatography (HPLC).

U.S. EPA cautions that Chl-a values derived from the different methodologies are not interchangeable (U.S. EPA 2000b–d). Spectrophotometry measures the amount of light

absorbance at specific wavelengths. One method of spectrophotometric analysis relies on trichromatic equations. U.S. EPA does not recommend the use of Chl-a values derived by trichromatic equations unless no other data exist (U.S. EPA 2000b–d). Fluorometry measures the amount of light emitted at a particular wavelength when exposed to light at a different wavelength. The presence of other chlorophyll pigments (e.g., pheophytin) can interfere with the Chl-a measurement using either the spectrophotometric or fluorometric methods. For this reason, U.S. EPA prefers data from methods that also incorporate an acid correction treatment (U.S. EPA 2000b). *Standard Methods for the Examination of Water and Wastewater* (APHA 1998), however, does not recommend the acidification step for freshwaters when using the fluorometric technique if pheopigments (Chl-b) are also present. Although HPLC accurately separates the pigments based on physical characteristics, it is an expensive and time-consuming method so is not used as often as the spectrophotometric and fluorometric methods (NC WRRI 2001).

According to an article in the *Water Resources Research Institute News of The University of North Carolina*, "No method of measuring chlorophyll a accounts for all sources of interference or variation. Every method addresses some interferences but ignores others. For this reason some experts support the reporting of 'total chlorophyll pigments.'" (NC WRRI 2001, p. 10). Furthermore, Carlson and Simpson (1996) state: "It is strongly recommended that the total chlorophyll pigment be reported in addition to chlorophyll *a*. This value, although flawed by interferences by other chlorophylls, phaeo-pigments, as well as a number of other possible interferences, is the only value that remains fairly independent of chlorophyll methodology. Therefore, it is the only measurement that provides historical consistency. Chlorophyll *a* methodologies have changed over the past 25 years, and with each change, the previous chlorophyll estimates became obsolete and non-comparable to the new methods. If everyone had reported total chlorophyll, at least there would be one consistent value that would allow comparison. In a monitoring program, where historical data consistency is absolutely necessary, this value should be reported."

Periphyton

Periphyton biomass is estimated by removing periphyton from a known or measured area of a substrate. The substrate can be natural, such as rocks in the streambed, or artificial, such as a clay tile onto which periphyton growth is allowed to accumulate for a controlled amount of time (e.g., two to four weeks). The periphyton is removed from its substrate by scraping with a brush, rubber spatula, knife, or other instrument. The content of Chl-a in the sample is measured as described in the above section. Chl-a periphytic data are recorded as milligrams per square meter (mg/m^2).

A relationship between the nutrient concentration in streams and the growth of periphyton has been observed in many studies but not all (see review in ENSR 2001). In lab studies (Horner et al. 1983) and field artificial enrichment studies (Bothwell 1989, Watson et al. 1990), nutrients were found to stimulate the growth of stream periphyton. Horner et al. (1983) obtained what they considered to be nuisance levels of periphytic biomass, 100 – 150 mg/m^2 of Chl-a (determined fluorometricly according to Strickland and Parsons 1972), at SRP levels between 0.015 and 0.025 mg/L. Perrin et al. (1987) increased the biomass of stream periphyton by more

than ten times that of the control section (< 10 mg/m^2) by raising stream SRP above background levels (0.001 mg/L) to maximum SRP levels of about 0.100 mg/L (average SRP being 0.015 – 0.025 mg/L), which yielded Chl-a levels around 90 – 150 mg/m^2.

In addition to a relationship between periphyton biomass and stream nutrient levels, specific taxa can be related to nutrient levels. Working in the riffle zones of 13 rivers in southern Ontario and western Quebec, Chételat et al. (1999) found the presence of green algae to dominate at moderately eutrophic sites while red algae taxa were most common at sites with the lowest Chl-a values. Specific taxa, *Cladophora* (green algae), *Melosira* (diatom), and *Audouinella* (red algae), were positively correlated with TP concentrations over the range of 0.006 – 0.082 mg/L ($r^2 = 0.39 – 0.64$, $p < 0.005$) and were dominant at sites with the highest nutrient concentrations (Chételat et al. 1999).

Factors other than nutrient levels also influence the growth and composition of periphyton, and may even exhibit more control than nutrient levels. For example, many studies suggest that light availability and stream flow may be the dominant control factors of periphyton accumulation in temperate streams (see ENSR 2001). Ponader and Charles (2003) state: "a strong correlation between nutrient and biomass is at best difficult to establish, and that other factors such as light (as a function of river basin size) and substrate must be taken into account when estimating nutrient-biomass relationships… Therefore, commonly used biomass measures (Chl *a*, AFDM [ash-free dry mass]) must be interpreted with caution, and inferences of nutrient levels in rivers based on these measures should be made only in conjunction with analyzing other variables" (p. 36). Thus, although very low nutrients levels in a stream system can be expected to result in low levels of periphyton, it does not necessarily follow that high nutrient levels will cause high levels of periphyton biomass.

Shading from vegetation, either growing along the stream bank or in the water, and water column turbidity caused by background coloration, sediments, etc. may reduce the amount of sunlight reaching the periphyton. Thus, shading may limit the growth of periphyton. In contrast, increases in light availability have been associated with excessive algal growth in streams (as cited in Mosisch et al. 1999: Lyford and Gregory 1975, Shortreed and Stockner 1983, Graham et al. 1995).

The depth and velocity of the water impacts periphyton accumulation and loss. Increasing velocity to a certain level can increase biomass growth by providing a constant supply of nutrients (Horner 1983, Welch et al. 1989), whereas higher water velocities can dislodge the periphyton community from the substrate. A number of studies suggest that the length of flood-free periods may be at least as important as nutrients in determining levels of periphyton biomass in streams (Biggs and Close 1989, Lohman et al. 1992, Biggs 1995, Biggs 2000a). For example, periphyton levels decreased at all study sites (22 sites on 12 Ozark streams) following floods (Lohman et al. 1992). Furthermore, growth of periphyton to nuisance levels often occurs during periods of low flow. Periphyton accumulation is limited in larger rivers by water depth, which decreases light availability.

Other factors, such as temperature, appear to control periphyton growth only at extremes (e.g., low, 0 – 1°C, and high, 35°C, temperatures) (ENSR 2001).

In general assemblages of periphyton grow poorly on sand, clay, and highly organic substrates, preferring rocks and other more stable substrate (Welch et al. 1992, ENSR 2001). In a study in eastern Ontario and western Quebec, Cattaneo et al. (1997) found that cobbles had the highest biomass of periphyton, while gravels had the lowest biomass, and sand and boulders were intermediate. Also, different types of periphyton did better on different substrates, e.g., filamentous algae prefer large rocks while cyanobacteria grew better in sand habitats (Cattaneo et al. 1997).

Grazing by snails, caddisfly larvae, mayfly larvae, and other organisms can control the growth of periphyton even when nutrient levels are high (Hill et al. 1992, Rosemond et al. 1993, Steinman 1996). For example, when working in a lowland stream (a stream with a low channel gradient) in New Zealand, Biggs et al. (1998b) found that grazing by snails reduced the mean periphyton biomass by 80% during periods of stable flows.

The biomass of periphyton may vary greatly from season to season, from year to year, and from one stream reach to another. For example, the biomass levels within a stream vary between shaded and open areas and between pool, run, and riffle habitats. Different stream reaches experience peaks of algal biomass at different times (Watson and Gestring 1996). The long-term patterns of periphyton biomass within a stream reach that are often observed include:

- constant, low periphyton biomass;
- cycles of accrual and sloughing of periphyton; and
- seasonal cycles of the growth of periphyton.

Seasonal patterns may result from changes in the light availability (more sunlight in summer), temperature (warmer water temperatures in the summer), and grazer activity (more grazing pressure in the summer). Seasonality in disturbances, such as floods associated with tropical storms and hurricanes, are also evident. Differences in dominant periphyton taxa are observed from the headwaters to the middle sections of rivers, with the upper reaches generally dominated by green algae (e.g., *Cladophora)* and the middle reaches generally dominated by diatoms (Watson and Gestring 1996).

Phytoplankton

The relative levels of phytoplankton are estimated by collecting a representative water sample (e.g., composite sample), filtering the sample, extracting the Chl-a pigment into a solvent, and measuring the Chl-a concentration in the sample. Phytoplankton Chl-a data are expressed in terms of mg/m^3 or µg/L (whereas periphyton biomass is expressed in mg/m^2 of the substrate). In this review, phytoplankton measurements are generally reported as micrograms per liter (µg/L).

Phytoplankton populations in streams and rivers develop from dislodged periphyton, phytoplankton from an upstream lake, or riverine phytoplankton (that which develops within the stream). Some algal species can live either as periphyton or as phytoplankton. For example, the diatoms *Fragilaria* (formerly *Synedra*) *ulna* and *Navicula tripunctata/lanceolata* are able to grow and reproduce whether attached to a surface or floating within the water column (Reynolds and Descy 1996). Based on a review of published year-long studies of 67 rivers worldwide, Rojo et al. (1994) found that about half of the phytoplankton species reported in these studies are

of benthic origin and half are truly riverine plankton. Phytoplankton that develop within lakes tend not to survive well under riverine conditions (Prygiel and Leitao 1994, Reynolds and Descy 1996, Yang et al. 1997).

The true riverine phytoplankton are thought to primarily develop in water storage zones (also referred to as dead zones or phytoplankton nurseries) that are found in the side arms and eddies within the river system. Such areas within the river are likely to have slower water flow and warmer temperatures, which promote phytoplankton cell growth (Reynolds 1995). Citing four studies at Leighton on the River Severn (U.K.), Reynolds and Descy (1996) reported that non-flowing waters had more than 40 times the algal concentration as the main stem of the river. Under normal flow conditions, the cells in these nursery areas are slowly leached into the main river channel.

Factors that impact the amount of phytoplankton biomass within a given stream reach include water velocity, solar radiation at the water surface, water column turbidity, temperature, nutrients, settling of cells out of the photic zone, and consumption by zooplankton (e.g., rotifers) and benthic suspension feeders (e.g., clams). Stream discharge, availability of light, and temperature have been reported as being the most important factors controlling phytoplankton biomass (Greenberg 1964, Gosselain et al. 1994, Kiss et al. 1994, Schmidt 1994). In general, more phytoplankton accumulate during periods of low flow, high light availability, and warm temperatures. Herbivorous consumption by organisms can negatively impact phytoplankton biomass levels. Cohen et al. (1984) reported that a particular reach of the Potomac River with the highest biomass of an introduced clam, *Corbicula fluminea,* also had the lowest phytoplankton biomass relative to other areas of the river.

According to Reynolds and Descy (1996), "…nutrient limitation of algal production probably never arises in most rivers and, with the exception of case studies on the Solmões and Negro tributaries of the Amazon (Hammer 1965), we are not aware of cases where it has been demonstrated otherwise" (p. 169). Nutrient enrichment from human activities over the past decades has brought about significant changes of biomass and composition of phytoplankton (Gosselain et al. 1994). Excess amounts of nutrients have been linked to algal blooms. For example, eutrophication of the River Ebro in north-east Spain has greatly increased since the 1970's along with increases in the orthophosphate levels (from 0.2 mg/L in the 1970's to 0.9 mg/L in the 1990's) and nitrate concentration (from 3.0 mg/L in the 1970's to 9.0 mg/L in the 1990's) (Ibañez et al. 1995).

Citing over 40 studies, Hynes (1970) concluded that phytoplankton in streams are almost always dominated by diatoms. Likewise, published studies from rivers around the world showed diatoms, primarily, and green algae, secondly, to dominate the stream species (Rojo et al. 1994). Furthermore, the species most often recorded in rivers from various parts of the world are generally not those that cause blooms (Table 1.). Of the phytoplankton causing blooms, *Stephanodiscus hantzschii* (5 studies) and *Cyclotella meneghiniana* (4 studies) populations were reported most frequently (*S. hantzschii* was reported as a group and not as a species) (Rojo et al. 1994).

Table 1. River algal species occurring in more than 50% of studies reviewed by Rojo et al. (1994), and species in this study reported to cause algal blooms. Only *Cyclotella meneghiniana* and *Pediastrum duplex* are high occurrence and blooming species.

High Occurrence Species	Blooming Species
Actinastrum hantzschii [green algae]	*Aulacoseira granulate* [diatom]
Ankistrodesmus falcatus [green algae]	*Aulacoseira italica* [diatom]
Asterionella formosa [diatom]	*Cylindrotheca closterium* [diatom]
Aulacoseira granulate [diatom]	***Cyclotella meneghiniana*** [diatom]
Cyclotella meneghiniana [diatom]	*Cyclotella pseudostelligera* [diatom]
Fragilaria capucina [diatom]	*Microcystis aeruginosa* [cyanobacteria]
Fragilaria ulna [diatom]	***Pediastrum duplex*** [green algae]
Melosira varians [diatom]	*Skeletonema costatum* [diatom]
Nitzschia acicularis [diatom]	*Skeltonema potamos* [diatom]
Pediastrum duplex [green algae]	*Stephanodiscus hantzschii* [diatom]
Scenedesmus acuminatus [green algae]	*Stephanodiscus parvus* [diatom]
Scenedesmus quadricauda [green algae]	*Stephanodiscus tenuis* [diatom]

Phytoplankton communities change from upstream to downstream. In general, diatoms replace green algae in dominance down river, but such change is not abrupt. The transition zone from dominance by green algae to dominance by diatoms migrates up and down the river, possibly depending on streamflow (Reynolds 1995).

Predictable seasonal changes have also been reported for the phytoplankton community. Working on the River Meuse (Belgium), Gosselain et al. (1994) found diatoms to dominate in spring, with green algae becoming more important in the summer. According to Köhler (1994), the few studies of river phytoplankton lasting longer than two years indicate a regular annual sequence of phytoplankton taxa occurring in rivers.

Phytoplankton biomass tends to be highly variable, changing from upstream to downstream within a stream system. By following a portion of water in the Rhine River from upstream to downstream, de Ruyter van Steveninck et al. (1992) found Chl-a concentrations to increase from 13.8 µg/L (at Maxau) to 170.5 µg/L (at Dusseldorf) in about 400 km. Further downstream, the Chl-a concentrations of phytoplankton declined sharply, possibly owing to increased grazing by zooplankton and filter-feeding organisms (Chl-a was determined photometrically, correcting for phaeopigments after acidification). As another example related to upstream-downstream levels, some downstream reaches in the Riddeau River in Ontario, Canada increased in phytoplankton biomass, while other reaches decreased in biomass (Basu and Pick 1995). Furthermore, Yang et al. (1997) found that the Rideau River reaches with the higher amounts of algal biomass were caused by an increase in the size of the plankton, not necessarily the abundance of phytoplankton.

Phytoplankton biomass can also differ by season and year. Seasonal changes in algal biomass in temperate rivers are obvious, with phytoplankton biomass levels tending to be lowest during winter. As an example of year-to-year differences, researchers of the lower River Rhine found

maximum Chl-a levels to be less than 25 µg/L during a cold and rainy summer; whereas in most years, the maximum Chl-a values reached between 50 – 100 µg/L, with values up to 170 µg/L (Chl-a was determined photometrically, correcting for phaeopigments after acidification) (de Ruyter van Steveninck et al. 1990, 1992). Similarly, Greenberg (1964) found the peak plankton population in June 1961 to be almost four times that found during the peak population in September 1960.

PROBLEMS CAUSED BY EXCESS NUTRIENTS AND EXCESS PRIMARY PRODUCTION

The effect of nutrient enriched waters on the biotic community depends on many factors. In some instances, nutrient inputs to streams can be utilized by the existing aquatic life without causing measurable changes in the community structure. In other situations, excessive levels of nutrients can increase the amount of primary production, which then may support more macroinvertebrates and other aquatic life. Unfortunately, excess nutrients and high levels of primary production can also have negative effects on the stream system. Most problems caused by excess nutrients are related directly or indirectly to the excessive growth of primary producers (periphyton, phytoplankton, and macrophytes).

Potential Impacts on Stream Use

Excessive nutrient levels may allow excessive increases in algae and other primary producers, which may in turn, prevent streams from meeting their designated uses. The adverse effects of either high nutrient levels or the nuisance growth of primary producers include, for example:

- Impairment of the aquatic life use
  -- Daily fluctuations in oxygen concentrations and pH values may negatively impact aquatic life (see explanations below).
  -- Toxicity may result if high ammonia levels (e.g., > 1 mg/L NH_3-N) contribute to the high nitrogen levels.
  -- Some algal blooms release toxic compounds (e.g., cyanotoxins) (see additional information below).
  -- A loss of diversity and other changes in the aquatic plant, invertebrate, and fish community structure may result.
- Negative impact on the drinking water and industrial water supply use
  -- Methemoglobinemia (blue-baby syndrome) may affect infants if nitrate levels >10 mg/L
  -- Diatoms and filamentous algae can clog intake screens and filters in water treatment plants.
  -- Decay of algae may lead to taste and odor problems of drinking water.
  -- Potentially carcinogenic disinfection by-products (trihalomethanes, THMs) may form during treatment of drinking water from eutrophic waters.
  -- Treatment costs may rise for waters drawn from eutrophic sources by requiring more backwashing, etc.
- Degradation of the aesthetic and recreational use

-- Unsightly algal growth is unappealing to many swimmers and other stream users.
-- Slippery streambeds caused by heavy growths of algae on rocks are difficult to walk on.
-- Fishing lures may become tangled in algae and macrophytes.
-- Boat propellers may get tangled by aquatic vegetation.

Dissolved Oxygen Depletion

Excessive growth of primary producers may lead to a depletion of dissolved oxygen. During the day, primary producers provide oxygen to the water as a by-product of photosynthesis. At night, however, when photosynthesis ceases but respiration continues, dissolved oxygen concentrations decline. Furthermore, as primary producers die, they are decomposed by bacteria that consume oxygen, and large populations of decomposers can consume large amounts of dissolved oxygen. Many aquatic insects, fish, and other organisms become stressed and may even die when dissolved oxygen levels drop below a particular threshold level (e.g., below 5 mg/L). Typical daily oxygen fluctuations in six enriched streams at low flow were reported to range from a high of about 25 mg/L at noon to a low of about 3 mg/L at night (Wong and Clark 1976). Excessive amounts of nutrients in streams and rivers may also negatively impact the dissolved oxygen levels of downstream receiving waters. For example, a zone of hypoxia (< 2 mg/L) in the Gulf of Mexico has been linked to high nutrient inputs from the Mississippi River (U.S. EPA 2000a).

pH Alterations

The pH of water is a measure of its acid-base condition (range: 0 – 14, with 7 being neutral, less than 7 indicating acidic conditions, and greater than 7 indicating basic conditions). The pH is controlled by the production of hydrogen ions (H^+) and hydroxyl ions (OH^-). Daily fluctuations of water column pH can be caused by excessive primary production.

When photosynthesis is occurring, the water column pH level tends to be more basic. During photosynthesis, carbon dioxide (CO_2) and water are converted by sunlight into oxygen and sugar (glucose, $C_6H_{12}O_6$). During the formation of glucose, hydroxyl ions are produced. These hydroxyl ions raise the water column pH (make it more basic). Furthermore, the removal of dissolved CO_2 for photosynthesis results in lower levels of carbonic acid (H_2CO_3) in the water column, which causes a shift to a less acidic condition (more basic condition).

More acidic conditions occur at night when photosynthesis ceases but respiration continues. Respiration results in the release of CO_2 into the water and thus increases the production of carbonic acid:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3.$$

Carbonic acid dissociates, producing hydrogen ions that lower the water column pH ($H_2CO_3 \rightleftharpoons HCO_3^- + H^+$).

Extremes in stream pH are stressful and can even be deadly to aquatic organisms. High pH levels increase the toxicity of some substances, such as ammonia, whereas low pH levels can make heavy metals in stream sediment more mobile. Water column pH also affects the

availability of phosphorus for algal intake, with phosphorus being unavailable to algae at high and low pH levels. High pH levels can damage fish gills, eyes, and skin. Low pH levels can interfere with fish reproduction. Levels of pH too high (e.g., >9) or too low (e.g., <5) can kill aquatic life.

Toxins

Some kinds of primary producers release toxins, which can kill fish and other organisms. These toxins can taint drinking water supplies and recreational waters. Livestock that drank water contaminated with cyanobacteria reportedly died (Bowling and Baker 1996 as cited in Dodds and Welch 2000). Humans who drink or swim in water that contains high concentrations of toxins from cyanobacteria may experience gastroenteritis, skin irritation, allergic responses, or liver damage (CDC 2004). In 1991, one of the largest recorded riverine blooms of toxic cyanobacteria occurred in the Murray-Darling River Basin in Australia, resulting in a state of emergency being declared to protect water supplies drawn from the river (Oliver et al. 1999). Here in the U.S., harmful algal blooms of the toxic dinoflagellate, *Pfiesteria,* have been tied to fish kills in several tidal tributaries of the Chesapeake Bay (U.S. EPA 2000a).

SECTION II -- APPROACHES TO SETTING NUTRIENT CRITERIA

The U.S. EPA's *Technical Guidance Manual for Developing Nutrient Criteria for Rivers and Streams* (2000a) offers three approaches for water quality managers to consider. The approaches include the use of (1) reference systems, (2) predictive relationships, and (3) established threshold values. U.S. EPA recommends using a combination of these approaches when developing numeric nutrient and algal criteria. Almost all examples used in this document incorporate more than one of the suggested approaches.

The steps outlined for the nutrient criteria setting process recommended by U.S. EPA (2000b) include:

(1) Develop reference conditions at a regional or watershed scale
(2) Evaluate historical data, published literature, and other information
(3) Use models to simulate physical and ecological processes and to determine empirical relationships for causal variables (e.g., TN, TP) and response variables (e.g., Chl-a, turbidity)
(4) Utilize expert review and consensus (best professional judgment)
(5) Evaluate downstream effects.

Clark Fork River Example

One of the best-known examples where nutrient thresholds were established is in the Clark Fork River in western Montana. This waterbody experienced heavy growths of filamentous algae and diatom algae that interfered with the recreation and irrigation uses of the river. Chl-a values commonly exceeded 100 mg/m^2 (Watson and Gestring 1996, Dodds et al. 1997). Because Montana does not have numeric nutrient criteria, target values were set for TN and TP from

studies that incorporated several of the approaches described above. The nutrient targets were set to keep mean Chl-a values below 100 mg/m^2 because levels above this value are generally considered undesirable (based on information by Horner et al. 1983; and 26 citations reported in Welch et al. 1988).

Using the reference approach, Dodds et al. (1997) sampled six sites on the Clark Fork River that typically exhibit Chl-a levels considered to be acceptable(< 100 mg/m^2) (stations 8.5, 9, 13, 15.5, 24, and 25 in Ingman 1992a, b as reported in Dodds et al. 1997). They established target TN and TP concentrations from the 1988 – 1992 summer means (21 June – 21 September) of these reference sites. The TN average summer concentration for the reference stations was 0.318 mg/L, and the mean TP concentration was 0.0205 mg/L (Dodds et al. 1997).

Watson et al. (1990) used artificial stream channels and water from the Clark Fork River in controlled experiments. The water was spiked with various concentrations of soluble nitrogen and phosphorus, and the growth rates and standing crop of algae were measured. They found that algal biomass would be reduced if soluble nitrogen levels were kept below 0.250 mg/L and SRP was kept below 0.030 mg/L (Watson et al. 1990).

A regression model using data from more than 200 river sites worldwide (Dodds et al. 1997) was used to predict TN and TP targets for keeping mean summer Chl-a values below 100 mg/m^2 in the Clark Fork River. The results from this regression yielded mean summer TN target concentrations of less than 0.350 mg/L and mean summer TP target concentrations of less than 0.0455 mg/L (U.S. EPA 1999).

Dodds et al. (1997) used a probabilistic modeling approach based on the methods developed by Heiskary and Walker (1988) for lakes. This model assessed the risk of exceeding 50, 100, and 200 mg/m^2 of Chl-a. For example, using the probabilistic model, they predicted that neither Chl-a seasonal mean nor maximum values would exceed 100 mg/m^2 more than 50 percent of the time if TN was kept below 0.200 mg/L. Similarly, if TP was kept below 0.050 mg/L, mean Chl-a levels would be less than 100 mg/m^2 and maximum Chl-a values would be below 150 µg/L 50 percent of the time (Dodds et al. 1997).

Dodds et al. (1997) also reported results from regression analyses of the Clark Fork River data set. In this study, they used iteration equations to find values of TN that would give 50, 100, and 200 mg/m^2 of Chl-a. The results of the TN values were then used to estimate corresponding TP values from the Redfield ratio of 7.23 N: 1 P by mass. Additional iterations were performed to determine TP values that would give 50, 100, and 200 mg/m^2 of Chl-a, with TN values set to the Redfield ratio. Holding the maximum Chl-a value at 100 mg/m^2, they predicted seasonal means of around 0.275 mg/L TN and 0.035 mg/L TP.

Based on the results from the studies reported in Dodds et al. (1997), the authors recommended setting targets at 0.350 mg/L for mean TN concentrations and 0.030 mg/L for mean TP concentrations to keep mean Chl-a values below 100 mg/m^2 in the Clark Fork River (150 mg/m^2 maximum). The Tri-State Implementation Council overseeing the Clark Fork River TMDL (total maximum daily load) accepted a summer mean Chl-a value of 100 mg/m^2 and peak Chl-a value of 150 mg/m^2 as the boundary between impaired and unimpaired water quality. To allow

for a margin of safety (as required in TMDL development), they set mean targets at 0.300 mg/L for TN, 0.020 mg/L for TP upstream of Missoula, and 0.039 mg/L for TP below Missoula. The Council also recommended monitoring for both total and soluble forms of nitrogen and phosphorus to continue the study of point source and nonpoint source impacts on the river (U.S. EPA 1999).

1. REFERENCE APPROACH

A group of national experts working with U.S. EPA in addressing nutrient criteria development issues recommended that U.S. EPA "not develop single criteria values for phosphorus or nitrogen applicable to all water bodies and regions of the country. Rather, the experts recommended that EPA put a premium on regionalization, develop guidance (assessment tools and control measures) for specific waterbodies and ecological regions across the country, and use reference conditions (conditions that reflect pristine or minimally impacted waters) as a basis for developing nutrient criteria" (U.S. EPA 2000b, p. 1).

With this advice, the U.S. EPA developed the *Technical Guidance Manual for Developing Nutrient Criteria for Rivers and Streams* (U.S. EPA 2000a), which included methods for establishing reference conditions and means for using the reference conditions to set nutrient targets. The reference conditions in the guidance manual are based on reference reaches defined as "relatively undisturbed stream segments that can serve as examples of the natural biological integrity of a region" (U.S. EPA 2000a, p. 94). The manual notes that reference *reaches* are only a section of a stream and differ from reference *streams*, which include the whole stream and have little or no human impacts in the entire catchment area. U.S. EPA recommends selecting at least three reference conditions for each stream class and suggests that all reference systems should be "minimally disturbed and should have primary parameter values (i.e., TN, TP, chl *a*, and turbidity) that reflect this condition" (U.S. EPA 2000a, p. 94).

The U.S. EPA technical guidance manual covers three methods by which nutrient criteria can be based on reference reaches for each stream class within a given region:

(1) Use best professional judgment to characterize reference reaches and set the criteria to equal the mean (or median) value for the water quality parameters of the reference reaches.
(2) Use a frequency distribution of data collected from reference reaches (e.g., TN, TP values) and set the criteria equal to the 75th percentile.
(3) Use a frequency distribution of data collected from all streams (reference and non-reference) or a random sample from all streams and set the criteria equal to the 5th to 25th percentile (Use the 5th percentile if most reference reaches are impacted by human activity to some extent.) (U.S. EPA 2000a).

Of the three reference methods, U.S. EPA prefers the frequency distribution that represents the reference reaches. U.S. EPA further recommends using both frequency distribution approaches, if possible, and selecting whichever criteria are most protective. U.S. EPA states that the use of the 75th, 25th, or 5th percentiles are only recommendations and stresses that the main reason to

chose a particular threshold should be based on the actual distribution of data for the given region (U.S. EPA 2000a).

The agency chose the 75th percentile of reference reaches for criteria setting because it is "likely associated with minimally impacted conditions, will be protective of designated uses, and provides management flexibility" (U.S. EPA 2000b, p. 10).

The 25th percentile of all streams was selected by U.S. EPA because studies indicate this boundary approximates the 75th percentile of reference streams, as illustrated in Figure 1 (U.S. EPA 2000b). In this example, the 75th percentile for the reference streams is at 20, and the 25th percentile for all streams is at 25. A line is drawn at 23, indicating the middle value, which could be used as the threshold point.

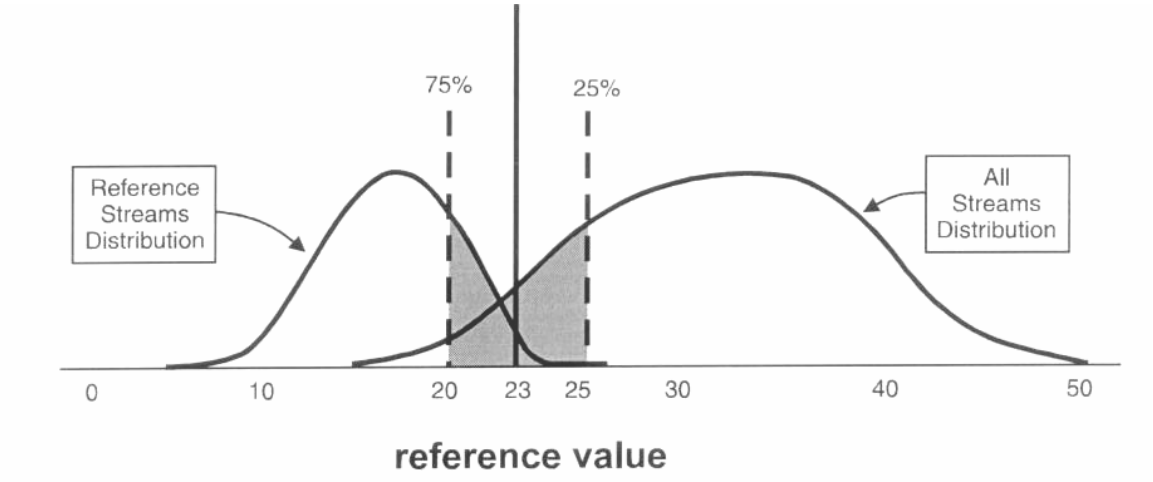


Figure 1. Selecting reference values for total phosphorus concentration (µg/L) using percentiles from reference streams and total stream populations.[1]

U.S. EPA recognizes that some streams, such as those that flow over bedrock with high phosphorus content, may not be able to meet the criteria because of natural causes. The agency also cautions that reference conditions could be highly variable if the data are lumped (from different stream orders, seasons, years, etc.) over a large geographical area (U.S. EPA 2000b). When using the reference approach, regions with streams that have high nutrient levels are likely to have higher criteria limits than those with mostly low nutrient levels. Consequently, the criteria may be too high in ecoregions with mainly high-nutrient reference conditions and too low for ecoregions with mostly low-nutrient reference conditions.

[1] Reprinted from Nutrient Criteria Technical Guidance Manual: Rivers and Streams, EPA-822-B-00-002, Office of Water and Office of Science and Technology, United States Environmental Protection Agency, Washington, DC, 152 pp., published 2000.

U.S. EPA's Reference Approach Using Aggregate Nutrient Ecoregions

Ecoregions are a means of classifying areas based on similarities of natural geographic features (e.g., geology, soils, climate, hydrology, vegetation, and wildlife) and land use patterns. U.S. EPA divided the continental United States into 14 nutrient ecoregions (aggregates of level III ecoregions) with similarities in characteristics expected to affect nutrient concentrations. Virginia is included in Aggregate Nutrient Ecoregions IX, XI, and XIV (Table 2, Figure 2). Although U.S. EPA promotes the use of the aggregate nutrient ecoregion, it also suggests that states and authorized tribes select reference conditions at smaller geographic scales, such as the level III ecoregion developed by Omernik (1987).

Table 2. Aggregate nutrient ecoregions and Omernik's (1987) level III ecoregions for streams in Virginia.

Aggregate Nutrient Ecoregion	Level III Ecoregion
IX	45, Piedmont
	64, Northern Piedmont
	65, Southern Plains
XI	66, Blue Ridge
	67, Ridge and Valley
	69, Central Appalachians
XIV	63, Middle Atlantic Coastal Plain

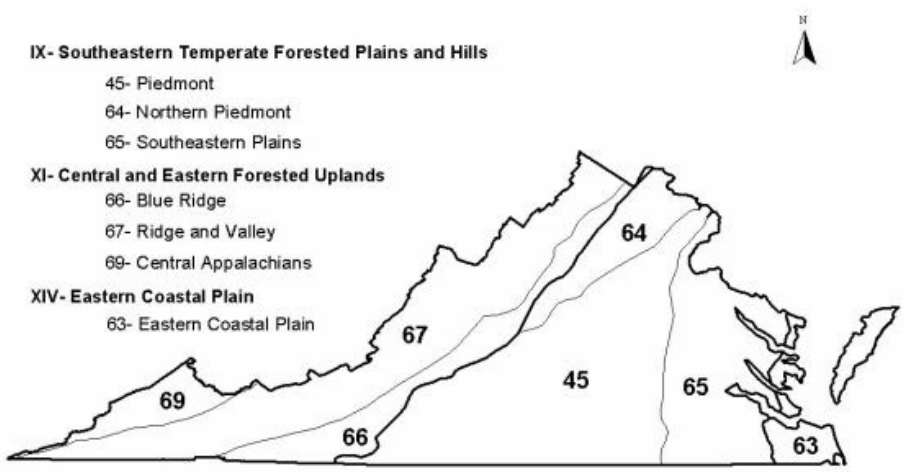


Figure 2. Map of Virginia showing U.S. EPA Aggregate Nutrient Ecoregions.

To define the reference condition, which could then be utilized in setting nutrient criteria, U.S. EPA used the following general procedures. Four median values, representing each season (winter, spring, summer, fall), were determined for each waterbody used. Within a given ecoregion, a frequency distribution of the median values for each season was plotted, and the 25th percentiles were selected (Figure 3). The reference condition for the ecoregion was then established as the median value of the seasonal 25th percentiles (U.S. EPA 2000b). Using this method, the U.S. EPA's calculated nutrient threshold recommendations applicable to Virginia's rivers and streams are listed in Table 3.

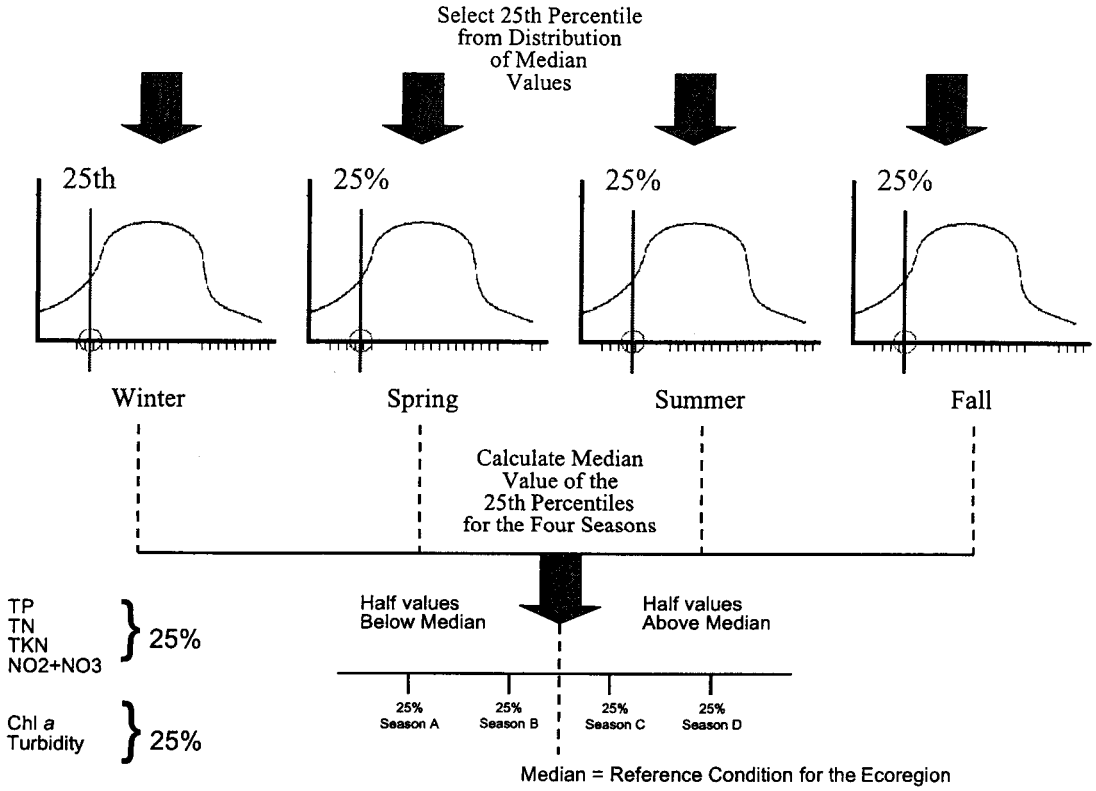


Figure 3. Illustration of reference condition calculation.[2]

[2] Reprinted from Ambient Water Quality Criteria Recommendations: Information Supporting The Development Of State And Tribal Nutrient Criteria For Rivers And Streams In Nutrient Ecoregion XIV, EPA 822-B-00-022, Office of Water—Office of Science and Technology Health and Ecological Criteria Division, United States Environmental Protection Agency. Washington, D.C. 79 pp., published 2000.

Table 3. U.S. EPA's nutrient threshold recommendations for Aggregate Nutrient Ecoregions and Level III Ecoregions applicable to Virginia's rivers and streams. Based on the median for all seasons' 25th percentiles.

	IX – 45 Piedmont	**IX – 64 Northern Piedmont**	**IX – 65 Southern Plains**	**IX**	**XI – 66 Blue Ridge**	**XI – 67 Ridge & Valley**	**XI – 69 Central Appalach .**	**XI**	**XIV – 63 Eastern Coastal Plain**	**XIV**
TKN (mg/L)	0.234	0.3	0.3		0.102	0.169	0.113		0.51	
$NO_2 + NO_3$ (mg/L)	0.177	0.995	0.095		0.058	0.23	0.177		0.04	
TN (mg/L) Calculated	0.411	1.295	0.395		0.16	0.399	0.29		0.55	
TN (mg/L) reported	0.615	2.225	0.618	**0.69**	0.28	0.214	0.502	**0.31**	0.87	**0.71**
TP (mg/L)	0.030	0.040	0.0225	**0.03656**	0.007125	0.010	0.007625	**0.01000**	0.0525	**0.03125**
Turb. (NTU)	5.713	2.825	6.2		1	2.4	2.175	**2.30**	3.89	
Turb. (FTU)	7.488	3.15	4.338	**5.7**	1.675	4.25	1.9	**1.7**	4.5	**3.04**
Turb. (JCU)	5.95	4.4	6.55		0.8	3.425	2.444		4.73	
Chl-a (µg/L) – F	3.3	-	1.438		1.625[zz]	-	-		0.44	
Chl-a (µg/L) – S	3.493	1.205	0.049	**0.93**	2	1.063		**1.61**	3.75	**3.75**
Periphyton Chl-a (mg/m^2)	-	20.35	-	**20.35**	-	32.75	-		-	

Chl-a – F = Fluorometric Method
Chl-a – S = Spectrophotometric Method
zz calculated medians from less than 3 seasons' data.

U.S. EPA recommends using its suggested reference conditions as a guide or "first step" in setting nutrient criteria. States and authorized tribes are encouraged to select reference conditions at smaller geographic scales (level III ecoregions or watershed scale) and refine their criteria through the use of models and published literature and in consideration of downstream effects and expert judgment (U.S. EPA 2000b).

Ponader and Charles (2003) applied the reference reach approach outlined by U.S. EPA to nutrient data from the Northern Piedmont ecoregion in New Jersey. They calculated a reference condition using the 25th percentile from all reaches in their study and compared their values with those reported by U.S. EPA (2000b) for the Northern Piedmont subecoregion (64). Ponader and Charles (2003) found fairly good agreement in the reference conditions for TN (1.28 mg/L to U.S. EPA's 1.30 mg/L) and TP (0.051 mg/L to U.S. EPA's 0.040 mg/L). In contrast, their 25th percentile Chl-a periphyton values (48.07 mg/m^2, determined by fluorescence) were substantially higher than U.S. EPA's Chl-a reference values (20.35 mg/m^2), showing that the reference reach approach can give very different results simply by using different data sets.

USGS Reference Approach Using Environmental Nutrient Zones

The U.S. Geological Survey (USGS), working as part of a Regional Technical Assistance Group (RTAG), evaluated and refined nutrient region boundaries in the Upper Midwest. The USGS followed the U.S. EPA reference approach in proposing nutrient criteria values but developed alternative nutrient regions, called *environmental nutrient zones*. The nutrient zones were established to address three main problems concerning the U.S. EPA's nutrient ecoregions. These issues include the following:

(1) The relative importance of the environmental characteristics used to delineate the U.S. EPA nutrient ecoregion boundaries is unknown. This lack of information makes it difficult to attribute differences in water quality among ecoregions to any specific environmental factor.

(2) The most important environmental characteristic used to delineate the nutrient ecoregion may be different than that which has the greatest impact on the water quality. If such occurs, it is possible that there will be greater variation of water quality within an ecoregion than between ecoregions.

(3) Although U.S. EPA states that classifications should be based on natural differences, land use was often used to delineate the nutrient ecoregions (Robertson et al. 2001).

In the USGS study, regression-tree analyses were used to determine the relative importance of various environmental characteristics (e.g., basin, climate, and soil characteristics) that affect nutrient concentrations. They then delineated the environmental nutrient zones based on the two or three most statistically significant characteristics that affect nutrient concentrations. For each zone, frequency distributions of the nutrient data were then used to propose nutrient criteria. Criteria were based on the 25th percentile of all the data within a given zone, and alternative criteria were proposed using the 75th percentile of data from sites within the zone that represent "reference" conditions.

The USGS study was based on TN, TP, and watershed characteristic data from 234 sites collected from 1961 to 1999. The data set incorporated data from 15 different studies or sources and included data from the New River and Big Sandy River in Virginia. The data set was first analyzed including land use as an environmental characteristic. Subsequently, as a way to represent only non-anthropogenic (natural) differences, the data set was analyzed excluding land use as a factor.

In the study, nutrient concentrations were most strongly correlated with land-use characteristics, particularly the percentages of forest and agriculture. The most important environmental factors for nitrogen included the amount forest cover (≥ 9 % and ≥ 33 %) and the amount of precipitation (≥ 30.1 inches per year). For total phosphorus, forest cover (≥ 30 %), percent clay (≥ 26 %), and amount of runoff (≥ 12.3 inches per year) were the most important factors.

When land-use characteristics were excluded, the most important environmental factors for nitrogen were soil slope (≥ 11 %), air temperature (≥ 43°F), and nonglacial sediments or exposed bedrock (≥ 99 %). Important non-land-use factors for total phosphorus included till deposits (≥ 59 %), amount of runoff (≥ 10.3 inches per year), and percent clay (≥ 26 %).

The total nitrogen concentrations were more affected by land use than were the total phosphorus concentrations. The nitrogen-based nutrient zones could be divided into low, moderate, and high amounts of agriculture. Furthermore, the delineated boundaries for the nitrogen nutrient zones that included land use as a factor were substantially different from those that excluded land-use characteristics. In contrast, the phosphorus-based nutrient zones were similar for those that included and excluded the land-use factor (Robertson et al. 2001).

Using the data set where land-use characteristics were excluded, USGS proposed TN and TP criteria based on the 25th percentile of all the data within a given zone and the 75th percentile for "reference conditions" in the nutrient zone. In this study, the "reference condition" was assumed to be sites in watersheds with less than 25 percent agricultural land use. It was noted, however, that sites with less than 25 percent agriculture were sometimes dominated by urban land use and therefore not representative of the natural condition (Robertson et al. 2001).

Based on the 25th percentile of all the data within a nutrient zone, TN criteria for the different nutrient zones in the Upper Midwest ranged from 0.51 mg/L to 1.75 mg/L (Table 4). The proposed TN criteria using the "reference" condition within the zone (the 75th percentile of the data for watersheds with less than 25 percent agricultural land use) ranged from 0.67 mg/L to 9.00 mg/L. For the proposed TP criteria, the 25th percentile of all the data within a zone ranged from 0.02 mg/L to 0.11 mg/L. The 75th percentile of the TP data for the "reference" conditions ranged from 0.05 mg/L to 0.16 mg/L (Robertson et al. 2001).

The Virginia waters (New River and Big Sandy River) included in this study are located in Environmental Nitrogen Zone-4 (ENZ-4) and Environmental Phosphorus Zone-2 (EPZ-2) (based on data when land-use characteristics are excluded from the analysis). From the 25th percentile of all the data for ENZ-4 and EPZ-2, a TN criterion of 0.51 mg/L and a TP criterion of 0.02 mg/L, respectively, could be expected (Robertson et al. 2001).

Table 4. Proposed nutrient criteria for different environmental nutrient zones in the Upper Midwest region based on results of regression-tree analyses when land-use characteristics were excluded from the analyses (-- means no data).

	Nutrient	**Zone 1** (mg/L)	**Zone 2** (mg/L)	**Zone 3** (mg/L)	**Zone 4** (mg/L)
25th percentile of all data	TN	0.91	1.75	1.29	0.51
75th percentile of 0-25% ag. land use	TN	1.10	9.00	--	0.67
25th percentile of all data	TP	0.06	0.02	0.06	0.11
75th percentile of 0-25% ag. land use	TP	0.06	0.05	0.06	0.16

As a part of the study, the USGS also compared the variability of nutrient concentrations within U.S. EPA's nutrient ecoregions to that within its nutrient zones (derived including land-use characteristics). The variability among the TN data within nutrient ecoregions and nutrient zones was similar for the two approaches. The within region variability in TP concentrations, however, decreased by about 50 percent using the nutrient zone approach compared to the U.S. EPA nutrient ecoregion approach (Robertson et al. 2001).

2. PREDICTIVE RELATIONSHIPS APPROACH

U.S. EPA recommends evaluating nutrient-algal relationships in streams to help predict algal responses to certain levels of nutrient input. In the technical guidance manual, U.S. EPA outlines three means by which to develop predictive relationships: the use of trophic state classification, models, and biocriteria (U.S. EPA 2000a).

A. TROPHIC STATE CLASSIFICATION

The term *trophic state* refers to a classification of the nutrient condition for a waterbody. Unfortunately, there are no standardized ways to classify the trophic state of a stream. There are also no standardized parameters to measure for determining the trophic state of the stream. Dodds (2003) suggests that because of biotic uptake and remineralization, trophic state is more appropriately represented by TN and TP concentrations than by inorganic nitrogen and inorganic phosphorus concentrations.

The trophic state classification method described in this section by Dodds et al. (1998) is similar to the frequency distribution methods described in the Reference Approach section. In the examples used in the previous section, the streams were divided into two classes: impaired streams and non-impaired streams. However, frequency distributions can be divided into more than two classes, such as reference streams, acceptable quality streams, and impaired streams.

With the trophic state classification method, the frequency distribution is generally divided into three states: (1) oligotrophic streams, (2) mesotrophic streams, and (3) eutrophic streams. In general terms, oligotrophic streams are those that are nutrient poor; mesotrophic streams have an intermediate level of available nutrients, and eutrophic streams are overly enriched by nutrients.

Trophic State Based on Photosynthesis/Respiration Ratios

Hornberger et al. (1977) used a subjective ranking of the eutrophic state of six river sites, five in Virginia and one in New Hampshire, based on their best professional judgment and knowledge of the land use; concentrations of nitrate, phosphate, and chlorophyll-a; and other information (Tables 5 and 6). They determined the productivity at each site from continuous measurements of dissolved oxygen, temperature, and solar radiation. The authors then compared their proposed eutrophic state with the results from their productivity study. They determined that productivity measurements can be used to classify the eutrophic state of rivers and developed a diagram (showing the relationship between productivity measurements and water quality) for use in classifying rivers of unknown trophic status. The authors concluded that the eutrophication potential for a river could be estimated by comparing several weeks of productivity data for summer, low-flow conditions with the data from the six streams in their study.

Table 5. Proposed eutrophy classification for river sites in Hornberger et al. (1977), the site location, potential sources of pollution or primary land use, and number of days that dissolved oxygen, temperature, and solar radiation measurements were collected.

Proposed Eutrophy Rank	River	Location	Primary Pollution Sources /Land Use	Days of data collection
High	Mechums River	Albemarle Co., VA; downstream of town of Crozet	PS: raw & partially treated sewage; NPS: agricultural runoff	78
High intermediate	South Fork Rivanna River	Albemarle Co., VA; 1 km downstream of Rivanna Reservoir	Moormans and Mechums Rivers	14
High	Rivanna River	Albemarle Co., VA; downstream of city of Charlottesville	PS: two secondary STPs; NPS: urban runoff	42
High	South River	Augusta Co., VA; upstream of the city of Waynesboro	NPS: agricultural runoff	38
Intermediate	Rappahannock River	Frederick Co., VA; upstream of the city of Fredericksburg	"…lower end of one of the longest reaches of large undisturbed river in the Eastern United States"	28
Very Low	Baker River	NH; in town of Rumney	Mainly forested; limited agriculture	23

Table 6. Physical and chemical conditions, and dates of sampling, for the rivers used in water quality analysis.[3]

Location and dates	NO_3 + $N0_2$(N) range mg/L	PO_4(P) range mg/L	Typical periphyton chlorophyll-a (mg/m^2)	Base flow (m^3/sec)	Mean channel width (m)	Mean water depth at base flow (m)	Mean Velocity at base flow (m/sec)
Mechums R. 19 Apr.-19 Aug.	0.4-0.6	0.08-0.30	60	0.80	16	0.33	0.15
South Fork Rivanna R. 5 May-25 Jul.	0.1-0.3	<0.003-0.01	135	0.55	26	0.37	0.06
Rivanna R. 20 Jul.-2 Sept.	0.3-1.0	0.02-0.08	50	2.40	36	0.33	0.20
South R. 16 Aug.-20 Sept.	0.4-0.8	0.03-0.04	500	1.40	11	0.40	0.30
Rapphannock R. 4 May-24 June	0.3-0.7	0.003-0.05		22.00	180	2.00	0.06
Baker R. 16 Jul. -2 Aug.	0.1-0.2	<0.003	16	1.80	18	0.48	0.30

In the study, the river representing the low eutrophic status, Baker River, had the lowest concentrations for NO_3+NO_2, PO_4, and typical periphyton chlorophyll-a (Table 6). The nutrient concentration ranges for the river representing the intermediate eutrophic status, Rapphannock River, overlapped with those of the high eutrophic rivers. It was also as high as or higher than the nutrient concentrations for South Fork Rivanna River, ranked high-intermediate. The nutrient concentrations for the three rivers rated high-eutrophic (Mechums, Rivanna, and South) ranged from 0.3 to 1.0 mg/L for NO_3+NO_2 and 0.02 to 0.30 mg/L for PO_4. Typical periphyton chlorophyll-a values (determined using procedures in Strickland and Parsons 1972) had a wide range, 50 – 500 mg/m^2, for the high eutrophic rivers.

The authors observed that the variability within the productivity measurements could be used as an indicator of the eutrophication status: the sites with more variability had a higher eutrophication ranking. Their findings supported the work by Ball et al. (1973), where the "cleanest sites on several Michigan rivers were most stable while disturbed sites showed the most variability" (Hornberger et al. 1977, p. 68).

The main purpose of the study by Hornberger et al. (1977) was to determine if cultural eutrophication and the resulting dissolved oxygen depletion could be evaluated from measurements of productivity and respiration. They calculated the mean gross production (P), net production (P - R), respiration (R), and the P:R ratio for each river site. Using discriminant analysis, the authors found that respiration and P:R ratio, used in conjunction, were good

[3] Reprinted from Water Research; Vol. 11; G.M. Hornberger, M.G. Kelly, and B.J. Cosby; Evaluating eutrophication potential from river community productivity; pages 65-69; copyright 1977; with permission from Elsevier.

indicators of water quality. When log R was plotted against the square root of P/R, the resulting graph could be divided into regions based on eutrophy (Figure 4). The lower left section of the graph was considered representative of "clean" (oligotrophic) waters, while the upper left area represented rivers with possible eutrophication even though the river seems "unpolluted" from the qualitative impressions of the authors. Rivers plotting in the upper right are eutrophic, and waters in the lower right (high respiration but low production) represent waters contaminated by organic material (Hornberger et al. 1977).

The authors stress that the regions of the graph should not be used as strict thresholds, but rather general indications of the trophic condition. They further recommended using the physical and chemical characteristics of the river as well as the biological community and habitat characteristics of the river in addition to their proposed method for determining trophic state.

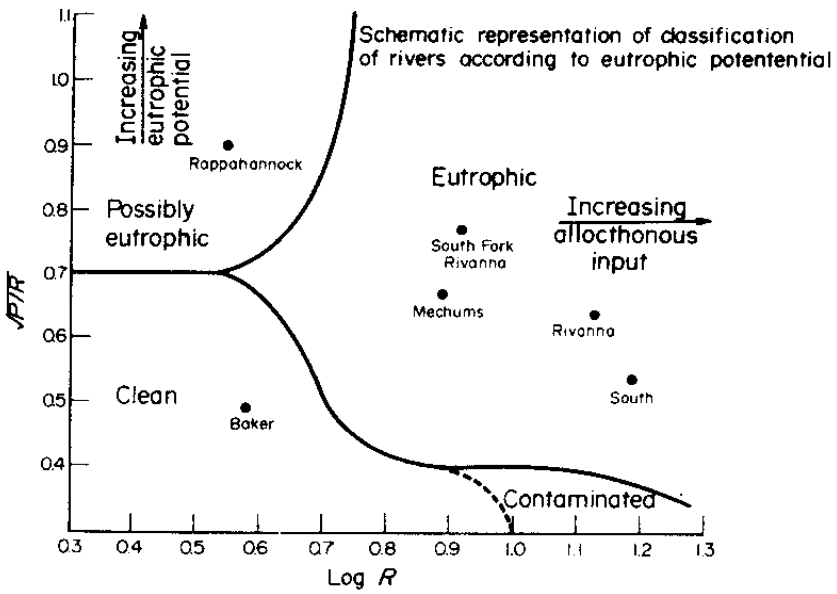


Figure 4. Schematic diagram showing relationship between production and respiration measurements and water quality.[4]

Trophic State Based on Geology and Land Use

Based on catchment geology and land use, Biggs (1995) divided 12 New Zealand watersheds (16 sites) into one of three classifications of potential enrichment: enriched, moderately enriched, or unenriched. He measured periphyton accumulation, algal community structure, and water column characteristics at each site every four weeks for one year. Chl-a was determined spectrophotometrically and corrected for phaeopigments by acidification.

[4] Reprinted from Water Research; Vol. 11; G.M. Hornberger, M.G. Kelly, and B.J. Cosby; Evaluating eutrophication potential from river community productivity; pages 65-69; copyright 1977; with permission from Elsevier.

Periphyton cellular nitrogen concentrations and conductivity correlated strongly with the apriori designation of the site enrichment groups. Inorganic N and P concentrations of the water column, however, did not correspond to the geology/land use apriori designation. The water column inorganic N and P concentrations also did not correlate significantly (at the 0.05 level) with cellular N and P concentrations respectively (Biggs 1995). Limited sampling, fluctuations in water column nutrient concentrations, and the ability of algae to store nutrients may account for these observations (Biggs 1995).

Biomass accrual appeared to be limited by the amount of nutrients in the unenriched streams; however, in the moderately-enriched and enriched categories, biomass accrual was controlled by flood frequency. Thus, Biggs (1995) concluded that the quantity of nutrients control the rate of biomass accrual during periods of stable flow. Data from this study are summarized in Biggs (1996) and provided in Table 7. Biggs (1996) observed that in the enriched streams, Chl-a values were higher than 100 mg/m^2 for about 40% of the year, whereas the moderately enriched streams exceeded 100 mg/m^2 less than 1% of the time during that year.

Table 7. Chlorophyll-a values, ash-free dry mass (AFDM), and conductivity for stream trophic states based on catchment geology and land use as determined from monthly monitoring for one year in 16 streams in New Zealand (data reported in Biggs 1996).

Trophic State of Monitored Sites	25th Percentile – 75th Percentile Chl-a (mg/m^2)	Median Chl-a (mg/m^2)	Median AFDM (g/m^2)	Mean Conductivity (µS/cm)
Unenriched	0.5 – 3	1.7	1.5	87
Moderately Enriched	3 – 60	21	4.8	106
Enriched	25 – 260	84	15	271

Trophic State Based on the Frequency Distributions of Nutrient Values

Dodds et al. (1998) proposed an initial means by which to characterize the trophic state of streams based on the frequency distributions of chlorophyll and nutrients. Because of the high variance observed in stream systems, Dodds et al. (1998) recommended using their method only as "a general first approach to categorizing stream ecosystems" (p. 1455). They used data from published studies (Omernik 1977, van Nieuwenhuyse and Jones 1996, and Dodds et al. 1997) that represented hundreds of streams, mostly in North America and New Zealand. The data included benthic Chl-a, water column Chl-a, TN, and TP. Using the distribution frequencies of each parameter, they divided the streams into categories with the lowest third (lowest values) of the distributions indicating oligotrophic conditions, the middle third suggesting mesotrophic conditions, and the top third (highest values) representing eutrophic conditions (Table 8). Dodds et al. (1998) caution, "Sharp, natural boundaries in trophic state and functional relations among the variables have yet to be identified for streams, so these boundaries should be viewed as provisional" (p. 1458).

Table 8. Suggested boundaries for trophic classification of streams from cumulative frequency distributions. The boundary between oligotrophic and mesotrophic systems represents the lowest third of the distribution, and the boundary between mesotrophic and eutrophic marks the top third of the distribution. [Dodds et al. (1998) reported TN and TP concentrations in µg/L; for consistency within this review, these values were converted to mg/L][5]

Variable (units)	Oligotrophic-mesotrophic boundary	Mesotrophic-eutrophic boundary	n
Mean benthic Chl-a (mg/m^2)[a]	20	70	286
Max. benthic Chl-a (mg/m^2)[a]	60	200	176
Sestonic Chl-a (µg/L)[b]	10	30	292
TN (mg/L)[a,c]	0.700	1.500	1070
TP (mg/L)[a,b,c]	0.025	0.075	1366

[a]Data from Dodds et al. (1997).
[b]Data from van Nieuwenhuyse and Jones (1996).
[c]Data from Omernik (1977).

Dodds et al. (1998) found their trophic categories to be analogous to those proposed by Biggs (1996). Approximately 90% of the benthic Chl-a values in Biggs' unenriched category were below 20 mg/m^2, the oligotrophic-mesotrophic boundary defined by Dodds et al. (1998). Similarly, about 90% of the benthic Chl-a values in Biggs' moderately enriched category were below 100 mg/m^2, which is comparable to the 70 mg/m^2 mesotrophic-eutrophic boundary proposed by Dodds et al. (1998).

An advantage of applying the frequency distribution method proposed by Dodds et al. (1998) to classify streams lies in its potential use in studying ecological processes within individual streams. For example, Dodds et al. (1998) hypothesized that if a stream has TP levels in the top 10% of the distribution but benthic or sestonic Chl-a values in the lowest 10%, other factors besides nutrients, such as benthic grazers or scouring floods, are likely affecting algal accrual.

There are also disadvantages to using this method. Of most significance, the method of classifying the trophic condition of streams as proposed by Dodds et al. (1998) rests on the assumption that the lowest third of the data represents oligotrophic conditions and the highest third signifies eutrophic conditions. Unfortunately, there is no basis for this assumption. It is unlikely that about 33% of streams are eutrophic, 33% mesotrophic, and 33% oligotrophic (ENSR 2001).

[5] Reprinted from Water Research; Vol. 32; W.K. Dodds, J.R. Jones, and E.B. Welch; Suggested classification of stream trophic state: Distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus; pages 1455-1462; copyright 1998; with permission from Elsevier.

B. MODELS

Mathematical models can be used to express relationships between different parameters, e.g., water column TP and algal biomass accumulation. Models are classified as empirical or process based. Empirical models identify patterns but do not explain them, whereas process-based models are explanatory. Empirical models use regression analysis, where the value of a known variable is used to predict the value of an unknown variable. For example, empirical models that correlate TN and/or TP with benthic algal biomass have been developed by Lohman et al. (1992), Dodds et al. (1997), Bourassa and Cattaneo (1998), Chételat et al. (1999), Biggs (2000a), and others. Empirical models have also been developed to identify relationships between nutrients and phytoplankton, as described, for example, by van Nieuwenhuyse and Jones (1996). Process-based models are made from equations that contain observable parameters. AQUATOX is an example of a process-based model developed by U.S. EPA with potential use in developing nutrient criteria for streams and rivers (http://www.epa.gov/waterscience/models/aquatox/index.html) (accessed January 16, 2006).

Empirical (Regression) Models

Statistics are useful in showing the relationships among sampling parameters and are therefore used in regression models. Simple descriptive statistics, such as the mean (average), median, and ranges for each measured parameter, are helpful in identifying the differences and similarities between streams. The *observed significance level*, known as the "p" value, expresses the probability of no relationship between variables (e.g., Chl-a and TP). A small p value (for example, $p < 0.001$) indicates that the variables are significantly related.

The *coefficient of determination* for a sample, r^2, reflects the proportion of variation explained by a regression model. It illustrates the fit of the data around the predicted relationship ($0 \leq r^2 \leq 1$). In general, the closer the r^2 value is to 1, the less scatter in the data and the higher the confidence that the data support the model relationship.

Empirical Models Relating Nutrients and Periphyton

--*Dodds, Smith, and Zander Study*

Dodds et al. (1997) used data from published literature representing approximately 200 sites in North America, New Zealand, and Europe to develop mathematical equations to predict benthic chlorophyll-a values. They recommend using the equations only when local relationships have not been developed. Regression equations were developed to estimate mean Chl-a or maximum Chl-a using TN, TP, or both TN and TP concentrations.

The model was used to predict benthic Chl-a values in the Clark Fork River in Montana. The r^2 values for the equations used in the Clark Fork River study ranged from 0.35 to 0.43. From the equations, Dodds et al. (1997) predicted that if seasonal mean TN concentrations in the Clark Fork River are reduced to 0.275 mg/L, the maximum chlorophyll-a values would be 100 mg/m^2. Additionally, if TN concentrations do not exceed 0.252 mg/L and TP concentrations are kept

below 0.035 mg/L, Chl-a values in the Clark Fork River would be expected to stay below 100 mg/m^2.

--Lohman, Jones, and Perkins Study

In a two-year study of 22 sites on 12 streams in the Ozarks, Lohman et al. (1992) developed regression equations to predict benthic Chl-a values from TN and TP concentrations. Benthic Chl-a was determined fluorometrically and corrected for phaeopigments by acidification. Chl-a was found to be positively correlated for both study years with respect to log TN (R^2 = 0.58, 0.60) and log TP (R^2 = 0.47, 0.60). Covariance analysis used to test for differences between years suggested that the slopes were not different but the intercepts were for both TN and TP data. Benthic Chl-a levels tended to be higher during the second year of the study. Lohman et al. (1992) credit the strength of their regression analysis on the use of long-term averages (a March – November "annual" average) and the wide range of TN (range of site annual means: 0.148 – 9.188 mg/L) and TP concentrations (range of site annual means: 0.006 – 3.264 mg/L).

Lohman et al. (1992) log-transformed the TP data from a New Zealand study (Biggs and Close 1989) and regressed it against Chl-a. A comparison of the regression equations in each of the two studies, suggested that neither the slopes nor intercepts were significantly different for the Ozark and New Zealand streams. From this analysis, Lohman et al. (1992) concluded that empirical models may be useful in assessing stream nutrient-periphyton relationships, at least under certain circumstances.

Lohman et al. (1992) also categorized the 22 Missouri stream sites as highly enriched, moderately enriched, or unenriched based on mean annual TP concentrations. These mean annual TP concentrations ranged from 0.212 to 3.264 mg/L for the enriched sites (n = 6), 0.014 to 0.125 mg/L for the moderately enriched sites (n = 10), and 0.006 to 0.019 mg/L for the low enrichment sites (n = 6). At one site, land use was taken into consideration to distinguish the category. This site was categorized as moderately enriched because it was known to receive point-source effluents, even though its mean annual TP concentrations were 0.017 mg/L in 1985 and 0.014 mg/L in 1986. Excluding this site, the mean annual TP concentrations for the moderately enriched sites ranged from 0.028 to 0.125 mg/L.

Growth curves for each trophic state category were developed from post-flood recovery data (Figure 5). From the growth curves, Lohman et al. (1992) predicted that the moderately and highly enriched sites would exceed Chl-a levels of 150 mg/m^2 within three or four weeks following a scouring flood. The unenriched sites, however, were not predicted to exceed 75 mg/m^2 of benthic Chl-a. Actual measurements were somewhat higher than predicted for the unenriched streams, but five of the six streams in this trophic class had maximum Chl-a values of less than 150 mg/m^2.

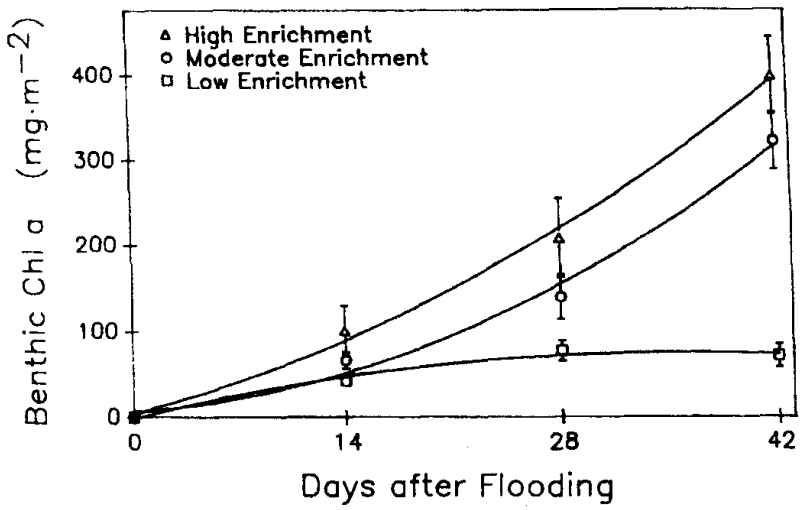


Figure 5. Growth curves fitted for stream sites classified as high-, moderate-, and low nutrient sites following a catastrophic flood in fall 1986. Points are means ± SE; n = 6 for high- and low-enrichment sites and n = 10 for moderate-enrichment sites.[6]

--*Chételat, Pick, Morin, and Hamilton Study*

Chételat et al. (1999) studied the relationship of nutrients to periphyton biomass and community composition in riffle areas from 13 rivers in southern Ontario and western Quebec during summer low flows (see Biocriteria section). Samples were collected once each year for three years at 33 sites. Chl-a was determined using a spectrophotometer and a trichromatic equation. The Chl-a, TP, and TN data were log transformed and regressed. Chl-a values were correlated with water conductivity ($r^2 = 0.71$), TP ($r^2 = 0.56$), and TN ($r^2 = 0.50$). TN and TP concentrations were also positively correlated with conductivity ($r^2 > 0.70$, $p < 0.001$). TP, TN, and conductivity were negatively correlated with catchment area, indicating that the smaller rivers in the Chételat et al. (1999) study had higher nutrient concentrations. The velocity measurements, ranging from 10 – 107 cm/s, explained less than 2% of the Chl-a variation. The authors suggest that this lack of a relationship between current velocity and algal biomass may be due to the relatively stable flows of the rivers and possibly due to the rough estimates made for velocity (Chételat et al. 1999).

For comparison, Chételat et al. (1999) log transformed and regressed the Chl-a and TP data from New Zealand rivers studied in Biggs and Close (1989) and Ozark streams described in Lohman et al. (1992) (Table 9). The slope of the Chételat et al. (1999) model was similar to that of Biggs and Close (1989) and was much greater than those of Lohman et al. (1992). Stream size and drainage area seemed to account for this finding, as the New Zealand rivers and catchment areas were comparable whereas the Ozark streams and drainage areas were smaller. Also, the impact from flooding in the Ozark streams could contribute to the differences observed in the slopes, whereas the two river studies had more stable flows.

[6] Reprinted from Canadian Journal of Fisheries and Aquatics Sciences; Vol. 49; K. Lohman,, J.R. Jones, and B.D. Perkins, Effects of nutrient enrichment and flood frequency on periphyton biomass in northern Ozark streams; pages 1198-1205, copyright 1992, with permission from National Research Council Canada.

Table 9. Regression of periphyton Chl-a (mg/m^2) as a function of TP concentration (µg/L) from the present study [Chételat et al. 1999] in comparison with other models obtained from the literature.[7]

Coefficients						
Intercept (±SE)	**log TP (±SE)**	**r^2**	**RMS**	**p**	**n**	**Source**
0.490 ±0.213	0.905 ±0.140	0.56	0.071	< 0.001	33	Present study
0.338 ±0.310	0.722 ±0.246	0.49	0.089	0.022	9	Biggs and Close 1989
1.207 ±0.115	0.247 ±0.056	0.46	0.038	< 0.001	22	(i) Lohman et al. 1992
1.383 ±0.095	0.228 ±0.047	0.52	0.025	< 0.001	22	(ii) Lohman et al. 1992

Note: Data sources: Biggs and Close (1989) (data are geometric annual means from nine New Zealand rivers) and Lohman et al. (1992) (data are annual means from 22 sites on 12 Northern Ozark streams for (i) 1985 and (ii) 1986). Data are log transformed. The coefficient of determination (r^2), residual mean square (RMS), p value, and number of observations (n) are presented for each model.

--Biggs Studies

Biggs (1995) divided 16 stream sites in New Zealand into one of three potential enrichment categories based on catchment geology and land use (see Trophic State Classification section). Every four weeks for a one-year period, he measured flooding disturbance, periphyton accumulation, Chl-a (determined spectrophotometrically and corrected for phaeopigments by acidification), algal cellular nutrient concentrations, water column total inorganic nitrogen, water column dissolved reactive phosphorus, and other parameters at each site. Cellular nitrogen (N_c) was measured as TKN, and cellular phosphorus (P_c) was measured as TP. The ash free dry mass of the sample was used to standardize the values to give percent cellular nitrogen (% N_c) and percent cellular phosphorus (% P_c) (Biggs 1995).

Using a stepwise multiple regression, Biggs (1995) determined that flood frequency (estimated as the number of annual events when the hydrograph exceeds 1 m/s), proportion of high-intensity agricultural land use, and proportion of alkaline rock could explain 89% of the variation in the mean monthly Chl-a levels. Combining the flooding disturbance factor (flooding frequency) and stream enrichment factor (as measured by cellular N concentrations), he developed a means to predict the growth of periphyton (n = 15, R^2 = 0.865). From this research, it appears that only streams having a combination of a few flood events (e.g., < 10) per year and enrichment of at least 3% N_c should yield benthic algal biomass (Chl-a) in excess of nuisance levels set at 100 mg/m^2 (Biggs 1995).

In 2000, Biggs (2000a) published a paper in which he combined and reanalyzed data from previous studies (Biggs 1995, Biggs et al. 1998b, 1999) and developed models for predicting mean monthly and maximum Chl-a as a function of SRP, SIN, and days of accrual. The dissolved nutrient-biomass models were derived for unshaded streams with gravel-cobble substrate and a flood threshold level of greater than three times (>3x) the median discharge. The models did not account for grazing benthic macroinvertebrates or site-specific characteristics

[7] Reprinted from the Canadian Journal of Fisheries and Aquatic Sciences; Vol. 56; J. Chételat, F.R. Pick, A. Morin, and P.B. Hamilton; Periphyton biomass and community composition in rivers of different nutrient status; pages 560-569; copyright 1999; with permission from National Research Council of Canada.

(e.g., local water velocities, differences in bed sediment for the sites), and therefore did not explain a large part of the variance in Chl-a values. The models, however, did explain up to 49% of the variation in mean monthly Chl-a (Table 10) and up to 74% of the variation in maximum Chl-a (Table 11).

Table 10. Regression statistics for $\log_{10}$ of mean monthly benthic algal biomass (mg/m^2 chlorophyll *a*) concentration as a function of mean days of accrual (d_a) and mean monthly soluble nutrient concentrations. SIN = soluble inorganic N, SRP = soluble reactive P, mg/m^3 (n = 30).[8]

Effect	Value / coefficient	SE	*p* (2-tail)	r^2
1. Constant	-0.888	0.434	0.050	
$\text{Log}_{10}\, d_a$	1.355	0.315	0.001	0.397
2. Constant	0.109	0.434	0.804	
Log_{10} SIN	0.483	0.245	0.057	0.122
3. Constant	0.468	0.192	0.022	
Log_{10} SRP	0.697	0.244	0.008	0.226
4. Constant	-1.229	0.494	0.019	
$\text{Log}_{10}\, d_a$	1.245	0.320	0.001	
Log_{10} SIN	0.284	0.206	0.179	0.437
5. Constant	-0.926	0.408	0.031	
$\text{Log}_{10}\, d_a$	1.152	0.310	0.001	
Log_{10} SRP	0.462	0.212	0.038	0.488

[8] Reprinted from Journal of the North American Benthological Society, Vol. 19(1), B.J.F. Biggs, Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae, pages 17-31, copyright 2000, with permission from the North American Benthological Society.

Table 11. Regression statistics for $\log_{10}$ of maximum monthly benthic algal biomass (mg/m^2 chlorophyll *a*) concentration as a function of mean days of accrual (d_a) and meant monthly soluble nutrient concentrations. SIN = soluble inorganic N, SRP = soluble reactive P, mg/m^3 (n = 30).[9]

	Effect	Value/ coefficient	SE	*P* (2-tail)	r^2
1.	Constant	-2.886	1.449	0.057	
	$\text{Log}_{10}\ d_a$	5.223	1.937	0.012	
	$(\text{Log}_{10}\ d_a)^2$	-1.170	0.624	0.072	0.618
2.	Constant	0.711	0.317	0.031	
	Log_{10} SIN	0.688	0.168	0.001	0.325
3.	Constant	1.400	0.184	0.001	
	Log_{10} SRP	0.797	0.233	0.002	0.295
4.	Constant	-2.946	1.217	0.023	
	$\text{Log}_{10}\ d_a$	4.285	1.649	0.015	
	$(\text{Log}_{10}\ d_a)^2$	-0.929	0.529	0.091	
	Log_{10} SIN	0.504	0.144	0.002	0.741
5.	Constant	-2.714	1.264	0.040	
	$\text{Log}_{10}\ d_a$	4.716	1.696	0.010	
	$(\text{Log}_{10}\ d_a)^2$	-1.076	0.545	0.059	
	Log_{10} SRP	0.494	0.160	0.005	0.721

Using the developed regression models for maximum biomass and the trophic state boundaries set by Dodds et al. (1998) – an oligo-mesotrophic boundary of 60 mg/m^2 Chl-a and a meso-eutrophic boundary of 200 mg/m^2 Chl-a – Biggs (2000a) developed a nomograph to predict oligo-, meso-, and eutrophic conditions (Figure 6). The nomograph shows the mean monthly soluble nutrient concentrations predicted to give maximum algal biomass for the different trophic states for varying days in accrual. Although Biggs warns that the models need additional testing, he concludes that "nutrient criteria for the prevention of benthic algal proliferations could be set in streams in relation to regimes of local flood frequency and expected time available for biomass accrual. The present analysis suggests that managing nutrient supply could not only reduce the magnitude of maximum biomass, but also reduce the frequency and duration of benthic algal proliferations in streams" (Biggs 2000a, p. 17).

[9] Reprinted from Journal of the North American Benthological Society, Vol. 19(1), B.J.F. Biggs, Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae, pages 17-31, copyright 2000, with permission from the North American Benthological Society.

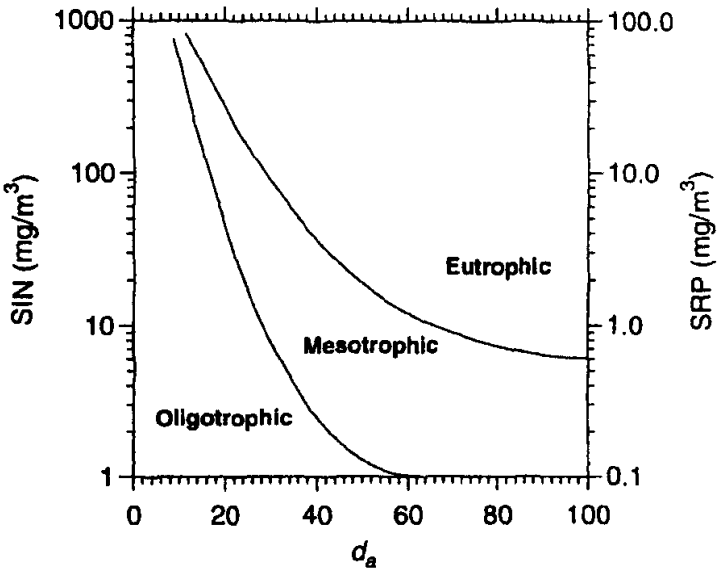


Figure 6. Nomograph of mean monthly soluble nutrient concentrations that are predicted to result in maximum benthic algal biomass indicative of oligotrophic, mesotrophic, and eutrophic conditions for varying days of accrual (d_a) in gravel/cobble-bed streams. The oligotrophic-mesotrophic boundary was set at 60 mg/m^2 chlorophyll *a* and the mesotrophic-eutrophic boundary was set at 200 mg/m^2 chlorophyll *a* (after Dodds et al. 1998). These boundaries also equate to maximum biomass criteria adopted for the protection of benthic biodiversity (oligo- to mesotrophic), aesthetics, and trout fishery values (meso- to eutrophic) in New Zealand streams (Biggs 2000[b]). The lines delineating the trophic boundaries were calculated using soluble inorganic N (SIN) equation 4 in [Table 11]. However, they also approximate P-limited communities by reference to the right-hand scale, which has been set at 0.1 x the SIN scale, because the mean ratio of biomass from the SIN and soluble reactive P (SRP) models was 10.8.[10]

--*TIAER Study*

The North Bosque River in central Texas has experienced elevated levels of Chl-a and nutrients, which have negatively impacted its contact recreation, aquatic life, and public water supply uses. Two segments of the river have been listed as impaired by Texas in its 303(d) report. Since the early 1990's, the Texas Institute for Applied Environmental Research (TIAER) has been performing water quality studies in the Bosque River watershed (Kiesling et al. 2001).

TIAER scientists modeled stream productivity – estimated by the lotic ecosystem trophic state index (LETSI) method – as a function of instream nutrient concentrations. The data fit a Monod type growth function (a model that relates algal growth to external nutrient concentrations). Using the half-saturation constant, McFarland et al. (2004) proposed a PO_4-P target of 0.023 mg/L for the North Bosque River. This phosphorus concentration was similar to the target

[10] Reprinted from Journal of the North American Benthological Society, Vol. 19(1), B.J.F. Biggs, Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae, pages 17-31, copyright 2000, with permission from the North American Benthological Society.

recommended by the North Bosque River Advisory Committee, 0.030 mg/L PO_4-P (McFarland et al. 2004). A description of the methodology used to reach this target is provided below.

TIAER scientists have applied LETSI, an in situ bioassay method developed at TIAER, to measure nutrient limitations and trophic status (Matlock et al. 1999, Kiesling et al. 2001, McFarland et al. 2004). The LETSI value is determined by relating the ratio of baseline primary productivity to maximum productivity. In other words, the researchers compare the Chl-a values from a control treatment to the Chl-a values from a N+P treatment. The LETSI values range from 0 to 1, with higher values representing nutrient enriched conditions. For example, a stream with a LETSI of 0.75 represents conditions exhibiting 75% of the potential growth response to nutrients. Streams with a LETSI value of 1.0 are at their maximum potential productivity.

In McFarland et al. (2004), analysis of variance (ANOVA) results indicated that when the LETSI values were greater than 0.5, neither N nor P were limiting. Conversely, LETSI values below 0.5 tended to be associated with N, P, or N+P limitation. The limiting nutrient can be identified by comparing the Chl-a values for treatments receiving solutions enriched with only one nutrient (N or P), a solution enriched with N+P, and the control solution. Using this method, Kiesling et al. (2001) observed that PO_4-P tended to be the limiting nutrient in the Bosque River watershed.

To determine the LETSI values, researchers deploy Matlock periphytometers in the study stream for a two-week period. The periphytometers work by allowing nutrients to diffuse from a bottle containing one of four treatment solutions across a semi-permeable membrane and onto a glass fiber filter that covers the mouth of the bottle and serves as a substrate for algal growth (Figure 7). Each bottle contains one of four treatment solutions: a control (deionized water), a N-treatment (NH_4NO_3), a P-treatment ($Na_2HPO_4 \cdot 7H_2O$), or a N+P-treatment (NH_4NO_3 and $Na_2HPO_4 \cdot 7H_2O$). An aluminum screen protects the algal growth from grazing invertebrates and fish. At the end of the deployment, the artificial substrate of each sample is analyzed for chlorophyll-a to estimate primary productivity. If algal growth is limited by nutrients, the samples receiving the nutrient solution treatments will be much higher compared to those receiving the control treatment.

Additionally, grab samples from the streams were collected for analyses of NH_3-N, NO_2-N+NO_3-N, TKN, PO_4-P, TP, and Chl-a. McFarland et al. (2004) found all N-limited LETSI values when DIN concentrations were below 0.05 mg/L and all P-limited LETSI values at or below PO_4-P concentrations of 0.01 mg/L.

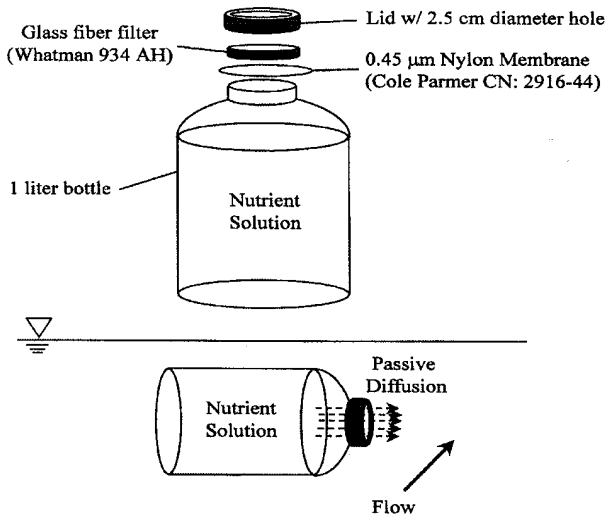
Data plotted for nutrient concentrations vs. LETSI values followed a Monod type growth pattern (Matlock and Rodriguez 1999, Kiesling et al. 2001, McFarland et al. 2004) (Figure 8). The Monod model is based on the following equation:

$$\mu = (\mu_{max} * S) / (K_s + S)$$

where μ = algal growth rate (represented by the LETSI value)
μ_{max} = maximum algal growth rate (represented by a normalized growth potential based on the general range of LETSI values)
S = external nutrient concentrations (represented by instream nutrient concentrations)

K_s = half-saturation constant (represented by the instream nutrient concentration when the LETSI is equal to half the maximum potential productivity).

McFarland et al. (2004) combined data from previous work (Matlock and Rodriguez 1999) with the results from their study and defined target concentrations based on the K_s value. They estimated a K_s value of 0.023 mg/L for PO_4-P, 0.145 mg/L for TP, 0.117 mg/L for DIN, and 1.34 mg/L for TN. The PO_4-P and TP data fit the Monod equation better than the DIN and TN data. Furthermore, the confidence intervals were tighter for the PO_4-P data than the TP data, indicating a closer biotic response of productivity to soluble P than to TP.



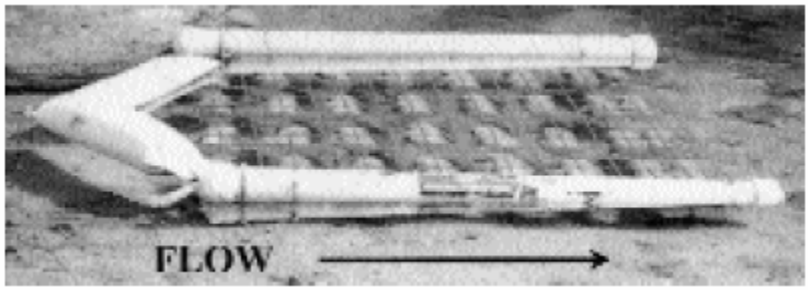


Figure 7. Diagrams of Matlock Periphytometer and photo showing Matlock Periphytometer treatment arrays.[11]

[11] Reprinted from Transactions of the ASAE; Vol. 42(3); M.D. Matlock, D.E. Storm, M.D. Smolen, M.E. Matlock, A.M.S. McFarland, and L.M. Hauck; Development and application of a lotic ecosystem trophic status index; pages 651-656; copyright 1999; with permission of the American Society of Agricultural and Biological Engineers.

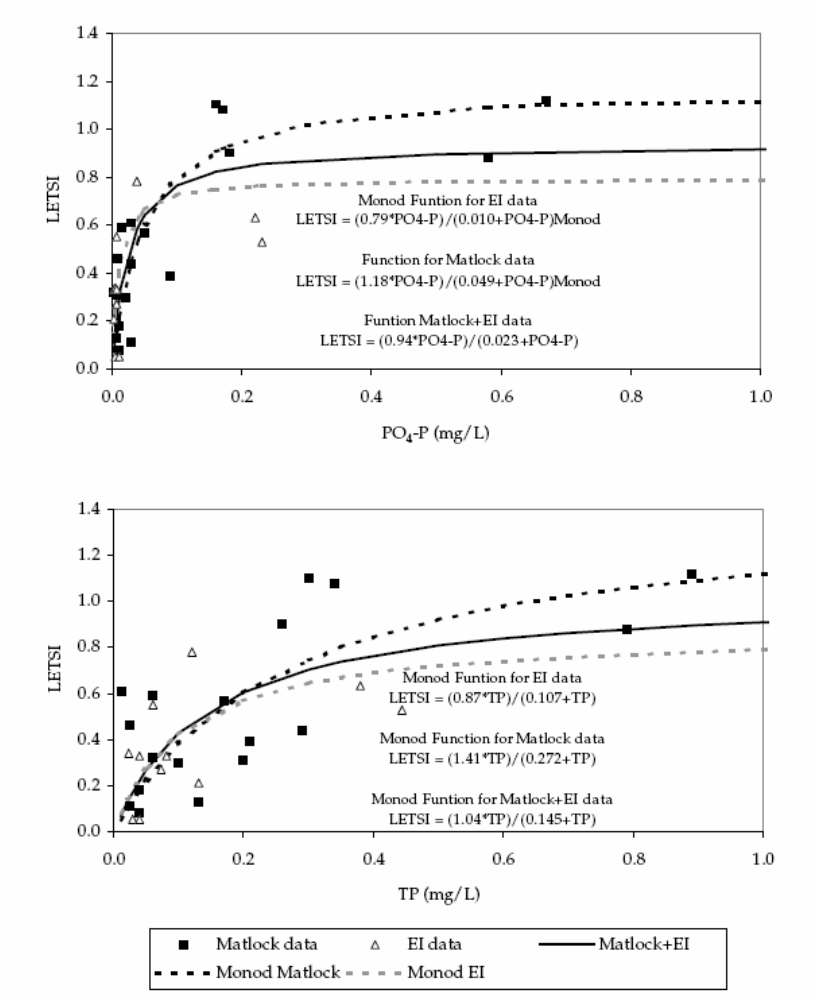


Figure 8. Monod curve fit of PO_4-P concentrations to LETSI values for P-limited and not limited results. EI = current study [McFarland et al. 2004] and Matlock = Matlock and Rodriguez (1999). X-axis truncated at 1.0 mg/L.[12]

--Ponader and Charles Study

The report by Ponader and Charles (2003) covers the first two years of a study to develop algal indicators for assessing cultural eutrophication. They used algal biomass and species composition plus water chemistry and physical stream measurements to develop models and metrics for determining the effects of nutrients in Piedmont streams in New Jersey (see Biocriteria section). Ponader and Charles (2003) found diatom inference models to be better predictors of nutrient concentrations and river eutrophication than either biomass measures or

[12] Reprinted from Using Periphytometers to Evaluate Impairment due to Nutrient Enrichment; TR0404; A. McFarland, R. Kiesling, and J. Back; 51 pp.; copyright 2004; with permission from Texas Institute for Applied Environmental Research, Tarleton State University, Stephenville, TX.

soft-algae inference models. For example, of all the parameters considered, Chl-a was only significantly correlated with visual estimates of *Cladophora* sp. coverage ($p = 0.01$, $r = 0.40$). Furthermore, with one exception (NH_3-N correlated at the 0.05 level with *Audouinella* sp.), there were no significant correlations between nutrients and soft algal species composition. In contrast, O-P and TP were found to have strong influence on diatom species distribution, and NO_3-N and NH_3-N expressed moderately strong influence on diatom species distribution.

Thus, nutrient inference models for each of the four nutrient variables were developed based on diatom species composition. The models were calibrated using two different data sets (complete 2000 data set [n = 85], 2001 data set [n = 54]) (Table 12). The best model, TP inference model, was tested by evaluating the distribution of observed TP values (from 2001 data set) versus the diatom-inferred TP values (model based on 2000 data set). The test showed that the TP model could be used to predict TP concentrations from other diatom samples collected in freshwater rivers in the New Jersey Piedmont ecoregion ($r = 0.78$, $r^2 = 0.61$, Ponader and Charles 2003). With this result, the authors recommended the use of the diatom inference model as a means to monitor eutrophication and outlined plans to improve the model to increase its predictive power.

Table 12. Predictive power of diatom inference models for total phosphorus (TP), orthophosphate (O-P), ammonia (NH_3-N) and nitrates (NO_3-N), as determined using weighted averaging (WA)-regression and calibration.[13]

	n = 85		**n = 54**	
Parameter	**r^2 (apparent)**	**$RMSEp_{(boot)}$ (log) µg/L**	**r^2 (apparent)**	**$RMSEp_{(boot)}$ (log) µg/L**
TP	0.72	0.33	0.69	0.22
O-P	0.69	0.42	0.67	0.31
NH_3-N	0.71	0.36	0.68	0.34
$N0_3$-N	0.68	0.26	0.64	0.21

Although predicting eutrophication was listed as a goal of the project, the study, as described in the report, has thus far focused on algal relationships to nutrients and not specifically to eutrophication. While use of diatoms to predict TP concentrations could be beneficial, there is still a need to relate TP concentrations to cultural eutrophication before setting nutrient criteria.

[13] Reprinted from Understanding the Relationship between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams, Final Report Year 2, Report No. 03-04, K. Ponader and D. Charles, New Jersey Department of Environmental Protection Division of Science, Research and Technology, copyright 2003, with permission of The Academy of Natural Sciences.

Empirical Models Relating Nutrients and Phytoplankton

--van Nieuwenhuyse and Jones Study

van Nieuwenhuyse and Jones (1996) developed a regression model to predict mean suspended chlorophyll values in small and large temperate streams. The model is based on summer (May – September) mean TP values (mg/m^3) and Chl concentrations (µg/L) from various literature sources for 292 temperate streams (Chl values were uncorrected for phaeophytin). Most of the data set is from North American streams (n = 231), particularly from tributaries of the Missouri and Mississippi rivers (n = 181). The mean TP concentrations ranged between 0.005 mg/L and 1.030 mg/L (5 mg/m^3 and 1,030 mg/m^3), and the mean Chl ranged between 0.4 µg/L and 170 µg/L (65% of Chl values ranged between 5 and 65 µg/L). Regression analysis showed that summer mean Chl values had a curvilinear relationship with summer mean TP concentrations (R^2 = 0.67, Figure 9).

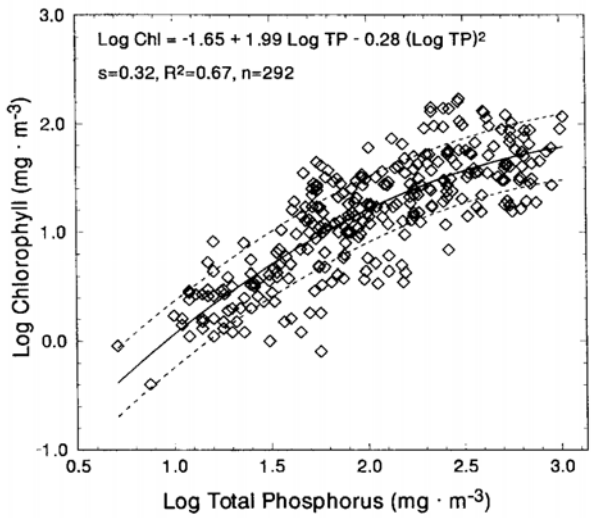


Figure 9. Log-log relation between mean total phosphorus concentration and mean chlorophyll concentration among streams. Dashed curves approximate 65% confidence interval for individual predicted values.[14]

Using a bivariate model, van Nieuwenhuyse and Jones (1996) also found that stream catchment area significantly affects the Chl concentrations at all levels of TP (Figure 10). Streams with larger catchment areas (10^5 km^2) had much higher Chl concentrations than those with smaller catchment sizes (10^2 km^2) for any given mean TP concentration. The authors interpret this finding as indicating that the hydraulic flushing rate or other physical factors may coregulate (regulate along with phosphorus) the amount of phytoplankton growth. They explain that the average phytoplankton biomass rate of loss decreases with increasing catchment size (van

[14] Reprinted from Canadian Journal of Fisheries and Aquatic Sciences, Vol. 53, E.E. van Nieuwenhuyse and J.R. Jones, Phosphorus-chlorophyll relationship in temperate streams and its variation with stream catchment area, pages 99-105, copyright 1996, with permission from National Research Council Canada.

Nieuwenhuyse and Jones 1996). Thus, phytoplankton biomass increases with increasing stream catchment area.

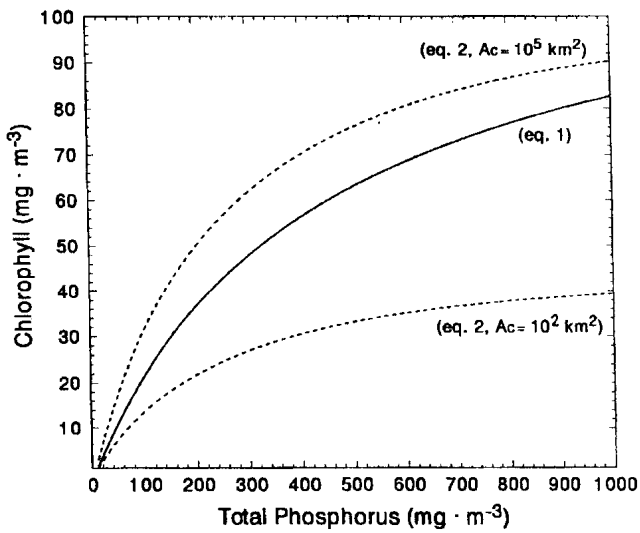


Figure 10. Arithmetic-scale plot of the phosphorus-chlorophyll relationship in streams and its variation with stream catchment area (A_c). The solid curve shows the predicted relationship for streams regardless of A_c; the broken curves show the predicted relationship for streams of widely differing A_c (100,000 and 100 km^2). Values are corrected for transformation bias.[15]

--Reckhow et al. Study

Reckhow et al. (2005) used structural equation modeling, SEM, to identify the relationships between nutrient-related parameters and the predictive use attainment for four waterbodies in the United States: Neuse Estuary, San Francisco Bay, Lake Washington, and Lake Mendota. They first identified the designated uses in each waterbody that could be potentially impacted by nutrients. They then used an expert elicitation approach, a well-established method to systematically obtain subjective judgments from experts. In this study, state officials or university scientists familiar with the particular waterbody were interviewed. Using the responses from the experts and water quality data, Reckhow et al. (2005) developed structural equation models to propose water quality criteria and relate the probability that the criterion would protect the designated use. Based on the results of their study, the authors found that the current Chl-a criterion of 40 µg/L and D.O. level of 5 mg/L in the Neuse Estuary has a 60% probability of attaining the use designations. Furthermore, the model predicts that assuming D.O. concentrations are at least 5 mg/L, Chl-a concentrations would need to be less than 10 µg/L to achieve a high level of attainment (Figure 11). The authors contend that realistically, this

[15] Reprinted from Canadian Journal of Fisheries and Aquatic Sciences, Vol. 53, E.E. van Nieuwenhuyse and J.R. Jones, Phosphorus-chlorophyll relationship in temperate streams and its variation with stream catchment area, pages 99-105, copyright 1996, with permission from National Research Council Canada.

criterion may not be attainable; however, quantifying the risk of nonattainment provides valuable information for use in setting criteria.

Structural equation modeling provides a means for studying a network of relationships among a set of correlated variables. An advantage to using the structural equation model includes its ability to test for indirect effects between two variables influenced by a third variable. For example, it can be used to study the impact that P has on zooplankton by affecting phytoplankton. Another advantage of the model is its ability to represent variables that are not directly measured, such as phytoplankton: often represented by Chl-a concentrations. One disadvantage of the current study is its use of only one expert for each waterbody. The authors recommend interviewing more than one expert in future studies and intend to make modifications to the described method.

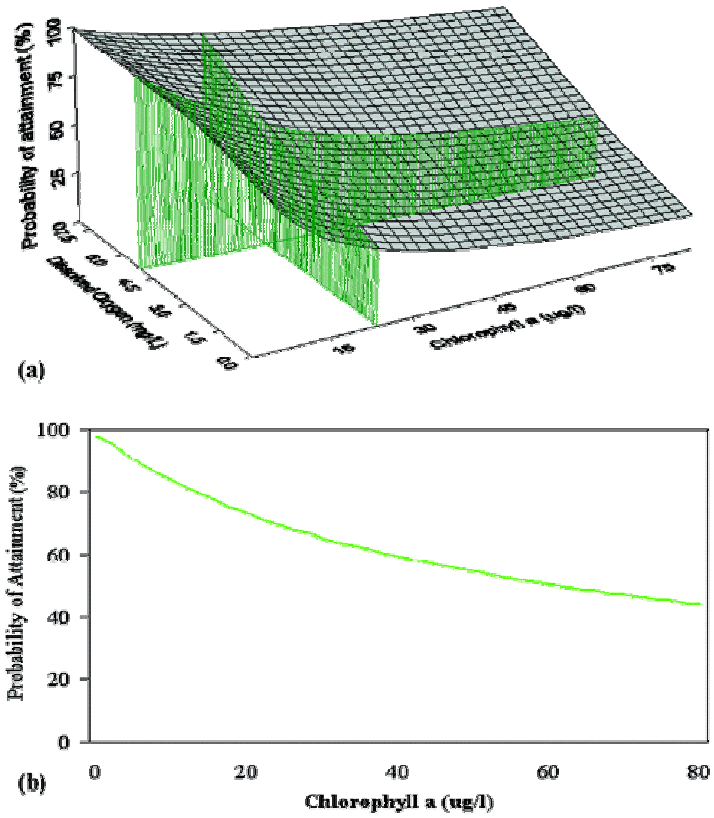


Figure 11. Application of the Neuse River Estuary structural equation model (SEM) for estimating the probability of attainment of the designated use (logit transformation of the expert's response) for the entire summer range of dissolved oxygen and chlorophyll *a* levels in the Neuse River estuary [Figure 11a]. The two surfaces correspond to specific levels of the two candidate criteria, i.e., dissolved oxygen = 5 mg/L and chlorophyll *a* = 20 µg/L (the latter is also shown in [Figure 11b]).[16]

[16] Reprinted from Environmental Science & Technology; Vol. 39(9); K.H. Reckhow, G.B. Arhonditsis, M.A. Kenny, L. Hauser, J. Tribo, C. Wu, K.J. Elcock, L.J. Steinberg, C.A. Stow, and S. J. Mc Bride; A predictive approach to nutrient criteria; pages 2913-2919, copyright 2005; with permission from the American Chemical Society.

Process-Based Models

AQUATOX

AQUATOX is a process-based model because it helps to identify and explain the cause and effect relationships between the chemical, physical, and biological components of aquatic ecosystems. The model, which was developed by U.S. EPA over a 30-year period (based on combining CLEAN, CLEANER, LAKETRACE, MACROPHYTE, PEST, and TOXITRACE models), predicts the fate of various environmental stressors (including nutrients) and their effects on algal, macrophyte, invertebrate, and fish communities. The model has been designed for streams and small rivers as well as ponds, lakes, and reservoirs. Potential applications of the model related to nutrient criteria development include simulations of nitrogen (total, nitrate, ionized and un-ionized ammonia), phosphorus (total, total soluble phosphate), chlorophyll-a, periphyton biomass, phytoplankton biomass, etc. AQUATOX has been successfully calibrated and validated against independent data in Walker Branch, Tennessee to simulate periphyton response to nutrients, light levels, grazing by snails, and variable flow (U.S. EPA 2001).

U.S. EPA developed a technical document that explains the individual components of the model so that the appropriateness of its applicability can be determined (Park and Clough 2004). To run the model, users must supply site, chemical, and biological characteristics. For example, to model the change in phytoplankton biomass, users must provide inputs for loadings of the phytoplankton, rate of photosynthesis, respiratory loss, photorespiration, nonpredatory mortality, herbivory, the loss or gain due to sinking, loss due to being carried downstream, and turbulent diffusion. AQUATOX can also be used with characteristics and loadings from BASINS data layers or from HSPF and SWAT watershed models (U.S. EPA models frequently used in TMDLs). The AQUATOX model uses differential equations to simulate numerous biological and ecological processes operating within the ecosystem. A simulation may cover a time period from several days to several years. Outputs from the model include biomass, biomass risk graphs, direct and indirect effects, etc.

The AQUATOX model may prove beneficial to state and tribal agencies in modeling processes to predict ecosystem effects from changes in streams and small rivers, e.g., reducing phosphorus loadings. An up-dated version of AQUATOX (Release 2.1) was published in 2005. U.S. EPA states that AQUATOX Release 2.1 offers improved simulation of periphyton and phytoplankton, particularly for river systems, and more realistically tracks the amount of nutrients in the waterbody. Testing of the model by U.S. EPA and improvements to the model are on-going. More information about AQUATOX can be found at http://www.epa.gov/ost/models/aquatox/ (accessed February 10, 2006).

C. BIOCRITERIA

The use of biocriteria to establish whether or not streams are impaired because of excess nutrients relies on the use of biological indicators (e.g., algal biomass, presence/absence of algal species, fish Index of Biotic Integrity [IBI]). Biocriteria can either be non-taxonomic or taxonomic. Non-taxonomic biocriteria rely on the mass or amount of the biological parameter

but make no distinction based on the species present or absent. Algal biomass can be expressed through measurements of periphyton Chl-a (mg/m^2), phytoplankton Chl-a (µg/L), AFDM (g/m^2), streambed coverage by filamentous algae (%), or turbidity (FTU, NTU). Taxonomic biocriteria, on the other hand, rely on species composition and knowledge of the tolerances and sensitivities of species.

Algal Bioindicators

Because in almost all instances nuisance levels of algae directly or indirectly cause the problems associated with excess nutrient input, U.S. EPA recommends using algal bioindicators to validate stream trophic classification (U.S. EPA 2000a). The use of algal bioindicators, however, is not yet common in the U.S. As of 2000, only Kentucky, Montana, and Oklahoma use algal data in their stream water quality assessments (U.S. EPA 2000a).

Indicator Algae

Indicator algal species are those with known sensitivities and tolerances and are therefore beneficial in describing water quality. For example, *Cladophora* is often associated with stream overenrichment and can thus be described as an indicator of nutrient overenrichment. The percent visual estimates of *Cladophora* sp. coverage (as determined from the rapid bioassessment method) were significantly correlated (at the 0.01 level) with Chl-a measurements ($r = 0.40$, Ponader and Charles 2003). Similarly, certain species of diatoms are used as indicator algae (as cited in U.S. EPA 2000a: KDOW 1993, Pan et al. 1996, Kelly et al. 1998). In a periphyton index of biological integrity developed by Hill et al. (2000) for streams in the Mid-Atlantic region, the authors identified one eutraphentic-diatom metric of particular importance for its association with nutrient impacted streams. Eutraphentic diatom genera identified in Hill et al. (2000) and included in the metric are presented in Table 13.

Table 13. Eutraphentic diatom algal genera present in streams of the Mid-Atlantic region as identified by Hill et al. (2000).

Eutraphentic Diatom Genera	
Amphora	*Melosira*
Asterionella	*Meridion*
Cocconeis	*Nitzschia*
Cymatopleura	*Rhoicosphenia*
Diatoma	*Stephanodiscus*
Fragillaria	*Synedra*
Gomphonema	*Thalassiosira*
Gyrosigma	

Algal Growth Studies

--*Heinonen Study*

Heinonen (1984) used algal growth plates in a nutrient study of a waterway situated between two lakes in southern Finland. Evidence showed that the discharge from a large fish breeding station located on the waterway was negatively affecting the aquatic life and recreational uses of the waterway by lowering the reproduction rate of the natural trout population, increasing the growth of periphyton on stones in some rapids, and causing slime growth on some beaches in the downstream lake. The mean loading to the waterway from the fish station was estimated at 93.4 kg/d N and 15.6 kg/d P during the study period. Heinonen (1984) placed growth plates upstream and downstream from the fish station and measured the amount of periphyton (represented by Chl-a) that grew during a three-week period (with three incubation times during the study period, July 20 – September 22, 1981). Significant differences were observed between the upstream and downstream plates, with the downstream growth plates having much more periphyton (Table 14). The mean nutrient concentrations in the water column collected during the study period, however, were low and not significantly different. The author concluded that Chl-a is more sensitive in identifying the early stages of eutrophication than are N and P water column concentrations (Heinonen 1984).

Table 14. Mean water quality and periphyton growth for a waterway in Finland impacted by a point source. Samples collected from July 20 – September 22, 1981 (adapted from Heinonen 1984).

	Immediately Upstream	**4 km Downstream**	**15 km Downstream***
TN (mg/L)	0.355	0.360	0.385
NO_3-N (mg/L)	0.043	0.043	0.043
NH_4-N (mg/L)	0.010	0.014	0.013
TP (mg/L)	0.009	0.011	0.012
PO_4-P (mg/L)	0.002	0.001	0.002
Chl-a (mg/m^2)	0.97	2.73	3.99

*located downstream of the second lake.

--*Tualatin River Study*

About 40 miles of the Tualatin River in the Portland, Oregon region were listed impaired for not supporting fishing, contact recreation, aesthetics, and aquatic life as a result of nuisance algal growths. Additionally, the downstream lake into which the Tualatin River flows was not supporting some of its designated uses because of excess algae. As part of the TMDL development for this river, a series of algal growth studies was performed. The purpose of the study was to set a TP limit for the river that would meet Oregon's established criteria of maintaining a mean suspended Chl-a level of 15 µg/L. From the results of the study, the researchers observed a reduction in periphyton when TP levels were held below 0.100 mg/L and

lower growth rates in periphyton at TP levels below 0.050 mg/L. Based on this study, a TMDL target limit, which included a margin of safety (i.e., a more conservative limit), was set as an average TP concentration of 0.070 mg/L during the growing season (based on monthly means from May 1 to October 31) (U.S. EPA 1999).

Biotic Indices

Biotic indices are based on the correlation of algal assemblages with current and past physical and chemical characteristics of the habitat. Indices are usually comprised of six to ten metrics that include a measure of the abundance of organisms, species richness, and trophic structure. The metrics are based on their sensitivity to human activities related to nutrient input to streams, their precision, and their usefulness among different regions and habitat types. Selected metrics should reflect the breadth of biological responses to varying nutrient levels (Hill et al. 2000, U.S. EPA 2000a). Indices generally rely on reference conditions to evaluate streams.

Periphyton Indices

--*Ponader and Charles Study*

Ponader and Charles (2003) tested benthic algal indices to indicate stream impairment owing to excess nutrients. They analyzed samples for diatoms and water chemistry from 1st – 6th order wadeable streams in the Piedmont ecoregion of New Jersey. A Spearman's rank-order correlation was used to determine if six different diatom metrics were correlated with different environmental variables used to signify stream impairment, e.g., land use, nutrient concentrations. The following three metrics: Siltation Index, centric diatoms/pennate diatoms, and number of diatom taxa in the sample showed moderately strong/strong ($r > 0.3$) and significant correlations (at 0.01 level) with basin size, NO_3-N, TP, and O-P. (The Siltation Index metric represents the frequency and severity of sedimentation and is indicated by the percent abundance of species in the genera *Navicula, Nitzschia, Cylindrotheca,* and *Surirella.*)

Furthermore, Ponader and Charles (2003) used the New Jersey Piedmont diatom data set to calculate three different diatom indices used in Europe (Trophic Diatom Index, Biological Diatom Index, and Specific Polluosensitivity Index). Because the flora in Europe differs somewhat from that in New Jersey, only about 80% of the diatom species in the Northern Piedmont samples were used in the calculations. Despite the species differences, the three European diatom indices were good predictors of TP and O-P in New Jersey Piedmont rivers and indicate a potential for their use in the U.S., particularly if they are modified to include North American diatom species (Ponader and Charles 2003).

--*Ponader, Flinders, and Charles Study*

Ponader et al. (2005) conducted a pilot study in Virginia in an attempt to develop algal indices and determine the best metrics for identifying streams with nutrient impairments. Thirty-seven stream sites from across the state were randomly selected using a probabilistic monitoring approach. The streams were located in four ecoregions of Virginia: Central Appalachians, Central Appalachian Ridge and Valley, Blue Ridge Mountains, and Piedmont. Within each

stream, during the period from late August to early November in 2004, samples were collected to determine algal biomass (estimated as Chl-a and AFDM) and algal species composition (diatoms and soft algae). Additionally, stream samples were analyzed for NH_3+NH_4, NO_2, NO_3, NO_2+NO_3 (reported as NO_3-N), TP, and O-P. Ordination analysis (CANOCO for Windows, version 4.5) was used to study patterns in the species composition data and to determine the strength of the correlation between environmental variables (e.g., nutrient concentrations, Chl-a values) and species composition. A Pearson correlation matrix was used to compare nutrient concentrations (TP and NO_3-N) with algal metrics, trophic indices, and Chl-a levels. In the study, NO_3-N was used to establish nitrogen categories because TN data were unavailable for the study period (Ponader et al. 2005).

Four hurricanes struck Virginia during the field season of this study and were believed to have greatly impacted the results of the study. For example, high flows forced the researchers to reduce the sampling sites from 50-60 streams to 37 streams. Furthermore, of the 343 diatom taxa identified, scour-resistant taxa were prevalent, suggesting that high flows may have impacted the algal species compositions. Also, the authors speculated that high flows resulted in lower levels of algal biomass than expected at the high nutrient sites. Additionally, the hurricanes likely impacted the nutrient concentrations (Ponader et al. 2005).

A small sample size, narrow range of nutrient concentrations, and uneven distribution of data points along the nutrient gradient limited the ability of the researchers to model the assemblage-nutrient relationships. The NO_3-N data ranged from less than 0.1 mg/L to 1.14 mg/L, with most of the samples being below 0.3 mg/L. The TP data ranged from less than the detection limit (0.01 mg/L) for 13 of the 37 streams to 0.16 mg/L. Most of the TP concentrations were below 0.05 mg/L (Ponader et al. 2005). The narrow and uneven distribution of the nutrient concentrations may have resulted from the hurricanes and/or the use of random sample sites, which tends to lead to a clumping of less extreme values.

Despite the drawbacks of this pilot study, the authors were able to draw several conclusions from the study:

- NO_3-N and TP concentrations explained significant variation in the diatom (particularly) and soft algae species assemblages.
- Algal assemblages differed among the four ecoregions, indicating a need to develop indicators at the ecoregion level.
- Significant ($P < 0.01$) and strong correlations were measured between (1) TP concentrations and the Siltation Index, (2) TP concentrations and the high TP Index developed from a USGS NAWQA data set, and (3) NO_3-N concentrations and Chl-a values.

From the observed changes in the diatom assemblages, the authors suggested threshold limits of 0.5 mg/L for NO_3-N and 0.05 mg/L for TP to protect against conditions they termed as nutrient impairment. The NO_3-N threshold was selected because benthic chlorophyll-a levels above 100 mg/m^2 occurred at NO_3-N levels above 0.5 mg/L, although several sites with NO_3-N levels above 0.5 mg/L did not exceed benthic chlorophyll-a levels of 100 mg/m^2. The TP threshold identification was based on the finding that several diatom species indices correctly assigned samples to the TP concentration categories 0.01-0.05 mg/L and 0.05-0.10 mg/L, indicating a change in the diatom species composition above and below 0.05 mg/L TP (Ponader et al. 2005).

Ponader et al. (2005) suggested conducting additional, multi-year sampling across all ecoregions in Virginia for all nutrient conditions. The new data set could then be used to develop diatom inference models and algal-based indicators for each ecoregion (or by combining data sets for ecroregions with similar characteristics).

--*Hill and Others Study*

Hill et al. (2000) developed a 10-metric periphyton index of biological integrity (PIBI) for streams in the Mid-Atlantic region. The PIBI is based on (1) algal genera richness, (2) relative abundances for diatoms and cyanobacteria, (3) Chl-a and AFDM data, and (4) alkaline phosphatase activity (Metrics include: richness, diatom, cyanobacteria, dominant diatom, acidophilic diatoms, eutraphentic diatoms, motile diatoms, chlorophyll, biomass, and phosphatase). The index was based on generic-level identifications so that non-experts in algal taxonomy can use it. The environmental preferences of the diatom genera were taken from published studies (as cited in Hill et al. 2000: Lowe 1974, Christie and Smol 1993, van Dam et al. 1994). One metric of particular importance for identifying nutrient impacted streams is based on eutraphentic diatoms, which are often associated with nutrient impacted streams (as cited in Hill et al. 2000: Palmer 1969, Lange-Berlatot 1979, Hall and Smol 1992, Christie and Smol 1993, Pan et al. 1996) (Table 13).

Data from 233 stream site-visits in the Mid-Atlantic region (including streams in Virginia) had PIBI scores that ranged from 48.0 to 85.1 (out of a possible 100), with an overall regional score ($\pm$ 1 SE) of 66.1 $\pm$ 0.5. Significant differences in the PIBI scores for lowland versus highland streams and for the different stream orders were observed. Hill et al. (2000) classified streams in the upper 25th percentile (PIBI $\geq$ 72) as acceptable and those in the lower 25th percentile (PIBI $\leq$ 60) as degraded.

Phytoplankton Indices

The Kentucky Division of Water (KDOW) is in the process of developing the environmental preferences for dominant and abundant phytoplankton taxa in Kentucky. For this process, the agency is analyzing samples for phytoplankton density (cells/mL) and/or phytoplankton community structure. If blooms are present, the KDOW field biologists record the bloom characteristics (e.g., odor if present, surface sheen if present, color of surface scum, thickness of bloom, etc.). The biologists also estimate and record the algal bloom coverage from bank to bank: absent (0%), sparse (1 – 29%), moderate (30 – 59%), dense (60 – 99%), and total (100%). In the laboratory, phytoplankton samples are allowed to settle (to concentrate the sample), identified to the lowest possible taxon, and quantified as algal density, biovolume, taxa richness, diversity, percent similarity, or other metrics (KDOW 2002).

The metric results are compared to those from control sites, other similar sites in the watershed, reference conditions, historic data, or data published in the scientific literature. Impairment is determined for each use—aquatic life, drinking water supply, and recreation. To determine if streams are supporting their aquatic life use, KDOW assesses the phytoplankton community and compares their findings with published information on the water quality conditions under which

different taxa thrive (based on studies from Whitford and Schumacher 1963, Bennett 1969, Lowe 1974, Palmer 1977, Patrick 1977, Collins and Weber 1978, van Landingham 1982, and others as cited in KDOW 2002). To assess the potential impact of phytoplankton on drinking water supplies, algae associated with taste and odor problems and potentially toxic taxa are identified and enumerated. To determine the impairment of recreational use, KDOW assesses algal blooms and bloom potential from Chl-a and/or cell density (KDOW 2002).

Saprobic Bioindicators

Species of algae, bacteria, protozoan, and fungi that obtain their energy from dissolved nutrients in the surrounding water, generally from decaying organic matter, are called *saprozoic organisms*. As a group, they form a community referred to as *sewage fungus,* a useful indicator of organic pollution.

The Saprobic System was developed in the early 1900's by Kolkwitz and Marsson (Friedrich et al. 1996). It is based on observations in changes of stream biota immediately downstream of highly organic point sources and recovery of the biota further downstream. Streams are divided into saprobic zones based on their physical, chemical, and biological characteristics. In Europe, the Saprobic System is used to monitor the effects of treated wastewater on streams (as cited in Hill et al. 2000: Lange-Bertalot 1979, Friedrich et al. 1992).

The first Saprobic Index was developed in 1955 by Pantle and Buck (Friedrich et al. 1996). In general, for each saprobic-indicator species at a given sampling site, the frequency of occurrence and its saprobic value are denoted numerically following the established methodology. The product of these two numbers yields the saprobic value for that particular indicator species at the given stream location. The Saprobic Index for a particular sampling station is calculated as the weighted mean of all the indicator species at that site. The index has been refined several times, e.g., by Sládecek (1973) and Friedrich et al. (1990). In the most recent refinement by Friedrich et al. (1990), the metrics pertaining to photoautotrophic species (those that can obtain energy through photosynthesis) were removed to "avoid interactions between indicating saprobity and trophic status" (Friedrich et al. 1996, p 192.).

Data obtained using the Saprobic Index, modified to only include North American species, might be helpful in the nutrient criteria development process, but no attempts to modify and use the index in this country are known. Alternatively, modifying the method to only consider the photoautotrophic indicator species might prove beneficial for identifying the trophic state of streams.

Bacterial Bioindicators

Lemly (2000) developed a method of identifying nutrient enriched streams with the use of bacterial growth on aquatic insect larvae (Figure 12). From laboratory studies, Lemly found that mayfly (*Epeorus* sp.) mid-instar larvae with more than 25% of their bodies covered by bacteria had mortality rates of about 100% while nearly all those uninfested with bacteria survived. Mayflies with 10 – 25% coverage by bacteria appeared healthy and unaffected.

Lemly (2000) tested his method in a field study in the Appalachian Mountains of Virginia (Craig Creek, Montgomery County). He measured TN and total orthophosphate concentrations in the water column, aquatic insect density, and bacteria coverage on insect larvae upstream and downstream of a cattle pasture. The nutrient concentrations were significantly higher downstream than upstream ($p < 0.01$). Downstream from the cattle pasture, the density of the insect community was lower, and the occurrence and amount of bacterial infestation was higher. Lemly (2000) proposed that bacterial coverage of aquatic insects provides a practical, quick, and easy way to screen for nutrient enrichment in streams.

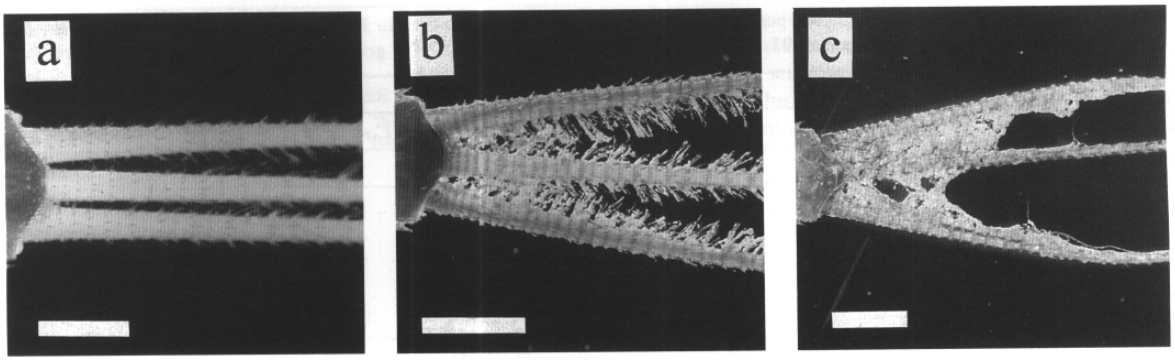


Figure 12. Characteristic appearance of bacterial growth on caudal cerci (tail filaments) of *Ephemerella* sp. immersed in 80% ethanol. Plate a (20X magnification) shows uninfested cerci with delicate, hair-like setae visible. Plate b (20X magnification) illustrates the appearance of heavy bacterial growth (>25% of body covered). Bacterial sheaths nearly fill the space between cerci and obscure the delicate setae. Plate c (15X magnification) shows an advanced stage of colonization in which bacterial filaments have become matted and partially covered by silt particles. Infestation of the degree shown in plates b and c was associated with 100% mortality in laboratory survival studies and reduced numbers of mayflies in the field. The condition can be easily diagnosed in the field using a hand lens with 10 – 20X magnification. Scale bars = 0.5 mm.[17]

Lemly and King (2000) applied the method described above to two third-order streams in low flowing, cypress-gum wetlands in the Cape Fear basin in North Carolina. The two watersheds were physically similar, and the land use was similar in both watersheds except that hog farms

[17] Reprinted from Wetlands, Vol. 20(1), A.D. Lemly and R.S. King, An insect-bacteria bioindicator for assessing detrimental nutrient enrichment in wetlands, pages 91-100, copyright 2000, with permission from BioOne.

were located in the watershed of one of the streams. Nutrient levels tended to be 2 to 10 times higher in the stream with hog farms located in the watershed. For instance, mean TN concentrations in the unenriched stream ranged from about 0.715 to 1.97 mg/L, whereas mean TN concentrations in the nutrient-rich stream ranged from 1.927 to 3.889 mg/L. Similarly, the mean TP concentrations ranged from 0.054 to 0.198 mg/L in the unenriched stream and ranged from 0.169 to 0.620 mg/L in the enriched stream. Although peak nutrient values in the unenriched streams sometimes exceeded the lowest concentrations in the enriched streams, the authors concluded that conditions must not have been favorable to cause bacterial blooms. All insect orders in the unenriched stream were free of bacterial growth, whereas bacteria (*Sphaerotilus* sp. and *Leptothris* sp.) colonized all orders of insect larvae in the stream with higher nutrient levels. Additionally, the abundance of mayflies was significantly higher in the unenriched stream compared to the enriched stream. Laboratory tests using mayflies (*Ephemerella* sp. and *Drunella* sp.) collected from the streams yielded a threshold of 25% body coverage by bacteria for survival (Lemly and King 2000).

The authors note that because field-collected insects were used in the laboratory studies, other factors, besides bacteria, could have accounted for the differences in survivorship. Also Lemly (2000) mentions that none of the field-collected nymphs with heavy bacterial growth were mature, while nymphs without bacterial growth were represented by all life stages. It may be possible that the life stage at collection could have influenced survivorship, i.e., older nymphs with no coverage of bacteria may have been better able to handle the stress of habitat change than younger nymphs.

Macroinvertebrate and Fish Bioindicators

Miltner and Rankin Study

Miltner and Rankin (1998) used physical, chemical, and biological data from streams throughout Ohio to study the relationship between nutrients and biotic integrity. Biotic integrity is defined as the "extent to which a community has a species composition, diversity and functional organization comparable to that expected for the natural habitat of a region." (Miltner and Rankin 1998, p. 2, citing Karr and Dudley 1981). For each study site, they assessed the fish community with the Index of Biotic Integrity (IBI, modified for Ohio), the macroinvertebrate community utilizing the Invertebrate Community Index (ICI), habitat using the Qualitative Habitat Evaluation Index (QHEI), and water samples for TP, TIN, and NH_3. The metrics for all indices (IBI, ICI, and QHEI) were scored against reference conditions, with higher scores denoting higher biotic integrity or higher quality stream habitat.

Stream size classes were divided into categories based on catchment area:

Headwaters:	< 20 mi^2
Wadeable streams:	21 – 200 mi^2
Small rivers:	201 – 1,000 mi^2
Large rivers:	$\geq$ 1,001 mi^2.

These stream classes were selected primarily because of observed changes in the patterns of the biological community and nutrient concentrations and because of theoretical expectations about nutrient concentrations for different stream classes (Rankin et al. 1999). Using the stream size

class and frequency distributions of the P and N concentrations (25th, 50th, 75th, and 90th percentiles), the streams were assigned one of six codes:

Code 1: both $\leq P_{25}$ N_{25}
Code 2: either $\leq P_{50}$ N_{50}
Code 3: $\leq P_{75} \leq N_{90}$
Code 4: $> P_{75} < > N_{90}$
Code 5: both $\geq P_{90}$ N_{90}
Code 6: $NH_3 \geq 1.0$ mg/L

Miltner and Rankin's Macroinvertebrate Results

Habitat scores (QHEI) generally explained between 20% to 30% of the variance in ICI scores (and IBI scores) across all stream sizes and were significant for all stream classes. In contrast, TP concentrations only explained from 2% to 16% of the variation, depending on stream size and model used.

Regression results from nutrient-and-habitat models and water-quality models indicated that ICI scores were, in general, negatively correlated with increasing TIN and TP concentrations in headwaters and wadeable streams. To some extent, macroinvertebrates were also shown to respond positively to intermediate levels of nutrient enrichment in wadeable streams. For example, when the TIN and TP concentrations in wadeable streams were between the 50th and 75th percentiles (TIN:1.65 – 3.63 mg/L; TP:0.12 – 0.32 mg/L), the abundance of mayflies was highest. High N concentrations, TIN > 75th percentile, had the largest effect on macroinvertebrates in headwater (TIN > 3.61 mg/L) and wadeable streams (TIN > 3.63 mg/L). For example, at the higher nutrient concentrations, the number of EPT taxa (mayflies, stoneflies, and caddisflies; indicators of "good" water quality) and relative abundance of Tanytarsini midges decreased while the relative abundance of other non-Tanytarsini dipterans and non-insects increased (Miltner and Rankin 1998).

Differences in the ICI scores as a response to TIN and TP concentrations were not evident for small and large rivers. However, Miltner and Rankin (1998) found lower mean ICI scores for all stream classes where the NH_3-N was greater than or equal to 1.0 mg/L, indicating the possibility of chronic NH_3 toxicity.

Miltner and Rankin's Fish Results

As with the macroinvertebrate ICI indices, habitat scores generally explained the majority of the variance in the fish IBI scores (across all stream sizes and models). Fish community indices, however, appeared to be more influenced by nutrient concentrations than were macroinvertebrate community indices. The IBI scores negatively correlated with higher TIN and TP concentrations in headwaters and wadeable streams. In small rivers, only high concentrations of TIN negatively affected the IBI scores, and in large rivers, no relationships for TIN and TP were apparent. As with the macroinvertebrate ICI scores, the fish IBI scores were significantly lower whenever NH_3-N exceeded 1.0 mg/L for all stream classes (Miltner and Rankin 1998).

More specifically, Miltner and Rankin (1998) found headwater streams with either TIN below 1.37 mg/L or TP below 0.17 mg/L (50th percentiles) to have significantly higher IBI scores than headwaters with higher nutrient concentrations. For wadeable streams, the mean IBI scores were significantly higher the lower the nutrient concentration ($25^{th} > 50^{th} > 75^{th} > 90^{th}$ percentiles), such that the highest IBI scores were for fish communities where TIN concentrations were less than 0.61 mg/L and TP was less than 0.06 mg/L (25th percentiles).

In headwaters and wadeable streams with low or intermediate nutrient concentrations (< 50th percentile; headwaters: TIN < 1.37 mg/L, TP < 0.17 mg/L; wadeable: TIN < 1.65 mg/L, TP < 0.12 mg/L), the number of sensitive fish species was significantly higher. Similarly, in nutrient-rich headwaters, wadeable streams, and small rivers, the relative abundance of tolerant and omnivorous fish increased significantly. In large rivers, there were no observed relationships between the fish community and nutrient concentrations, except that top carnivores were positively related to higher nutrient levels. A comparison of the macroinvertebrate data with the fish data suggested that a loss in EPT taxa corresponded with a decrease in the number of sensitive fish. Also, an increase in the number of dipterans and non-insects related to a decrease in insectivorous fish and an increase in omnivorous fish.

Miltner and Rankin (1998) found that TP concentrations in Ohio were highest where habitat quality is lowest. They cautioned that the decreases observed in ICI and IBI scores along a TP gradient may also be reflecting degraded habitat conditions. In wadeable streams, however, the IBI differences behaved independently of habitat conditions (at least up to the 50th percentile), supporting "a cause and effect relationship between nutrients and biotic integrity" (Miltner and Rankin 1998, p. 156).

Ohio EPA Study

In an Ohio EPA publication, *Association Between Nutrients, Habitat, and the Aquatic Biota in Ohio Rivers and Streams*, Rankin et al. (1999) documented the localized effects of nutrients on the aquatic organisms of streams and rivers in Ohio and proposed statewide criteria for nitrate+nitrite nitrogen and TP. The authors also present a method for using the nutrient concentrations in grab samples to rank the relative risk to aquatic life use attainment.

Water column nutrient concentrations, collected from June 15th to October 15th during 14 field seasons, were correlated with IBI, ICI, and QHEI scores. Higher IBI and ICI values signify higher biotic integrity. An approximate narrative description of the scores suggests:

< 20 = very poor
20 – 29 = poor
30 – 39 = fair
40 – 49 = good
50 – 60 = exceptional.

Similarly, higher QHEI scores reflect higher quality stream habitats, with scores in the range of 61 to70 reflecting the highest quality stream habitats reported in this study (Rankin et al. 1999).

The data were separated by ecoregion (HELP: Huron/Erie Lake Plain; ECBP: Eastern Corn Belt Plains; WAP: Western Allegheny Plateau; EOLP: Erie-Ontario Lake Plane; and IP: Interior

Plateau) as well as being divided into stream class by catchment size (using the same stream classes as Miltner and Rankin 1998). Data from reference sites (the REF database) were utilized in setting expected values for least impacted sites in streams designated as warmwater habitats (WWH) and exceptional warmwater habitats (EWH), or best attainable in streams designated as modified warmwater habitats (MWH; channelized, impounded, or otherwise significantly altered waterways). Furthermore, a database that represents a wide range of expected anthropogenic impacts (the ALL database) was used to develop relationships between nutrient concentrations and impacts to the biological community. Rankin et al. (1999) theorized that measured parameters with strong effects would show a strong correlation, and parameters with weak effects or those that covary with other factors would yield weaker correlations.

Summary statistics of nitrate+nitrite nitrogen concentrations obtained from the REF database, the oligo-mesotrophic and meso-eutrophic boundaries given by Dodds et al. (1998), and suggested statewide criteria are shown in Table 15. The concentrations of NO_3-N in the reference streams varied by ecoregion and stream size. The highest concentrations were generally found in the large rivers. Rankin et al (1999, p.2) state: "While nutrient concentrations are expected to increase in the larger mainstem rivers, the concentrations considered as 'reference' are themselves indicative of enrichment that is largely the product of anthropogenic sources and activities."

Analysis of the ALL database indicated that only the highest median NO_3-N values (>3 – 4 mg/L) had a negative relationship with the IBI and ICI scores, and consistently only in headwater streams (IBI only) and small rivers (IBI and ICI). In large rivers, median NO_3-N seldom exceeded 4 mg/L (ALL database), and no relationship between nitrogen and the biological indices were observed for this stream class (Rankin et al. 1999).

Table 16 shows the median total phosphorus concentrations from the REF and ALL databases for different IBI ranges, analysis of variance (ANOVA) significance levels for differences in IBI scores between TP categories, and proposed statewide TP criteria to protect aquatic life. For most ecoregions and stream sizes, the sites with the highest water quality, as determined from its REF classification or high IBI and ICI scores, had lower TP medians. Median TP concentrations at REF sites were frequently less than 0.10 mg/L (except in the HELP ecoregion and in large rivers). An analysis of the ALL data (excluding large rivers) yielded median TP concentrations less than 0.15 mg/L at sites receiving exceptional IBI and ICI scores (50 – 60), and the ALL sites with IBI and ICI ratings of "good" (40 – 49) generally had TP concentrations less than 0.20 mg/L (Table 16) (Rankin et al. 1999).

Additionally, TP concentrations were higher at locations with low quality habitats. These findings suggest that TP, or a covariate (e.g., sediment, toxins), are directly affecting the biological community. For example, Rankin et al. (1999) found that TP is frequently a covariate with other factors, particularly where point source discharges occur. These factors are known to result in an increased incidence of fish with deformities, eroded fins, lesions, and tumors — characteristics associated with chronic stress.

Table 15. Median and seventy-fifth percentile nitrate+nitrite nitrogen concentrations [mg/L] by stream size and ecoregion for reference sites [in the following Ohio ecorgeions: HELP, Huron/Erie Lake Plain; IP, Interior Plateau; EOLP, Erie-Ontario Lake Plane; WAP, Western Allegheny Plateau; and ECBP, Eastern Corn Belt Plains], oligo-mesotrophic and meso-eutrophic boundaries given by Dodds et al. (1998), and proposed statewide criteria for WWH [warmwater habitat], EWH [exceptional warmwater habitat], and MWH [modified warmwater habitat] streams. Values corresponding to the IBI [Index of Biotic Integrity] range typical of the MWH use best attainable concentrations for MWH streams.[18]

	Ecoregional Criteria					Oligo-mesotrophic Meso-eutrophic boundaries†	State-wide Criteria		
	HELP	IP	EOLP	WAP	ECBP		WWH	EWH	MWH*
Headwaters (drainage area < 20 mi^2)									
median	0.38	0.49	0.42	0.15	0.98	0.7			
75th %	2.26	1.18	1.00	0.34	2.24	1.5	1.0	0.5	1.0
20 - 29	1.22	3.15	0.56	0.21	0.86				
Wadable (drainage area 20 mi^2 < 200 mi^2)									
median	0.16	0.24	0.43	0.22	0.84	0.7			
75th %	0.60	0.54	1.05	0.47	2.80	1.5	1.0	0.5	1.6
20 - 29	0.68	1.42	1.60	0.50	1.34				
Small Rivers (drainage area 200 mi^2 < 1000 mi^2)									
median	1.88	0.43	1.00	0.64	1.65	0.7			
75th %	3.24	0.96	1.42	1.02	3.06	1.5	1.5	1.0	2.2
20 - 29	2.01	-	1.97	1.55	1.88				
Large Rivers (drainage area > 1000 mi^2)									
median	1.47	2.63	-	1.50	3.08	0.7			
75th %	2.76	2.93	-	2.20	4.14	1.5	2.0	1.5	2.4
20 - 29	1.73	-	-	2.60	3.98				

† Oligotrophic-mesotrophic and mesotrophic-eutrophic boundaries are given by Dodds et al. (1998) and were derived from data sets covering a wide range of stream sizes.

* MWH criteria are the statewide median concentrations from the ALL database for an IBI range of 20 - 29.

[18] Reprinted from Association between Nutrients, Habitat, and the Aquatic Biota in Ohio Rivers and Streams; Ohio EPA, Technical Bulletin MAS/1999-1-1; E. Rankin, B. Miltner, C. Yoder, and D. Mishne; 70 pp; published 1999.

Table 16. Median total phosphorus concentrations [mg/L] by IBI range (from the ALL data set), ANOVA results, and suggested criteria for the protection of aquatic life.[19]

	Ecoregion Criteria						Statewide Criteria		
IBI Range[a]	HELP	IP	EOLP	WAP	ECBP	ALL[c]	WWH[†]	EWH[†]	MWH
Headwaters (drainage area < 20 mi^2)									
20 – 29	0.42	2.88	0.19	0.05	0.58	0.34			
40 – 49	-	0.13	0.05	0.05	0.07	0.06			
50 – 60	-	0.05	-	0.05	0.05	0.05			
ANOVA[b]	ns	ns	0.05	ns	0.05	0.05	0.08	0.05	0.34
Wadable (drainage area 20 mi^2 < 200 mi^2)									
20 – 29	0.33	0.50	0.25	0.07	0.22	0.28			
40 – 49	-	0.15	0.07	0.05	0.11	0.09			
50 – 60	-	0.07	0.05	0.05	0.08	0.07			
ANOVA	ns	ns	0.10	0.10	0.10	0.05; 0.10	0.10	0.05	0.28
Small Rivers (drainage area 200 mi^2 < 1000 mi^2)									
20 – 29	0.25	-	0.20	0.25	0.25	0.25			
40 – 49	-	0.33	0.12	0.08	0.16	0.18			
50 – 60	-	0.15	0.08	0.05	0.17	0.14			
ANOVA	ns	ns	0.10	0.10	ns	ns	0.17	0.10	0.25
Large Rivers (drainage area > 1000 mi^2)									
20 – 29	0.22	-	-	0.51	0.60	0.32			
40 – 49	-	0.35	-	0.18	0.41	0.34			
50 – 60	-	-	-	0.15	0.46	0.24			
ANOVA	ns	ns	-	ns	ns	ns	0.30	0.15[*]	0.32

[a]Median total phosphorus concentrations for the given IBI range are from Appendix Table 2 [in Rankin et al. (1999)].

[b]ANOVAs were run on three categories of total phosphorus concentrations, ≤ 0.05, 0.06 ≤ 0.10, and > 0.10, total phosphorus concentrations listed in ANOVA rows show concentrations where difference in IBI scores between categories were significant.

[c]For IBI ranges, ALL is the average of all ecoregions. Data were pooled across ecoregions for the ALL ANOVAs, otherwise ANOVAs were stratified by ecoregion and drainage area.

[†]Values in the WWH and EWH columns represent suggested total phosphorus concentrations that are protective of aquatic life.

[*] TP concentration chosen to reflect N:P ratio ≥ 10.

[ns] ANOVAs for the stream size and ecoregion were not significant (P > 0.05)

[19] Reprinted from Association between Nutrients, Habitat, and the Aquatic Biota in Ohio Rivers and Streams; Ohio EPA, Technical Bulletin MAS/1999-1-1; E. Rankin, B. Miltner, C. Yoder, and D. Mishne; 70 pp; published 1999.

Rankin et al. (1999) also developed a system for ranking the relative risk to aquatic life based on nutrient concentrations in grab samples (Table 17). The predictive model is based on the probability of departing from the nutrient concentrations of (1) the REF database or (2) the subset of the ALL database that correlates with IBI and ICI scores supporting the aquatic life use for warmwater habitats or exceptional warmwater habitats (e.g., IBI scores of 40+). To determine the risk, the frequency distribution of the sampling results is compared to the distribution for the reference sites (REF) or to the distribution of the subset of the ALL data that reflects minimally impacted conditions. Specifically, the 50th (median) and 90th percentiles of the water samples are compared to the 50th, 75th, or other percentiles of the REF data and ALL data that support the desired biocriteria. The more the sample distribution deviates from these reference values, the higher the probability that the tested stream reach will not attain the criteria associated with the warmwater habitat or exceptional warmwater habitat aquatic life uses.

From the results of the study, statewide criteria for nitrate+nitrite and TP were proposed (Tables 15 and 16). Because streams with high nutrient concentrations sometimes have exceptional biological communities, Rankin et al. (1999) warn against using the proposed criteria in the same way as criteria for toxic substances are used. Instead, they recommend: "a tiered or multicriteria approach, especially in light of the importance of habitat. In other words, a single exceedance should not necessarily trigger a violation of water quality standards…. Moreover, nutrient values should not be interpreted in a vacuum of biological information given that high values of both can co-occur. Instances where biological index scores meet the biological criteria but nutrient concentrations are high, implies that nutrients are not *locally* problematic" (Rankin et al. 1999, p. 3).

Problems may still occur downstream owing to nutrients from upstream sources. Rankin et al. (1999) suggests sampling all along the stream reach or river system to determine whether nutrients are assimilated before causing a problem. Furthermore, they recommend a determination of the trophic state of the stream from certain measured values, such as Chl-a or the composition of the periphyton community, in response to nutrient levels. Secondary effects, such as diel dissolved oxygen variations or the presence of cyanotoxins, should also be monitored according to Rankin et al. (1999).

Table 17. A process for determining the risk to aquatic life use attainment based on TP and NO_3-N concentrations in Ohio rivers and streams. The risk of aquatic life impairment increases as sample statistics increasingly deviate from reference site results (REF) or observed associations between biological community performance and nutrients (ALL data).[20]

Risk of Impairing Aquatic Life	Sample Median	Sample 90th Percentile
None	Less Than REF Median for Aquatic Life Use	Less Than ALL or REF Median for Aquatic Life Use
Minimal	Less Than REF Median for Aquatic Life Use	Less Than ALL or REF 75th %tile for Aquatic Life Use
Low	Greater Than REF Median for Aquatic Life Use	Less Than ALL or REF 75th %tile for Aquatic Life Use
Moderate	Greater Than REF 75th %tile for Aquatic Life Use	Less Than ALL or REF [Median + 2* Interquartile Range] for Aquatic Life Use
Mod./High	Greater Than REF [Median + 2* Interquartile Range] for Aquatic Life Use	Less Than ALL or REF [Median + 2* 75th %tile - Median] for Aquatic Life Use
High	Greater Than ALL or REF [Median + 2* Interquartile Range] for Aquatic Life Use	
Extreme		Greater Than ALL or REF [Median + 2* Interquartile Range] for Aquatic Life Use

3. PUBLISHED NUTRIENT CRITERIA AND THRESHOLD APPROACH

In addition to using either the reference reach approach or the predictive relationship approach, U.S. EPA offers that literature values may be used as criteria "if a strong rationale is presented that demonstrates the suitability of the threshold value to the stream of interest (i.e., the system of interest should share characteristics with the systems used to derive the threshold, published values)" (U.S. EPA 2000a, p. 100). U.S. EPA furthermore suggests that published values could

[20] Reprinted from Association between Nutrients, Habitat, and the Aquatic Biota in Ohio Rivers and Streams; Ohio EPA, Technical Bulletin MAS/1999-1-1; E. Rankin, B. Miltner, C. Yoder, and D. Mishne; 70 pp; published 1999.

serve as temporary limits until other criteria can be established from reference reaches or other means.

ESTABLISHED GUIDELINES AND CRITERIA

United States Criteria

In the United States, criteria are the part of the water quality standards designed to support the uses of the waterbody. Criteria can be expressed as numeric concentrations or levels, or as narrative statements.

There are no national phosphorus or nitrogen criteria for fresh waters to protect against eutrophication. However, U.S. EPA warns that exceeding the nitrate criterion for drinking water (set to protect human health) and ammonia criterion (set to protect aquatic life from toxicity) may cause problems of enhanced algal growth (U.S. EPA 1986 as cited in U.S. EPA 2000a). The national water quality criteria to protect public drinking water is 10 mg/L for nitrate-N, 1 mg/L for nitrite-N, and 10 mg/L for nitrate-N + nitrite-N (40 CFR 141.62). U.S. EPA has also established ammonia criteria for freshwaters to protect aquatic life. These criteria are based on toxicity data on aquatic organisms, with the toxicity level varying depending on water pH and temperature. Virginia's established ammonia criteria can be found in the Virginia Water Quality Standards – 9 VAC 25-260-155 (http://www.deq.virginia.gov/wqs/) (accessed August 1, 2005).

Presently, all states have narrative criteria related to nutrients (U.S. EPA 2003a). Examples of narrative criteria include:

> Indiana—All waters shall be free from substances "… that will cause or contribute to the growth of aquatic plants or algae to such degree as to create a nuisance, be unsightly or deleterious or be harmful to human, animal, plant, or aquatic life or otherwise impair the designated uses" (327 IAC 2-1-6).
>
> New Mexico—"Plant nutrients from other than natural causes shall not be present in concentrations which will produce undesirable aquatic life or result in a dominance of nuisance species in surface waters of the state" (20 NMAC 6.4.12).
>
> Virginia—"State waters, including wetlands, shall be free from substances attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards or interfere directly or indirectly with designated uses of such water or which are inimical or harmful to human, animal, plant, or aquatic life. Specific substances to be controlled include, but are not limited to: …substances which nourish undesirable or nuisance aquatic plant life" (9 VAC 25-260-20).

Examples of numeric nitrogen, phosphorus, and turbidity criteria and guidelines for streams and rivers (and U.S. EPA's drinking water standards for nitrates) are reported in Tables 18, 19 and 20. Although not a complete list, these summaries show the variation of criteria and guidelines within individual states and between states.

Table 18. Examples of freshwater numeric values (excluding lakes and reservoirs) for nitrogen criteria and guidelines set by U.S. EPA and U.S. states.

Form of Nitrogen	State and Waters	Nitrogen Criteria Values	Reference
Total N	**Arizona** River specific:	Annual mean: 0.3 - 1.0 mg/L 90th percentile: 0.75 - 2.50 mg/L Single sample max.: 0.10 - 10.00 mg/L	AAC R18-11-109
	Hawaii Inland streams	Geometric mean, not to exceed 0.25 mg/L --Wet season (Nov. 1-Apr. 30) 0.18 mg/L -- Dry season (May 1-Oct. 31)	HAR 11-54-5.2
Nitrate-N + nitrite-N	**Hawaii** Inland streams	Geometric mean, not to exceed 0.07 mg/L -- Wet season (Nov. 1-Apr. 30) 0.03 mg/L -- Dry season (May 1-Oct. 31)	HAR 11-54-5.2
	Ohio Ohio River	Maximum limit: 10 mg/L (Based on toxicity effects)	OAC 3745-1
Nitrate-N	**U.S. EPA** Public drinking water	Maximum limit: 10 mg/L (Based on toxicity effects)	40 CFR 141.62
	Nevada* River specific	Mostly maximum limit: 10 mg/L; Maximum: 90 mg/L for Virgin River (at mouth of Lake Mead to Mesquit)	NAC 445A. NAC 445A.177
	New Jersey Pineland waters	Maximum: 2 mg/L, unless shown that a lower level is needed to protect existing water quality.	NJAC 7:9B-1.14(b)
	North Dakota Class 1 streams	Maximum limit: 1.0 mg/L (interim guideline limit)	NDAC 33-16-02-09
	Utah Streams and rivers to protect aquatic wildlife; 3B, 3C waters	Maximum limit: 4 mg/L (Used as pollution indicator; when exceeded, further investigations are conducted)	UAC R317-2 (Table 2.14.2)
	Vermont Class A1 & A2 (> 2,500 ft); Class A1 & A2 (< 2,500 ft); Class B	Maximum limit: 0.20 mg/L at low median monthly flow 2.0 mg/L at low median monthly flow 5.0 mg/L at low median monthly flow	VWQS 3-01-B3
Nitrite-N	**Nevada*** River specific	Mostly maximum: 0.06 mg/L or 1.0 mg/L Maximum: 5 mg/L for Virgin River (at mouth of Lake Mead to Mesquit)	NAC 445A NAC 445A.177
	Ohio Ohio River	Maximum limit: 1.0 mg/L (Based on toxicity effects)	OAC 3745-1

* Different requirements may exist to maintain existing higher quality streams.

Table 19. Examples of numeric criteria and guidelines for total phosphorus in the United States.

State and Waters	Phosphorus Criteria Values	Reference
Arizona River Specific	Annual mean: 0.05 - 0.20 mg/L 90 percentile: 0.10 - 0.33 mg/L Single sample maximum: 0.20 - 1.0 mg/L	AAC R18-11-109
Arkansas All waters	Maximum limit: 0.100 mg/L (guideline)	2 AAC 2.509
Hawaii Inland streams	Geometric mean, not to exceed 0.050 mg/L -- Wet season (Nov. 1-Apr. 30) 0.030 mg/L -- Dry season (May 1-Oct. 31)	HAR 11-54-5.2
Illinois Streams at entrance to reservoir or lake with surface area of 8.1 hectares or more	Maximum limit: 0.05 mg/L	35 IAC 302.205
Nevada* River specific	Mostly, average: 0.1 mg/L	NAC 445A
New Jersey Streams	Maximum limit: 0.1 mg/L, unless demonstrate TP is not a limiting nutrient and will not render the waters unsuitable for designated uses.	NJAC 7:9B-1.14(c)
New Mexico Perennial reaches of specific waters in Rio Grande, Pecos River, and San Juan River basins	Maximum limit (single sample): 0.1 mg/L	20 NMAC 6.4.109 20 NMAC 6.4.208 20 NMAC 6.4.404 20 NMAC 6.4.407
North Dakota Class I, IA, II and III streams	Maximum limit: 0.1 mg/L (interim guideline limit)	NDAC 33-16-02-09
Oregon Yamhill River and its tributaries	Monthly median: 0.070 mg/L as measured during summer low flow	OAR 340-041-0350
Utah Streams and rivers to protect aquatic wildlife; 3B, 3C waters	Maximum limit: 0.05 mg/L (used as pollution indicator; when exceeded, further investigations are conducted)	UAC R317-2 (Table 2.14.2)
Vermont Upland streams (> 2,500 ft.)	Maximum limit: 0.010 mg/L at low median monthly flow	VWQS 3-01-B2
Washington Spokane River (river mile: 34-58)	Average euphotic zone: 0.025 mg/L (during June 1 to October 1)	WAC 173-201A-130

* Different requirements may exist to maintain existing higher quality streams.

Table 20. Examples of numeric turbidity criteria for U.S. states applicable to freshwater streams and rivers. Source of information came from individual state surface water quality regulations located at the following website: http://www.epa.gov/waterscience/standards/states/.
NTU = Nephelometric Turbidity Units

State and Waters	Criteria
Alabama All use classes, except "limited warm water fishery"	Not to exceed 50 NTU above the background turbidity
Connecticut Class AA, A, and B	Not to exceed 5 NTU over ambient levels and none exceeding level necessary to protect and maintain all designated uses
Delaware Freshwater	Not to exceed natural levels by more than 10 Nephelometric or Formazin turbidity units
Florida All classes Mixing zones	Not to exceed 29 NTU above natural background conditions at any time Average ≤ 41 NTU above natural background conditions
New Hampshire Class B	Not to exceed naturally occurring conditions by more than 10 NTUs
New Jersey Freshwater with discharge	Maximum 30-day average of 15 NTU. Maximum of 50 NTU at any time
North Carolina Streams (not trout waters) Streams designated as trout waters	Not to exceed 50 NTU; if exceeded due to natural conditions, the existing turbidity level cannot be increased Not to exceed 10 NTU; if exceeded due to natural conditions, the existing turbidity level cannot be increased
Pennsylvania Neshaminy Creek Basin	Not to exceed 100 NTU to protect potable water supply, warm water fishes, and migratory fishes Not to exceed 40 NTU for May 15-Sept. and not more than 100 NTU for Sept.16-May 14 to protect cold water fishes in addition to potable water supply, warm water fishes, and migratory fishes
Rhode Island Class A Class B, B1, and C	Not to exceed 5 NTU over natural background Not to exceed 10 NTU over natural background
South Carolina Trout waters	Not to exceed 10 NTU or 10% above the natural conditions, provided existing uses are maintained
Vermont Class A(1) and A(2) Class B-cold water fish habitat Class B-warm water fish habitat	Not to exceed 10 NTU Not to exceed 10 NTU Not to exceed 25 NTU
West Virginia All waters	Not to exceed 10 NTU's over the background turbidity for waters with 50 NTU or less; Not to exceed more than a 10% increase in turbidity (plus 10 NTU minimum) when the background turbidity is more than 50 NTU's

Only two states, North Carolina and Oregon, were found to have numeric chlorophyll-a criteria applicable to freshwater rivers and streams. The criteria for these states are listed below:

> North Carolina—"Chlorophyll a (corrected): not greater than 40 µg/L for lakes, reservoirs, and other waters subject to growths of macroscopic or microscopic vegetation not designated as trout waters, and not greater than 15 µg/L for …waters subject to growths of macroscopic or microscopic vegetation designated as trout waters…" (15A NCAC 02B.0211).
>
> Oregon—"The following average Chlorophyll a values must be used to identify water bodies where phytoplankton may impair the recognized beneficial uses: …rivers: 0.015 mg/L [15 µg/L]" (OAR 340-041-0019).

British Columbia, Canada Guidelines

The Ministry of Water, Land and Air Protection in British Columbia set *guidelines*—safe levels of substances for the protection of a given water use—to protect some or all of the following uses: (1) drinking water, (2) aquatic life, (3) agriculture (livestock watering and irrigation), (4) wildlife, (5) recreation and aesthetics, and (6) industry (food processing water). The guidelines, in conjunction with specific site characteristics, are considered in developing water quality *objectives*, which are used to set waste discharge limits. Neither guidelines nor objectives have legal standing, however, the discharge limits derived from them do have legal standing (BC Guidelines 1998).

British Columbia has established maximum periphyton-biomass guidelines, measured as Chl-a, to protect aesthetic, recreational, and aquatic life uses. To protect the first two uses, a 50 mg/m^2 Chl-a criterion was set. This criterion was based on "literature and experiences reported from British Columbia" (Nordin 1985). To protect aquatic life, a maximum Chl-a level of 100 mg/m^2 was suggested: "This criterion is designed primarily to protect fish habitat and changes in communities or organisms such as invertebrates which are important themselves or which may be important fish-food organisms" (Nordin 1985).

In explaining why algal biomass is used as an indicator of recreational and aesthetic protection instead of nutrients, the ministry offers that nutrients "…are not likely to cause any direct problems in terms of body contact or visual deterioration. The more likely problem would be eutrophication-related problems when high concentrations of nitrogen (and accompanying phosphorus) cause heavy accumulations of algae" (Nordin and Pommen 1986). Furthermore, there are other conditions, "…water velocity, light, temperature and invertebrate grazing pressure which must be satisfied before nutrients become the most important factor limiting stream algal growth" (Nordin 1985).

United Kingdom Nutrient Status

The United Kingdom assesses its river quality using its General Quality Assessment whereby four aspects of river quality—biology, chemistry, nutrients, and aesthetic quality—are measured at about 7,000 sites representing about 40,000 km of rivers and canals in England and Wales.

The latest river quality assessment for nutrients was conducted in 2004. For this assessment, nitrates greater than 30 mg/L in rivers and phosphates greater than 0.1 mg/L in rivers were considered to be high concentrations (www.environment-agency.gov.uk) (accessed August 1, 2005).

Australian and New Zealand Guidelines

Narrative and numeric guidelines to protect the water quality of freshwater streams from excessive enrichment have been published in *Australian and New Zealand Guidelines for Fresh and Marine Water Quality* (2000), referred to in this literature review as the A-NZ Guidelines. The two councils that developed and published the guidelines strongly caution: "***These Guidelines should not be used as mandatory standards*** because there is significant uncertainty associated with the derivation and application of water quality guidelines" (A-NZ Guidelines 2000, p. 1-1).

The guidelines pertaining to nuisance organisms, such as algae, are designed to protect primary contact (e.g., swimming), secondary contact (e.g., boating, fishing), and visual (aesthetics) use. According to the guidelines pertaining to nuisance organisms: "Macrophytes, phytoplankton, scums, filamentous algal mats, sewage fungus … should not be present in excessive amounts. Direct contact activities should be discouraged if algal levels of 15,000 – 20,000 cells/mL are present, depending on the algal species" (A-NZ Guidelines 2000, p. 5-3). To protect secondary contact, the guidelines state: "To protect water-skiers from injury and boating vessels from damage, the water should be free from …excessive growth of algae and other aquatic plants. The quality of the water should be maintained so that there is minimal alteration of the fish habitat" (A-NZ Guidelines 2000, p. 5-3). Furthermore, the narrative guidelines protecting the visual use state that waters should be free from "undesirable aquatic life, such as algal blooms, or dense growth of attached plants…." (A-NZ Guidelines 2000, p. 5-4).

In addition to the general statements, the councils also define low-risk guideline trigger values. Below is a section from the A-NZ Guidelines (2000, p. 3.3-5) that describes how these values are to be used.

> The guideline trigger values are the concentrations (or loads) of the key performance indicators, below which there is a low risk that adverse biological effects will occur. The physical and chemical trigger values are not designed to be used as 'magic numbers' or threshold values at which an environmental problem is inferred if they are exceeded. Rather they are designed to be used in conjunction with professional judgment, to provide an initial assessment of the state of a water body regarding the issue in question. They are the values that trigger two possible responses. The first response, to continue monitoring, occurs if the test site value is less than the trigger value, showing that there is a 'low risk' that a problem exists. The alternative response, management/remedial action or further site-specific investigations, occurs if the trigger value is exceeded — i.e. a 'potential risk' exists. The aim with further site-specific investigations is to determine whether or not there is an actual problem. Where, after continuous monitoring, with or without site-specific investigations, indicator values at sites

are assessed as 'low risk' (no potential impact), guideline trigger values may be refined. The guidelines have attempted as far as possible to make the trigger values specific for each of the different ecosystem types.

For the trigger values reported in the guidance document, the 80th and/or 20th percentiles of the reference data from slightly disturbed ecosystems within geographical regions of Australia and New Zealand were used. The nutrient-related Australian and New Zealand water quality guidelines for fresh waters are summarized in Table 21, however, the trigger values associated with tropical Australia (northern Queensland, the Northern Territory and north-west Western Australia) and low rainfall areas (South Australia) are not included. The reported trigger values can be made more or less stringent depending on the level of protection desired by the stakeholders and in consultation with experts. The document recommends using biological and ecological effects data, reference system data, predictive modeling, and professional judgment when proposing changes to the trigger values (A-NZ Guidelines 2000).

Table 21. Default trigger values for slightly disturbed ecosystems as reported in *Australian and New Zealand Guidelines for Fresh and Marine Water Quality* (2000). Values for some specific rivers, alpine streams, and glacial and lake-fed streams differ from those reported here. Chl-a = chlorophyll-a, TN = total nitrogen, NO_x = oxides of nitrogen, NH_4^+ = ammonium, TP = total phosphorus, FRP = filterable reactive phosphate, D.O. = dissolved oxygen, and Turb. = turbidity.

Region	**Ecosystem type**	**Chl-a** (µg/L)	**TN** (mg/L)	**NO_x** (mg/L)	**NH_4^+** (mg/L)	**TP** (mg/L)	**FRP** (mg/L)	**D.O. lower limit** (% sat.)	**D.O. upper limit** (% sat.)	**Turb.** (NTU)
S.E. Aus.	Upland rivers	na	0.250	0.015	0.013	0.020	0.015	90	110	2-25
S.E. Aus.	Lowland rivers	5	0.500	0.040	0.020	0.050	0.020	85	110	6-50
S.W. Aus.	Upland rivers	na	0.450	0.200	0.060	0.020	0.010	90	na	10-20
S.W. Aus.	Lowland rivers	3-5	1.200	0.150	0.080	0.065	0.040	80	120	10-20
N.Z.	Upland rivers	na	0.295	0.167	0.010	0.026	0.009	99	103	4.1
N.Z.	Lowland rivers	no data	0.614	0.444	0.021	0.033	0.010	98	105	5.6

--South-east Australia applies to Victoria, New South Wales, south-east Queensland, the Australian Capital Territory, and Tasmania.
--South-west Australia applies to southern Western Australia
--Upland streams are defined as those > 150 m altitude.
--na = not applicable; for Chl-a, monitoring of periphyton biomass is recommended in upland rivers, and periphyton biomass (Chl-a, mg/m^2) levels were not developed at publication.

Biggs (2000b) published periphyton guidelines for New Zealand streams and rivers (Table 22). The guidelines were developed to prevent degradation of aesthetic, recreation, and aquatic life uses. The guidelines for aesthetics and recreation only apply during the summer months (November 1 – April 30 in New Zealand) when recreational use is likely. The guidelines for benthic biodiversity and fishing apply year-round.

In the New Zealand guidelines, percent periphyton coverage that protects the aesthetic and primary contact uses refers to the part of the streambed that is generally visible from the stream bank during summer low flows (usually < 0.75 m deep) or walked on. Biggs (2000b) notes that it is not an aesthetic problem for individual rocks to have thick slime, but when 60% of the streambed is covered by a thick slime, visual and recreational uses are negatively impacted. Biggs and Price (1987) reported that when filamentous algae exceed 40 percent cover, it is very conspicuous. However, experience in New Zealand has shown that water users find this level too high (Biggs 2000b). A guideline of 30 percent filamentous coverage was therefore suggested (Biggs 2000b). Corresponding biomass, in terms of filamentous green algae, was set at 35 g/m^2 AFDM and about 120 mg/m^2 Chl-a (estimated from work by Horner et al. 1983, Nordin 1985, Welch et al. 1988, Welch et al. 1989, and Dodds et al. 1998). Biggs (2000b) states, "However, because these biomass criteria have only been arrived at subjectively, it would be worthwhile to carry out a quantitative study to define human perception of algal proliferations and what might constitute 'too much' algae for recreational and aesthetic use of streams" (p. 93).

Table 22. Provisional biomass and cover guidelines for periphyton growing in gravel/cobble bed streams for three main instream values (AFDM = ash-free dry mass).[21]

Instream value/variable	Diatoms/cyanobacteria	Filamentous algae
Aesthetics/recreation (1 November - 30 April) Maximum cover of visible stream bed	60 % >0.3 cm thick	30 % >2 cm long
Maximum AFDM (g/m^2)	N/A	35
Maximum chlorophyll-a (mg/m^2)	N/A	120
Benthic biodiversity Mean monthly chlorophyll-a (mg/m^2)	15	15
Maximum chlorophyll-a (mg/m^2)	50	50
Trout habitat and angling Maximum cover of whole stream bed	N/A	30 % >2 cm long
Maximum AFDM (g/m^2)	35	35
Maximum chlorophyll-a (mg/m^2)	200	120

[21] Reprinted from New Zealand Periphyton Guideline: Detecting, Monitoring and Managing Enrichment of Streams, B.J.F. Biggs, 122 pp, copyright 2000, with permission from Ministry for the Environment.

To set guidelines to protect benthic biodiversity, Biggs (2000b) analyzed data from paired periphyton-invertebrate samples collected at about 30 sites in more than 20 New Zealand streams. From this work, he found, for example, that EPT taxa (indicators of "good" water quality) decreased substantially when periphyton biomass exceeded about 5 g/m^2AFDM, and streams that exceeded this level tended to have more midges, worms, and snails (indicators of "poor" water quality). Using AFDM values and the regression equation:

$$\text{Ln Chl-a } (mg/m^2) = 0.338 + 1.396 \times \text{Ln AFDM } (g/m^2) \ (r^2 = 0.790, n = 170),$$

Biggs (2000b) recommended Chl-a values of about 13 – 20 mg/m^2. To determine maximum Chl-a values, Biggs (2000b) analyzed the peak biomass of 16 oligotrophic streams with diverse benthic invertebrate communities having "good" water quality indicator taxa. The mean peak biomass, as measured from Chl-a, for these streams was 47 mg/m^2. Higher biomasses were generally only found on a one-time occurrence. Based on these studies, Biggs (2000b) set provisional guidelines to protect benthic biodiversity at 15 mg/m^2 for mean monthly Chl-a and 50 mg/m^2 for maximum Chl-a.

The guidelines for filamentous algal levels to protect benthic biodiversity are less certain. Biggs (2000b) states, "… the relationship between the biomass of filamentous green algae and invertebrates is poorly defined and requires considerably more research" (p. 97). Based on one study and "in the absence of any more specific research on the effects on benthic invertebrate communities of filamentous green algal proliferations, the diatom/cyanobacteria guidelines are also recommended for use with filamentous communities where biodiversity is an issue" (Biggs 2000b, p. 97).

To develop guidelines to protect trout habitat and angling uses, Biggs (2000b) considered scientific data from New Zealand and North America. For example, he examined Chl-a data from 10 river reaches in New Zealand known for their trout fisheries. To avoid low D.O. levels that could negatively impact fish, Quinn and McFarlane (1989) recommended that *Cladophora* biomass in the Manawatu River (New Zealand) should stay below 34 g/m^2 AFDM during summer. Based on these findings, Biggs (2000b) recommended maximum biomass of 35 g/m^2 AFDM (corresponding to about 200 mg/m^2 Chl-a for diatom-dominated communities and 120 mg/m^2 Chl-a for filamentous algal communities). The impact of filamentous algae on the fishing experience was also considered based on the appearance and smell of the water and in regard to the amount of fouling of lures and wet flies that filamentous algae could cause. Biggs also comments that periphyton proliferations can negatively affect the taste of fish. Biggs (2000b) concludes: "In the absence of any other information, the aesthetics/contact recreation guidelines for percent cover of filamentous algae should be adopted" to protect the angling use (p. 101).

Using the guidelines proposed in Table 22, Biggs (2000b) derived mean monthly guidelines for specified average days of accrual expected to prevent maximum Chl-a levels (i.e., 50 mg/m^2 to protect benthic biodiversity; 120 mg/m^2 to protect aesthetics, trout habitat, and angling in communities dominated by filamentous green algae; and 200 mg/m^2 to protect trout habitat and angling in communities dominated by diatoms) (Table 23). The guidelines were primarily derived from a regression model that Biggs developed. This model combines days of accrual and mean monthly soluble nutrient concentrations (see Models section). The regression

equations were developed from 30 New Zealand rivers sampled every 2 – 4 weeks for at least 13 months:

$$\text{Log}_{10}\,(\text{max. Chl-a}) = 4.285 \times (\text{Log}_{10}\, d_a) - 0.929 \times (\text{Log}_{10}\, d_a)^2 + (0.504 \times \text{Log}_{10}\, \text{SIN}) - 2.946 \quad (r^2 = 0.741)$$

$$\text{Log}_{10}\,(\text{max. Chl-a}) = 4.716 \times (\text{Log}_{10}\, d_a) - 1.076 \times (\text{Log}_{10}\, d_a)^2 + (0.494 \times \text{Log}_{10}\, \text{SRP}) - 2.741 \quad (r^2 = 0.721)$$

where Chl-a is in mg/m^2, d_a refers to days of accrual, and SIN and SRP are in mg/m^3.

Biggs (2000b) cautions that because the guidelines were derived from an empirical model, they will contain some error. He further warns, "It is important not to get bound up in minor breaches of the recommended nutrient levels," acknowledging that under natural conditions, mean monthly nutrient concentrations may exceed the guidelines without causing excess proliferations of periphyton (Biggs 2000b, p. 104). Particularly in reference to protecting benthic diversity (Chl-a = 50 mg/m^2), Biggs (2000b) suggests that resource managers, "focus on 'outcomes' rather than 'inputs' as measures of success" (p. 104) or in other words, determine if the benthic macroinvertebrate community is being supported under the existing water quality conditions.

Table 23. Soluble inorganic nitrogen (SIN = NO_3-N + NO_2-N + NH_4-N) and soluble reactive phosphorus (SRP) concentrations [mg/L] predicted to prevent maximum periphyton biomass from exceeding the given levels. The nutrient concentrations were determined as mean monthly concentrations over a year. Limits of detection are assumed to be around [0.005 mg/L] for SIN and [0.001 mg/L] if analyses are carried out using standard autoanalyser techniques. The chlorophyll-a at 120 mg/m^2 refers to filamentous green algae dominated communities whereas the chlorophyll-a at 200 mg/m^2 refers to diatom dominated communities. AFDM = ash-free dry weight. [Biggs (2000b) reported SIN and SRP concentrations in mg/m^3; for consistency within this review, these values were converted to mg/L.][22]

Study	Chlorophyll-a= 50		AFDM=35 Chlorophyll-a= 120 Chlorophyll-a= 200	
Days of accrual	SIN	SRP	SIN	SRP
20	< 0.020	< 0.001	< 0.295	< 0.026
30	< 0.010	< 0.001	< 0.075	< 0.006
40	< 0.010	< 0.001	< 0.034	< 0.0028
50	< 0.010	< 0.001	< 0.019	< 0.0017
75	< 0.010	< 0.001	< 0.010	< 0.001
100	< 0.010	< 0.001	< 0.010	< 0.001

[22] Reprinted from New Zealand Periphyton Guideline: Detecting, Monitoring and Managing Enrichment of Streams, B.J.F. Biggs, 122 pp, copyright 2000, with permission from Ministry for the Environment.

NUTRIENT THRESHOLDS OR RECOMMENDED ALGAL LIMITS

A nutrient threshold can be defined as the concentration at which an effect, such as eutrophication or biological impairment, begins to occur. This section of the literature review focuses on threshold values derived from studies conducted within Virginia and outside the state (mainly). Chl-a values published in the literature have been suggested as "thresholds" for over-enrichment effects. Most of the published nitrogen and phosphorus values discussed in this section, however, were not presented in the literature as "threshold values." These studies are included in the review to show similarities and differences between results from studies that examine the impact of nutrients on aquatic life. Threshold values derived from arbitrary distinctions within frequency distributions (e.g., one third of streams are oligotrophic, one third are mesotrophic, and one third are eutrophic) are not included.

Chlorophyll-a Thresholds

Periphyton

Suggested benthic Chl-a thresholds to identify eutrophic conditions in streams are in the range of 50 – 200 mg/m^2 (Table 24). Citing several studies (e.g., Horner et al. 1983; Welch et al. 1988, 1989; Biggs 1996, 2000b; and Dodds et al. 1998), Biggs (2000a) states, "Biomass levels > 150 – 200 mg/m^2 chlorophyll *a* are very conspicuous in streams, are probably unnaturally high, and can compromise the use of rivers for contact recreation and productive sports fisheries" (p. 26).

Table 24. Suggested criteria from various studies for maximum benthic biomass (as Chl-a) levels to avoid problems for recreational and aesthetic use in streams (adapted from a table in Dodds et al. 1998).

Suggested Chl-a value or range (mg/m^2)	Comment	Reference
100-150	Based on 19 enrichment cases and surveys	Horner et al. 1983, Welch et al. 1988
50-100	British Columbia Environment Guideline	Nordin 1985
150-200	Based on perceived impairment	Welch et al. 1989
150 maximum 100 mean	Guidelines for Clark Fork River, MT	Tristate Implementation Council 1996
50-200 maximum	New Zealand Periphyton Guideline	Biggs 2000b

The U.S. EPA's technical guidance manual (2000a) states: "While [a] 150 mg/m^2 [Chl-a] level cannot be supported as an absolute threshold above which adverse effects on water quality and benthic habitat readily occur, it nonetheless is a level below which an aesthetic quality use will probably not be appreciably degraded by filamentous mats or any other of the adverse effects attributed to dense mats of filamentous algae (e.g., objectionable taste and odors in water supplies and fish flesh, impediment of water movement, clogging of water intakes, restriction of

intro-gravel water flow and D.O. replenishment, D.O./pH flux in the water column, or degradation of benthic habitat) (Welch 1992). Avoidance of these problems in many stream systems may be achieved with a maximum of 150 mg/m^2 chl *a* criterion" (U.S. EPA 2000a, p. 102). Furthermore, the U.S. EPA's technical guidance manual (2000a) cautions that studies establishing an approximate 150 mg/m^2 benthic Chl-a threshold may only be applicable for northern to mid-temperate, cobble-bottom streams since these were the stream types generally used to establish the threshold. Streams in southern temperate zones, especially those with sandy bottoms, may not respond similarly. Also, intermittent streams and streams with flow from primarily effluent sources may have different benthic Chl-a threshold levels.

Filamentous algal coverage of the streambed by about 20% (Welch et al. 1988) and 30% (Biggs 2000b) has also been proposed as thresholds for identifying nuisance conditions.

Phytoplankton

Oregon has a Chl-a criterion for phytoplankton of 15 µg/L for rivers (OAR 340-041-0019), but information was not found to determine how this value was derived. For lakes, reservoirs, and other waters likely to have nuisance vegetation, North Carolina set a maximum planktonic Chl-a criterion of 40 µg/L for non-trout waters and a 15 µg/L criterion for trout waters (15A NCAC 02B.0211). These criteria were based on a literature review, a study of nutrient enrichment in 69 North Carolina lakes, and professional judgment by government and university scientists (NC WRRI 2001). Reckhow et al. (2005) estimated that the 40 µg/L Chl-a criterion has a 60% probability of supporting the designated uses of the Neuse Estuary and recommended that a 10 µg/L criterion would be more suitable from a use-protection perspective. Australia and New Zealand have a criterion of 15,000 – 20,000 cells/mL (depending on algal species) for recreational waters to protect swimmers and boaters, however, the decision-making process for setting this value was not found during the current search of the literature.

Nitrogen and Phosphorus Thresholds

TMDL Target Limits

TMDLs developed for nutrient related problems have set target limits for TN and TP that could be considered as thresholds. Because TMDLs must allow for a margin of safety, it is possible that these concentrations are lower than needed to prevent nuisance growth. The TMDL target values for TN and TP in the Clark Fork River, Montana were set to keep mean Chl-a values below 100 mg/m^2. The Tri-State Implementation Council overseeing the Clark Fork River TMDL set mean targets at 0.300 mg/L for TN, 0.020 mg/L for TP upstream of Missoula, and 0.039 mg/L for TP below Missoula (see the introduction to Section II -- Approaches to Setting Nutrient Criteria for more information). For the Tualatin River in Oregon, a TMDL TP limit of 0.070 mg/L was set. This value was derived from results of a series of algal growth studies to determine the TP target that would achieve Oregon's planktonic Chl-a criterion of 15 µg/L. Algal growth was noticeably reduced at 0.100 mg/L of TP and was low at 0.050 mg/L of TP. Using this information and input from stakeholders, a TP target of 0.070 mg/L of TP was set (U.S. EPA 1999).

Total Nitrogen

In their paper entitled "Establishing Nutrient Criteria in Streams," Dodds and Welch (2000) consider numerous scientific studies conducted primarily in the U.S. From these studies, they conclude that water column TN concentrations should remain below 0.470 mg/L to keep benthic mean Chl-a values around 50 mg/m^2 (and thereby ensuring that Chl-a values stay below 100 mg/m^2 most of the time). They also proposed that lower criteria could be set for more pristine streams. The proposed 0.470 mg/L TN threshold was developed, in part, from earlier work using probabilistic modeling based on the methods developed by Heiskary and Walker (1988) to assess the risk of exceeding certain Chl-a levels (based on data from 205 streams or sites throughout North America and New Zealand) (Dodds et al. 1997). The authors found that when the mean TN concentrations were kept below 0.500 mg/L, the benthic algal biomass exceeded 150 mg/m^2 only 5% of the time (Dodds et al. 1997). In later work using breakpoint regression and a two-dimensional Kolmogorov-Smirnov statistical technique, Dodds et al. (2002) suggested a much lower breakpoint for TN (0.040 mg/L). They primarily attributed the differences to additional data from other river systems that altered the regression equations and therefore cautioned: "care should be taken in using any specific set of published equations for management decisions" (Dodds et al. 2002, p. 871).

In a study conducted in Missouri, Lohman et al. (1992) designated six stream sites as "low enrichment," ten stream sites as "moderate enrichment," and six sites as "high enrichment" based on mean "annual" (March – November) TP concentrations and land use (see Models section). The "low enrichment sites" had mean "annual" TN concentrations less than 0.330 mg/L. At these sites, the Chl-a values exceeded 150 mg/m^2 from 0% to 2.6% (average 0.4%) of the dates sampled (n = 38 for each site). The "annual" TN values for the ten "moderately enriched streams" ranged from 0.186 mg/L to 0.773 mg/L (median = 0.507 mg/L) and exceeded a Chl-a threshold of 150 mg/m^2 on average 5.3% of the time (0% – 10.5%). The mean TN concentrations at the "high enrichment sites" exceeded 0.872 mg/L, and the Chl-a threshold of 150 mg/m^2 was exceeded at these sites, on average, 18.9% (7.9% – 42.1%) of the dates sampled (n = 38 for each site) (Chl-a was fluorometrically determined and corrected for phaeopigments by acidification). Although the authors stated no such conclusions, their findings suggest that to keep Chl-a values below 150 mg/m^2 between 80% to 90% of the time during the summer months, the stream TN concentrations should be kept below about 0.800 mg/L.

A preliminary study in Virginia by Hill and Devlin (2003) that incorporated nutrient concentration and benthic macroinvertebrate community data suggest a TN threshold for benthic impairment somewhere between 0.35 mg/L and 0.90 mg/L. In this study, 18 reference streams that supported a viable and diverse benthic community had a mean TN concentration of 0.33 mg/L and a median TN concentration of 0.34 mg/L (n = 59). In contrast, the 19 streams with benthic impairments had a mean TN concentration of 1.82 mg/L and a median TN concentration of 0.90 mg/L (n = 69) (Hill and Devlin 2003).

Another study that also included macroinvertebrate community data suggests a higher threshold. Laboratory studies by Lemly (2000) and Lemly and King (2000) demonstrated a direct linkage between bacterial growth on benthic macroinvertebrates and macroinvertebrate mortality (see

Biocriteria section). In the study by Lemly and King (2000), a stream classified as unenriched had mean TN concentrations between 0.715 – 1.97 mg/L and macroinvertebrate orders that were free of bacterial growth. The enriched stream in this study had mean TN concentrations in excess of 1.93 mg/L (1.93 – 3.89 mg/L) and macroinvertebrate orders that were colonized by bacteria (Lemly and King 2000).

Inorganic Nitrogen

Based on observed changes in the diatom assemblages found in 37 streams in four ecoregions of Virginia during the fall of 2004, Ponader et al. (2005) propose a NO_3-N threshold of 0.5 mg/L. In their pilot study, the authors found benthic Chl-a values to exceed 100 mg/m^2 only when NO_3-N concentrations exceeded 0.5 mg/L. This study, however, was based on a small sample size and had a narrow range in the NO_3-N concentration data. The study was also influenced by four hurricanes that struck the region during the sampling season. Therefore, the authors recommend additional sampling to obtain multi-year data sets within each ecoregion (or aggregate ecoregion for ecoregions with similar characteristics) that cover a larger range in nutrient concentrations under normal flow conditions. These larger data sets could then be used to determine threshold limits with a higher level of confidence (Ponader et al. 2005).

A review of studies considering the effects of inorganic nitrogen on macroinvertebrates and fish indicate mixed results. A preliminary study described in a Virginia DEQ memorandum reports that a set of 18 non-impaired biological reference stations for second, third, and fourth order streams had mean NO_3-N monthly concentrations of 0.17 mg/L (median = 0.14 mg/L), while 19 stations with benthic impairment had monthly mean NO_3-N concentrations of 1.35 mg/L (median = 0.40 mg/L) (Hill and Devlin 2003). Miltner and Rankin (1998) noted that headwater streams in Ohio with TIN concentrations below 1.37 mg/L had significantly higher (better) fish IBI scores than those with higher TIN concentrations. For wadeable streams, TIN concentrations less than 0.61 mg/L had the highest IBI scores (Miltner and Rankin 1998). Rankin et al. (1999) found that only median NO_3-N concentrations above 3 – 4 mg/L affected the fish IBI scores in headwater streams and the fish IBI and macroinvertebrate ICI scores in small rivers. The NO_3-N concentrations seldom exceeded this concentration in large rivers, and no relationship between nitrogen and the ICI and IBI scores was observed for this stream class.

Total Phosphorus

Studies seeking to identify changes in the algal community generally suggest a TP threshold of 0.020 – 0.060 mg/L. Dodds and Welch (2000) suggested that TP concentrations of 0.060 mg/L would keep benthic Chl-a values below 100 mg/m^2 most of the time. From a pilot study in Virginia streams, Ponader et al. (2005) suggest a change in the diatom species composition above a TP level of 0.05 mg/L. Dodds et al. (2002) proposed a TP breakpoint of 0.030 mg/L to keep mean benthic Chl-a values low. Chételat et al. (1999) found that the filamentous green algae, *Cladophora,* could dominate in streams exceeding 0.020 mg/L TP. Stevenson reported at the March 2005 Mid-Atlantic Nutrient Criteria Development workshop that *Cladophora* can become a frequent nuisance when TP exceeds 0.030 mg/L (Stevenson 2005). He further reported that TP concentrations of 0.030 mg/L would likely represent stream conditions with moderate changes in the structure of the biotic community and minimal changes in the function

of the community. He suggested that TP concentrations of 0.010 mg/L or less should reflect minimal changes in both the structure and function of the biotic community (Stevenson 2005).

Studies of changes in benthic macroinvertebrate and fish communities generally suggest TP threshold levels higher than the 0.020 – 0.060 mg/L range cited above. For example, the stream classified as unenriched in a North Carolina study by Lemly and King (2000) had mean TP concentrations less than 0.200 mg/L (range of mean TP: 0.054 – 0.198 mg/L), and all insect orders in this stream were free of bacterial growth. Rankin et al. (1999) reported that macroinvertebrate ICI and fish IBI scores were typically *good* (40 – 49) in waters with TP concentrations between 0.10 and 0.20 mg/L and tended to be *exceptional* (50 – 60) when TP concentrations were below 0.10 mg/L. A set of 18 reference reaches in Virginia without macroinvertebrate impairments had a mean TP concentration of 0.06 mg/L (median = 0.07 mg/L, n = 59), whereas 19 sites with benthic impairments had a mean TP value of 0.28 mg/L (median = 0.10 mg/L, n = 69) (Hill and Devlin 2003).

Phosphates

Studies included in this review that could be used to identify phosphate thresholds have found that waters with less than 0.020 mg/L phosphates tend not to be nutrient impaired, whereas those with phosphate concentrations above 0.030 mg/L tend to be impaired. The highly-eutrophic rivers in the study by Hornberger et al. (1977) had PO_4-P concentrations that range from 0.02 – 0.08 mg/L. An advisory committee in Texas recommended a phosphate limit of 0.030 mg/L PO_4-P to control excess algal growth in the North Bosque River, and McFarland et al. (2004) proposed a similar, although somewhat stricter, phosphate limit (0.023 mg/L) for this same river. Furthermore, McFarland et al. (2004) found P-limited conditions only when phosphate levels were less than 0.010 mg/L.

DOWNSTREAM EFFECTS

U.S. EPA regulations require that in "designating uses of a waterbody and the appropriate criteria for those uses, the State shall take into consideration the water quality standards of downstream waters and shall ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters" (CFR Part 131.10[b]). Therefore, the U.S. EPA's technical guidance manual (2000a) calls for consideration of downstream receiving waters when developing nutrient criteria for freshwater streams.

The U.S. EPA's technical guidance manual (2000a) specifically suggests that more stringent nutrient criteria may be required for streams that feed into lakes. For example, van Nieuwenhuyse and Jones (1996) suggest that the average abundance of sestonic algae per unit TP tends to be lower in streams than in lakes (Figure 13). Thus, nutrient concentrations that cause no problems in streams may cause nuisance levels of algae in lakes.

The impact of nutrients in streams and rivers draining to estuarine waters must be considered as well. Virginia's streams and rivers drain into such nutrient rich waters as the Chesapeake Bay, Albemarle Sound, and the Gulf of Mexico. Mulholland et al. (in print) have also recorded

blooms of *Aureococcus anophagefferens* (abundances reached 500,000 cells/mL) in Virginia's coastal estuarine waters (Chincoteague Bay). As an example of the potential for nutrients carried by streams and rivers to influence downstream waters, nutrient-related criteria for the Chesapeake Bay and its tidal tributaries are described below.

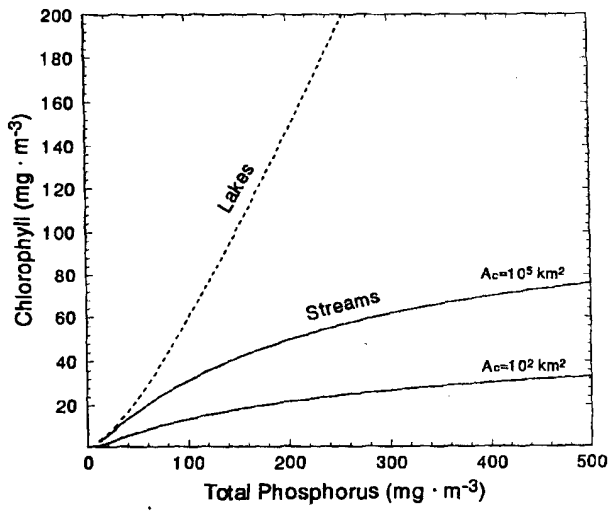


Figure 13. Comparison of the phosphorus-chlorophyll relationship in temperate streams and lakes. Solid curves show predicted Chl concentrations in streams of widely differing catchment area (100,000 and 100 km^2). The broken curve depicts the trajectory of expected Chl concentration in P-limited lakes (i.e., total nitrogen to TP ratio = 25, Forsberg and Ryding 1980) and is calculated from McCauley et al.'s (1989) model on the basis of data compiled form the literature (n = 382, R^2 = 0.80, s = 0.23 assumed for bias correction).[23]

Chesapeake Bay Example

The Chesapeake Bay and its tidal tributaries have been listed by U.S. EPA as impaired for not supporting balanced populations of aquatic life (9 VAC 25-260-10) and are classified as "nutrient enriched waters" (9 VAC 25-40). The signatories of *Chesapeake 2000* (the governors of Maryland, Pennsylvania, and Virginia, the mayor of the District of Columbia, the chair of the Chesapeake Bay Commission, and the Administrator of the U.S. EPA) committed to "continue efforts to achieve and maintain the 40 percent nutrient reduction goal agreed to in 1987, as well as the goals being adopted for the tributaries south of the Potomac River" (Chesapeake 2000). Furthermore, by 2010, the nutrient-related problems of the Bay and its tidal tributaries are to be corrected so that these waters can be removed from the list of impaired waters.

In an effort to meet these goals, Virginia designated the following uses for the Bay and its tidal tributaries (9 VAC 25-260-10):

[23] Reprinted from Canadian Journal of Fisheries and Aquatic Sciences, Vol. 53, E.E. van Nieuwenhuyse and J.R. Jones, Phosphorus-chlorophyll relationship in temperate streams and its variation with stream catchment area, pages 99-105, copyright 1996, with permission from National Research Council Canada.

- The migratory fish spawning and nursery designated use
- The shallow-water bay grass designated use
- The open-water fish and shellfish designated use
- The deep-water seasonal fish and shellfish designated use
- The deep-channel seasonal refuge designated use

Virginia also developed nutrient-related water quality criteria for the Chesapeake Bay and its tidal tributaries (9 VAC 25-260). These uses and criteria went into effect June 24, 2005.

Impact of Excess Nutrients in the Bay

Excess nutrients promote blooms of phytoplankton in the Chesapeake Bay and its tributaries. Based on studies of light and nutrient limitations to phytoplankton growth in the Chesapeake Bay, Fisher and Gustafson (2003) identified a limiting threshold of DIN at 0.07 mg/L and a limiting threshold for PO_4 at 0.007 mg/L. Nutrients were found to be in excess of phytoplankton needs throughout the James River and in the tidal fresh waters of the Rappahannock River (as cited in Butt 2004: Haas and Webb 1998, Fisher and Gustafson 2003).

U.S. EPA (2003b) suggests that blooms of *Microcystis aeruginosa*, a colonial species of cyanobacteria, in excess of 10,000 cells/mL adversely affect zooplankton communities. Levels of *M. aeruginosa* have averaged over 10,000 cells/mL during the summer months in the tidal fresh James River. The highest reported levels of *M. aeruginosa* to the Bay exceeded 2,000,000 cells/mL (found in the Potomac River in 2000 by the Maryland Department of Natural Resources) (Butt, in print).

Nuisance levels of algae reduce the available sunlight to the submerged grass beds and grow on the surface of the grass blades, both of which reduce the ability of the grasses to photosynthesize. Grasses unable to adequately photosynthesize will die. U.S. EPA (2003b) states: "The loss of underwater bay grasses from shallow waters of the Chesapeake Bay, which was first noted in the early 1960s, is a widespread, well-documented problem. The primary causes of the decline of these underwater bay grasses are nutrient over-enrichment and increased suspended sediments in the water, and associated reductions in light availability" (p. xiii).

Nuisance levels of algae in the Chesapeake Bay promote excess bacterial production that leads to a decrease in the dissolved oxygen concentrations. As nuisance levels of algae die and sink to the bottom of the Bay, bacteria break down the dead algal material. Large amounts of dying algae support large populations of decomposing bacteria. These bacteria use the available dissolved oxygen in the Bay, which reduces the amount of oxygen available for other organisms. Low oxygen levels (hypoxia) and cultural eutrophication have been linked in the Chesapeake Bay.

Non-tidal Freshwater Nutrient Levels to the Bay

As a part of a monitoring program in major rivers draining to the Chesapeake Bay, river stations were established at the most downstream location unaffected by tides. These sites are called *river input stations* and include, for example:

- Potomac River at Chain Bridge, Washington, D.C.

- James River at Cartersville, Va.
- Rappahannock River near Fredericksburg, Va.
- Appomattox River at Matoaca, Va.
- Pamunkey River near Hanover, Va.
- Mattaponi River near Beulahville, Va.

Water samples were collected monthly or bimonthly and during storms at each station for periods that span 8 to 14 years (1985 – 1998). The median and data range for TN and TP concentrations are shown for the major rivers draining catchments in Virginia (Table 25) (Sprague et al. 2000). The percent contribution of flow, total nitrogen, and total phosphorus to the Chesapeake Bay were calculated for each basin or sub-basin draining catchments in Virginia (Table 26) (Belval and Sprague 1999). Nutrient concentrations (mg/L), loads (lbs/year), and yields (lbs/mi^2) are shown for all the major river basins (Susquehanna, Potomac, James, Rappahannock, York, Patuxent, and Choptank) in Figure 14 (Belval and Sprague 1999).

Table 25. Median concentrations of total nitrogen and total phosphorus and the range of the concentration data for the non-tidal, most downstream monitoring station of the major rivers of Virginia that drain to the Chesapeake Bay (data from Sprague et al. 2000).

Basin	River	Median TN (mg/L)	Range of TN (mg/L)	Median TP (mg/L)	Range of TP (mg/L)	Data Collected
Potomac	Potomac	1.8	0.35 - 11.4	0.06	0.01 - 3.29	1985 - 1998
James	James	0.68	0.03 - 3.30	0.13	0.02 - 1.40	1988 - 1998
Rappahannock	Rappahannock	0.93	0.12 - 4.21	0.06	0.008 -1.50	1988 - 1998
James	Appomattox	0.56	0.10 - 1.12	0.05	0.01 - 0.20	1988 - 1998
York	Pamunkey	0.67	0.16 - 2.23	0.06	0.02 - 0.50	1989 - 1998
York	Mattaponi	0.54	0.03 - 1.57	0.05	0.005 - 0.26	1989 - 1998

Table 26. The flow, total nitrogen, and total phosphorus contribution to the Chesapeake Bay as determined from the non-tidal, most downstream monitoring station of the Bay's main feeder rivers in Virginia (data from Belval and Sprague 1999).

Basin	River	Flow Contrib. to Bay	TN Load Contrib. to Bay	TP Load Contrib. to Bay	Data Collected
Potomac	Potomac	20 %	28 %	33 %	1985 - 1998
James	James	12 %	5 %	20 %	1988 - 1998
Rappahannock	Rappahannock	3 %	2 %	8 %	1988 - 1998
James	Appomattox	2 %	<1 %	1 %	1989 - 1998
York	Pamunkey	2 %	<1 %	2 %	1989 - 1998
York	Mattaponi	<1 %	<1 %	<1 %	1989 - 1998

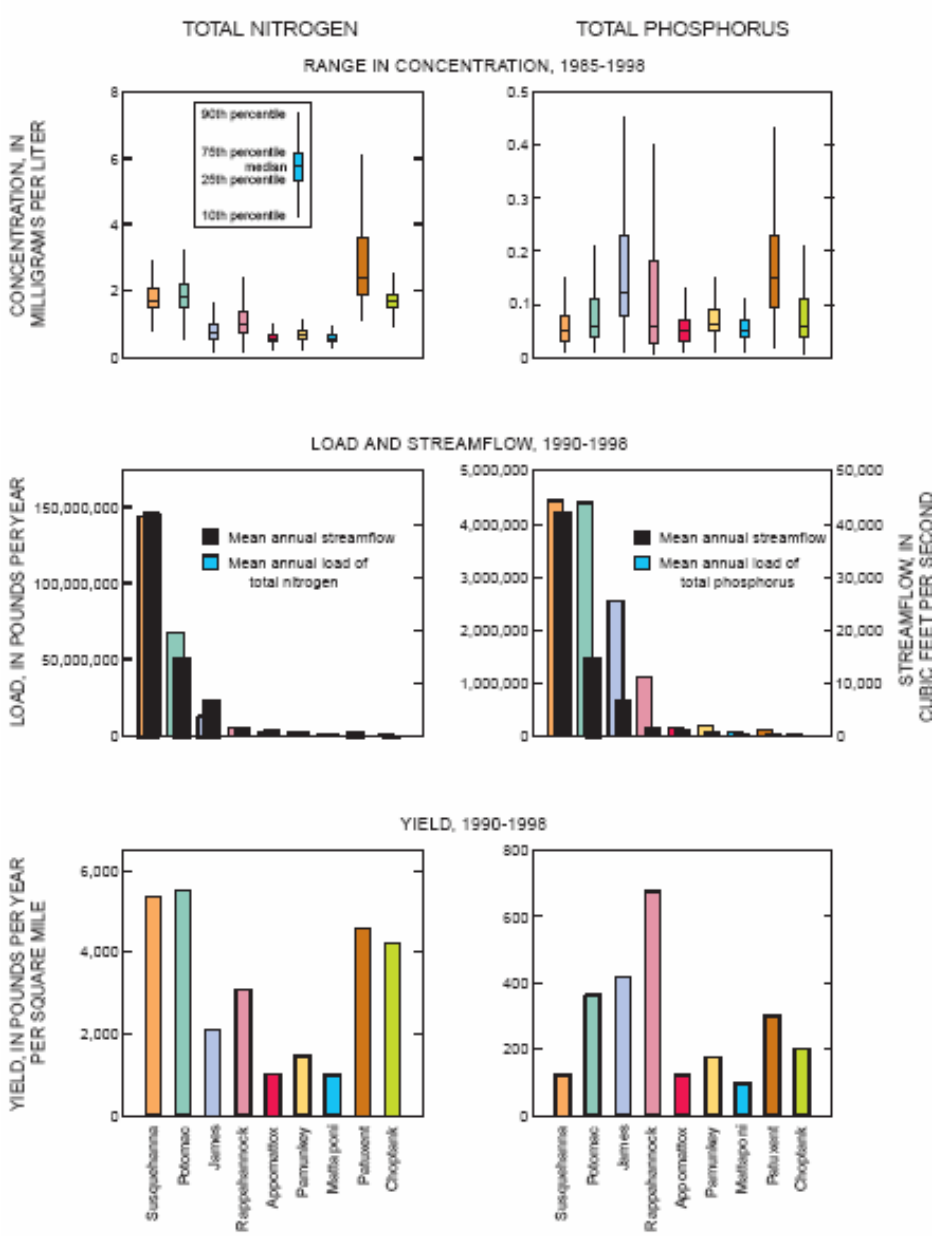


Figure 14. Nutrient concentration ranges, nutrient loads, streamflow, and nutrient yields for the River Input Monitoring stations. [Concentration graph shows 90th percentile, 75th percentile, 25th percentile, and 10th percentile. Load graphs shows mean annual streamflow (left and right), mean annual load of total nitrogen (left), and mean annual load of total phosphorus (right).][24]

[24] Reprinted from Monitoring Nutrients in the Major Rivers Draining to Chesapeake Bay, U.S. Geological Survey Water-Resources Investigations Report 99-4238, D.L. Belval and L.A. Sprague, 8 pp., published 1999.

Criteria Development for the Chesapeake Bay and Tidal Tributaries

U.S. EPA Region III developed a guidance document: *Ambient Water Quality Criteria for Dissolved Oxygen, Water Clarity and Chlorophyll* a *for the Chesapeake Bay and Its Tidal Tributaries* for use by the Bay states and the District of Columbia in adopting water quality standards to address nutrient pollution in the Bay and its tidal tributaries (U.S. EPA 2003b). For example, in the guidance document, U.S. EPA (2003b) proposed numeric dissolved oxygen criteria for the Bay waters designed to protect aquatic life and to reflect the natural environments of the Bay. Following the guidance, Virginia set dissolved oxygen criteria for the part of the Bay and its tidal tributaries that lie within the state's jurisdiction (9 VAC 25-260-50).

U.S. EPA (2003b) suggested water clarity criteria for low and high salinity habitats based on published literature, Chesapeake Bay-specific field studies, and models. The agency proposed that water clarity criteria apply only during the bay grass growing season: April 1 – October 31 for tidal-fresh, oligohaline, and mesohaline waters; and from March 1 – May 31 and September 1 – November 30 for polyhaline waters. Furthermore, the U.S. EPA (2003b) suggested that criteria meet minimum light penetration levels: 13 percent for tidal-fresh and oligohaline waters; and 22 percent for mesohaline and polyhaline waters. Virginia followed this guidance in setting water clarity criteria for different segments of the Bay (9 VAC 25-260-185).

The U.S. EPA Region III guidance document offered narrative criteria for chlorophyll-a and recommended that states "adopt numeric chlorophyll *a* criteria for application to tidal waters in which algal-related designated use impairments are likely to persist even after attainment of the applicable dissolved oxygen and water clarity criteria" (U.S. EPA 2003b, p. xvi). Baywide numeric criteria were not proposed because of "the regional and site-specific nature of algal-related water quality impairments" (U.S. EPA 2003b, p. 104). U.S. EPA (2003b, p. 104) did however provide Chl-a values "as a synthesis of the best available technical information" for consideration by states. The regional guidance document suggests using Chl-a concentrations derived from a number of different means:

- historical data,
- literature values related to trophic status,
- characteristics of phytoplankton growth-limiting water quality conditions,
- characteristics of potentially harmful algal blooms,
- characteristics of trophic-based conditions, and
- values protective against water quality impairments.

Virginia DEQ and U.S. EPA Region III determined that Virginia needs numerical criteria for Chl-a only in the tidal James River. According to Virginia DEQ, "…the tidal James has the most 'unbalanced' phytoplankton community compared to Virginia's other tidal waters with numerous observations of over-abundances of 'undesirable' plant life.... While the Chesapeake Bay and other major tidal tributary waters may also have algal-related impairments due to eutrophication, EPA and the seven watershed jurisdictions have determined that numerical chlorophyll-a criteria are not required for these other tidal waters at this time" (Virginia DEQ 2005, p. 4). Instead, a narrative criterion for Chl-a, which covers the growing period from March 1 – September 30, was adopted for the other waters (9 VAC 25-260-185).

Mean Chl-a concentration criteria during spring and summer for specific sites within the tidal James went into effect January 12, 2006 (Table 27). The sites include tidal-fresh (JMSTF2, JMSTF1), oligohaline (JMSOH), mesohaline (JMSMH), and polyhaline (JMSPH) reaches. These numeric criteria were developed through analysis of water quality data since the 1950s, U.S. EPA recommendations (presented to the Chesapeake Bay Water Quality Standards Ad Hoc Committee; March 24, 2004), reference conditions, models, threshold concentrations of harmful algal blooms, and best professional judgment.

Table 27. Site specific numerical chlorophyll-a criteria applicable March 1 – May 31 and July 1 – September 30 for the tidal James River segments JMSTF2, JMSTF1, JMSOH, JMSMH, JMSPH (excludes tributaries) (9 VAC 25-260-310).

Designated Use	Chlorophyll *a* µg/L	Chesapeake Bay Program Segment	Temporal Application
Open-Water	10	JMSTF2	March 1 - May 31
	15	JMSTF1	
	15	JMSOH	
	12	JMSMH	
	12	JMSPH	
	15	JMSTF2	July 1 - September 30
	23	JMSTF1	
	22	JMSOH	
	10	JMSMH	
	10	JMSPH	

A brief description of the reasoning behind the numeric criteria for the tidal-fresh waters is provided. The James River tidal fresh region is divided into two segments because of different natural river bathymetric and hydrodynamic characteristics, which likely affect the algal communities. Thus, different criteria were set for these two regions. The more upstream segment (JMSTF2) was given lower Chl-a concentration criteria, as supported by 1986 – 2003 monitoring data (Virginia DEQ 2005).

Because the Chesapeake Bay and its tidal tributaries have most likely never been oligotrophic, Virginia set a goal of meeting a mesotrophic status (Virginia DEQ 2005). Virginia DEQ based its tidal freshwater numeric criteria, in part, on Wetzel's (2001) definition of mesotrophic freshwater: those waters with Chl-a concentrations in the range of 2 – 15 µg/L (however, this range is based on lake data). Based solely on phytoplankton reference communities (in the York River), average Chl-a concentration criteria of less than 14 µg/L in the spring (March 1 – May 31) and less than 12 µg/L in the summer (July 1 – September 30) would be set. These values lie near the mesotrophic-eutrophic boundary proposed by Wetzel (2001) and are higher than the

levels observed in the 1950s (mean spring Chl-a = 3.7 µg/L; mean summer Chl-a = 7.0 µg/L) (Virginia DEQ 2005).

To protect against dominance of undesirable, nuisance algal species, Virginia DEQ considered studies of *Microcystis aeruginosa*. Zooplankton are known to avoid ingesting both toxic and nontoxic strains of *M. aeruginosa* so this species contributes little to the food web. In tidal-fresh regions, blooms of *M. aeruginosa* can "cover certain Bay tributaries for miles during the summer" (Virginia DEQ 2005, p. 17). Data from the Chesapeake Bay Phytoplankton Monitoring Program showed that chlorophyll-a concentrations above 15 µg/L are associated with *M. aeruginosa* blooms in excess of 10,000 cells/mL (U.S. EPA 2003b). Furthermore, zooplankton communities are adversely affected by *M. aeruginosa* blooms exceeding 10,000 cells/mL (U.S. EPA 2003b citing Lampert 1981, Fulton and Paerl 1987, Smith and Gilbert 1995).

Because the summer conditions naturally result in higher Chl-a conditions (due to lower flows, warmer temperatures, and greater light levels), higher criteria were set for the summer months. Although the summer criterion for JMSTF1 and JMSOH exceeds the 15 µg/L limit often suggested, Virginia DEQ states: "…this higher criterion concentration is justified based on naturally higher chlorophyll a concentrations observed in this section of the tidal fresh reach of the James River" (Virginia DEQ 2005, p. 23).

SECTION III -- ALTERNATIVE APPROACH TO SETTING NUMERIC NUTRIENT CRITERIA

The Kansas Department of Health and Environment (KDHE) is proposing to U.S. EPA an alternative strategy to reducing the impact of nutrients in its waters and downstream waters (KDHE 2004). It should be stressed, however, that the U.S. EPA has not yet commented on this approach and may or may not support it.

The KDHE strategy is to implement nutrient reductions by developing and utilizing a *nutrient export budget*. They propose implementation of nutrient reductions through such means as technology-based biological nutrient removal limits for wastewater, voluntary best management practices in prioritized watersheds, and trading nutrient credits between sources within a watershed. They do not consider nutrient criteria to be a part of the immediate solution, but instead, propose developing criteria in the future once more data have been collected and analyzed (KDHE 2004).

With regard to using the specific criteria proposed from the U.S. EPA aggregate nutrient ecoregions (see Reference Approach section), KDHE states: "The criteria methodology utilizes a statistical approach that attempts to predict a relatively unimpacted condition rather than relying on a more traditional approach that seeks to establish criteria based on protecting against health, aquatic life, or recreational impairments" (KDHE 2004, p.6). The USGS predicts background total phosphorus levels will exceed the criteria proposed through the U.S. EPA aggregate nutrient ecoregions in about half the stream reaches throughout the nation (Smith et al. 2003 as

cited in KDHE 2004). Furthermore, "KDHE staff asserted the EPA-developed ecoregional criteria were believed to be unachievable, and would likely never be met in Kansas reservoirs or streams, even if elaborate measures were taken to control sewage treatment plants and nonpoint sources" (KDHE 2004, p. 3). KDHE adds: "Many states also expressed the concern that moving forward with poorly developed nutrient criteria would cause loss of credibility for the state agencies and lead to prolonged legal and political conflicts that would further delay effecting any appreciable reduction in waterborne nutrients" (KDHE 2004, p.5).

The KDHE expressed great concern about the impact that numeric nutrient criteria could have on wastewater treatment facilities (WWTF). Federal regulations (40 CFR 122.44[d]) require discharge permits to limit pollutant levels so that water quality standards are not exceeded. Therefore, because point sources must meet permit limits while nonpoint sources do not, WWTFs and other permitted facilities would be the first impacted by numeric nutrient criteria. A question of fairness thus arises because point sources are estimated to be responsible for 5% to 30% of the nutrients, while nonpoint sources of pollution (runoff) are estimated to contribute to the majority of nutrient input to surface waters (KDHE 2004). KDHE asks: "Should a minority of the problem bear the entire cost of mitigation?" (KDHE 2004, p. 5).

KDHE cites additional problems faced by WWTF if the U.S. EPA proposed numeric criteria are adopted in Kansas.

(1) – Because many receiving waters will not meet the water quality criteria, the discharges from sewage treatment plants will also need to meet the criteria limits.

(2) – The tertiary treatment technologies currently used at many facilities may not reduce the nutrient concentrations to the proposed criteria levels. For example, EPA's ecoregional TN criteria range from 0.56 to 2.18 mg/L in Kansas, while the best expected treatment performance is estimated at 3.0 mg/L for TN (Oldham and Rabinowitz 2002 as cited in KDHE 2004).

(3) – For small municipalities, the wastewater treatment needed to meet the criteria would be beyond their technological and financial means.

Concept of KDHE's Alternative Approach

KDHE proposed an alternative approach to developing numeric nutrient criteria that includes three main components:

(1) – Develop an inventory of nutrients entering and leaving the waters of the state;
(2) – Establish a nutrient reduction target; and
(3) – Establish and implement a plan to meet the nutrient reduction target.

To determine the nutrient budget of the state, Kansas estimated the TN and TP from point sources and nonpoint sources entering and leaving the state. As a result of this process, KDHE found that point sources contribute about 18% of the TN and about 25% of the TP exiting the state (KDHE 2004). KDHE then proposed nutrient reduction targets for TN and TP but stressed that while the targets provide measurable objectives, they differ from legally enforceable criteria. KDHE proposed nutrient reduction targets of 30% for both TN and TP. Based on work by Brezonik et al. (1999), KDHE estimated that a 30% reduction in TN could substantially improve the dissolved oxygen levels in the Mississippi River and the Gulf of Mexico, and a 30%

reduction in TP could "bring about a 10% increase in the number of flowing waters in the Mississippi River basin that would meet a proposed phosphorus criteria for flowing water of 0.1 mg/L" (p. 11). Furthermore, Kansas estimates that the 30% reduction targets are attainable (KDHE 2004).

KDHE proposed specific plans for meeting the established nutrient reduction targets. For point sources, the agency suggested using National Pollutant Discharge Elimination System (NPDES) permits (with best available technology for municipal wastewater treatment facilities). Because KDHE (2004) found that about 85% of the nutrients from point sources were attributable to discharges with at least one million gallons per day (1 MGD), they suggest initially focusing on these facilities. The agency projected that biological nutrient removal at these facilities could bring down annual average effluent levels to 8 mg/L for TN (a 67% reduction in the current levels) and to 1.5 mg/L for TP (a 65% reduction in the current levels). Implementation of this aspect of the plan alone would reduce the state's export levels of TN by about 10% and the export levels of TP by 14%. For nonpoint sources, the agency suggests setting priority areas based on high potential nutrient export levels and TMDLs. KDHE suggests focusing federal grant monies on voluntary reductions through BMPs within these priority regions. Nutrient trading between point and nonpoint sources would also be promoted to more economically reach the nutrient targets (KDHE 2004).

By implementing the point source and nonpoint source strategies and focusing on priority sources, KDHE estimates that the proposed approach will meet or exceed the 30% reduction targets for TN and TP. The agency cites the European Union, Mississippi River/Gulf of Mexico Watershed Nutrient Task Force, Connecticut, and North Carolina as entities having success in addressing nutrient control by establishing nutrient reduction targets and implementing practices to meet the established targets (KDHE 2004).

SUMMARY

The U.S. EPA has identified nutrients as a major reason for impaired water quality in the nation's streams and rivers (U.S. EPA 2000a). Excess nutrients in streams and rivers can negatively impact the use of the waterbody. Most problems with excessive nutrients are related directly or indirectly to too much growth of primary producers (periphyton, phytoplankton, and macrophytes). To protect the designated uses of streams and rivers, U.S. EPA is directing states and authorized tribes to develop numeric criteria for nutrients.

The purpose of this review is to provide background information for the interested public, the Academic Advisory Committee (AAC), and the Virginia Department of Environmental Quality (DEQ) as discussions begin concerning nutrient criteria development in Virginia. Virginia DEQ requested specifically that the AAC conduct a comprehensive literature search on the following topics:

- Investigate methods for defining undesirable (nuisance) levels of periphytic algae in wadeable streams, and what such studies have concluded as undesirable (nuisance) levels.

- Investigate a corresponding approach to planktonic algae in non-wadeable streams.

This literature review should not be used to select numeric values for freshwater streams in Virginia simply based on the research or criteria of others. Instead, much work and thorough discussions between all stakeholders are needed before nutrient criteria can satisfactorily be set for the freshwater streams and rivers in Virginia.

The technical guidance manual developed by U.S. EPA (2000a) recommends using a combination of the (1) reference approach, (2) predictive relationship approach, and (3) established threshold approach to develop nutrient criteria. These approaches are described in this document.

Reference Approach

Criteria development using the reference system approach can be initiated using a combination of reference reaches—stream segments that reflect the water quality conditions suitable for meeting all designated uses of the stream—and best professional judgment. Criteria can also be developed from the frequency distribution of the reference reaches in a given region, and/or the frequency distribution of the general population of a class of streams in a particular area (the 25th percentile of all data was found to approximate the 75th percentile of data from reference reaches).

U.S. EPA divided the continental U.S. into 14 nutrient ecoregions with similarities in geographic and land-use characteristics expected to affect nutrient concentrations. The aggregate nutrient ecoregions applicable to Virginia are shown in Figure 15. Using the reference approach, U.S. EPA developed recommended nutrient criteria for the aggregate nutrient ecoregions (U.S. EPA 2000b–d). Within each ecoregion, U.S. EPA developed a frequency distribution of the median values for each season (spring, summer, fall, and winter) and selected the 25th percentile from these seasonal values. U.S. EPA then calculated the median value of the four seasonal 25th percentile values to set its recommended criteria. The recommended criteria for streams and rivers in Virginia are listed in Table 28.

U.S. EPA recommends using reference conditions as a guide or “first step” in setting nutrient criteria. States and authorized tribes are encouraged to select reference conditions at smaller geographic scales (level III ecoregions or watershed scale) and refine their criteria through the use of models and published literature and in consideration of downstream effects and expert judgment (U.S. EPA 2000b).

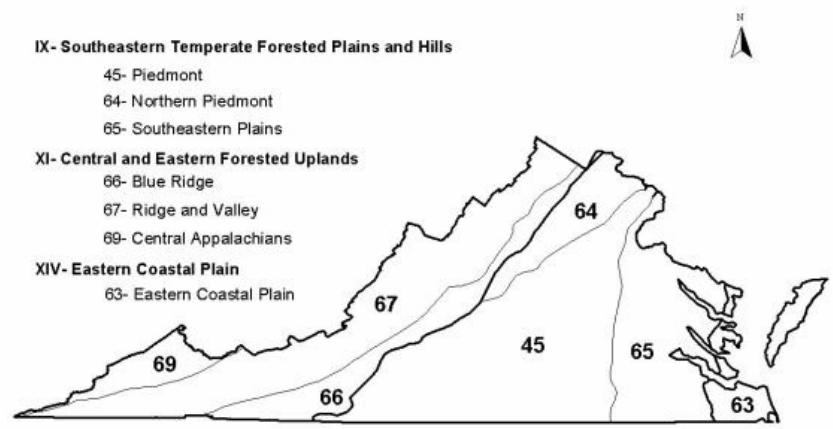


Figure 15. Map of Virginia showing U.S. EPA Aggregate Nutrient Ecoregions

Table 28. U.S. EPA's nutrient threshold recommendations for aggregate nutrient ecoregions applicable to Virginia's rivers and streams. Chlorophyll-a determined by the spectrophotometric method, and periphyton Chl-a values are listed separately.

Rivers and Streams	Aggregate Nutrient Ecoregions		
Parameter	**IX**	**XI**	**XIV**
TP (mg/L)	0.037	0.010	0.031
TN (mg/L)	0.69	0.31	0.71
Chl-a (µg/L) - S	0.93	1.61	3.75
Periphyton Chl-a (mg/m^2)	20.35	-	-
Turbidity (NTU)	-	2.30	-
Turbidity (FTU)	5.70	1.7	3.04

Predictive Relationship Approach

Trophic State Classification

Frequency distributions of water quality characteristics for streams in a given region can be utilized when developing nutrient criteria with the predictive relations approach. In this application, the frequency distributions are applied to classify streams according to their trophic state: oligotrophic (unenriched), mesotrophic (moderately enriched), or eutrophic (enriched). One of the best known examples of the use of frequency distributions is the work by Dodds et al. (1998) (Table 29). These authors used data from published studies (Omernik 1977, van Nieuwenhuyse and Jones 1996, and Dodds et al. 1997) that represented hundreds of temperate streams, mostly in North America and New Zealand. Using the distribution frequencies of each parameter, they divided the streams into categories with the lowest third (lowest values) of the distributions indicating oligotrophic conditions, the middle third suggesting mesotrophic

conditions, and the top third (highest values) representing eutrophic conditions. The authors recommend using this method only as a first step in categorizing the trophic status of streams.

Table 29. Suggested boundaries for trophic classification of streams from cumulative frequency distributions. The boundary between oligotrophic and mesotrophic systems represents the lowest third of the distribution, and the boundary between mesotrophic and eutrophic marks the top third of the distribution. [Dodds et al. (1998) reported TN and TP concentrations in µg/L; for consistency within this review, these values were converted to mg/L][25]

Variable (units)	Oligotrophic-mesotrophic boundary	Mesotrophic-eutrophic boundary	n
Mean benthic Chl-a (mg/m^2)[a]	20	70	286
Max. benthic Chl-a (mg/m^2)[a]	60	200	176
Sestonic Chl-a (µg/L)[b]	10	30	292
TN (mg/L)[a,c]	0.700	1.500	1070
TP (mg/L)[a,b,c]	0.025	0.075	1366

[a]Data from Dodds et al. (1997).
[b]Data from van Nieuwenhuyse and Jones (1996).
[c]Data from Omernik (1977).

Models

Mathematical models are often employed to identify patterns in the water quality data, which are then used to predict numeric levels to prevent eutrophication. For example, Dodds et al. (1997) developed regression equations to estimate mean benthic Chl-a or maximum benthic Chl-a using either TN, TP, or both TN and TP concentrations. Applying their model to data from the Clark Fork River (Montana), Dodds et al. (1997) predicted that if seasonal mean TN concentrations are reduced to 0.275 mg/L, the maximum chlorophyll-a values would be 100 mg/m^2. Additionally, if TN concentrations do not exceed 0.252 mg/L and TP concentrations are kept below 0.035 mg/L, Chl-a values would be expected to stay below 100 mg/m^2.

In another study, scientists used a Monod model, which relates algal growth to external nutrient concentrations, to set a nutrient target in the North Bosque River in central Texas. This river has experienced elevated levels of Chl-a and nutrients, which have negatively impacted its contact recreation, aquatic life, and public water supply uses. Because phosphorus tended to be the limiting nutrient in the Bosque River watershed (Kiesling et al. 2001), McFarland et al. (2004) proposed using the half-saturation constant of PO_4-P (0.023 mg/L) as the target that would likely control algal growth in the North Bosque River.

[25] Reprinted from Water Research; Vol. 32; W.K. Dodds, J.R. Jones, and E.B. Welch; Suggested classification of stream trophic state: Distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus; pages 1455-1462; copyright 1998; with permission from Elsevier.

Many studies indicate that factors besides nutrients greatly influence algal biomass accumulation, e.g., flood frequency and drainage area are also important. For example, Biggs (2000a) found that flooding frequency ($p < 0.001$) was more important than in-stream nutrient levels ($p = 0.045 – 0.337$) in determining algal biomass accumulation. Periphytic biomass in streams with long accrual periods (e.g., > 100 d) reacted to small increases in dissolved nutrients (e.g., SRP > 0.005 mg/L). In fact, whenever average accrual periods exceeded about 50 days, the frequency of high-biomass events increased greatly. In contrast, periphyton accrual in streams with frequent flooding was affected much less by nutrient concentrations. As an application of this finding to nutrient criteria development, Biggs (2000a) suggests using flood frequency (i.e., >3x the median flow) as a basis for classifying streams regionally; he then recommends developing local or regional nutrient target concentrations.

In another study, van Nieuwenhuyse and Jones (1996) developed a regression model to predict mean suspended chlorophyll values (µg/L) for use in small and large temperate streams. These researchers found that stream catchment area significantly affects the Chl-a concentrations, with increases in phytoplankton biomass occurring with increasing stream catchment area. van Nieuwenhuyse and Jones (1996) also found that hydraulic and other physical factors may co-regulate (regulate along with phosphorus) the amount of phytoplankton growth.

Reckhow et al. (2005) used structural equation modeling to identify the relationships between nutrient-related parameters and the predictive use attainment. They identified the designated uses that could be impacted by nutrients and used an expert elicitation approach, using the responses from the experts and water quality data. Reckhow et al. (2005) developed structural equation models to propose water quality criteria and relate the probability that the criterion would protect the designated use. Based on the results of their study, the authors found that the current Chl-a criterion of 40 µg/L and D.O. level of 5 mg/L in the Neuse Estuary has a 60% probability of attaining the use designations. Furthermore, the model predicts that assuming D.O. concentrations are at least 5 mg/L, Chl-a concentrations would need to be less than 10 µg/L to achieve a high level of attainment.

Biocriteria

A third means by which the predictive relation approach to setting nutrient criteria can be directed is through biocriteria, the use of biological parameters to define nutrient limits. For example, several biotic indices for periphyton or phytoplankton are being studied for possible use in the eastern United States (e.g., Hill et al. 2000, KDOW 2002, Ponader and Charles 2003), and such biotic indices are commonly used in Europe. Indices are usually comprised of six to ten metrics that include a measure of the abundance of organisms, species richness, and trophic structure. The metrics are based on their sensitivity to human activities related to nutrient input to streams, their precision, and their usefulness among different regions and habitat types. The indices can be used to identify streams not meeting their designated uses, particularly in regard to supporting a diverse and productive aquatic life community.

Studies by Lemly (2000) and Lemly and King (2000) illustrated the use of identifying nutrient enriched streams from bacterial growth on aquatic insect larvae. In laboratory studies, mayfly larvae with more than 25% of their bodies covered by bacteria had mortality rates of about 100%

while nearly all those uninfested with bacteria survived. Mayflies with 10 – 25% coverage by bacteria appeared healthy and unaffected. Field studies conducted in a mountainous stream in Virginia and in a lowland stream in North Carolina, whereby nutrient concentrations within the water column and bacteria infestation of insects were measured, supported the laboratory findings. Lemly concluded that bacterial coverage of aquatic insects provides a practical, quick, and easy way to screen for nutrient enrichment in streams.

Miltner and Rankin (1998) used metrics for fish (IBI), macroinvertebrates (ICI), and habitat (QHEI) to study the effects of nutrients on the aquatic life in streams and rivers in Ohio. Building on this work, Rankin et al. (1999) developed a system for ranking the relative risk to aquatic life based on nutrient concentrations in grab samples. The predictive model is based on the probability of departing from the nutrient concentrations determined from (1) a reference database or (2) the subset of all the data that correlates with IBI and ICI scores supporting the aquatic life use for warmwater habitats or exceptional warmwater habitats (e.g., IBI scores of 40+). The more the sample distribution deviates from these values, the higher the probability that the tested stream reach will not attain the criteria associated with the warmwater habitat or exceptional warmwater habitat aquatic life uses. From the results of the study, statewide criteria in Ohio for nitrate+nitrite and TP were proposed (Table 30).

Table 30. Proposed statewide criteria for warmwater habitat (WWH), exceptional warmwater habitat (EWH), and modified warmwater habitat (MWH) streams in Ohio (data from Rankin et al. 1999).

	Nitrate + Nitrite (mg/L)			**Total Phosphorus (mg/L)**		
Stream Type	**WWH**	**EWH**	**MWH**	**WWH**	**EWH**	**MWH**
Headwaters	1.0	0.5	1.0	0.08	0.05	0.34
Wadable	1.0	0.5	1.6	0.10	0.05	0.28
Small Rivers	1.5	1.0	2.2	0.17	0.10	0.25
Large Rivers	2.0	1.5	2.4	0.30	0.15*	0.32

* TP concentration chosen to reflect N:P ratio ≥ 10.

Published Nutrient Criteria and Threshold Approach

There appears to be a general consensus that benthic chlorophyll-a values between 100 – 200 mg/m^2 are associated with eutrophic conditions. Chl-a criteria proposed in other countries for periphyton have generally been 200 mg/m^2 or lower. The criteria or guidelines proposed by British Columbia (Canada), Australia, and New Zealand for summer conditions designed to protect various stream uses are shown in Table 31.

Table 31. Criteria or guidelines used to protect stream uses in various provinces or countries.

Stream Use	Criteria or Guideline	Province or Country
Aesthetics, Swimming, and Boating	50 mg/m^2 maximum Chl-a	British Columbia
	100 mg/m^2 maximum Chl-a	New Zealand
	15,000 - 20,000 cells/mL (depending on algal species)	Australia and New Zealand
	60% maximum cover (> 0.3 cm thick diatom/cyanobacteria dominant)	New Zealand
	30% maximum cover (> 2 cm long filamentous algae dominant)	New Zealand
Fishing	200 mg/m^2 maximum Chl-a	New Zealand
	30% maximum cover (> 2 cm long filamentous algae dominant)	New Zealand
Aquatic Life	100 mg/m^2 maximum Chl-a	British Columbia
	50 mg/m^2 maximum Chl-a	New Zealand

Downstream Effects

Because phytoplankton is not attached to the streambed but is transported in the water column to downstream waters (e.g., reservoirs and estuaries), upstream planktonic Chl-a criteria must be set to meet both local and downstream water quality needs. The U.S. EPA's technical guidance manual (2000a) specifically suggests that more stringent nutrient criteria may be required for streams that feed into lakes. Likewise, of consequence to setting nutrient criteria for streams and rivers in Virginia is the impact of nutrients to downstream estuarine waters. The nutrient criteria for such waters as the Chesapeake Bay and its tidal tributaries may therefore drive the nutrient criteria development process in non-tidal, freshwater streams and rivers in Virginia.

An Alternative Approach

The Kansas Department of Health and Environment (KDHE) is proposing to U.S. EPA an alternative approach to establishing nutrient criteria in freshwaters. At the time of publication of this literature review, the U.S. EPA has yet to comment on this alternative approach. The KDHE strategy is to implement nutrient reductions by developing and utilizing a *nutrient export budget*. Their approach includes three main components:

(1) – Develop an inventory of nutrients entering and leaving the waters of the state;
(2) – Establish a nutrient reduction target; and
(3) – Establish and implement a plan to meet the nutrient reduction target (KDHE 2004).

To determine the nutrient budget of the state, Kansas estimated the TN and TP from point sources and nonpoint sources entering and leaving the state. KDHE then proposed nutrient reduction targets of 30% for both TN and TP based on studies that these reductions would substantially improve the dissolved oxygen levels in the Mississippi River and the Gulf of

Mexico and meet a proposed phosphorus criteria of 0.1 mg/L for the Mississippi River (KDHE 2004). KDHE proposes implementation of technology-based biological nutrient removal limits for wastewater, voluntary best management practices in prioritized watersheds, and trading nutrient credits between sources within a watershed. By implementing the point source and nonpoint source strategies and focusing on priority sources, KDHE estimates that the proposed approach will meet or exceed the 30% reduction targets for TN and TP. Furthermore, they believe this approach will adequately reduce the impact of nutrients in its waters and downstream waters without having to develop legally enforceable nutrient criteria (KDHE 2004).

Concluding Remarks

This review demonstrates that a number of different approaches to developing nutrient criteria are possible. Numerous studies have been conducted to determine nutrient and/or algae levels that demonstrate measurable effects on stream communities, and a wide range of results have been obtained. Such thresholds for benthic algae levels vary by a factor of four, and thresholds for both nitrogen and phosphorus concentrations vary by an order of magnitude. These results emphasize the importance of assuring that Virginia sets numeric nutrient criteria that are science-based and meet the goals of the Clean Water Act.

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