Nutrient Reference Condition Identification and Ambient Water Quality Benchmarck Development Process

Rivers and Streams within EPA Region 7

DRAFT

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of the following: the USEPA Region 7 RTAG members from the various agencies, tribes, organizations and universities within Iowa, Kansas, Nebraska and Missouri. Additional invited water quality and biological specialists attended and contributed at meetings on topics within their range of expertise.

The Regional Technical Assistance Group (RTAG) for the development of nutrient criteria in Environmental Protection Agency (EPA) Region 7 formed in 1999. The group consisted of state, federal, tribal and academic members. The RTAG's mission was to develop nutrient benchmarks (surrogate criteria) for midcontinent streams and rivers. The benchmarks developed were designed to protect aquatic life against anthropogenic eutrophication (excess nutrients beyond natural nutrient levels).

The RTAG used EPA guidance for developing nutrient criteria and the process consisted of several iterations of data gathering and assessment, stream classification, and statistical analysis and modeling. Stream data gathering and assessment were conducted by the Central Plains Center for BioAssessment (CPCB) with the assistance of RTAG members. Several stream classification methods were pursued including classifying and analyzing midcontinent streams by Level III ecoregions, Strahler stream order, and land use-land cover. RTAG members developed a selection process for identifying reference streams, and reference streams were identified in each state. Statistical analyses were performed on reference stream data to generate reference stream conditions. Reference stream conditions were also modeled using two statistical approaches: percentiles of all stream data and trisection modeling based on chlorophyll-*a* levels.

Nutrient benchmarks for streams were determined by the RTAG using a weight-of-evidence approach and operating on group consensus basis. Nutrient stream benchmarks were selected by the RTAG after examination and discussion of stressor and response values derived the analysis of four different assessment approaches: 1) *a prior* determined reference method, 2) quartile method, 3) trisection method to define reference 4) stressor-response method (e.g. linear and non-linear regressions) and 5) examination of scientific literature. The RTAG's final benchmark numbers were developed for the entire Region 7 area that includes the state of Kansas (KS), Iowa (IA) Missouri (MO) and Nebraska (NE).

Benchmarks for streams occurring in Region 7 are as follows:

- **0.9 mg/L for total nitrogen (TN)**
- **0.075 mg/L for total phosphorus (TP)**
- **8 mg/L for sestonic chlorophyll-*a* (Chl*a*)**
- **40 mg/M^2 for benthic (Chl*a*)**

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(University of Nebraska, Lincoln) and Dr. Gary Welker (USEPA) were integral members of the RTAG and their collective knowledge and insight were primary drivers in the development of this document and resulting regional benchmarks.

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INTRODUCTION

Background

Nutrients are essential to sustain life and fuel growth. However, in life, too much of a good thing can lead to deleterious effects. In general, excessive amounts of nutrients lead to increased cyanobacteria and algal production resulting in increased availability of organic carbon within an ecosystem, a process known as eutrophication (Bricker et al., 1999). Nutrients in excess of natural conditions, termed cultural eutrophication, impair aquatic life and can lead to harmful human health effects. Results of excess nutrient levels include production of phytoplankton blooms (cyanobacteria, eukaryotic algae) leading to decreased oxygen concentrations, shifts and loss of aquatic species including fish (e.g., Leach et al., 1977; Eminson and Phillips, 1978; Persson et al., 1991; Schupp and Wilson, 1993, Egertson and Downing, 2004) and excessive macrophyte growth (Daldorph and Thomas 1978). Further consequences of high nutrient levels are decreases in water clarity (i.e., murky water), drinking water taste and odor problems and human health effects from the production of cyanobacteria ('blue green algae') and their resultant toxins (see review by Chorus et al., 2000).

Nutrients not only affect autotrophic state of flowing waters, but can also alter heterotrophic state (Dodds, 2006). Research in this area is sparse, but researchers have demonstrated that nutrient enrichment of forested streams can influence litter decomposition (Abelho and Graca, 2006), secondary production of invertebrates (Cross et al., 2006), and production of vertebrates (salamanders) that depend upon streams (Johnson and Wallace, 2005; Johnson et al., 2006). Thus, protecting biotic integrity likely will require nutrient control even in systems where autotrophic processes are not dominant (e.g., turbid rivers or streams with a dense canopy cover).

Biotic integrity can also be influenced by the toxic effects of nitrate (Smith et al., 2006). Invertebrate biodiversity is negatively correlated with the nutrient content of rivers and streams (Wang et al., 2007). Some species of unwanted cyanobacteria can be stimulated by nutrients in streams (Perona and Mateo, 2006), and some species of stream cyanobacteria produce toxic microcystins (Aboal et al., 2005 Makarewicz et al., 2009). Research is still needed to directly link eutrophication in rivers and streams to algal toxin production.

Sources of excess nutrients include agricultural runoff, municipal wastewater, urban runoff and atmospheric deposition (USGS, 1999; Mueller and Spahr, 2006). Impairment from excess nutrients in lakes, streams and wetlands has been documented in virtually all 50 States. 'Dead zones' in coastal waters resulting from cultural eutrophication have been documented as well. The Gulf of Mexico hypoxic (i.e., low oxygen) zone, an area along the Louisiana-Texas coast, is believed to be the resultant impairment from nutrient contributions in the Mississippi, Missouri, and Ohio River watersheds.

Nutrient Benchmark Development Process for Streams

On the 25th anniversary of the 1972 Clean Water Act, former Vice President Al Gore called for the development of an action plan that would fulfill the original "fishable and swimmable" waters goal of the Act. The result of the call to action was the *Clean Water Action Plan* (February, 1998) which provided a 'blueprint for restoring and protecting the nation's waters' by building upon past water quality accomplishments and proposed new challenges for the protection of the nation's waters. One of the challenges proposed was the reduction of nutrient over-enrichment, and the Environmental Protection Agency (USEPA) was called upon to develop numerical criteria – acceptable levels of nutrients (i.e., nitrogen and phosphorus) in water. Nutrient criteria would be different than typical water quality criteria and would be a "menu" of different numeric values' based on waterbody types (i.e., stream, lake, wetland) and ecoregional, physiographic or other spatial classifications.

In June of 1998, the *National Strategy for the Development of Regional Nutrient Criteria* was produced by EPA's Office of Water and provided an approach for assessing nutrient information and working with States and Tribes in the development of protective nutrient criteria. Key elements of the strategy were to take a geographic and waterbody approach, development of technical guidance, the use of regional nutrient teams and the development of criteria by States and Tribes.

Ecoregional and *waterbody-type* specific approach

Rather than develop nation-wide criteria for nutrients, the national nutrient strategy called for the development of criteria based on regional geographic basis as defined by geology, soils, topology, vegetation and climatic conditions. One suggested geographic approach was the 'ecoregion' framework (Figure 1) developed by James Omernik (Omernik, 1987) that was used as the basis for his later development of nutrient ecoregions (Omernik, 2000) as illustrated in this document (Figure 2). The size of ecoregions can vary from watershed size to continental in scale. For the purposes of developing nutrient criteria the scale or size the ecoregions should be dictated by regional nutrient conditions and availability of data. In addition to Omernik's ecoregion work, other geographic regions and ecoregions have been developed by a number of researchers (e.g., Kuchler, 1964; Bailey, 1995; Maxwell et al., 1995; Abel et al., 2000). While a number of regionalization approaches are available, Omernik's ecoregions are often used in proposed approaches that could be used in development of nutrient criteria (Rohm et al., 2002; Dodds and Oakes, 2004; TDEC, 2004; Stoddard, 2005; VWRRC, 2005).

A major element of the national nutrient strategy was to develop nutrient criteria by waterbody type (i.e., lakes, streams and wetlands). The focus of this document is on the development of nutrient benchmarks for streams. The technical advisory groups will determine or adapt a classification of streams for criteria development based, whole or in part, on observed relevant nutrient relationships found for stream classes. The strategy also calls for the development of nutrient criteria for lakes, wetlands, estuaries and coastal marine waters and highlights the need to keep in mind the inter-relationship of all water-body types.

Technical guidance

In July of 2000, the EPA Office of Water published the *Nutrient Criteria Technical Guidance Manual: Rivers and Streams* (USEPA 2000a). This 'streams document' provides technical guidance to develop water quality criteria for the protection aquatic life from excess nutrients in lotic systems. It builds upon the national nutrient strategy and provides guidance on establishing appropriate databases, causal and response variables, characterization of reference condition, nutrient modeling, criteria development process and use of nutrient criteria to protect water quality. In addition to the stream document the Office of Water has published the following waterbody specific guidance: *Nutrient Criteria Technical Guidance Manual: Lakes and Reservoirs* (USEPA, 2000b) and *Nutrient Criteria Technical Guidance Manual: Wetlands* (USEPA, 2008).

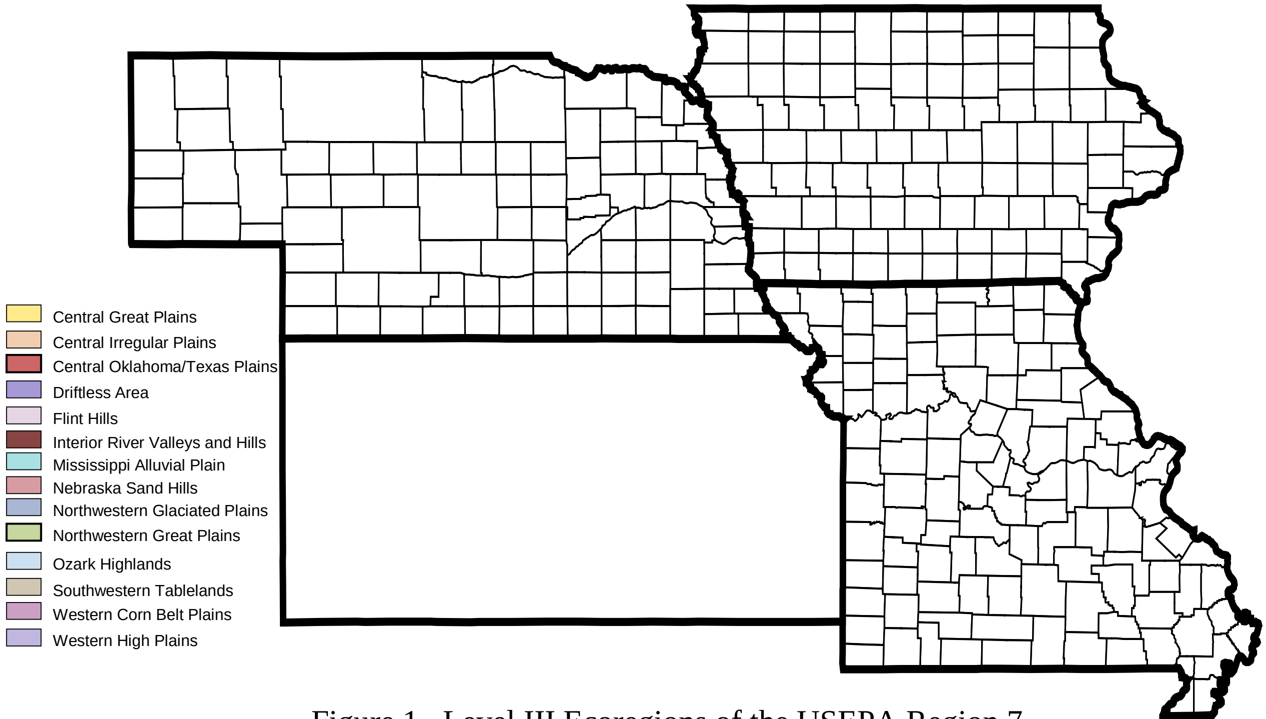


Figure 1. Level III Ecoregions of the USEPA Region 7

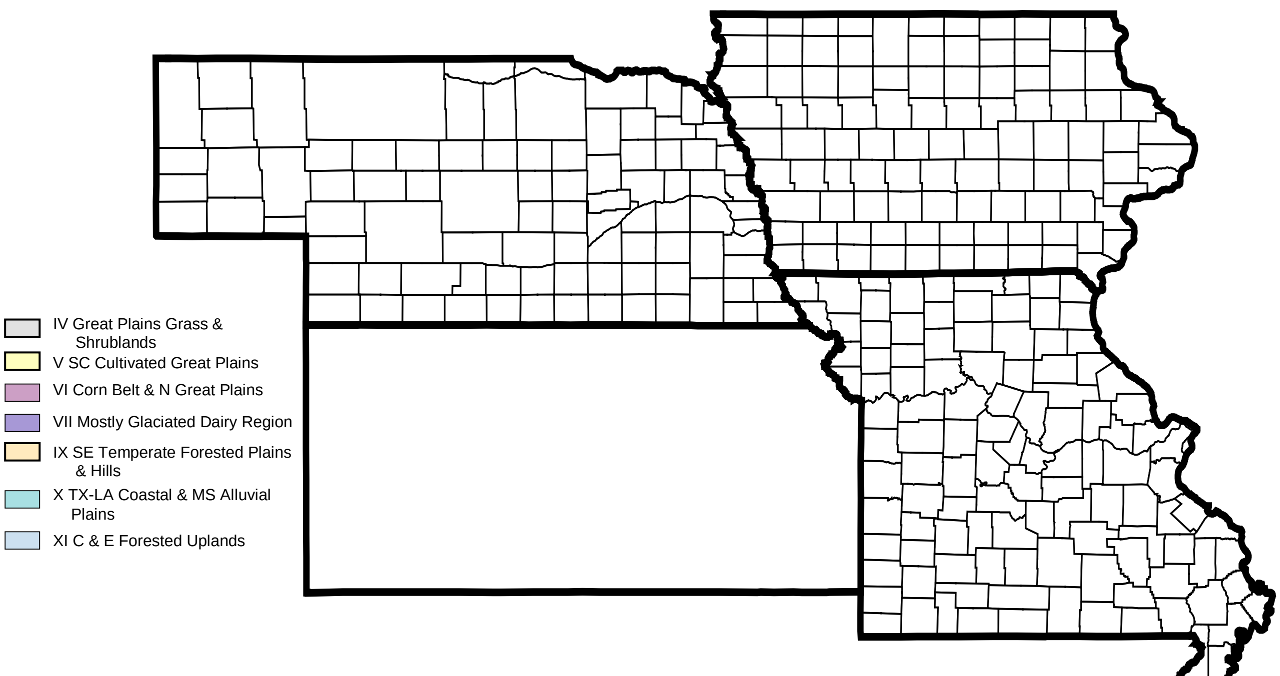


Figure 2. Nutrient Ecoregions of the USEPA Region 7.

In December of 2000, the EPA Office of Water published river and stream nutrient criteria recommendations and associated documents for each of the 14 ‘nutrient regions’ in the continental United States. Seven of the 14 documents pertain to nutrient regions occurring within the geopolitical boundaries of EPA Region 7 (Figure 2): *Ambient Water Quality Criteria Recommendations Rivers and Streams in Nutrient Ecoregion* IV, V, VI, VII, IX, X and XI (USEPA 2000c-f, 2001c-e). Nutrient criteria recommendations published in the aforementioned documents are to provide guidance for State and Tribes in developing water quality criteria and provide benchmarks to EPA when federal promulgation of nutrient standards is deemed necessary. Other uses of the recommendations cited in the documents are the identification of status and trends and the use as yardsticks or benchmarks for over-enrichment assessment in rivers and streams. In addition to these regional stream documents, EPA's Office of Water has also produced a similar series of documents for lakes and reservoirs.

Technical Advisory Groups

Regional Technical Advisory Group (RTAG)

The RTAG (i.e., regional nutrient workgroup) for EPA Region 7 was first established in 1999. The regional workgroup was coordinated by the Region 7 Regional Nutrient Coordinator (Dr. Gary Welker) and facilitated by the Central Plains Center for BioAssessment (Dr. Don Huggins, Director). The workgroup was comprised of individuals from governmental, tribal and academic institutions having technical expertise in nutrients and water quality standards. Membership has change slightly since the formation but has essentially been comprised of scientists from: Iowa Department Natural Resources, Kansas Department of Health and Environment, Missouri Department of Natural Resources, Nebraska Department of Environmental Quality, the Prairie Band of Potawatomi Indians, Iowa State University, University of Kansas, Kansas State University, University of Missouri-Columbia, University of Nebraska-Lincoln, United States Geological Survey, United States Department of Agriculture, Central Plains Center for BioAssessment, and the United States Environmental Protection Agency.

The mission of the workgroup was to 'develop scientifically defensible numeric nutrient benchmarks for lakes/reservoirs, streams/rivers and wetlands in the Central Great Plains (Iowa, Kansas, Missouri and Nebraska).' The RTAG's role was to develop benchmarks for nutrients to aid the States and Tribes in their responsibility to develop nutrient criteria. Workgroup operational ground-rules for the development of nutrient benchmarks for rivers and streams were as follows:

- Nutrient benchmarks are to protect rivers and streams and down stream receiving waters against adverse impacts of cultural eutrophication (excess nutrient levels above natural or minimally impaired conditions).

- Nutrient benchmarks are to be protective of aquatic life. Economics, technology, attainability, social values are not part of the benchmark development process.

- Benchmarks developed by the RTAG are by group consensus and are developed for the purpose of assisting and providing guidance to States and Tribes in the development of their own nutrient criteria.

River and stream variables

EPA requires that nutrient criteria developed by States and Tribes be comprised of both causal and response variables (Grubbs, 2001). Nitrogen and phosphorus have long been known to be primary causes of cultural eutrophication (National Academy of Science, 1969; Smith, 1982; Elser et al., 1990; Correll, 1999; Jeppesen et al., 2000; National Research Council, 2000) and have been selected as the two primary nutrient causal variables. The linkage between causal variables (e.g., TP and TP) and

Chl*a* (response variable), a commonly used indicator of algal biomass, is well known (USEPA, 2000a, b). Both sestonic and benthic Chl*a* could be used algae response variables. Macroinvertebrate and fish communities can also be examined for possible indirect effects related to ecosystem-level changes related to nutrient enrichment (i.e. increases in nitrogen and phosphorus). Macroinvertebrate and fish community metrics quantify changes in community structure and function with changes in trophic state. Other examples of response variables that a State or Tribe could develop into criteria are periphyton metrics, biological oxygen demand, and macrophyte metrics. Emphasis should be placed on developing both causal (TN and TP) and a response variable(s).

Development of Criteria by States and Tribes

The Office of Water has produced nutrient criteria recommendations (sometimes referred to as 304(a) criteria), which are based on rather large geographic areas (i.e., nutrient regions). The result of the RTAG effort and this document is the development of 'nutrient benchmarks' on a smaller geographic scale as compared to the geographic scale used to develop the 304(a) criteria. However, the States and authorized Tribes are ultimately responsible for developing causal and response nutrient criteria for their State/Tribe on a Statewide/Reservation-wide approach or using sub-sets of geographic areas (i.e., ecoregions) within their State/Tribal borders. Thus the States and Tribes, on a geographic scale, will further refine the nutrient benchmarks developed within this document. It is hoped that the States and Tribes will use this document and the lessons learned from the process the RTAG experienced in the development of their nutrient criteria. Alternatively, they may may chose to adopt the nutrient benchmarks within this document as numeric criteria for streams in their state.

OVERVIEW OF THE NUTRIENT PROBLEM

Eutrophication is an established water quality management concept and concern reaching as far back as the 1600's in America (Capper et al., 1983). However, extensive public recognition of this form of pollution in coastal water bodies is relatively recent. The publication "Eutrophication, Causes, Consequences, and Correctives" (National Academy of Science, 1969) is often perceived as the technological beginning of American nutrient pollution awareness and is centered on the understanding and abatement of this problem primarily in freshwater lakes and reservoirs. We have since come to better understand the problem in streams, rivers, and estuaries, with a focus on: (1) recommending ways to help watershed managers achieve meaningful reductions in the impacts of nutrient over-enrichment in the near-term and (2) identifying areas where future efforts hold the promise of long-term reductions in nutrient over-enrichment and its effects (Nürnberg, 1996; Smith et al., 1999; Anderson et al., 2002; Smith, 2003).

Eutrophication in rivers and streams has received far less attention than that in lakes. For example, only a few schemes exist to classify trophic state in rivers and streams (Dodds et al., 1998; Dodds, 2006). In contrast, entire books have been written about controlling and classifying trophic state in lakes (e.g., Ryding and Rast 1989). Thus, most regions are starting at a more fundamental level to set nutrient criteria for rivers and streams. The need to specifically identify indicators of over-enrichment and reference conditions in the region before proceeding with setting nutrient criteria is clear, since nutrient condition is intimately tied to ecosystem structure and function as well as water quality in rivers and streams (Dodds, 2006). Several challenges exist in defining trophic state for streams and rivers. The most daunting is the non-equilibrium nature of lotic ecosystems that increases variance in the relationship between nutrients in the water column and benthic metabolic activity. Another obstacle is that conditions in rivers and streams reflect processes across a large and heterogeneous landscape. Relationships between nutrients and primary producers and the possible consequences on higher tropic levels are confounded by a number exogenous and endogenous factors including rapid nutrient spiraling (Mulholland et al., 1995), light limitation due to turbidity and shading (e.g., Lowe et al., 1986; Hill 1996), frequency and duration of spates (e.g., Briggs 1995; Lohman et al., 1992), habitat heterogeneity (Briggs, 1996; 2000), duration of nutrient enrichment (Elsdon and Limburg, 2008).

Moreover, it is oftentimes difficult to find reference sites with respect to nutrient conditions in streams, and ever more so in rivers where anthropogenic impacts are almost certain to occur at multiple locations within the watershed with rivers most often suffering cumulative effects across the whole of their larger drainage area.

In response to this growing awareness nutrient impacts, USEPA's National Nutrient Criteria Program encouraged the development of this technical manual to be used by States and Tribes in the reduction of eutrophication of the Nation's freshwaters. This document concentrates on the effort to identify reference conditions of streams within the USEPA Region 7, which can be used as benchmarks to evaluate eutrophication and as starting points for nutrient criteria derivation.

GEOGRAPHIC AREA COVERED BY THIS DOCUMENT

The following section provides a general description of Nutrient Ecoregions and Level III Ecoregions examined in this report, and their geographical boundaries. The boundaries and extent of both the Nutrient Ecoregions and Level III Ecoregions occurring partially or wholly within USEPA Region 7 are shown in Figure 1 and Figure 2. Portions of seven Nutrient Ecoregions are found in Region 7 all of which have been studied by USEPA to determine Nutrient Ecoregion-level benchmark values for TN, TP and Chl*a* (see USEPA 2000c-f, 2001c-e). The published benchmark values for the seven Nutrient Ecoregions are used in comparing literature values with RTAG benchmark values

USEPA Region 7 is comprised of fifteen Level III Ecoregions, all of which had stream data. However, the Mississippi Valley Loess Plains contains only one site with one record. A detailed description of the characteristics of each of these subecoregions is provided below for each of the seven aggregate Nutrient Ecoregions covered by this report. The following are brief descriptions provided by Rohm et al. (2002) of the climate, vegetative cover, topography, and other ecological information pertaining to these ecoregions.

Aggregate nutrient ecoregion IV: Great Plains Grass and Shrublands.

26. Southwestern Tablelands (Omernik Level III)
Unlike most adjacent Great Plains ecological regions, little of the Southwestern Tablelands is in cropland. Much of this elevated tableland is in sub-humid grassland and semiarid range land. The potential natural vegetation in this region is grama-buffalo grass with some mesquite-buffalo grass in the southeast and shinnery (midgrass prairie with open low and shrubs) along the Canadian River.

28. Flint Hills (Omernik Level III)
The Flint Hills is a region of rolling hills with relatively narrow steep valleys, and is composed of shale and cherty limestone with rocky soils. In contrast to surrounding ecological regions that are mostly in cropland, most of the Flint Hills region is grazed by beef cattle. The Flint Hills mark the western edge of the tallgrass prairie, and contain the largest remaining intact tallgrass prairie in the Great Plains.

43. Northwestern Great Plains (Omernik Level III)
The Northwestern Great Plains ecoregion encompasses the Missouri Plateau section of the Great Plains. It is a semiarid rolling plain of shale and sandstone punctuated by occasional buttes. Native grasslands, largely replaced on level ground by spring wheat and alfalfa, persist in rangeland areas on broken topography. Agriculture is restricted by the erratic precipitation and limited opportunities for irrigation.

44. Nebraska Sand Hills (Omernik Level III)

The Nebraska Sandhills comprise one of the most distinct and homogenous ecoregions in North America. One of the largest areas of grass stabilized sand dunes in the world; this region is generally devoid of cropland agriculture and is treeless except for some riparian areas in the north and south. Few streams drain this ecoregion but large portions of the region contain numerous lakes and wetlands.

Aggregate nutrient ecoregion V: South Central Cultivated Great Plains

25. Western High Plains (Omernik Level III)
Higher and drier than the Central Great Plains to the east, and in contrast to the irregular, mostly grassland or grazing land of the Northwestern Great Plains to the north, much of the Western High Plains comprises smooth to slightly irregular plains having a high percentage of cropland. Grama-buffalo grass is the potential natural vegetation in this region as compared to mostly wheatgrass-needlegrass to the north, Trans-Pecos shrub savanna to the south, and taller grasses to the east. The northern boundary of this ecological region is also the approximate northern limit of winter wheat and sorghum and the southern limit of spring wheat.

27. Central Great Plains (Omernik Level III)
The Central Great Plains is slightly lower, receives more precipitation, and is somewhat more irregular than the Western High Plains to the west. Much of this ecological region is now cropland but was once grassland with scattered low trees and shrubs in the south. The eastern boundary of the region marks the eastern limits of the major winter wheat growing area of the United States.

Aggregate nutrient ecoregion VI: Corn Belt and Northern Great Plains.

47. *Western Corn Belt Plains* (Omernik Level III)
Once covered with tallgrass prairie, over 75 percent of the Western Corn Belt Plains is now used for cropland agriculture and much of the remainder is in forage for livestock. A combination of nearly level to gently rolling glaciated till plains and hilly loess plains, an average annual precipitation of 63-89 cm, which occurs mainly in the growing season, and fertile, warm, moist soils make this on of the most productive areas of corn and soybeans in the world. Major environmental concerns in the region include surface and groundwater contamination from fertilizer and pesticide applications as well as impacts from concentrated livestock production.

Aggregate nutrient ecoregion VII: Mostly Glaciated Dairy Region

52. *Driftless Area* (Omernik Level III)
The hilly uplands of the Driftless Area easily distinguish it from surrounding ecoregions. Much of the area consists of a deeply dissected, loess-capped, bedrock dominated plateau. The region is also called the Paleozoic Plateau because the landscape’s appearance is a result of erosion through rock strata of Paleozoic age. Although there is evidence of glacial drift in the region, the influence of the glacial deposits have done little to affect the landscape compared to the subduing influences in adjacent ecoregions. Livestock and dairy farming are major land uses and have had a major impact on stream quality.

Aggregate nutrient ecoregion IX: Southeastern Temperate Forested Plains and Hill

29. Central Oklahoma/Texas Plains (Omernik Level III)
The Central Oklahoma/Texas Plains ecoregion is a transition area between the once prairie, now winter wheat growing regions to the west, and the forested low mountains of eastern Oklahoma. The region

does not possess the arability and suitability for crops such as corn and soybeans that are common in the Central Irregular Plains to the northeast. Transitional “cross-timbers” (little bluestem grassland with scattered blackjack oak and post oak trees) is the native vegetation, and presently rangeland and pastureland comprise the predominant land cover. Oil extraction has been a major activity in this region for over eighty years.

40. Central Irregular Plains (Omernik Level III)
The Central Irregular Plains have a mix of land use and are topographically more irregular than the Western Corn Belt Plains (47) to the north, where most of the land is in crops. The region, however, is less irregular and less forest covered than the ecoregions to the south and east. The potential natural vegetation of this ecological region is a grassland/forest mosaic with wider forested strips along the streams compared to Ecoregion 47 to the north. The mix of land use activities in the Central Irregular Plains also includes mining operations of high-sulfur bituminous coal. The disturbance of these coal strata in southern Iowa and northern Missouri has degraded water quality and affected aquatic biota.

72. Interior River Lowland (Omernik Level III)
The Interior River Lowland is made up of many wide, flat-bottomed terraced valleys, forested valley slopes, and dissected glacial till plains. In contrast to the generally rolling to slightly irregular plains in adjacent ecological regions to the north (54), east (55) and west (40, 47), where most of the land is cultivated for corn and soybeans, a little less than half of this area is in cropland, about 30 percent is in pasture, and the remainder is in forest. Bottomland deciduous forests and swamp forests were common on wet lowland sites, with mixed oak and oak-hickory forests on uplands. Paleozoic sedimentary rock is typical and coal mining occurs in several areas.

Aggregate nutrient ecoregion X: Texas-Louisiana Coastal and Mississippi Alluvial Plains.

73. Mississippi Alluvial Plain (Omernik Level III)
This riverine ecoregion extends from southern Illinois, at the confluence of the Ohio River with the Mississippi River, south to the Gulf of Mexico. It is mostly a broad, flat alluvial plain with river terraces, swales, and levees providing the main elements of relief. Soils are typically finer-textured and more poorly drained than the upland soils of adjacent Ecoregion 74, although there are some areas of coarser, better-drained soils. Winters are mild and summers are hot, with temperatures and precipitation increasing from north to south. Bottomland deciduous forest vegetation covered the region before much of it was cleared for cultivation. Presently, most of the northern and central parts of the region are in cropland and receive heavy treatments of insecticides and herbicides. Soybeans, cotton, and rice are the major crops.

74. Mississippi Valley Loess Plains (Omernik Level III)
This ecoregion fell into the study area of USEPA Region 7 in 2002 with revisions of the ecoregions boundaries. This ecoregion stretches from near the Ohio River in western Kentucky to Louisiana. It consists primarily of irregular plains, some gently rolling hills, and near the Mississippi River, bluffs. Thick loess is one of the distinguishing characteristics. The bluff hills in the western portion contain soils that are deep, steep, silty, and erosive. Flatter topography is found to the east, and streams tend to have less gradient and siltier substrates than in the Southeastern Plains ecoregion (65). Oak-hickory and oak-hickory-pine forest was the natural vegetation. Agriculture is now the dominant land cover in the Kentucky and Tennessee portion of the region, while in Mississippi there is a mosaic of forest and cropland.

Aggregate nutrient ecoregion XI: Central and Eastern Forested Uplands.

39. Ozark Highlands (Omernik Level III)
The Ozark Highlands ecoregion has a more irregular physiography and is generally more forested than adjacent regions, with the exception of the Boston Mountains (38) to the south. The majority of this dissected limestone plateau is forested; oak forests are predominant, but mixed stands of oak and pine are also common. Karst features, including caves, springs, and spring-fed streams are found throughout the Ozark Highlands. Less than one fourth of the core of this region has been cleared for pasture and cropland, but half or more of the periphery, while not as agricultural as bordering ecological regions, is in cropland and pasture.

DATA COLLECTION FOR STREAMS AND RIVERS IN EPA REGION 7

The USEPA Region 7 RTAG and outside experts comprised water quality specialists, colleges, water resource managers and scientists, representing a variety of state and tribal agencies and universities, who were selected in accordance with National Nutrient Criteria Strategy Document guidelines (USEPA, 1998). Database compilation and data analysis were performed by Central Plains Center for BioAssessment (CPCB).

In agreement with the EPA's National Strategy, the RTAG initially recommended four primary variables for data collection: Total Nitrogen (TN), Total Phosphorus (TP), and Chlorophyll-*a* (Chl*a*, sestonic and benthic). These variables were selected because they are early indicators of causal and biological response indicators of nutrient loadings. Early in the RTAG process it was recognized that more nutrient data needed to be collected and analyzed for reference streams to increase their sample size and allow better chacterization of this population. USEPA Region 7 tasked CPCB to sample a select number of reference streams for TN, TP and Chla from both sestonic and benthic algal communities. Spring, summer and fall samples were collected and analysed for each of three years to enhance the reference stream database. Auxiliary data (e.g., ecoregion, stream order, turbidity, biological information) were also collected to link trophic state to other potential abiotic drivers and biotic responses.

Data sources

CPCB compiled available water quality data for streams and rivers in Iowa, Kansas, Missouri, and Nebraska (Figure 3 and Figure 4). These data were collected between 1965 and 2003 by a variety of agencies and individuals with established internal quality assurance procedures (Table 1). All data were ultimately combined into a single Microsoft Access® relational database, and checked for accuracy and quality. See Appendix B for references to original field and laboratory methods, as well as for details on data handling, database compilation, and quality assurance methods.

Though the data came from reliable sources, the methods of recording the data, units of measure, common and specific names and resolution of geospatial data sometimes varied from agency to agency. Therefore, CPBC identified differences in data attributes and reporting and standardized all information within the RTAG databases. Sites that were geospatially similar but having differing information (e.g., fish, water quality, macroinvertebrate) were linked by a proximity rule. All sites along the same stream channel that were within 2 km of each other were given the same numeric site code, if there were no tributaries, wastewater treatment plants, or other conditions between them that might greatly affect their biological and physiochemical similarity. See Appendix 8.1 for the rules of grouping sites together.

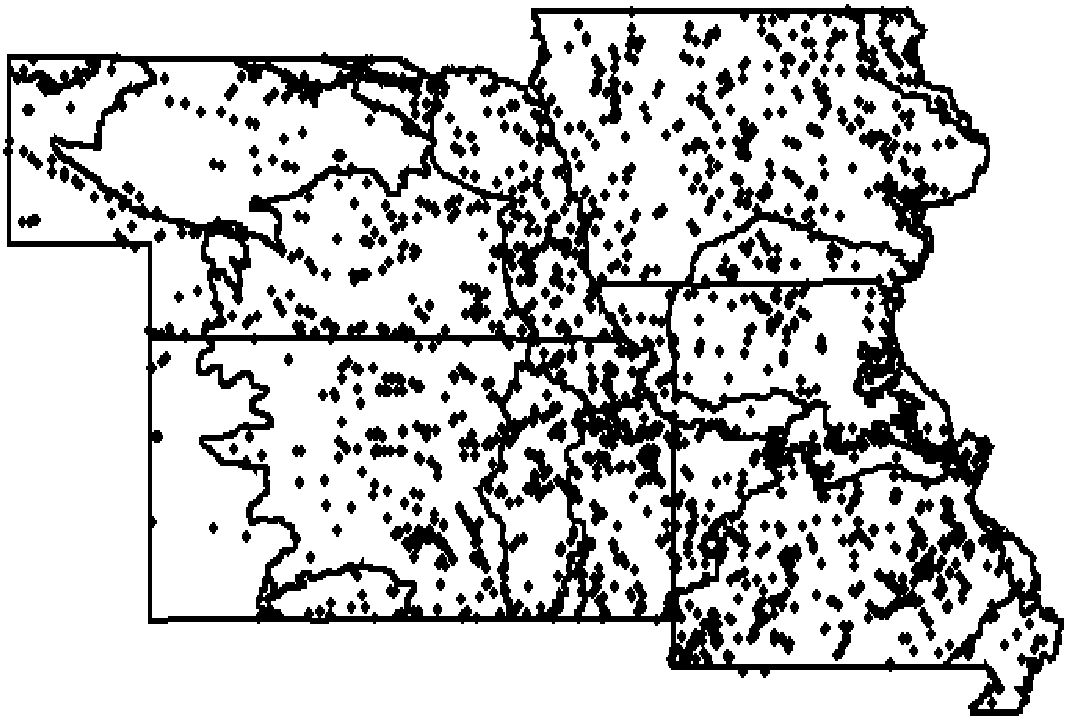


Figure 3. All water quality and biological monitoring stations for streams and rivers within USEPA Region 7 from which data were extracted and used in this study. Omernik's Level III ecoregions are outlined.

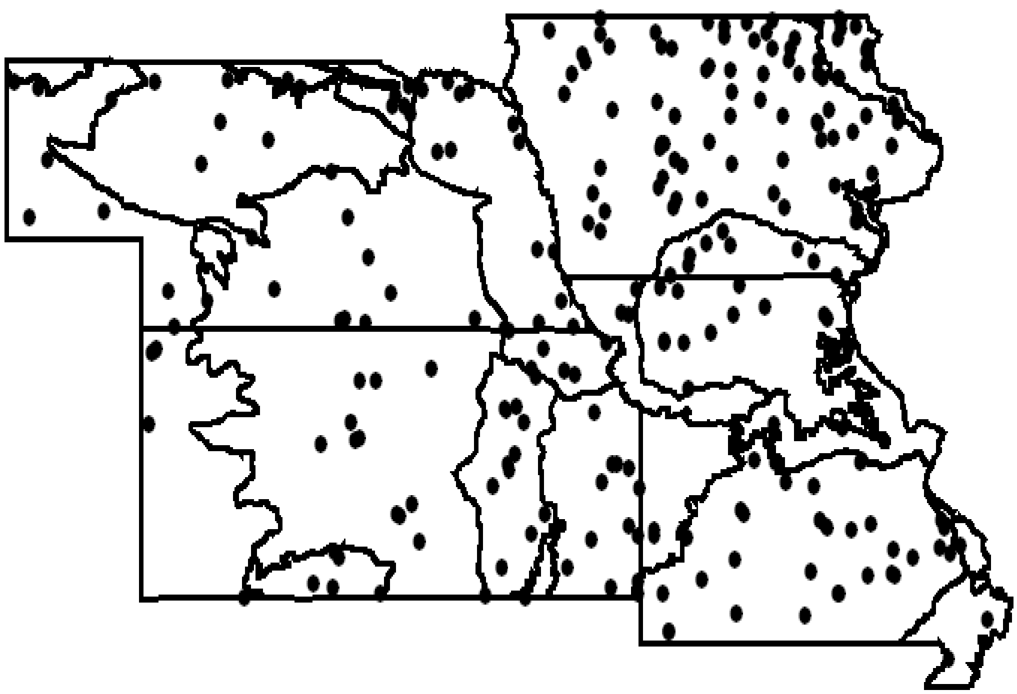


Figure 4. The *a priori* reference water quality and biological monitoring stations for streams and rivers within USEPA Region 7 from which data were extracted and used in this study. Omernik's Level III ecoregions are outlined.

Table 1. Datasets comprising the USEPA Region 7 stream database analyzed in this document, with states (I = Iowa, K = Kansas, M = Missouri, N = Nebraska), number of sample sites and records of total nitrogen (TN), total phosphorus (TP), chlorophyll-*a* (Chl*a*), fish, and macroinvertebrates (Inv).

Dataset name	Agency	Project	State	Years	Sites	TN	TP	Chl*a*	Fish	Inv
CPCB DO	CPCB	DO flux	I,K,N	1999-2002	52	177	177	177	0	46
CPCB nutrient	CPCB	reference streams	I,K,MN	2000-2002	106	726	726	726	0	348
CPCB TMDL Streams	CPCB	impaired streams	K	1999-2001	42	198	198	198	0	0
CPCB model	CPCB	KS lake tributaries	K	2001-2002	22	243	243	0	0	0
CPCB tristate	CPCB	KBS Tristate	I,K,N	1992-1994	80	1972	2079	1911	540	521
IA STORET	USEPA	various	I	1990-1999	268	yes	yes	yes	0	0
IADNR	IDNR	IDNR	I	1994-2001	207	286	285	0	334	328
IAREMAP2002	IDNR	IDNR	I	2002	65	176	176	176	885	3984
IAUniv	Univ. IA Hyg. Lab	IDNR	I	1999-2003	99	4104	4388	3858	0	0
KDHE	KDHE	KDHE	K	1999-2003	318	4422	5822	98	0	0
KDHE ref	KDHE	KDHE	K	1990-2002	20	0	0	0	0	131
KS STORET	USEPA	various	K	1990-1997	491	900	11075	222	0	0
KDWP	KDWP	KDWP w/REMAP2	K	1994-2001	~399	562	566	0	727	304
MDNR James	MSU	MDNR James R.study	M	2001-2002	11	341	334	373	0	0
MDNR streams	MDNR	All MDNR data	M	1965-2002	618	9177	13065	0	0	0
MO STORET	USEPA	various	M	1990-1997	177	1141	1700	257	0	0
MO Jones	UMC	Jack Jones, UMC	M	1985-1986	16	577	579	579	0	0
MOREMAP2	MDOC	REMAP 2002	M	2002	83	0	0	0	83 sites	74 sites
MDNR ref	MDNR	REMAP 2002	M	2002	61	0	0	0	0	61 sites
NDEQ20022003	NDEQ		N	2002-2003	153	3335	3335	0	0	0
NDEQpre2002	NDEQ	NDEQ	N	1983-2001	194	198	159	0	205 sites	120 sites
NE EPA Storet	USEPA	various	N	1990-1998	153	1164	1789	0	0	0

The Primary Database – Raw Data

The chemistry database used in developing this report was created from the sources detailed above. In the chemistry database, each record represented a single sampling event, and its relevant source information (agency, sampling date) was preserved. As noted above, the RTAG initially identified five parameters as relevant to Nutrient Criteria development: Total Nitrogen (TN), Total Phosphorus (TP), sestonic chlorophyll-*a* (Chl*a*), benthic chlorophyll-*a* (natural substrates), and turbidity. The RTAG also identified two ratios as being of interest, TN:TP and Chl*a*:TP, which were calculated for each sampling event. This chemistry database contained raw sample records of 54,393 sampling events from 1965 to 2003, at 2400 waterbodies. The number of sampling events per waterbody ranged from 1 to 452. Each record in the database did not necessarily include values for all five parameters, however, most of the records in the database contained some information relevant to Nutrient Criteria development: 32,364 TN values, 51,176 TP values, 8007 sestonic Chl*a* values, 1203 benthic Chl*a* values, and 19,087 turbidity values.

The RTAG also directed CPCB to assembled a database of fish collections (2369 sampling events, 1325 sites, 1984 to 2003, >200 taxa) and a database of macroinvertebrate collections (1874 sampling events, 1151 sites, 1984 to 2003, >1200 taxa) in USEPA Region 7 (Table 1) as potential indicators of biotic integrity. The raw specimen data was distilled into several metrics, then the metrics for each biological sampling event was paired with chemistry data collected ≤30 days prior to the biological data. Because so few biological events co-occurred exactly with water quality sampling dates a series of water quality sampling periods were created (i.e. ≤30, ≤60, ≤90 , ≤120 days) and correlated with fish and macroinvertebrate metrics using both Spearman's rank correlation and Pearson's correlation (NCSS, 2004). After examination of these correlations the RTAG concluded the "best" sampling window was the ≤30-day sampling window given this window consistently produced the higher correlation coefficients between water quality variables (e.g. TN, TP, turbidity, chlorophyll) and biotic metrics. Metrics calculated were taxa or species richness and percent sensitive taxa for both fish and macroinvertebrate. Also for the fish we calculated Simpson's Diversity Index (Simpson's D), and for the macroinvertebrate we calculated percent EPT (Ephemeroptera, Plecoptera and Trichoptera). Using the 30-day window, we paired 1179 chemistry sampling events with the fish samples, and 507 chemistry sampling events with macroinvertebrate samples.

Macroinvertebrate indices calculations:
Total Taxa Richness: Count of all taxa found at that site on that date.
Percent EPT: Count of all EPT taxa found at that site on that date/total taxa richness.
Percent Sensitive: Count of all sensitive taxa found at that site on that date/total taxa richness. Sensitivity was assigned based on values taken from Appendix B: Regional Tolerance Values in in the USEPA document entitled "Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2nd edition" (Barbour et al., 1999). In that document, for five geographic regions taxa were assigned tolerance values on a scale from 0 (extremely sensitive or not tolerant) to 10 (tolerant). We averaged the literature scores for each taxon and then divided each mean taxa tolerance value by three (3) to to produce three tolerance classes (≈ sensitive or intolerant, intermediate or facultative, tolerant) as used in the tolerance scheme for fishes (see below). Therefore the re-scaling of the macroinvertebrate tolerance scores produced the following tolerance scheme: ≤ 3.67 indicated sensitive taxa; the intermediate class was 3.68 to 7.34 and taxa having adjusted tolerances scores greater than 7.35 were considered to be pollution tolerant taxa.

Fish indices calculations:
Total Taxa Richness: Count of all taxa found at that site on that date.
Simpson's D: Measures the probability that two individuals randomly selected from a sample will belong to the same species, 0 represents infinite diversity and 1 represents no diversity. The formula is $D = [\Sigma n(n-1)]/N(N-1)$ where n = the total number of organisms of a particular species and N = the total number of organisms of all species.
Percent Sensitive: Count of all sensitive taxa found at that site on that date/total taxa richness. Taxa were marked as sensitive based on two documents that list fish sensitive values: The first list was Appendix C: Tolerance and Trophic Guilds of Selected Fish Species which is also found in USEPA's Rapid Bioassessment Protocols document (Barbour et al., 1999). In this document, taxa were assigned tolerance values of I = intolerant (sensitive), M = intermediate, or T = tolerant. The second list was the Autecology table developed by Dave Peck of USEPA's Western Ecology Division Laboratory in Corvallis, OR. Mr. Peck compiled information for this table on the autecology of North American fishes for use in EPA's EMAP program studies. If the tolerance values for the two lists differed for a taxon, the more sensitive category or the category for the "corn belt" region was selected for use in our analyses.

Classification factors

The RTAG also identified five factors as potentially important classification variables for use in the initial data analyses (see below). Other classification factors (e.g. discharge, stream gradient, dominant substrate, geomorphic stream type) were considered by the RTAG but these data were seldom known for streams and sites in the RTAG database which prevented exploring their usefulness. The first factor attempts to describe similar geophysical regions that have potential similar nutrient and biological features. The second factor addresses temporal classes (e.g sampling season) and their potential effects on benchmark values. The other three factors were quantitative measures of morphometry and hydrology (Strahler stream order, watershed size, and precipitation). All factors and their relationships with other variables were analyzed using both the primary database and the medians database. Details of these analyses are described in Section 5.3.

Level III Ecoregion (15 categories): Fifteen Level III Ecoregions (Omernik, 1987) are found in US EPA Region 7 however only fourteen had sufficient data for analyses. River or stream sampling sites were classified according to EPA-revised ecoregion boundaries (Chapman et al. 2001; 2002). These Ecoregions may be reclassified if needed into seven Nutrient Regions (USEPA, 1998), but this regional classification scheme was not used in our analyses.

Sampling Season (all dates, 4 seasons, monthly, growing season): The use of individual sampling date data was dismissed early in the RTAG process as the RTAG could not envision a use for the evaluation of daily effects in establishing benchmarks and because sampling events for stressor and receptor indicators were almost never coordinated between streams, watershed, ecoregions or states. Thus analysis of stressor data and receptor data by stream population (e.g. large watershed, ecoregion, Region 7) by sample date was considered impractical due to the highly variable sample size when testing for small scale temporal effects (i.e datewise). Assessment of temporal effects by month, season or even growning season were thought to be more feasible when generating benchmark values that might have practical application. Four “seasonal periods” were created by coding sampling date into four seasons: Winter (22 Dec-21 Mar), Spring (22 Mar-21 Jun), Summer (22 Jun-21 Sep), and Fall (22 Sep-21 Dec) while the “growning season” within Region 7 was estimated to start around the third week of March and end the second week of September. Upon initial evaluation of the raw data, the RTAG decided not to classify stressor data into any temporal groupings but decided to uses all available TP and TN data in determining the overall stressor concentrations of streams and rivers in the medians database. These descriptive statistics were used to calculate various stressor properties of the total stream and river population as well as reference groups defined by various approaches (e.g. tri-section).

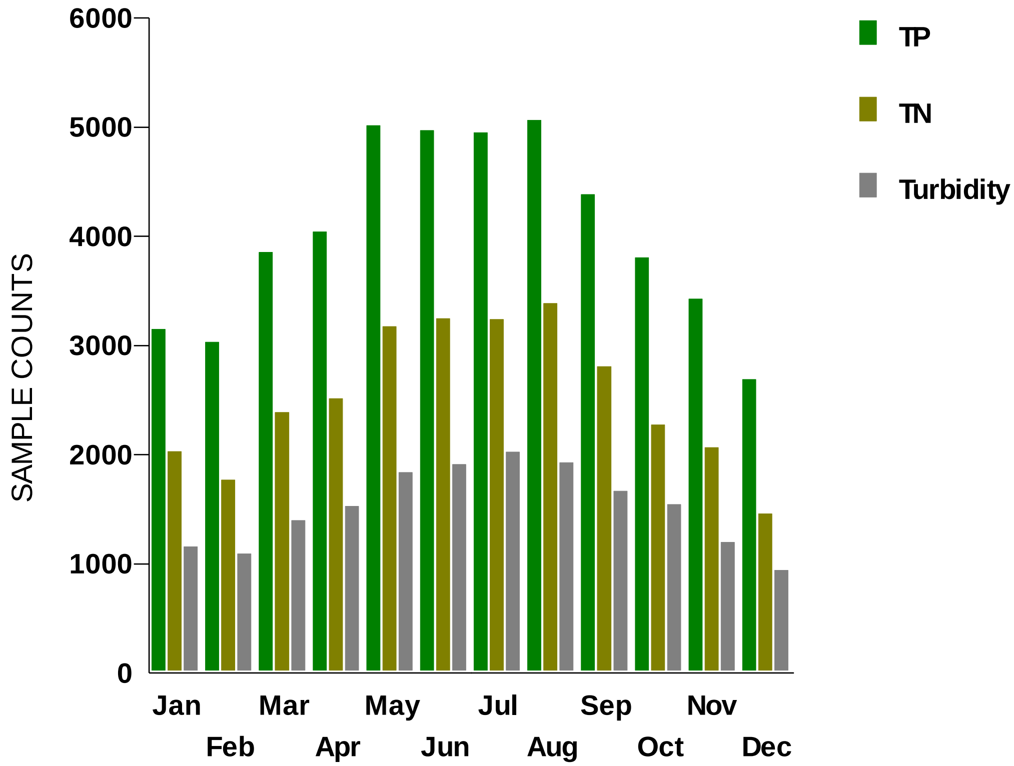


Figure a. Monthly distribution of TP, TN and Turbidity samples within the nutrient database.

Most RTAG attempts to discern temporal differences across additional classification categories (e.g.ecoregions, watershed size) were not practical due to the sometimes limited and often temporally clumped nature of the samples within other categories.. Aditionally, it wasrecognized that the north/south and east/west clines in temperature, rainfall and other factors made the defining months or seasons by specific dates or periods assumed that the climate conditions within Region 7 were fairly homogenious which they are not. Conversely, only by pooling the temporal data for streams could the RTAG gain a reasonable sample size for analysis and assessment of the other identified classification factors.

Use of temporially-related variables of interest when examining stressor – receptor relationships was also discussed at length by the RTAG. As previously stated seldom were stressor/receptor data obtained on the same day or week or even at precisely the same site in the system yet properly linking receptors to current or prior existing stressor concentrations was recognized as important in attempting to identify stressor/receptor relationships. This was overcome by examining and creating a 30-day sampling window that linked these two groups of variables (see Primary Data -

Raw Data Section). Receptor groups examined were fish, macroinvertebrates and algae (i.e chlorophyll *a* concentrations) all of which were most often sampled in a more restricted portion of the annual cycle that stressor data. Examination of the monthly distribution of receptor samples showed that in most instances these data were confined to the warmer months and most occurred within the proposed "growning season" (Figures b and c). There is some regional evidence macroinvertebrate community structure exhibits temporally differences with summer and autumn clusters being more similar to each other than the winter cluster (late December to mid-April) (Kosnicki and Sites 2011). Nearly all of our paired mcroinvertebrate/chemistry samples occurred within the summer/autumn period of this study.

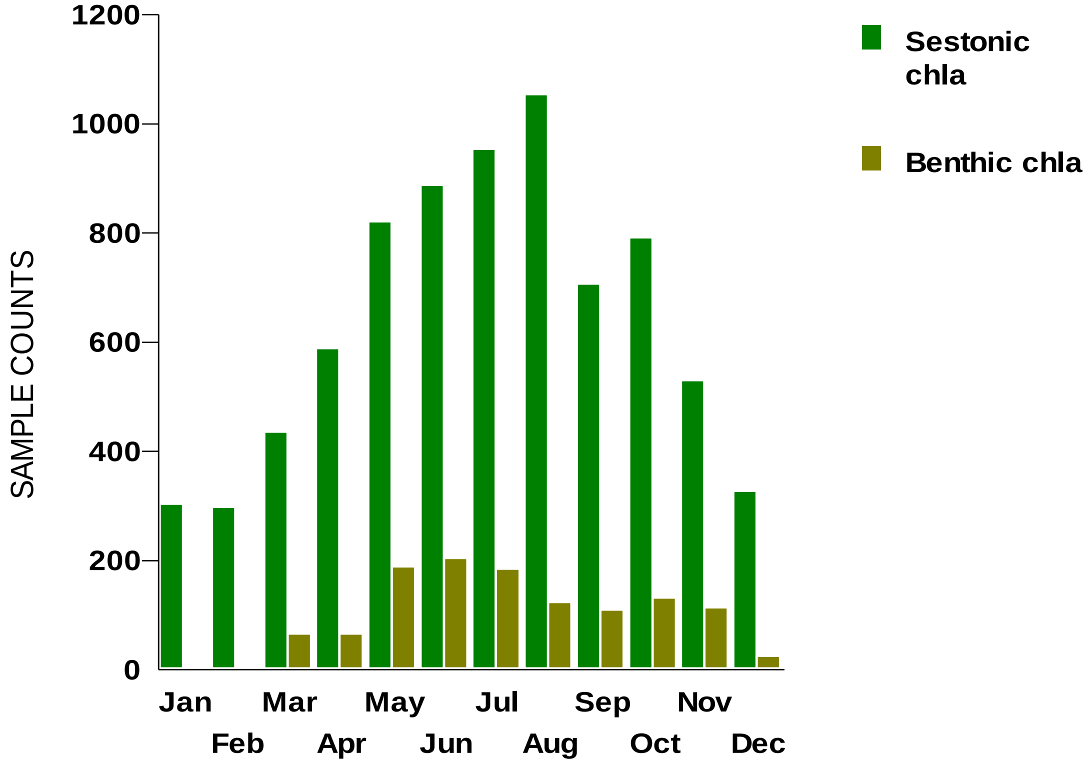


Figure b. Monthly distribution of sestonic and benthic chlorophyll a samples within the nutrient database.

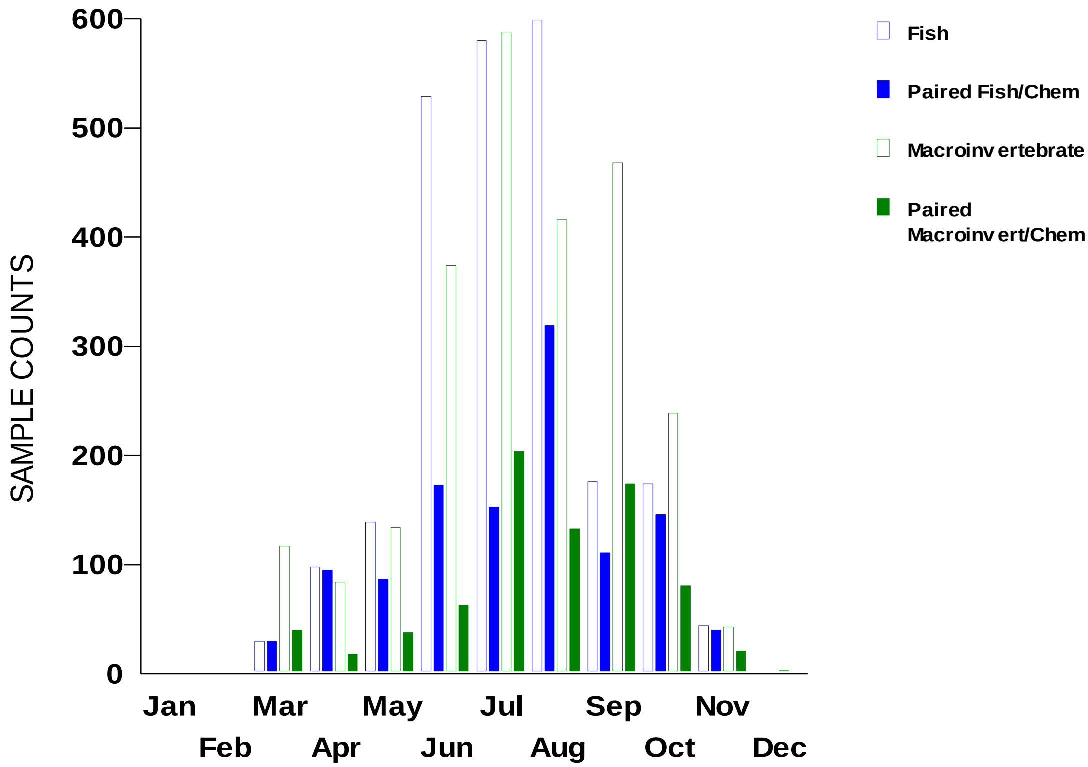


Figure c. Monthly distribution of all fish and macroinvertebrate samples, and those fish and macroinveretebrate samples that were paired to exisiting stressor samples within the nutrient database. (see Primary Data - Raw Data Section for discussion of sampling window linking abotic and biotic variables)

Strahler Stream Order (orders 1 – 7): Strahler Stream order was calculated for 1239 stream sites. The consistent identification of the stream order of the sampling sites was eventually determined to be unfeasible because of the variable resolution among base maps used for this purpose. The USGS's National Hydrography Dataset (http://nhd.usgs.gov/chapter1/index.html#_Toc474479766) was one of the datebases assessed to see if stream order could be accuately and consistently determine thoughout USEPA Region 7. Within the NHD inconsistancies in mapped stream densities were identified both within and between Region 7 states finally forcing the RTAG to look at other stream classification variables such as watershed size

Watershed Size (size in hectares, 4 categories): Watershed size was calculated for drainages to 2149 of the stream sites in the database. A synthetic drainage network was first developed using digital elevation maps (DEMs) and delineation algorithms developed by staff of the Kansas Applied Remote Sensing Program of KBS. Two watershed size variables were used in subsequent evaluation of the effects of watershed size on nutrient and biological variables of interest. Both a continuous variable (size in hectares) and categorical variable (size classes) were examined as potential watershed classification variables. Four watershed size classes were selected: Class 1, <3200 hectares (<12.2 mi^2); Class 2, 3200-32,000 (12.3-123.3 mi^2); Class 3, 32000-320000 ha (123.4-1233.5 mi^2); and Class 4, >320,000 ha (>1233.5 mi^2).

Potential Area Discharge (PAD): Because broad precipitation differences are common between various geographic regions in USEPA Region 7 an attempt was made to add a serogote for runoff to watershed area. PAD represents the watershed area weighted by mean precipitation. It was calculated by first determining the 15 year (1989-2003) precipitation average for each watershed pixel in an Arc View GIS coverage using estimates derived from PRISM (Parameter-elevation Regressions on Independent Slopes Model) (www.prism.oregonstate.edu). Because some of the watersheds in the database were fairly small the 4 km PRISM data was resampled into 1 km pixel estimates. The mean precipitation for each watershed (to the nearest 0.001 mm) was calculated from the pixel data that comprised the watershed. The mean watershed precipitation estimate (cm) was then multiplied by the total watershed area (M^2) and the resulting weighted area divided by 10,000 to get a hectare equivalent. This watershed variable was dubbed potential area discharge (PAD) and considered a very rough approximation of potential watershed runoff (discharge) under average annual rainfall conditions.

STATISTICAL ANALYSES

Effect of sample size on parameter means and spatial distribution of sites.

The number of samples per site ranged from 1 to 449 samples depending on parameter of interest. The number of sites and samples for each site was very limited for both sestonic and benthic Chl*a*, which was of greatest concern as they represented possible response variables. The use of site data with a large number of samples was previously recommended by the RTAG so that the calculated median would better reflect the longer-term temporal conditions associated with each stream parameter of interest at a particular site.

The instream variability of algal biomass (i.e., Chl*a*) was anticipated to be high due to algal growth dynamic, variable light conditions, effects of stream flow and other factors unrelated to nutrient concentrations. Similar variability might also be anticipated with nutrient values and other stream chemistry as these parameters would also change due to time of season and flow conditions (i.e. run off conditions, base flow conditions). Therefore, site means or medians would offer a better estimate of long-term parameter levels associated with a particular site or stream. However, the selected use of sites with higher numbers of temporal samples reduces the number of sites used to estimate the parameter values for a particular stream class (e.g., stream order) or ecoregion or other geographic region. In order to investigate the potential effects of sample size on estimating the population mean and spatial coverage of the data set, we generated a series of plots that show the change in mean values and number of sites (count) used in estimating the mean for both stressors (Figure 5) and response variables of algae (Figure 6). In most instances there was minimal change (≤20%) in population means between <u>all</u> sites and sites with 3 to 7 values, which was deemed a necessity when calculating median values. However, using only sites with 3 or more measures of a parameter often resulted in a loss of as much as one half of the sites in any one analyses of the data relationships thus compromising some of the robustness of the datasets. Greater changes in the parameter means and greater loss of sites occurs using sites with more than 7 samples in the database. Therefore it was determined that using sites with ≥5 samples would minimizes changes in the population means and sample size while allowing construction of box plots, five-number summaries (http://en.wikipedia.org/wiki/Box_plot) in examining statistical relationships within and between variables of interest.

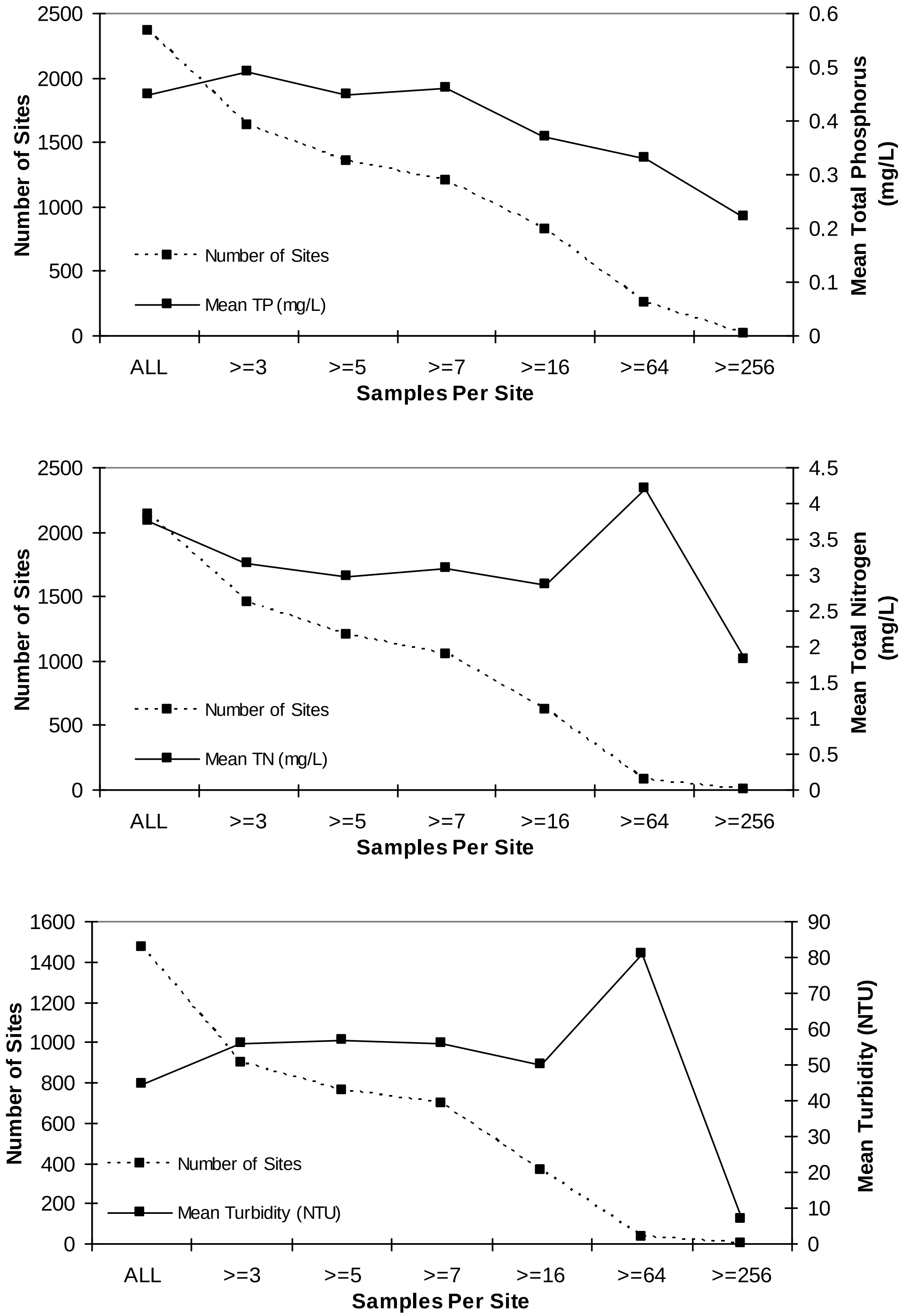


Figure 5. Frequency distribution showing the number of sites and the mean of the parameter corresponding to the given number of samples for total phosphorus, total nitrogen and turbidity.

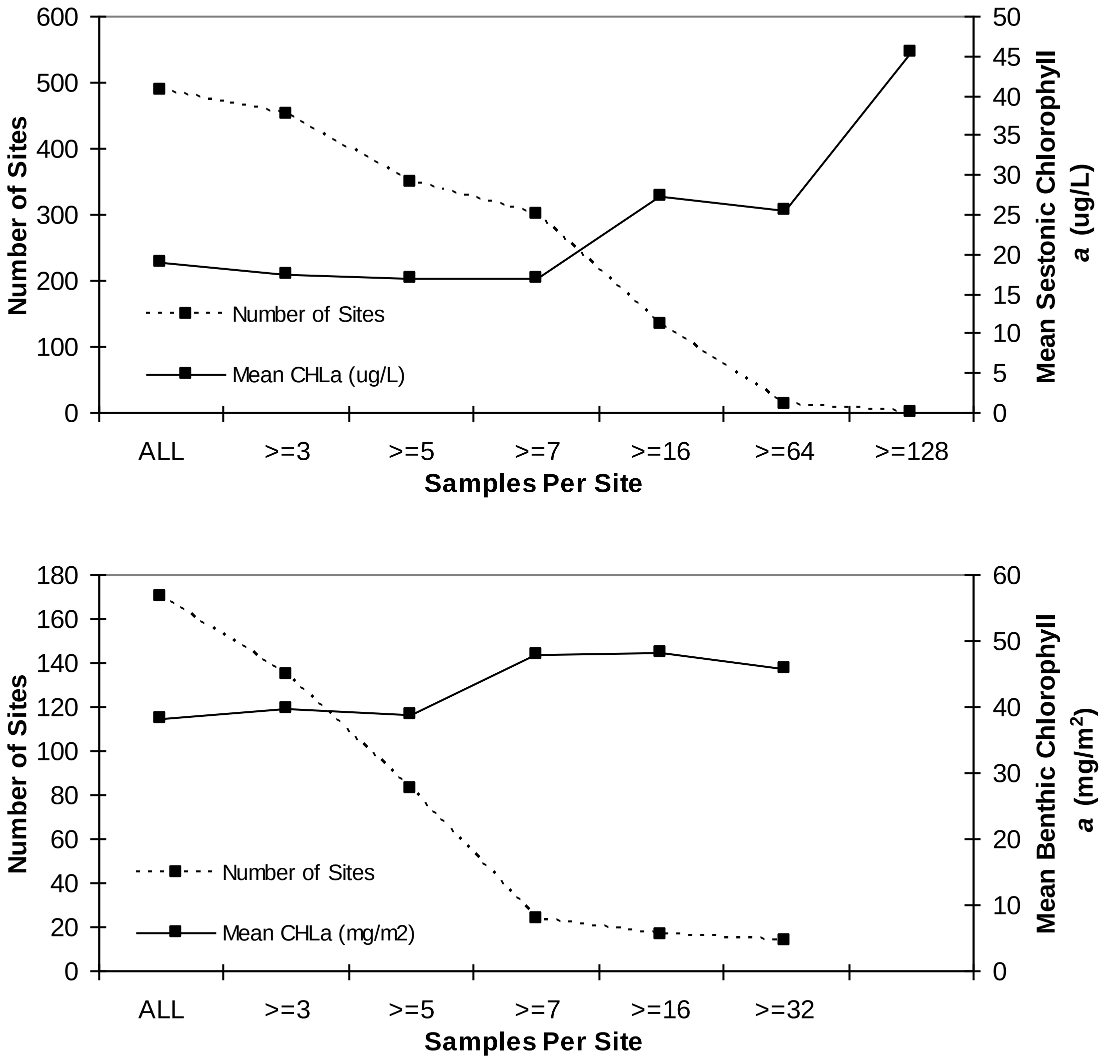


Figure 6. Line plots of the distribution of means and site counts for all database sites and sites with varying sample sizes for Chl*a* variables.

. In this way sites with enough samples to generate medium and other central tendency values could be used to build the recommended medians database. Trimming the median database to sets having larger numbers of samples per site limits the number of sites that would be available for analysis and greatly alters the population means when compared to the means for all sites population.

The Medians Database

The previous assessment of relationships between sample size (n) and the RTAG's desire to select a sites with a sample size that: 1) allowed the development of a medians database as suggested in the nutrient guidance documents; 2) allowed calculation of a box or violin plots (e.g five –number summaries), 3) maximize site numbers for analyses, and limit changes in site means compared to the site mean determined using all data. The RTAG relied on the visual interpretation of the above line plots (Figures 5 and 6) to select a sample size that minimized the loss of stream sites and change in site means and met the five sample reqirement of a box plot. A sample size of five or more appeared to best fit these criteria and was selected for use in development of the medians database. However, RTAG members expressed concerns regarding the loss of sites with chlorophyll data expecially benthic Chl*a* if the ≥5 sample size rule was applied to these parameters. The RTAG compromise was to retain all sites with ≥3 sestonic or benthic Chl*a* samples because both the ≥3and ≥5 sample site means were very similar and yet the ≥3 rule preserved many more Chla sites for analyses (see Figure 6). Additionally, the RTAG used all fish and macroinvertebrate data when examining possible causal relationships between TN, TP and Chl*a* parameters. In most cases the RTAG analyses of fish, macroinvertebrate and nutrient relationships were based on site means for faunal and nutrient variables measures within the 30-day samling window. The group acknowledged the fact that box or violin plots could not be constructed for some sites because of the limited number of samples.

Overall the medians database allowed the RTAG to characterize and quantify most sites, nearly all ecoregions and all of Region 7 using parameter medians for TN, TP, sestonic and benthic Chl*a* which was consistent with data reduction methods used in the USEPA's regional guideline documents (USEPA 2000a-f, 2001b-d, 2002). Other correlation and regression analyses as well as most descriptive statistics used either all data values, site means or site medians depending upon test restrictions.

It should be noted that a waterbody in the Medians database may be represented by median TN and TP values that were derived from measurements taken from the same or different sample events. However, median TN:TP ratios were *not* constructed as a quotient of the corresponding median TN and median TP values, but rather was the median of all calculated TN:TP values recorded for that waterbody. Sampling metadata (e.g., the name of data collector, date, and other information about specific sampling events) were not preserved with the parameter values for each waterbody in the Medians database. The Medians database comprised 2232 sites from approximately 1900 waterbodies.

Water Quality Characteristics and Relationships

A number of nutrient and response variables were examined using several statistical and graphic analysis techniques to identify potentially useful physical classification or geographic factors that might facilitate identification of ecologically similar stream groupings. Initial examinations were done using all stream data available in the regional streams database and tests were performed using three datasets; all data (all dates), and only data collected during an *a priori*-determined plant-growing season and non-growing season. The RTAG agreed that the period starting about the third week of March and ending mid-September would encompass the growing season associated with EPA Region 7 states and many other states within the Central Plains region. A series of one-way ANOVAs were preformed to test for possible temporal differences within TN, TP, sestonic Chl*a* and benthic Chl*a* data using the raw data (Table 2A). Only TP and sestonic chlorophyll were found to have significant seasonal differences XXXSAY SOMETHING ABOUT PERCENT DIFFERENCE

Table 2A. Results of one-way ANOVA tests (i.e. GLM ANOVA) to examine for possible temporal effects on selected nutrient variables. All response variables were log transformed (log + 1) but differences between the means (x diff) are in original measurement units. Non significance (NS) in group means was noted when alpha of ≤ 0.05 was exceeded.

Test Factors	TN (mg/L)		TP (mg/L)		Sestonic Chl*a* (µg/L)		Benthic Chl*a*^{2} (mg/M^2)	
	p value	x diff	p value	x diff	p value	x diff	p value	x diff
All dates vs. growing season	0.3213	NS	>0.0000	+ 0.060	>0.0000	+ 3.9	0.5093	NS
All dates vs. non-	0.1748	NS	>0.0000	- 0.088	>0.0000	- 7.2	0.2888	NS

growing season								

It was assumed that both nutrient and response variables would be more likely to display geographic and stream class affiliations during the growing season when available nutrients might stimulate and support plant growth and then all trophic levels within lotic ecosystems are biological most activity. However, using only growing season data greatly reduced the number of streams available for analysis and thus analytic differences associated with the use of data collected over a broad temporal range (all the dates) verse growing season data were examined first. Many of the response variables were not normally distributed and power transformations were preformed to achieve normality in the data. Then a series of ANOVAs (Analysis of Variance) and ANCOVAs (Analysis of Covariance) were conducted on total phosphorus, total nitrogen, chlorophyll-*a* and turbidity data to help identify potential treatment (i.e. ecoregions, stream order) and temporal effects (i.e., all dates, growing season). Omernik's level III ecoregions (Chapman, et al., 2001; 2002) and Strahler stream orders (Strahler, 1952) were used as geographical and stream classification variables, respectively.

Results of both the ANOVA tests indicated that significant ecoregion groupings were common among all nutrient and response variables (Table 2B). Stream order was not a significant covariate except for the ANCOVA test for sestonic chlorophyll-*a*, TN:TP and Chl*a*:TP ratios. In all cases ANCOVA and ANOVA results were nearly identical both for analyses using all response data (all dates) and only data collected during the growing season dates **(**mid-March through mid-September) suggesting that the use of all the data would be appropriate and would increase our sample size and the spatial coverage. Based on results summarized in Table 2B, it appears that using only growing season data is of little advantage since there are no statistical differences between ANOVAs using all dates or growing season except for turbidity difference for stream orders. Thus data from all seasons were retained for all other analyses.

Table 2B. Results of one-way ANOVA (i.e. GLM ANOVA) tests to examine for possible ecoregion and stream order effects were performed using the median values of response variables for streams having 5 or more samples. Non significance (NS) when alpha of ≤ 0.05 was exceeded.

Test factor	**Index Period**	**Response Variables**						
		TN	**TP**	**Sestonic Chl*a***	**Benthic Chl*a*[1]**	**TN:TP**	**Chl*a*:TP**	**Turb[2]**
Ecoregion	All Dates	0.000	0.000	0.000	0.000	0.000	0.001	0.000
Ecoregion	Grow. Season	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Stream Order	All Dates	0.000	0.001	0.000	0.064	0.000	0.000	0.036
Stream Order	Grow. Season	0.000	0.009	0.000	0.058	0.000	0.000	NS

[1] Benthic chlorophyll-*a* estimates were obtained from sampling periphyton attached to natural substrates in the streams

[2] Turb = turbidity

Ecoregional characteristics

Descriptive statistics of each parameter in each of fourteen ecoregions are summarized in Table 3. In general, few streams in the Central Oklahoma/Texas Plains, Mississippi Alluvial Plain, Northwestern Glaciated Plains, Northwestern Great Plains and Southwestern Tablelands ecoregions could be included in the medians databases based on the number of chlorophyll samples for these streams. Examination of Table 3 shows that the small stream sample size in ecoregions MAP, NGP and ST for streams with median seston or benthic chlorophyll values prevent there inclusion in ANOVA testing for ecoregions or stream order effects. Additionally, streams in ecoregions CGP, COT, IRL and NGL could not be included in the ANOVA testing for classification effects (i.e. ecoregion and stream order) on benthic chlorophyll-*a* again due to the small number of streams available for testing (≤2 steam median values). Thus statistical testing for ecoregions or stream order effects on chlorophyll variables could only be done on 50 to 75 percent of the ecoregions occurring in EPA Region 7.

Differences due to stream order were found for TP, TN and TN:TP as well as sestonic chlorophyll-*a* and the ratio of sestonic chlorophyll-*a* to TP. Periphyton (i.e., benthic chlorophyll) differences among stream orders were not significant for either the growing season or all samples.

Examination of the Tukey-Kramer multiple-comparison test results (*post hoc* group mean testing from ANOVA tests) and the violin plots for the various nutrient variables suggest that regional differences were attributed to several different ecoregional groupings and not individual ecoregions (Figure 8 **- Figure 11**). Interpretation of these statistical groups is difficult as groups often change memberships in response to the test variable (e.g. TP, TN, sestonic chlorophyll-*a*). It is clear that regional differences do occur when considering all streams (i.e. reference and non-reference) but interpretation of the meanings of these groups may be difficult or of limited value. Similar results were noted when the effects of stream order were assessed for the various nutrient variables. Based on *post hoc* multiple comparison test (Tukey-Kramer test) results there often appeared to be two stream order groups. First through fifth and sixth order streams generally formed one group while a second group was typically composed of sixth and larger streams and rivers. However, benthic algal biomass (i.e., chlorophyll) differences were not found between stream orders but the sample size for benthic chlorophyll was small and limited to only a few ecoregions (see Tables 2 and 3).

Table 3. Descriptive statistics for nutrient variables by ecoregion within USEPA Region 7 calculated from the medians dataset for sites with known watershed sizes and five or more samples for the given parameter. TP and TN are in mg/L, sestonic Chl*a* is µg/L and benthic Chla is in mg/M^2**.**

Parameter	Ecoregion	n	Mean	Minimum	25th quartile	Median	75th quartile	Maximum
TN	CGP	172	2.10	0.71	1.25	1.78	2.33	16.01
TN	CIP	182	1.59	0.02	0.79	1.12	1.57	32.80
TN	COT	3	0.30	0.17	--	0.35	--	0.40
TN	DA	17	4.88	1.20	3.83	5.20	6.00	9.16
TN	FH	60	1.02	0.13	0.53	0.81	1.42	4.37
TN	IRL	50	2.44	0.12	1.17	1.79	2.99	9.21
TN	MAP	5	0.48	0.19	0.30	0.46	0.68	0.78
TN	NGL	12	3.14	0.28	1.04	1.59	4.79	12.34
TN	NGP	13	1.19	0.60	0.69	1.00	1.55	2.64

Parameter	Ecoregion	n	Mean	Minimum	25th quartile	Median	75th quartile	Maximum
TN	NSH	30	1.46	0.39	0.70	0.99	1.25	6.37
TN	OH	220	1.32	0.09	0.39	0.66	1.55	16.05
TN	ST	14	1.02	0.34	0.58	0.64	1.49	2.50
TN	WCB	237	5.91	0.79	2.78	5.53	8.50	16.34
TN	WHP	44	2.21	0.39	0.99	1.61	3.41	5.85
TP	CGP	199	0.32	0.04	0.15	0.24	0.37	2.00
TP	CIP	218	0.30	0.02	0.09	0.13	0.20	18.20
TP	COT	3	0.04	0.04	--	0.04	--	0.04
TP	DA	15	0.08	0.03	0.06	0.08	0.10	0.13
TP	FH	71	0.13	0.01	0.06	0.10	0.17	0.46
TP	IRL	65	0.48	0.02	0.10	0.19	0.30	7.01
TP	MAP	6	0.16	0.03	0.11	0.17	0.21	0.25
TP	NGL	13	0.14	0.02	0.10	0.14	0.18	0.23
TP	NGP	13	0.17	0.04	0.11	0.15	0.20	0.48
TP	NSH	30	0.21	0.11	0.14	0.18	0.22	0.52
TP	OH	238	0.52	0.00	0.02	0.04	0.14	8.80
TP	ST	15	0.07	0.03	0.04	0.06	0.08	0.14
TP	WCB	261	0.24	0.03	0.13	0.20	0.29	2.04
TP	WHP	46	0.09	0.02	0.04	0.06	0.11	0.63
Seston Chl*a*	CGP	8	20.79	4.78	8.11	14.80	38.56	42.65
Seston Chl*a*	CIP	43	10.24	2.10	6.08	9.26	13.00	24.87
Seston Chl*a*	COT	2	8.98	5.50	--	*	--	12.46
Seston Chl*a*	DA	10	4.65	0.90	2.48	4.50	7.63	8.57
Seston Chl*a*	FH	15	14.90	1.20	9.37	13.05	19.10	43.45
Seston Chl*a*	IRL	9	32.14	2.95	5.07	13.15	52.00	127.00
Seston Chl*a*	MAP	2	3.54	2.58	--	*	--	4.50
Seston Chl*a*	NGL	4	13.98	5.96	--	6.46	--	37.06
Seston Chl*a*	NGP	1	--	3.15	--	--	--	3.15
Seston Chl*a*	NSH	5	4.67	1.95	2.47	3.88	7.28	8.85
Seston Chl*a*	OH	39	3.84	0.19	1.06	2.04	3.20	46.86
Seston Chl*a*	ST	2	4.09	3.93	--	*	--	4.26
Seston Chl*a*	WCB	167	10.74	0.45	1.62	4.00	10.00	105.00
Seston Chl*a*	WHP	8	6.93	1.30	1.60	3.18	9.31	29.24
Benthic Chl*a*	CGP	2	30.77	21.24	-	-	-	40.30
Benthic Chl*a*	CIP	15	12.22	4.60	8.56	11.04	12.61	34.65

Parameter	Ecoregion	n	Mean	Minimum	25th quartile	Median	75th quartile	Maximum
Benthic Chl*a*	COT	0	--	--	--	*	--	--
Benthic Chl*a*	DA	4	51.75	26.30	--	54.50	--	71.70
Benthic Chl*a*	FH	6	21.07	11.91	15.97	20.09	27.88	29.70
Benthic Chl*a*	IRL	2	22.61	20.31	--	*	--	24.90
Benthic Chl*a*	MAP	0	--	--	--	--	--	--
Benthic Chl*a*	NGL	1	--	27.71	--	--	--	27.71
Benthic Chl*a*	NGP	1	--	23.63	--	--	--	23.63
Benthic Chl*a*	NSH	5	35.55	12.00	21.13	39.50	48.00	49.66
Benthic Chl*a*	OH	16	30.72	4.06	8.12	23.23	40.28	144.25
Benthic Chl*a*	ST	1	--	62.54	--	--	--	62.54
Benthic Chl*a*	WCB	15	34.88	10.79	23.90	26.82	43.44	84.92
Benthic Chl*a*	WHP	5	36.16	13.80	14.49	29.58	61.13	61.52
Turbidity	CGP	157	26.48	3.35	11.00	18.00	27.75	154.25
Turbidity	CIP	117	31.07	3.50	11.60	17.00	30.00	530.00
Turbidity	COT	3	20.50	8.00	--	8.5	--	45.00
Turbidity	DA	19	10.14	2.90	4.00	5.70	12.00	39.00
Turbidity	FH	56	13.07	0.64	7.05	11.00	16.75	50.00
Turbidity	IRL	20	33.08	10.00	18.25	30.00	40.88	93.70
Turbidity	MAP	3	23.97	4.50	--	18.90	--	48.50
Turbidity	NGL	12	54.92	3.00	5.45	31.55	111.63	149.00
Turbidity	NGP	12	81.40	1.56	6.97	13.85	45.86	718.00
Turbidity	NSH	28	21.65	2.75	7.58	22.55	28.43	62.90
Turbidity	OH	46	8.93	0.50	2.39	4.98	8.00	88.20
Turbidity (	ST	15	13.42	2.80	6.55	11.75	17.00	36.00
Turbidity	WCB	205	30.28	3.33	12.50	20.00	40.43	140.50
Turbidity	WHP	31	17.73	1.80	4.40	11.20	23.10	63.00

* mean and median are the same if there are only two values

Violin plots allowed the RTAG to visualize the similarities and differences among ecoregions based on a number of important characteristics for each parameter of interest. The violin plot (such as Figure 7) combines the basic summary statistics of a box plot (Tukey, 1977) with the visual information provided by a local density estimator. They are standard box plots surrounded by an outline indicating the data density estimated by a kernel method (NCSS, 2004). The plots show the median value with a circle and the 25^{th} to 75^{th} quartile values by the interior lines on either side of the median circle. This is then "boxed" by the mirrored density curves for all data used in the analysis. The goal is to not only define the typically box plot measures but to reveal the distributional structure in a variable. Examination of the density curve shape can be used to distinguish departures from normal distributions. While there are many variations of the box plot, five or more data points are needed to correctly calculate and construct a box plot. Therefore in this document a violin plot is provided when a parameter is recorded for five or more sites that are represented by their median values. For example, to generate plots by parameter(s) by ecoregion(s), only those ecoregions having data for ≥ 5 or more sites (e.g., streams) which have ≥ 5 or more parameter measurements could be plotted. The use of median in representing sites and ecoregion values prevents data distortions caused by uneven sampling efforts and yet provides a good estimate of prevalent conditions. For the purposes of this document, outliers are cut off to better show the violin plots.

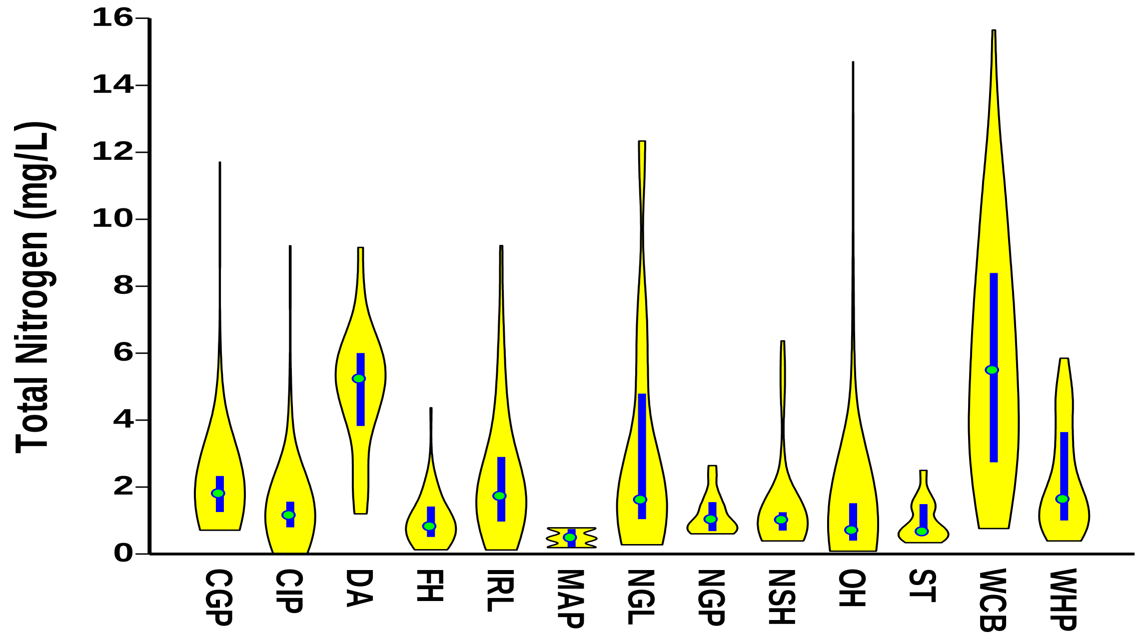


Figure 7. Violin plots of total nitrogen by ecoregion for sites with known watershed sizes, ≥ 5 sites and ≥ 5 samples for TN per site.

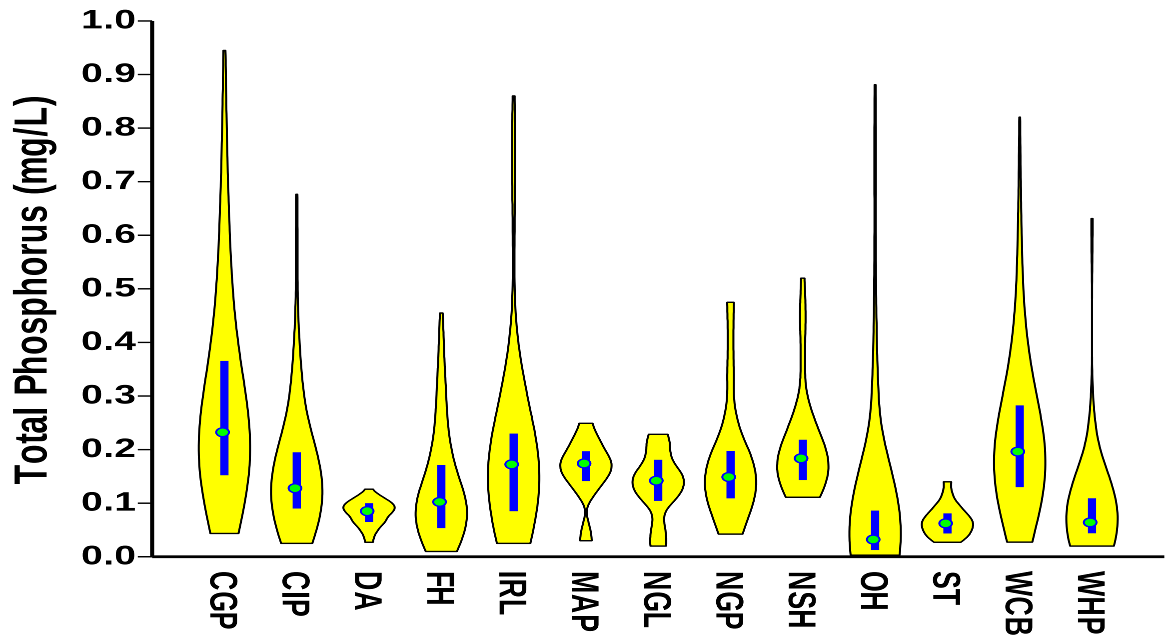


Figure 8. Violin plots of total phosphorus by ecoregion for sites with known watershed sizes, ≥ 5 sites and ≥ 5 samples for TP per site.

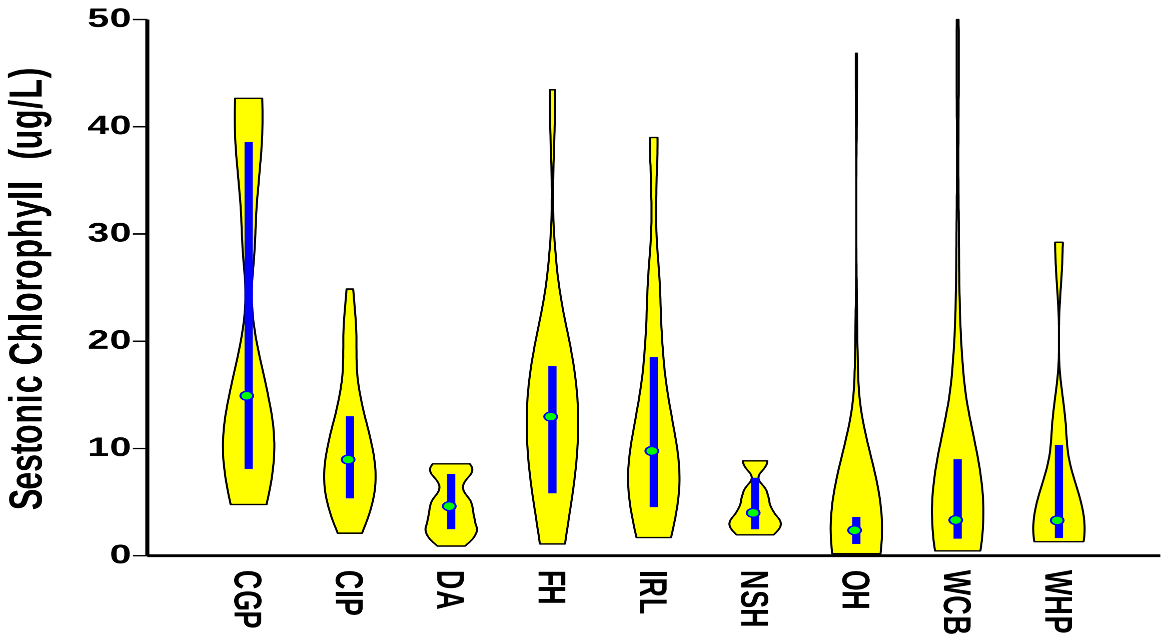


Figure 9. Violin plots of sestonic chlorophyll-*a* by ecoregion for sites with known watershed sizes, ≥ 5 sites and ≥ 5 samples for sestonic chlorophyll.

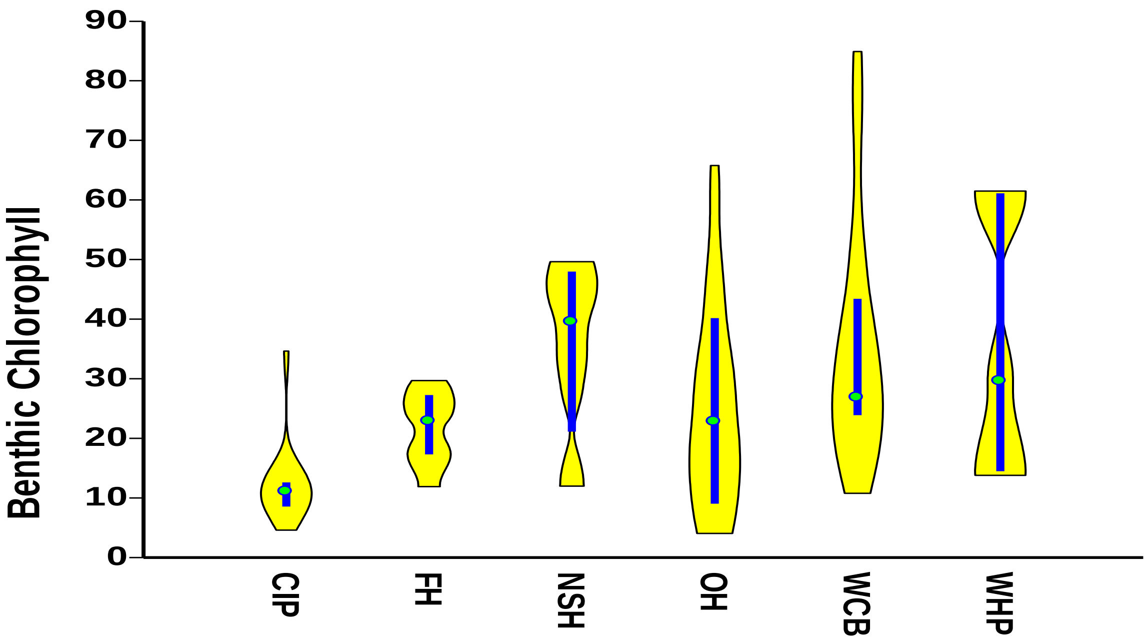


Figure 10. Violin plots of benthic chlorophyll-*a* (on natural substrate) by ecoregion for sites with known watershed sizes, ≥ 5 sites and ≥ 5 samples for sestonic chlorophyll.

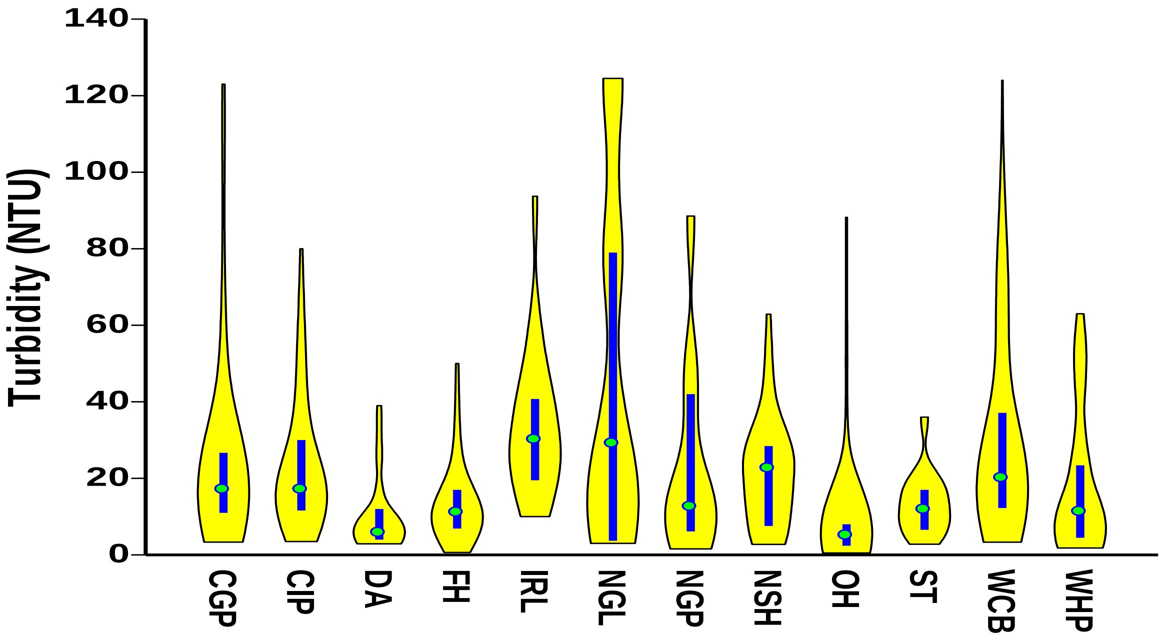


Figure 11. Violin plots of turbidity by ecoregion for sites with known watershed sizes, ≥ 5 sites and ≥ 5 samples for turbidity.

In general most of the nutrient variables are right-skewed or "skewed to the right" indication that most of these variables have long tails of high values. The few exceptions are the more normal appearing distributions of TN in DA ecoregion and TP in DA and NGL ecoregions. In addition apparent bimodal distributions occurred in TP within MAP ecoregion, sestonic Chl*a* within DA and NSH ecoregions and in benthic Chl*a* in FH, NSH and WHP ecoregions. However, all of these parameters that show bimodal distributions within specific ecoregions are characterized by having low sample sizes (i.e. number of streams). Small sample sizes can cause the density curves to take on a bimodal or wavey appears since the limited sample size tends to creat breaks and dips in the curve fitting process.

Stream Size Classification

The systematic determination of stream orders within US EPA Region 7 was attempted then abandoned because of inconsistencies in mapped stream features and variability in the resolution of the source maps. It was clear that not all stream segments, especially small stream reaches (e.g., first order, intermittent stream segments) were always mapped on individual USGS 7.5 quad maps which were the primary maps used in determining stream order. Instead of classifying streams size by stream order the RTAG investigated the use of watershed size as a surrogate measure of stream size.

Watershed size

Watershed sizes were delineated using a synthetic stream system developed from DEMs of the region. Using the synthetic stream system allowed researchers to use the stream location of each collection site as the watershed outlet and then generate a watershed area that captured all of the upstream area that drained to the collection site. A regional data set of samples sites with both stream order (Strahler, 1964) and watershed size (ha) was generated to examine the potential relation between these two variables. We checked these data for consistency and quality, and corrected all errors that were apparent in the screening process. Then simple linear and robust regression tests (NCSS 2004) were preformed on the 1232 paired watershed size/stream order values (Figure 12). Both the simple and robust linear models produced highly significant models that explain 78 to 86 percent of the variance between these variables, respectively. Based on these results and the inter-regional problems of accurately determining stream order values, we adopted watershed size as a potential stream classification approach.

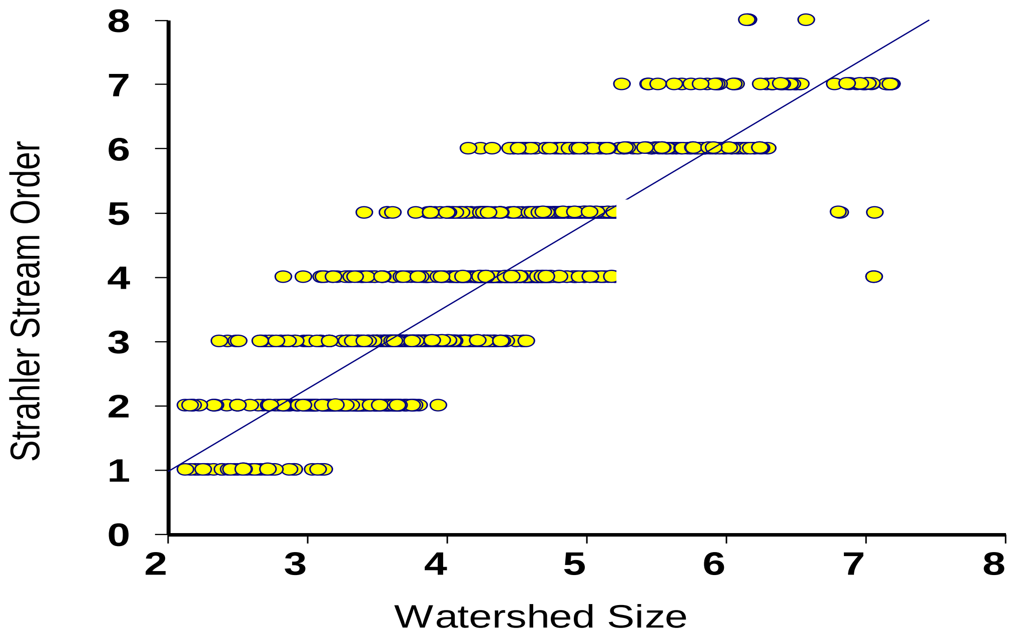


Figure 12. Scatter plot and linear trend line of watershed sizes in hectares (powers of ten) and Strahler stream order values for 1,232 sample sites and their attending watersheds.

Next a series of robust regressions were run between watershed size (i.e. stream classification variable) and a number of stressor (abiotic) and response (biotic) variables (Table 4). These results suggested that about 42% of the sestonic chlorophyll concentrations and 22% of fish richness was explained by watershed size with larger watersheds (and streams and rivers) having greater sestonic chlorophyll-*a* concentrations and higher fish richness. However, in most instances there appeared to be little or no relationship between the size of a watershed and either nutrient concentrations or other water quality or biotic variables. While total phosphorus had a significant and positive relationship with watershed size, very little of the variance of TP was explained by this independent variable. It should be noted that the large sample sizes associated with most of variables of interest often causes regressions to meet the conditions of significance (low p values).

Table 4. Results of robust regression analysis (NCSS, 2004) for a select number of abiotic and biotic variables and watershed size (independent variable).

Dependent variable (log values)	**Sample Size (n)**	**Significant model (p value)**	**R^2**	**Relationship (slope)**
Total Nitrogen (mg/L)	1862	No (0.1242)	--	--
Total Phosphorus (mg/L)	2049	Yes (0.0000)	0.03	+0.0649
Turbidity (NTU)	1371	Yes (0.0000)	0.01	+0.0512

Seston chlorophyll-*a* (µg/L)	447	Yes (0.0000)	0.42	+0.3600
Benthic chlorophyll-*a* (mg/M^2)	155	No (0.3722)	--	--
Macroinvertebrate total richness	471	No (0.5486)	--	--
Fish total species richness	1148	Yes (0.0000)	0.22	+0.1670

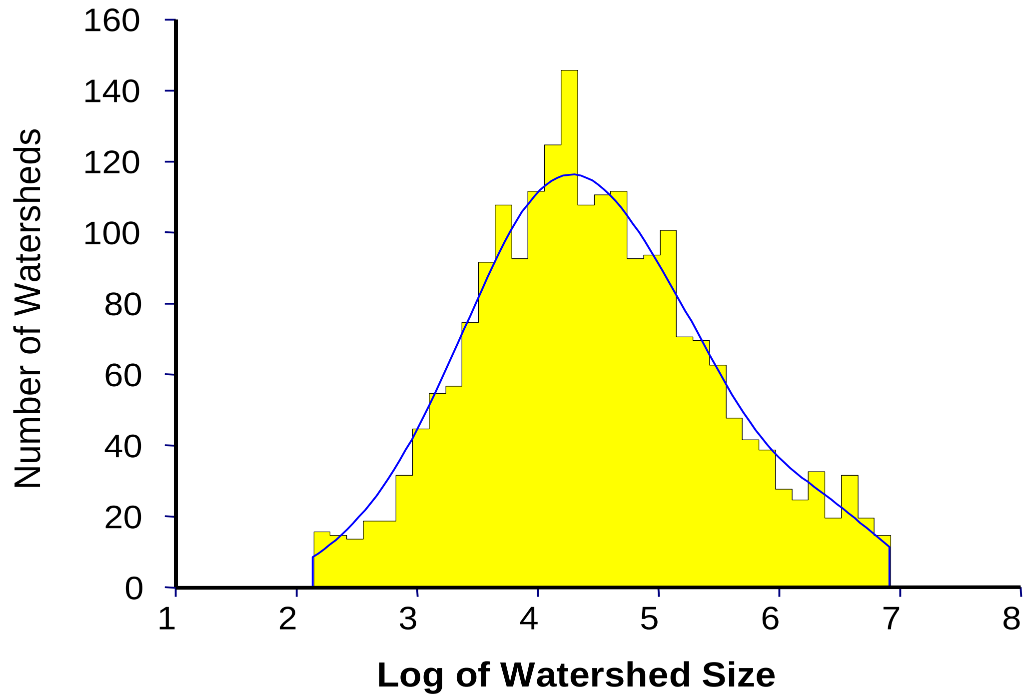


Figure 13. Frequency distribution of all watersheds draining sites for which data was available for assessment in this study.

Four watershed size classes were subjectively determined from the distribution of all the watersheds examined in this study (Figure 13) so that the statistical characteristics of nutrients and algal chlorophyll for each group could be calculated. The largest and smallest watershed for which data were available was 8,159,804 to 136 hectares, respectively (Table 5).

Table 5. The four watershed size classes selected for use in this study and their area in hectares (square miles).

Watershed size class	Number of watersheds	Class size range	Minimum size	Maximum size
1	353	< 3,200 (< 2.4)	136	3,198
2	824	3,200 to 32,000 (12.4 to 123.6)	3,207	31,985
3	657	32,001 to 320,000 (123.6 to 1235.5)	32,090	319,743

4	315	> 320,000 (> 1235.5)	323,178	8,159,804

An examination of the median and 25th quartile values for nutrients, turbidity and sestonic and benthic chlorophyll-*a* and fish variables indicated that most watershed classes had similar nutrient and chlorophyll-*a* attributes (Table 6). Only one strong trend was noted and that was for sestonic chlorophyll-*a*, which appeared to have increasingly higher median, and 25th quartile values as watershed class size increased. Median and 25th quartile values for sestonic chlorophyll in watershed class 4 were over twice those found for watershed class 3.

Table 6. Median and 25th quartile values (number of sites = n) for the stressor parameters total nitrogen, total phosphorus, and turbidity in each of four watershed classes found within US EPA Region 7 (Iowa, Nebraska, Kansas, and Missouri).

Watershed size class	**Total Nitrogen (mg/L)**			**Total Phosphorus (mg/L)**			**Turbidity (NTU)**		
	n	Median	25%	n	Median	25%	n	Median	25%
1	150	1.720	1.004	168	0.125	0.056	62	21.250	10.813
2	328	1.431	0.800	385	0.121	0.068	232	14.000	7.562
3	366	1.245	0.659	398	0.140	0.070	262	15.000	9.608
4	215	1.745	1.174	242	0.198	0.122	168	19.000	11.775

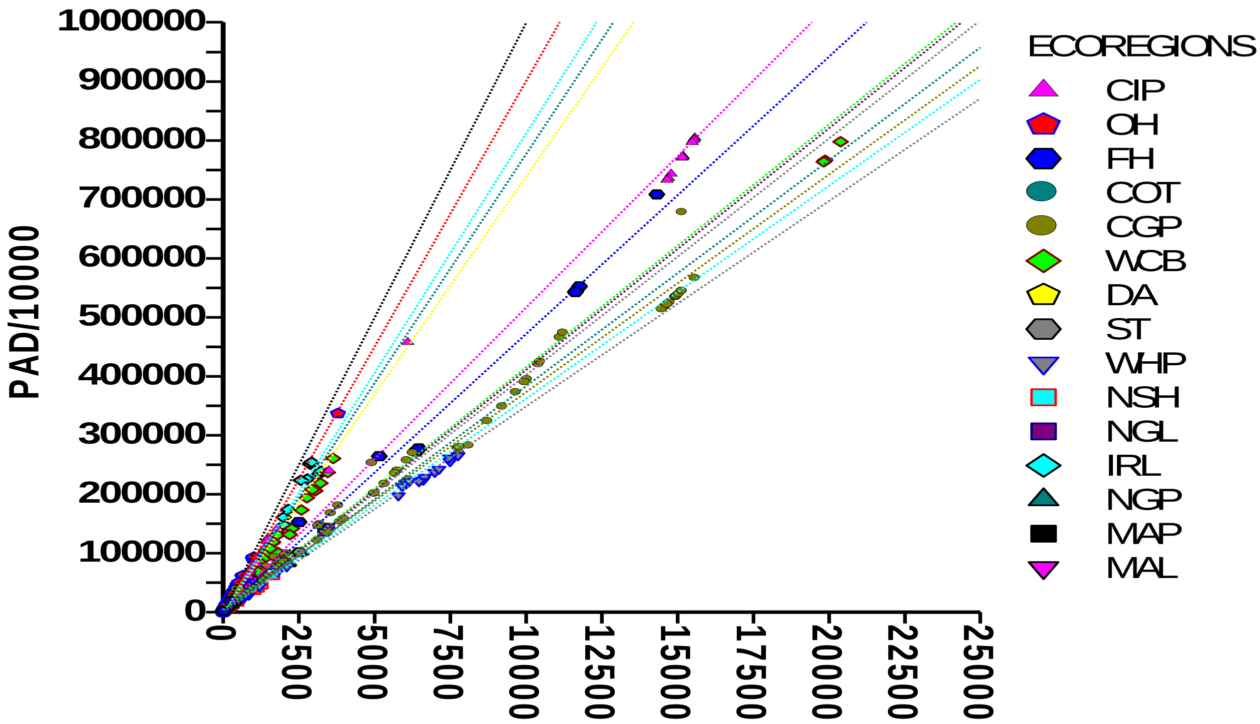
Table 7. Median and 25th quartile values (number of sites = n) for the response parameters sestonic and benthic chlorophyll-*a* and macroinvertebrate and fish richness in each of four watershed classes found within US EPA Region 7 (Iowa, Nebraska, Kansas, and Missouri).

Watershed size class	**Sestonic Chlorophyll (µg/L)**			**Benthic Chlorophyll (mg/M^2)**			**Macroinv Richness (count)**			**Fish Richness (count)**		
	n	Median	25%	n	Median	25%	n	Median	25%	n	Median	25%
1	56	1.62	1.09	11	26.30	17.37	73	36	18	122	7	6
2	121	2.95	1.70	42	22.04	11.41	257	45	33	286	13	8
3	97	7.00	4.30	16	26.90	11.93	157	37	26	159	16	11
4	41	23.00	10.50	4	35.29	17.92	48	24	14	67	12	9

Potential Annual Discharge

To account for precipitation gradient that occurs from the western to eastern portion of the central plains, the RTAG explore the relationships between PAD which attempts to account for differences in potential runoff and flow and biological communities that might be most affected by flow and discharge (e.g. macroinvertebrates and fish). A comparison of watershed size and their corresponding PAD values for watersheds in the various ecoregions indicated that nearly all ecoregion relationships were statistically significant ($p < 0.05$ for simple linear regression, GLM model). Examination of the linear relationships between PAD and watershed size for ecoregions occurring generally from east to west in Region 7 had corresponding lower slopes. It was concluded that while the regression slopes did change among ecoregions, the significant positive linear relationships found between PAD and watershed size for all ecoregions were highly explanatory ($R^2 > 0.70$) and thus either factor could be used as a stream classification variable. Because watershed size was simpler to calculate and understand as a stream classification variable, the RTAG decided not to pursue the use of PAD as a classification factor in this effort.

A.



B.

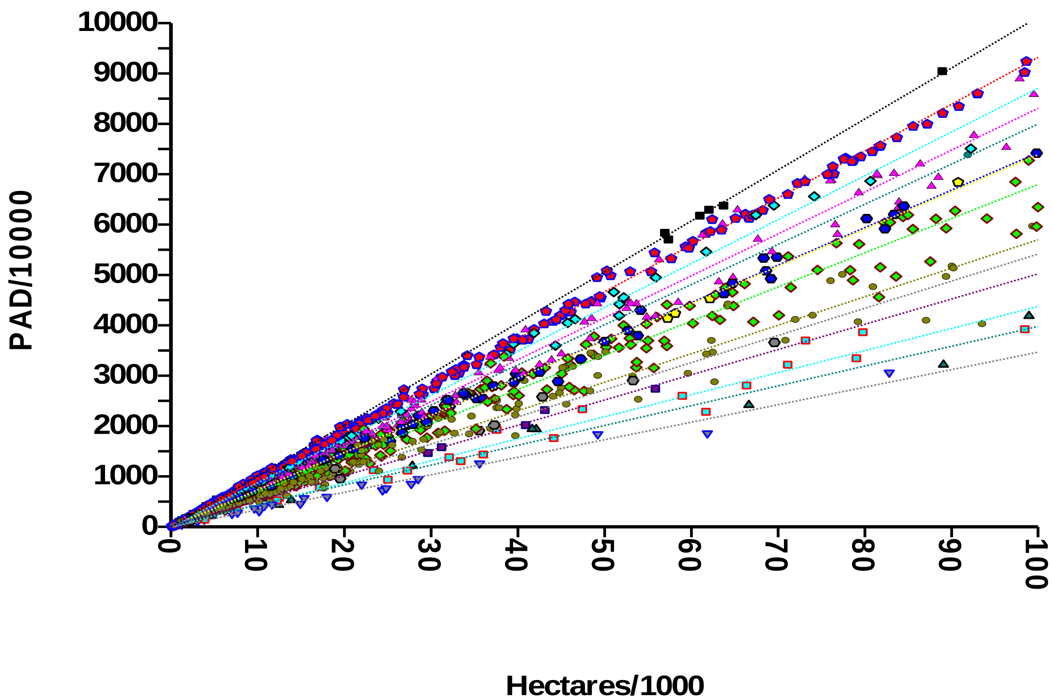


Figure 14 A and B. A. Scatter plot of Potential Annual Discharge (PAD) in cubic meters (divided by 10000) and watershed size in hectares (divided 1000). B. Same graph with smaller watersheds detailed.

However, before PAD was dropped from consideration as a stream classification factor the relationships between these two classification variables and both macroinvertebrate and fish richness variables were explored using simple least squares regression and LOWESS smoothed trend lines (Figures 15 and 16). In both cases the relationships observed between biological variables and PAD and watershed size were, for all practical purposes were identical thus confirming that using PAD as a classification variable was unnecessary. These figures also show that both macroinvertebrate and fish richness tended to peak in watersheds that are from 10^4 to 10^5 hectares in size

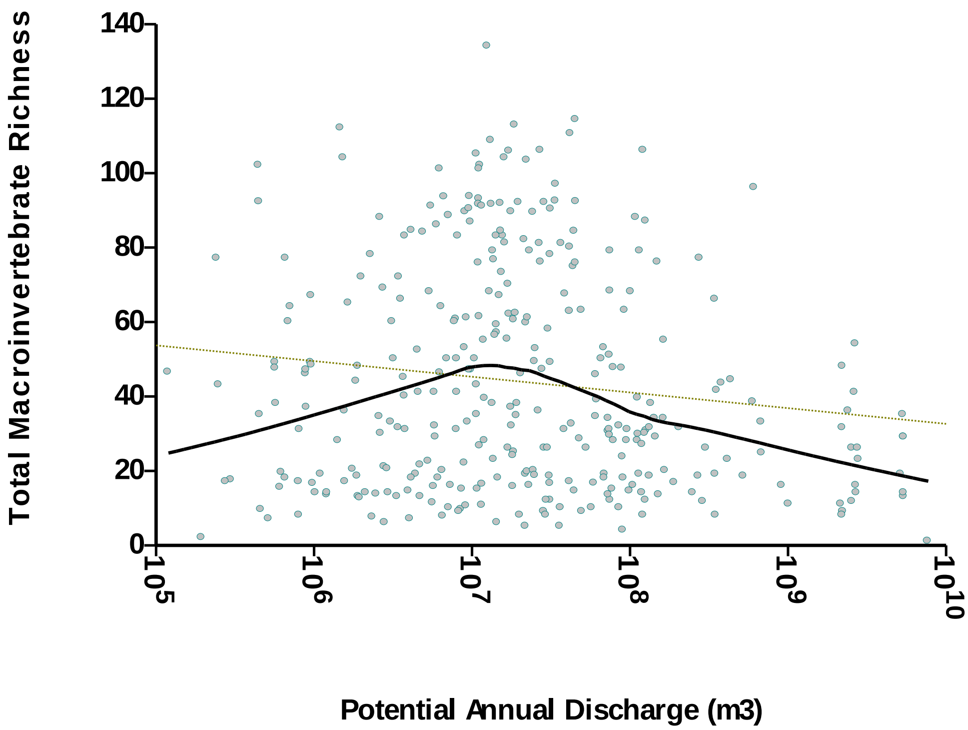
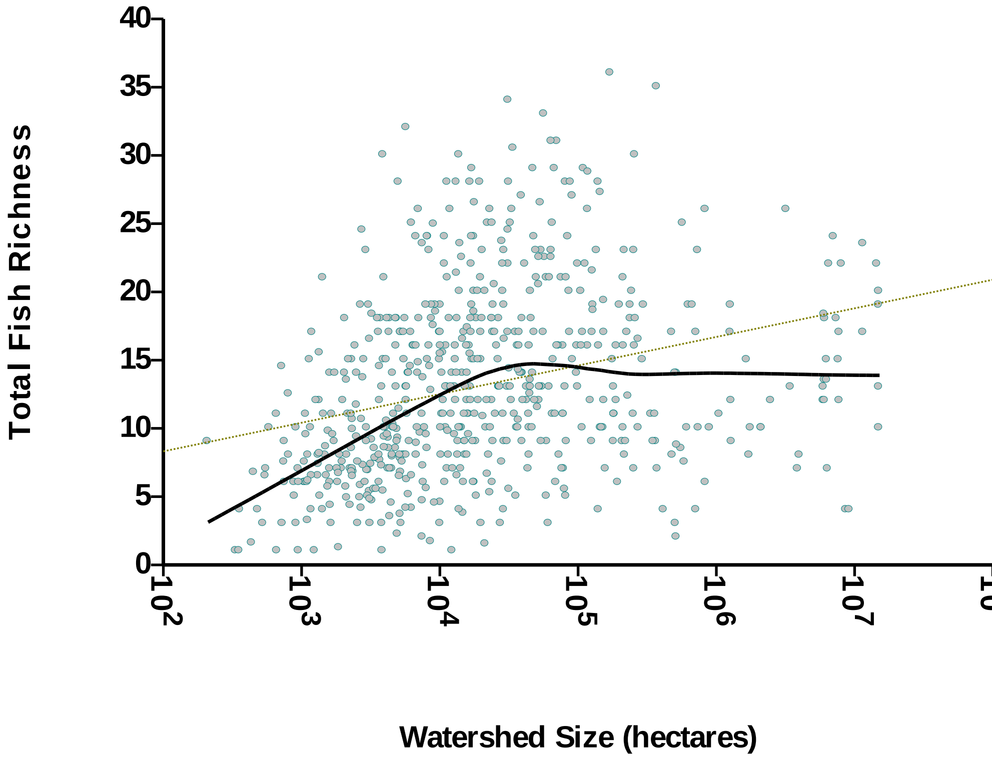


Figure 15. Regression (dashed line=least-squares trend line) and smoothed (solid line=LOWESS) fit relationships between total macroinvertebrate richness and watershed size and Potential Annual Discharge (PAD). Percent LOWESS used was 80%.



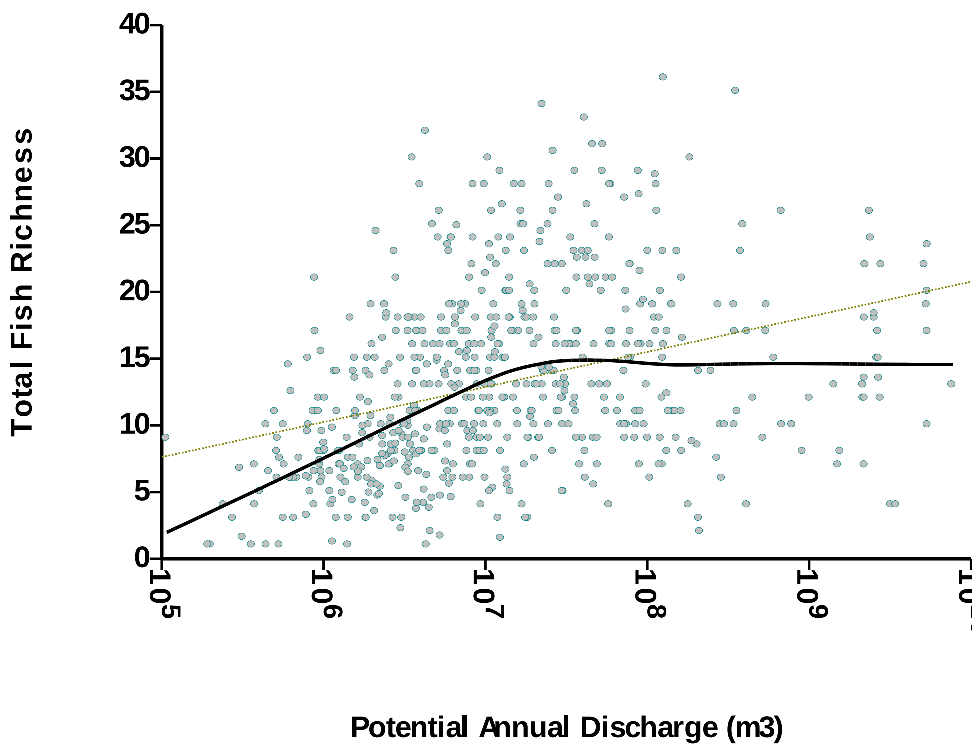


Figure 16. Regression (dashed line=least-squares trend line) and smoothed (solid line=LOWESS) fit relationships between total fish richness and watershed size and Potential Annual Discharge (PAD). Percent LOWESS used was 80%.

Nutrient Limitation

Limitation by nutrients can be indicated by deviations from Redfield ratios of nitrogen and phosphorus (Dodds et al., 2002). A ratio of 16:1, N:P by moles, or 7:1 by mass indicates growth. Ratios substantially less than this indicate nitrogen limitation, and substantially greater indicate limitation by phosphorus. Grimm and co-workers suggested that an N:P ratio less that 15 indicate that their study streams were nitrogen limited while N:P ratios greater than 15 were phosphorus limit (Grimm, et al., 1981). Other studies (Grimm and Fisher, 1986; Lohman et al., 1991) have also noted that N:P ratios can be used to predict which major nutrient might be limiting to plant growth in streams. For a river community, Schanz and Juon (1983) determined that nitrogen was limiting at N:P <10, phosphorus was limiting at N:P > 20 and in the inter-range of 10 to 20 neither nutrient could be assumed to be limiting with any level of certainty.

About half of the streams examined in the regional database had TN:TP ratios of 10 or less. Most of the ecoregions had N:P ratios close to 7 (from 5.5 to 11.3). The MAP ecoregional values indicated nitrogen limitation (N:P by mass = 2.7) and OH (16.5), WCB (29), and WHP (161) had values potentially indicating phosphorus limitation. It should be kept in mind that these ratios were determined from all data, including impacted sites, and they could not indicate the reference condition. However, most sites seemed to be balanced close to nitrogen and phosphorus limitation, which justifies including both in the nutrient criteria process.

REFERENCE CONDITION DETERMINATION WITHIN USEPA REGION 7

In order to assess the impacts of human mediated disturbances, scientists often identify sites that experience relatively minimal levels of impairment and therefore represent "healthy or acceptable" conditions. These reference conditions can then be used as benchmarks for ecosystem health in the development of nutrient criteria (USEPA, 1996; USEPA, 2000a; Stoddard, et al., 2006). USEPA has suggested that the following general approaches can be used to assist in identifying and defining reference conditions in streams and rivers:

- Biological survey of sites (determination of reference sites).
- Evaluation of historical data.
- Prediction of expected conditions using models (simulation, statistical, hybrid models).
- Expert opinion/consensus

However, the question arises as to the meaning of reference condition and the extent of disturbance, or lack of disturbance, it represents. Varying levels of human disturbance found in the environment require the need for a range of reference condition definitions (Hughes, 1995; Bailey et al., 2004; Stoddard et al., 2006). *Historical condition,* the condition of the ecosystem at some point in the past that was totally undisturbed by human activity that is they were in absolutely 'natural" or pristine condition. Waterbodies in this type of condition are unlikely to be found and are difficult to define but knowing this condition or approximate condition allows us to better describe all current conditions and the extent of change. The *minimally disturbed* condition (MDC), or the absence of significant human disturbance, if this can be determined, may serve as a benchmark in comparing other definitions of reference condition such as *least disturbed condition (LDC),* which may change over time as climate, land use, and management practices change. *Least disturbed condition* describes the "best" condition of water bodies occurring in moderately to heavily altered landscapes such as the agroecosystem landscapes of EPA Region 7. *Best potential condition* (BPC) is defined as least disturbed ecological conditions with best management practices in place for a time period that yields results. Most states in the Central Plains region refer to the *least disturbed* reference condition due to the extent and nature of modern land use in the plains region. The RTAG examined several different methods of defining a reference condition that represents the least disturbed condition, or what is considered to be the *best potential* condition. This section compares these methods.

The RTAG used several alternative methods to derive at possible "reference" or benchmark conditions (e.g., concentrations, values) and these were compared to each other in a weight-of-evidence approach. Some of these methods were literature derived (back-calculation to ambient nutrient concentration), others were essentially biotic indices (e.g., diversity versus nutrient thresholds). Each of these will be described in the following sections.

A Priori-Determined Reference Sites

Another mandate of the Clean Water Act is to set narrative biological criteria as part of State water quality standards. When implemented, biological criteria will expand and improve water quality standards programs, help identify impairment of beneficial uses, and help set program priorities. The USEPA Region 7 Biocriteria Workgroup was formed to work on the development of regionwide guidelines to identifying biological indicators, characterizing biological condition and establishing biological criteria or benchmarks. One outcome of the workgroup's efforts was a set of guidelines to consider when evaluating reference conditions, the "Core Factors to Consider in the Selection of Reference Condition in Central Plains Streams" (Table 8). The four states in Region 7 submitted lists of stream sites that reflect reference condition, or minimal anthropogenic impact. This list of 308 sites was last updated in 2004 (Appendix 8.D).

Table 8. Summary of the factors used in identifying and defining reference sites and conditions within USEPA Region 7 (Biocriteria Workgroup, 2000).

Factors	Primary or secondary evaluator
Wastewater treatment plants and other point sources	Primary
Animal feeding/grazing operations	Primary
Instream habitat	Primary
Riparian habitat	Primary
Land use and land cover – broad scale	Primary
Land use and land cover – site-specific	Primary
Physical and chemical parameters	Primary
Altered hydrologic regime	Primary
Biological metrics	Secondary/ confirmatory
Biotic assemblages	Secondary/ confirmatory
Representativeness	Primary

Nebraska sites were selected by the NDEQ who evaluated sites sampled for the 1997-2001 Regional Environmental Monitoring and Assessment Program (REMAP). Only the REMAP sites were considered because this program resulted in the best and most complete suite of site data. After choosing sites based on the best habitat scores, NDEQ secondarily used the IBI and ICI scores to verify the best sites. This resulted in 50 reference sites.

Iowa sites were submitted by the IDNR which chose 111 reference sites that are regionally representative and that are least disturbed by human activities (Wilton 2004). IDNR staff developed guidelines that specify the target number of sites for each ecoregion and the range of stream sizes to be considered for reference site nomination (IDNR 1992). The population of candidate streams included wadeable rivers and streams currently designated for protection of *Biological Assessment of Iowa's Wadeable Streams Bioassessment Framework* warm water or cold water aquatic life uses. Intermittent headwater streams classified as general use waters and large, non-wadeable interior or Border Rivers were excluded. In reviewing candidate reference sites, IDNR staff considered five major factors: 1) animal feeding operations; 2) channel alterations; 3) land cover/land use; 4) riparian and instream habitat characteristics; 5) wastewater discharges.

Kansas sites were selected by KDHE who considered water chemistry data, stream flow (trend) data, and available information on watershed land-use, municipal and industrial point sources, confined animal feeding operations, impoundments and other channel obstructions/modifications, oil field development activities, and irrigation development activities. In the past few years, KDHE also has considered the extent to which the contemporary biological condition deviates from the historical biological condition, where known (limited primarily to an assessment of fish and shellfish communities).

MDNR used the six-step selection process of Hughes et al., (1986), which provides a flexible and consistent method of evaluating reference suitability (Table 9). Topographic maps, water quality staff at the MDNR and the MDOC, and fisheries management biologists at MDOC were consulted during steps 1, 3, 4, and 5 of the reference stream selection process (Sarver et al., 2002). Water quality violations and fish kill reports were examined to help in the process. This process resulted in the selection of 72 reference sites in Missouri that were included in this effort.

Table 9. The six-step process for selecting reference sites for rivers and streams as described by Hughes et al., (1986).

Evaluate human disturbance	Eliminate watersheds with concentrations of human influence, point source pollution, channelization or atypical sources of pollution (e.g., acidification, mine waste, overgrazing, clearcuts).
Evaluate stream size	Use watershed area and mean annual discharge instead of stream order. Watershed areas and discharges of impacted and reference sites should differ by less than an order of magnitude.
Evaluate stream channel	Locate influent streams, springs and lakes; determine drainage pattern, stream gradient, and distance from major receiving water. Retain the stream type most typical of the region.
Locate refuges	Unless the refuge results from local natural features atypical of the region, consider parks, monuments, wildlife refuges, natural areas, state and federal forest, grasslands and wilderness areas.
Determine migration barriers, historical connections among streams, known zoogeographical patterns	Such information helps to form reasonable expectations of species presence and richness.
Suggest reference sites	Reject degraded or atypical watersheds and rank candidates by

	level of disturbance.

Table 10. Total number of stream sites in the Region 7 database, along with number of reference stream sites that were a part of each state's totals.

State	All sites	Reference sites
Iowa	419	94
Kansas	595	53
Missouri	772	60
Nebraska	444	47

The population of *a priori* selected sites was designated as the 'reference' population. Descriptive statistics for TN, TP, and sestonic and benthic Chl*a* from these sites were examined and are shown in Table 11. Region wide, the mean concentration for each indicator was 14 to 30% higher than corresponding median values indicating the data was skewed toward higher values by a few sites with very high values. When examining the relationships between levels of nutrients associated with the reference population (Table 11) it must be remembered that the a priori reference population in essence represents stream/watershed systems that are believed to be minimally impacted by a number of factors (e.g., point source pollutants, nutrients, altered riparian condition, instream habitat, land use) and may or may not represent sites experience minimal nutrient impacts. Similarly these reference streams do not represent streams with the best biological metrics or biological condition. This is illustrated when we compare the median values for fish and macroinvertebrate richness in the a priori reference population with the median richness values for the Trisection method based the same two endpoints for all sites (Table 14). The very best one-third streams based on fish or macroinvertebrate richness had median values that were 16 to 26 percent higher richness values, respectively, than the a priori reference streams.

Table 11. Descriptive statistics for selected parameters for the *a priori*-defined population of reference streams (from sites with known watershed sizes and five or more samples for the given parameter).

Parameters	n	Mean	25th quartile	Median	75th quartile
Total N (mg/L)	97	2.48	0.55	1.08	3.25
Total P (mg/L)	98	0.17	0.05	0.08	0.14
Sestonic Chl*a* (µg/L)	50	5.26	1.95	3.32	6.93
Benthic Chl*a* (mg/M^2)	28	28.58	13.11	24.20	35.24
Fish richness	163	16	11	16	69
Macroinvertebrate	189	55	37	49	23

The Quartile Method to Establish a Reference Condition

USEPA's nutrient technical manuals (USEPA, 2000a; 2000b; 2001b) describe two ways of establishing a reference condition. The first method is to choose the upper quartile value (75^{th} percentile) of the distribution of an a priori reference population of sampling stations (such as those determined in Section 6.1). When reference conditions are not identified, the second method is to determine the opposite end of the distribution or lower quartile value (25^{th} percentile) of the population of all available sampling stations (some of which are presumed to be degraded) within a region (Figure 17).

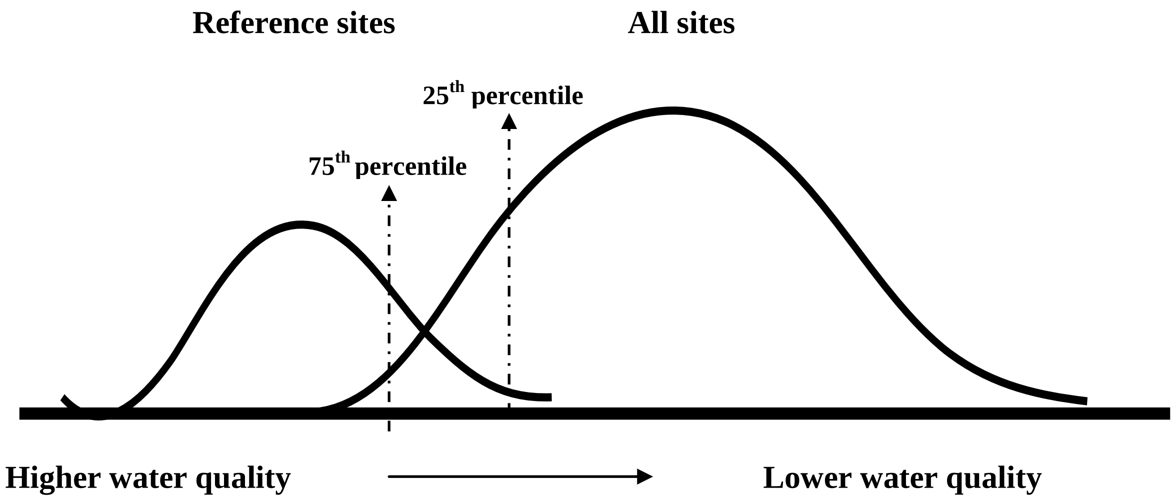


Figure 17. Reference values as selected from a theoretical dataset of streams, proposed by the USEPA Nutrient Criteria Guidance Manual (2000a). The reference value is selected from either the 75th percentile of a reference population or the 25th percentile of the entire waterbody population.

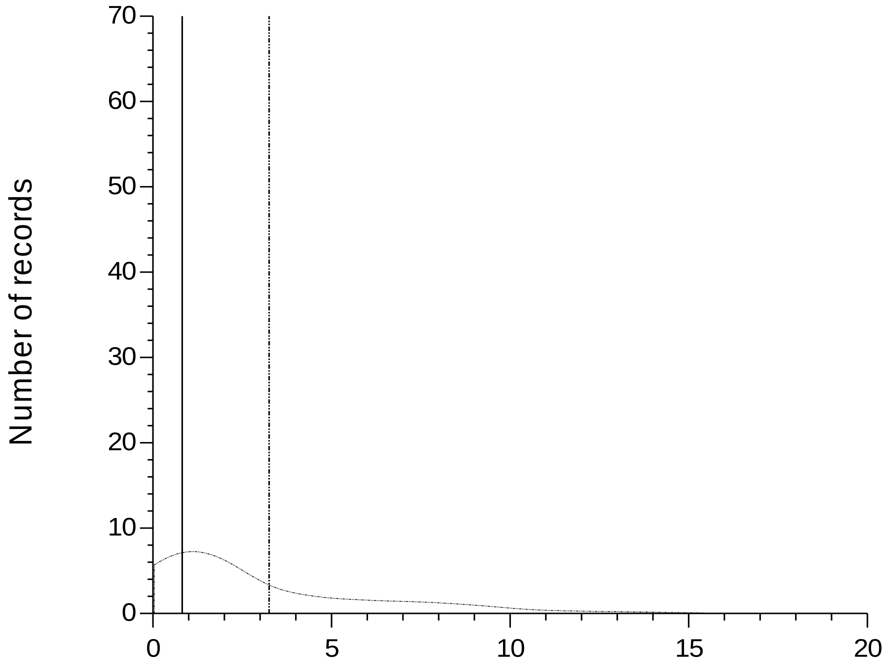
Using the medians dataset, the 25^{th} percentile of the entire stream population was calculated for each parameter (Table 12 and Table 13). This cut-off was later compared to other potential benchmark values. The values also show that this dataset of USEPA Region 7 streams does not fit the theoretical model proposed by the USEPA, since the 25^{th} percentile of the parameters from the entire population is below that of the 75^{th} percentile of the reference population. Histograms of the values further illustrate this point (Figure 18a – c). The model most likely exaggerates what one would find for any set of waterbodies, greatly pulling the reference population out of the entire population. In reality, the reference population is a subset of the entire population and its bell curve should greatly overlap the left side of the bell curve of the entire population.

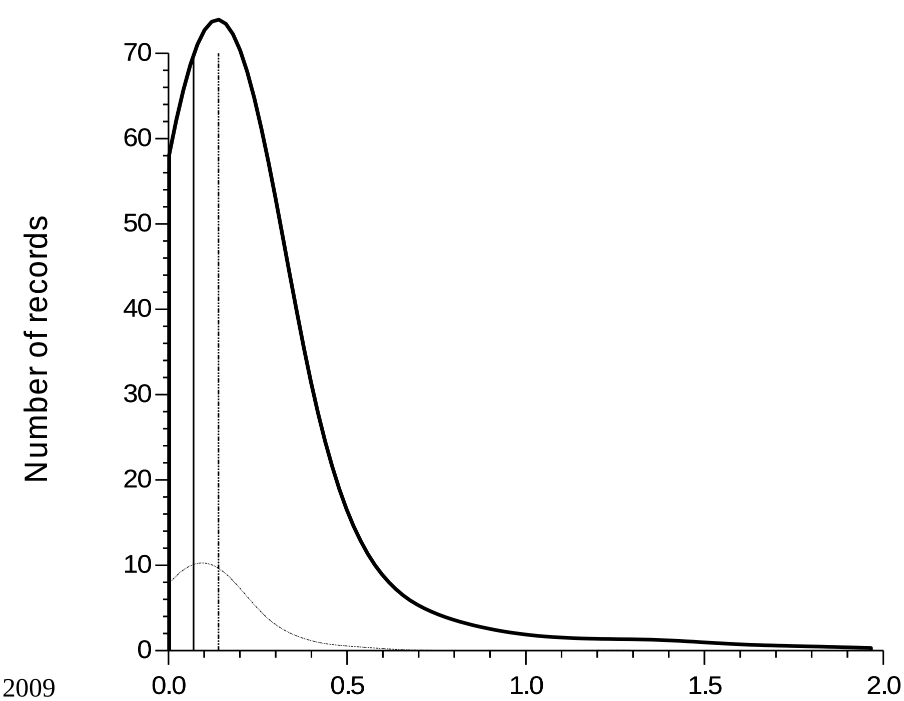
Table 12. Descriptive statistics for selected parameters for the entire population of Region 7 stream sites (from sites with known watershed sizes and five or more samples for the given parameter).

Parameters	**n**	**Mean**	**25^{th} quartile**	**Median**	**75^{th} quartile**
Total Nitrogen (mg/L)	1059	2.66	0.82	1.49	3.10
Total Phosphorus (mg/L)	1193	0.31	0.07	0.14	0.25
Sestonic Chl*a* (μg/L)	315	10.42	2.00	5.00	11.00
Benthic Chl*a* (mg/M^2)	73	28.91	11.92	23.90	40.24

Table 13. Comparison of the 75th percentile of the a priori-defined reference population and the 25th percentile of the entire stream population.

Parameters	**75th percentile Reference sites**	**25th percentile All sites**
Total N (mg/L)	3.25	0.82
Total P (mg/L)	0.14	0.07
Sestonic Chl*a* (μg/L)	6.93	2.00
Benthic Chl*a* (mg/M^2)	35.24	11.92





However, most waterbodies in Region 7 have already been strongly impacted by anthropogenic nutrient loading. In cases where a group of waterbodies already shows evidence of human impact, a percentile other than 25% than can be used in an effort to approximate previous natural conditions (USEPA Nutrient Criteria Guidance Manual April, 2000). The RTAG thus chose to explore a third procedure termed the Trisection method.

6.3 The Trisection Method to Establish a Reference Condition

The Trisection method (USEPA, 1998) designates the group of streams that fall in the best third for a specific response metric as the reference condition for that metric (Figure 19). This population of reference streams can then be used as a standard of comparison for the entire population of streams. Some methods recommend trimming off the worst 5% of streams before sectioning the population into thirds, but the RTAG ultimately decided not to do this creating a more conservating estimate.

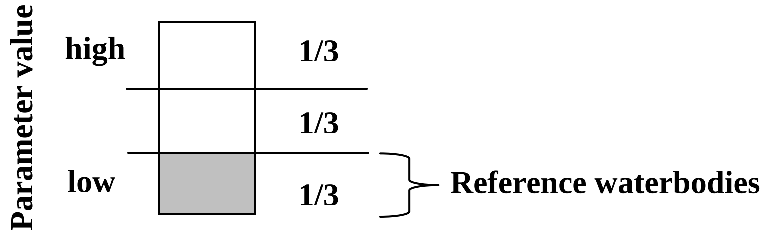


Figure 18. Illustration of reference condition calculation using the Trisection Method for chlorophyll-*a* response parameters.

The RTAG decided to examine TP and TN values associated with the Trisection reference populations determined using four different biological response variables; fish species richness, macroinvertebrate taxa richness, and benthic and sestonic chlorophyll-*a* concentration. The population of data for each response parameter in the Medians dataset was first divided into thirds, and then the waterbodies in the best (i.e., highest values for richness, lowest values for chlorophyll) one-third of each parameter were designated as the 'reference' population. Median values for both stressor and response parameters were then calculated for the reference waterbody population determined for each response parameter (Table 14). For all but taxa richness, calculations were based on data from sites that had five or more samples collected at each site. Fish and macroinvertebrate richness values for each site were the mean value for that site as many sites had less than five fish or macroinvertebrate samples recorded for the site. The best fish and macroinvertebrate populations had median richness values of 19 and 66, while median concentration of Chl*a* for the reference population based on the lower one-third sestonic or benthic Chl*a* levels were about 11 and two, respectively. It was noted that the reference population based on one biological response variable did not necessarily have the correspondingly best value for other biological parameters (Table 14). For example the reference population based on fish richness had a median richness value for macroinvertebrates that was 35 percent less that the median reference value for macroinvertebrate streams. However, while the reference streams for either sestonic or benthic chlorophyll-*a* had low fish richness (median ≈ 7), the median macroinvertebrate richness was quite high (median = 77 to 90). Median TN and TP values associated with the different reference populations identified with each response parameter varied from 5.73 to 0.64 mg/L and 0.07 and 0.12 mg/L, respectively. Reference populations based on macroinvertebrate richness and benthic Chl*a* had the most restrictive median levels for both nutrients, but the number of streams comprising the reference population for benthic chlorophyll-*a* was only 23 (Table 14).

Table 14. Median values of stressor and response parameters based on best third of the sites determined for a given biological response parameter.

	Response parameters							
	Fish total richness		**Macroinvertebrate total richness**		**Benthic Chl*a* (µg/L)**		**Sestonic Chl*a* (mg/M^2)**	
	n	**median**	**n**	**median**	**n**	**median**	**n**	**median**
Total N (mg/L)	51	2.09	44	0.81	23	0.64	100	5.73
Total P (mg/L)	56	0.12	43	0.07	23	0.07	96	0.12
Turbidity (NTU)	49	16.00	38	10.53	22	18.00	95	17.67
Sestonic Chl*a* (µg/L)	24	6.96	31	2.80	23	3.94	100	1.46
Benthic Chl*a* (mg/M^2)	10	25.82	17	20.31	23	10.79	20	23.23
Macroinvertebrates (sample richness)	208	43	169	66	10	77	19	90
Fish (sample richness)	217	19	111	17	3	--	71	7

6.4 Regression and Threshold Methods

Potential causal relationships between nutrient stressors and various biotic characteristics of lotic ecosystems in the region were explored with robust regression analysis a linear modeling technique. This regression technique was used because it is less affected by heteroskedasticity and outliers than ordinary least squares regression (see http://en.wikipedia.org/wiki/Robust_regression; Western, 1995;

Rousseeuw and Leroy, 2003). Only two of four robust regression models were found to be statistically significant (alpha ≤ 0.05) when chlorophyll variables were identified as the dependant variables (Table 15 A – find and use old regression talbe from prior work). Sestonic chlorophyll levels were positively related to TP values indicating that increases in this nutrient is a contributer to increases in algal boiomass. This regression was highly signifcant and explained 20 percent of the variation in water column chlorophyll values. Conversely, TN was the sole nutrient linked to changes in benthic chlorophyll concentrations (i.e. periphyton). The TN model showed a positive relationship between chlorophyll and TN levels explaining somewhat over 10 percent of the variation in benthic chlorophyll. In northern Ozark streams in Missouri Lohman *et al.* (1992) and Lohman and Jones (1999) found much stronger linear regression relationships ($R^2 \geq 0.47$) between benthic algae and both TP and TN summer means, as well as mean summer sestonic chlorophyll and TP ($R^2 \geq 0.67$). More recently researchers again fund that in low-level nutrient streams the Ozark Highland ecoregion fish-, macroinvertebrate- and algal-based biotic indices all showed significant negative relationships with nutrients (Justus *et al.,* 2010). These strong stressor/response relationships in low-nutrient Ozark streams were not found when analysing our regional stream data suggest that most streams in Region 7 have such high nutrient levels that linear stressor/response relationships are masked by numerous high stressor values that lay well beyond the stressor/response thresholds (see threshhold discussion below). Using data from a large series of temporate streams Van Niewenhuyse and Jones (1996) found that TP and mean summer benthic chlorophyll values were positively related with simple linear regression explaining about 67 percent ($R^2 = 0.67$)of the variance in chlorophyll concentrations. Identifying strong stream nutrient and plant relationships is often hampered by a number of other stream factors that affect algal biomass accroal and standing crop. One set of factors of concern by the RTAG was flow conditions since virtually all of the stream data used in these assessments lacked associated discharge and flow measurements. Current and prior flow conditions especially stream velocities can both increase and decrease production and bimass periphyton regardless of nutrient conditions (Horner and Welch 1981, Humphrey and Stevenson 1992, Biggs 2000).

Existence of biological thresholds could indicate reference conditions, or conditions under which biological integrity can be compromised. This method makes the assumption that organisms in rivers and streams (for practical reasons related to available data and length of life cycle, aquatic macroinvertebrates) have evolved under a range of nutrient conditions. Thus, maximum diversity of organisms can be found within the range of water column nutrient concentrations under which these organisms evolved. If there are thresholds in diversity (e.g., an abrupt change in the relationship between diversity and nutrients) related to an environmental parameter, this indicates environmental conditions outside those typical of a reference condition. Recently a number of researchers have identified water quality threshold values for several biological groups and metrics (Paul and McDonald 2005,

Several methods can be used to indicate the presence of thresholds. A simple method is to visually inspect locally weighted regressions lines (LOWESS, Cleveland 1979) for regions of abrupt change. Several statistical methods also exist to indicate thresholds, non-linearities or breakpoints in relationships. Breakpoint regression finds the two best lines that fit 2 dimensional data. The point of transition from one predictive line to the next indicates a threshold. Two-dimensional Kolomgorov-Smirnov test is a nonparametric method to indicate breakpoints in variance (Garvey et al., 1998). Polynomial regression can indicate if a non-linear fit explains more variance than a linear fit, but does not establish the breakpoint in the relationship. Piecewise regression has been recognized a statistical tool for identifying ecological threstholds (Tom and Lesperance 2003). Breakpoint values for a number of different response variables were determined using a two-segment piecewise, nonlinear regression technique (Table 15) given in SigmaPlot 2000 (version 6.00. SPSS, Inc., Chicago, IL). This was done using TP, TN and turbidity as stressor variables. Trimmed versions of both TP and TN were also tested against various dependant variables (see foot notes 1-4, Table 14). Trimmed values were visually identified as outliers and no formal outlier tests were conducted to determined statistical outlier values. Macroinvertebrate richness and ratio of sensitive taxa values from Iowa were dropped from analysis because larvae of the family Chronomidae were not taken to the genus level as was done for other states thus altering the taxonomic resolution of the Iowa data.

Table 15. Breakpoint values for several significant ($p \leq 0.05$) nonlinear, piecewise regression models based on different stressor (independent) and response (dependant) variables taken from the USEPA Region 7 RTAG stream database.

Dependant variable	Independent variable	Sample size	P value	R^2	Breakpoint for independent (mg/L)
Sestonic chlorophyll-*a*	TP (mg/L)	463	<0.0000	0.11	0.32
Sensitive fish species	TP (mg/L)[1]	406	0.0006	0.04	0.16
Macroinvertebrate richness (genus-level resolution)	TP (≤ 1.6 mg/L)[2]	230	<0.0001	0.36	0.12
Sestonic chlorophyll-*a*	TN (≤ 3.0 mg/L)[3]	233	<0.0001	0.11	0.07
Fish richness	TN (≤ 25.0 mg/L)[4]	1091	<0.0001	0.08	12.10
Macroinvertebrate richness (genus-level resolution)	Turbidity (≤ 200 NTU)[5]	163	<0.0001	0.47	13.70

1 includes only sites having at least one sensitive species
2 one site (TP = 1.82 mg/L) was excluded from the analysis
3 228 sites (TN >3 mg/L) were excluded from the analysis
4 four sites (TN >25 mg/L) were excluded from the analysis
5 four sites (NTU > 200 NTU) were excluded from analyssi

6.5 Published information related to nutrient criteria in EPA Region 7

Several peer-reviewed publications have calculated estimates for reference levels of nutrients in ecoregions that occur in EPA Region 7. Smith et al. (2003) used modeling approaches to estimate reference levels, Dodds and Oakes (2004) used an analysis of co-variance and extrapolation to remove the influence of human land use. These data are compared to the EPA method that simply chooses the 25th percentile of all sites. The methods roughly agree, with the largest disparity occurring in the Corn Belt, with the 25% method yielding substantially higher TP and TN estimates, probably because of the absence of extant true reference sites in this ecoregion.

Literature review of levels of algal biomass indicates thresholds that may occur in benthic chlorophyll which may link nutrient levels in the water column to algal biomass. Data suggest that there is a level of nutrients above which there is less increase in algal biomass (Table 17). The existence of these thresholds indicates that management of nutrients that does not bring water column concentration below the threshold is unlikely to result in decrease in algal biomass. An alternative way to view the same data set is to look at the proportion of cases that exceed some level of chlorophyll concentration (i.e. mean and maximum value) in the water column (Figure 20). These data suggest threshold concentrations for TP (levels at which the probability that benthic chlorophyll will exceed 100 mg m^{-2} sharply increases) occur in the range of 20-80 µg/L total P and 200-800 µg/L total N (Fig 20). These plots allow visualization of the probability that benthic chlorophyll will exceed some level given a specific concentration of nutrient in the water column. In general, mean values of chlorophyll exceeding 100 mg m^{-2} and maximum above 150 mg m^{-2} are considered excessive (Dodds and Welch 2000).

Table 16. Comparison of results from Dodds and Oakes (2004, indicated as D&O) with 25% values suggested by the United States Environmental Protection Agency (USEPA 2000a-f, 2001b-e) and values modeled by Smith *et al.* (2003, indicated as Smith) corrected for atmospheric N loading. All values reported in µg/L.

Nutrient Ecoregion number	Nutrient Ecoregion Name	TP (D&O)	TP (USEPA)	TP (Smith)	TN (D&O)	TN (USEPA)	TN (Smith)
IV	Great Plains Grass and Shrublands	59	23	60	659	560	95
V	Central Cultivated Great Plains	23	67	58	566	880	258
VI	Corn Belt and Northern Great Plains	23	76	54	215	2180	355
VII	Mostly Glaciated Dairy Region	23	33	22	565	540	147
IX	Southeastern Temperate Forested Plains and Hills	31	37	48	370	690	150
X	Texas-Louisiana Coastal and Mississippi Alluvial Plains	112	128	48	745	760	439
XI	Central and Eastern Forested Uplands	43	10	20	1102	310	156

Table 17. Corrected breakpoints from Dodds *et al.* (2002) analysis of breakpoints from regression and two-dimensional Kolomgorov-Smirnov (2DKS) tests based on total nitrogen (TN), total phosphorus (TP) and mean and maximum chlorophyll-*a* (mean and maximum Chl*a*, respectively) relationships using the literature dataset. $P < 0.0002$ for all 2DKS determinations.

Dependent variable	Independent variable	Breakpoint from regression (µg/L)	Breakpoint from 2DKS (µg/L)
log (mean Chl*a*)	log (TP)	43	27
log (mean Chl*a*)	log (TN)	537	515
log (max Chl*a*)	log (TP)	62	27
log (max Chl*a*)	log (TN)	602	367

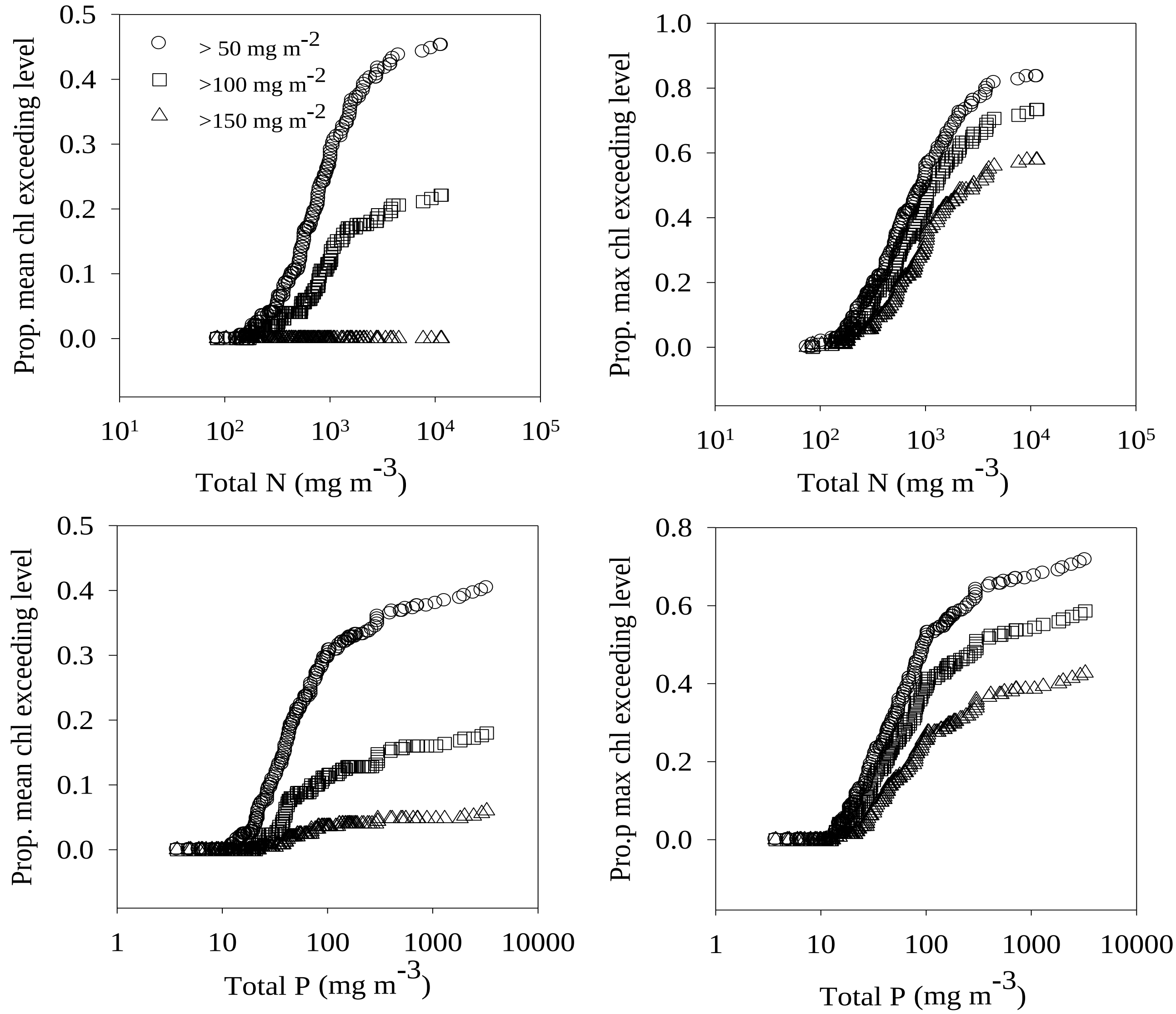


Figure 19. Relationships between seasonal mean water column nutrients (TN and TP) and proportion of instances in which seasonal mean and maximum chlorophyll exceed 50, 100 or 150 mg m-3. Data from literature sources compiled in Dodds *et al*., (2002). This compilation previously had incorrect values for data reported by Lohman *et al*., (1992), however these incorrect values have now been corrected for use in this figure. Sample size for TN and TP was n = 199 and 250, respectively. (Figure reproduced from Dodds 2006).

FRAMEWORK FOR ESTABLISHING NUTRIENT CRITERIA FOR USEPA REGION 7

The Region 7 RTAG met on December 12, 2006, and agreed on benchmarks to protect uses and biotic integrity. Several issues were discussed related to how suggested levels should be applied. Issues discussed were: 1) Should stream size matter in criteria? 2) Should criteria be ecoregion-specific? 3) What role should observed thresholds in nutrient versus biotic responses play? 4) Should turbidity be considered in nutrient criteria? and 5) How do the various methods (thresholds, reference streams, trisection, and EPA 25%) compare?

The first issue is should criteria be set specific to stream or river size? In general there was little relationship to watershed size and response variables (TN, TP, turbidity, benthic chlorophyll). The only exception was that sestonic chlorophyll was greatest in the largest watersheds. The consensus was that there was little to be gained from separating out larger rivers from others for recommending nutrient criteria levels. Sample size for rivers was also relatively small for all nutrient variables since few states had active large river monitoring programs in place.

The second issue approached was the question of ecoregion-specific criteria. The main hurdle in this discussion was the fact that some ecoregions are highly impacted and have few reference sites. Trisection and EPA 25% methods could be greatly influenced by lack of good reference sites and a preponderance of highly nutrient-enriched sites. The question then becomes can we determine reference levels in the absence of pristine or native sites? Several literature approaches have allowed this, and the literature data were discussed. The ecological thresholds are also important in this determination. We could find no scientific justification for different responses to nutrients across ecoregions, and this was buttressed at least in part by the literature (Dodds et al., 2002) which only found deviations from chlorophyll/ TN response in subtropical ecoregions. While basic stressor/responses may not differ significantly between ecoregions, regional differences in stressor concentrations both anthropogenic and natural may require establishment of benchmanrk/criteria at some finer geographical scale (e.g. Rohm *et al.* 2002)

An issue of attainability related to the ecoregion question has come up repeatedly as part of the discussions of nutrient criteria. Some regions may not be able to reach nutrient criteria levels because they are already so heavily impacted by human uses that the technology does not exist or socioeconomic factors would make itimpractical to lower nutrients to levels protective of uses or aquatic life. The RTAG did not consider this point because their specific charge was to determine the scientific basis behind levels for nutrient criteria, and the feasibility of regulating to reach some set level of nutrient criteria in terms of what were historical regions. Socioeconomic and implementation issues related to the adoption of nutrient criteria were issues that fell beyond the expertise and charge of the RTAG and is better addressed by economists, engineers, stakeholders and decision-makers. We simply could not address technical limitations of nutrient criteria except what would be attainable give historic conditions.

In the end the group could find little scientific justification for proposing criteria that varied by ecoregions within the EPA region VII states and tribal lands. The point was made that reference conditions set a lower bound on nutrient criteria, because you cannot hope to lower nutrient concentrations below those that were historically present before the advent of widespread anthropogenic influences (e.g., before widespread fertilization of cropland, numerous large confined animal feeding operations, and substantial populations releasing sewage effluent into waterways).

The third issue discussed was what the threshholds mean. The group decided that threshholds levels of TN and TP above which chlorophyll no longer increases substantially provide an indication of regions where nutrient control may be effective. That is, there will be limited biological response to different nutrient criteria with respect to benthic algal biomass if nutrients remain above threshold levels. Numerous thresholds were also identified where macroinvertebrate biodiversity decreased with increasing nutrients, but then stabilized at low levels above some threshold nutrient concentration.

Given the probability that this threshold level indicates a range above which most organisms have little exposure over evolutionary time, macroinvertebrate thresholds were viewed as an upper level for protecting aquatic life. Development of nutrient thresholds for fish variables were limited as few significant piecewise regressions were identified, those that were significant had low R^2 values and threshold values two to 12 times higher that breakpoints for macroinvertebrate variables (Table 15). However, in Ohio streams Miltner and Rankin (1998) found a negative correlation between nutrients and their biotic index for fish. Increasing nutrient concentrations in low order streams were associated with deleterious effects on the fish community especially when nutrient levels exceeded background (total inorganic nitrogen and phosphorus > 610 and 60 ug/L, respectively). These numbers are similar to RTAG benchmark numbers for TN and TP. Based on data from 70 wadeable streams within watersheds exhibiting a large gradient of agricultural land use practices a TN threshold of 480 ug/L was identified by Maret *et al.* (2010) where eutrophic index scores based on aquatic plant metrics abruptly became less responsive to increasing TN concentrations.

The role of stream turbidity in either altering the relationships between nutrient stressors and receptors could not be determined. Consistent relationships between turbidity and other factors related to nutrient criteria were poorly delineated in our dataset, except the very strong positive relationship between TP and turbidity. Subsequently, the group decided not to consider turbidity in their final recommendations for benchmark values, but observed that methods to control turbidity also control non-point sources of total phosphorus.

The final area of discussion was how the various methods compare (Table 18). The group felt that there was general concordance across methods, and chose values for TN, TP, sestonic Chl*a*, and benthic Chl*a* that were obtainable given baseline nutrient concentrations for the ecoregions, and were consistent with the numbers determined from reference streams and trisection. Values were less than the thresholds for biodiversity, consistent with the group's understanding of how these thresholds should be used. The point was made with regard to these benchmarks that they apply only to streams and rivers. The values are higher than those chosen for lakes in Region 7, and managers may need to set more restrictive values if protection of downstream waterbodies is required.

Table 18. Summary of values relevant to setting benchmark values in streams and rivers.

Parameter	**Literature[1, 2, 3] (ranges)**	**Nutrient Regions[4] (range)**	**Reference Streams (median)**	**Trisection[5] (median)**	**25%**	**MEANS (all methods)**
TN (mg/L)	0.7 – 1.5[1] 0.15 - 1.10[2] 0.51 - 0.54[3]	0.54 – 2.18	1.08	0.81	0.82	0.964
TP (mg/L)	0.025 – 0.075[1] 0.023 - 0.060[2] 0.027 - 0.043[3]	0.01 – 0.128	0.08	0.07	0.07	0.052
Sestonic Chl*a* (µg/L)	10 – 30[1]	0.9 – 3.0	3.3	2.8	2	6
Benthic Chl*a* (mg/M^2)	20 - 70[1]	NA	24.2	20.3	11.9	25.4
Turbidity (NTU)	NA	1.7 – 17.5	12	10.5	9.5	10.4

[1]Dodds *et al*.,1998
[2]Dodds and Oakes 2004 and Smith *et al*., 2003
[3]Dodds *et al*., 2002
[4]From EPA 822-B-00-017, -18, -019, -020; EPA 822-B-01-013, -014, -016.
[5]Trisection values are for upper one-third streams in US EPA Region 7 having highest total richness for macroinvertebrates.

Table 19. The final proposed nutrient benchmark values and response values most likely to protect use and integrity of lotic waters for median values of TN, TP, and sestonic and benthic chlorophyll *a* for EPA Region 7.

Parameter	Benchmark value
TN (mg/L)	0.9
TP (mg/L)	0.075
Sestonic Chl*a* (μg/L)	8
Benthic Chl*a* (mg/M^2)	40

Summary

As mentioned in Section 1.2, information on five weight-of evidence factors (reference conditions, historical data and trends, models, RTAG expert review, and consensus, and downstream effects), as well as information from the literature, are urged to be used in establishing nutrient water quality criteria. These elements, as expressed in EPA's technical manuals (USEPA, 2000a; 2000b; 2001b) should ideally be incorporated in the criteria development process. The RTAG of the USEPA Region 7, States, and Tribes are the most knowledgeable parties for the optimal incorporation of this information for comprehensive criteria development. In the absence of this effort, EPA may be obliged to rely extensively on the reference condition values presented in this report for any necessary nutrient quality management decision-making. Thus, States are strongly encouraged to use this information as their basis for more geographically specific and refined criteria development. With these benchmarks for decision-making, EPA-State cooperation can be established to protect our rivers and streams.

A series of violin plots were produces to examine the relationships between proposed regional benchmark values and all sites (e.g., streams) and *a priori* reference sites within ecoregions having enough sites (≥5) with five or more samples (Figure 20 - Figure 22).

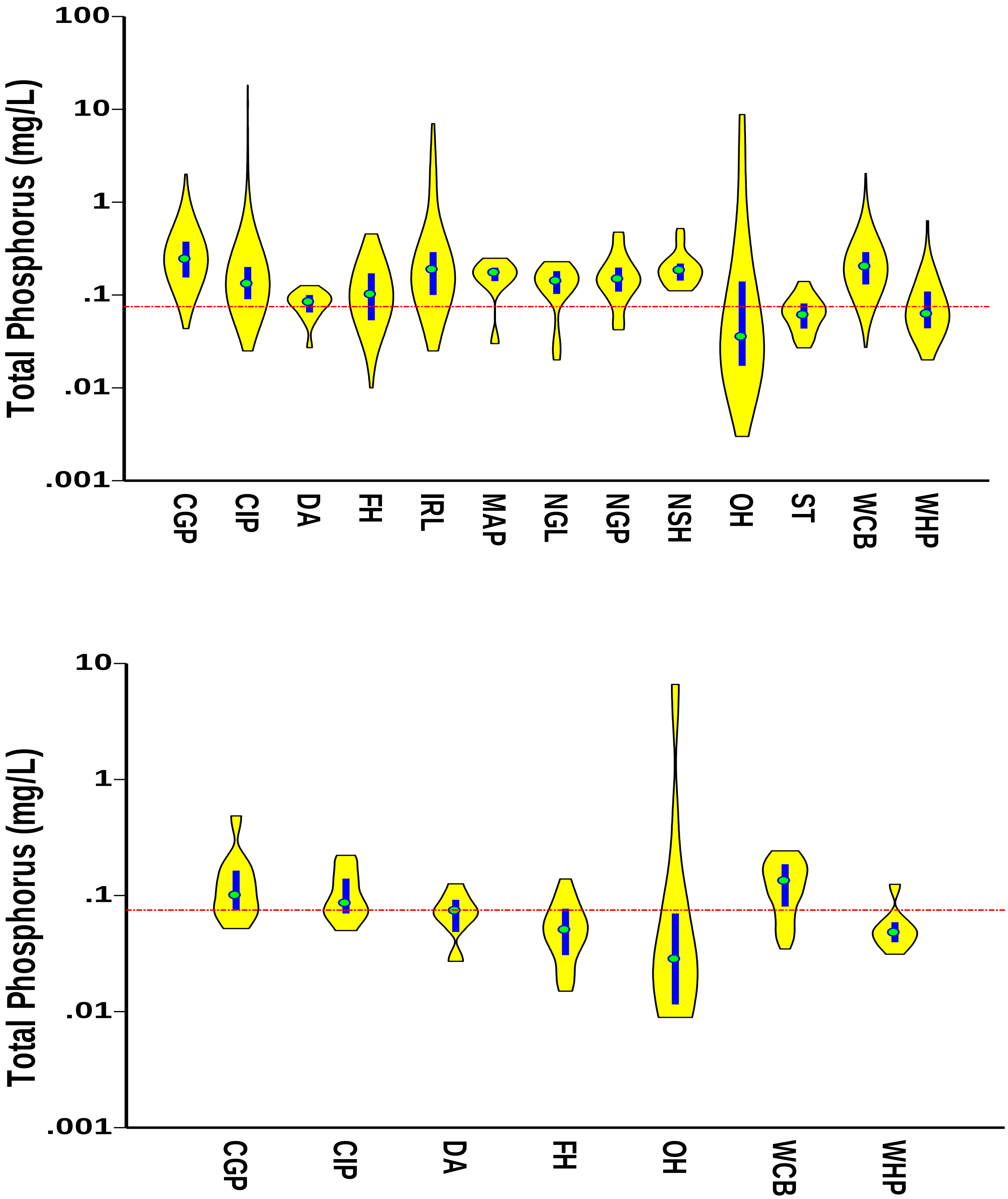
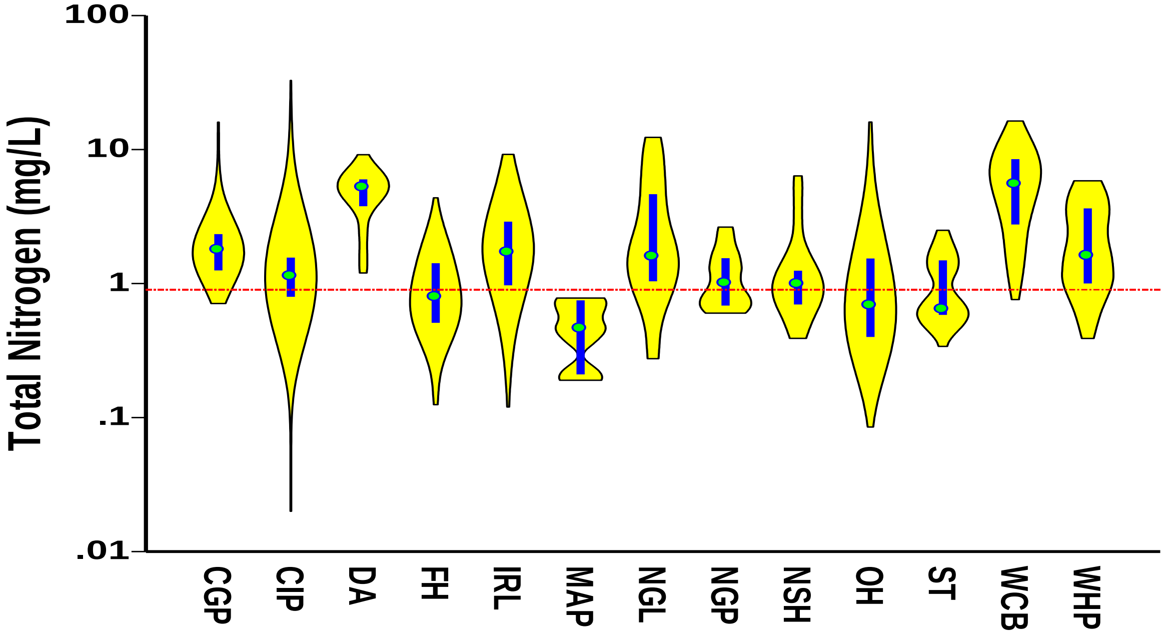


Figure 20. Violin plots of TP (mg/L) for all streams (a) and a priori reference streams (b) within ecoregions. The benchmark value for TP (Section 7) of 0.075 mg/L is represented by the dashed line.



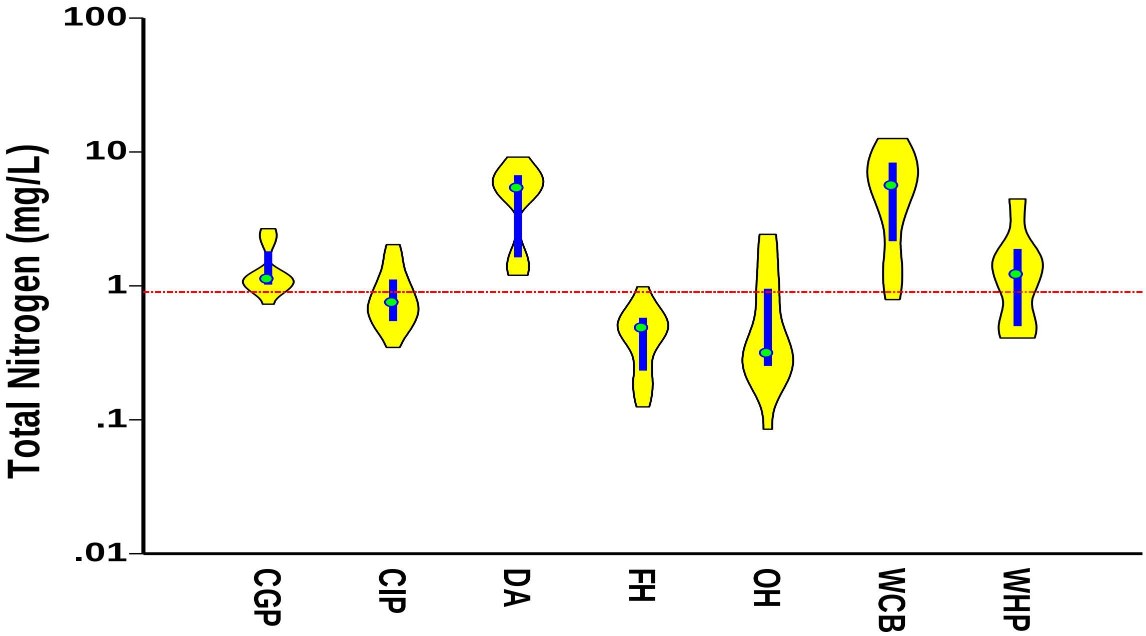
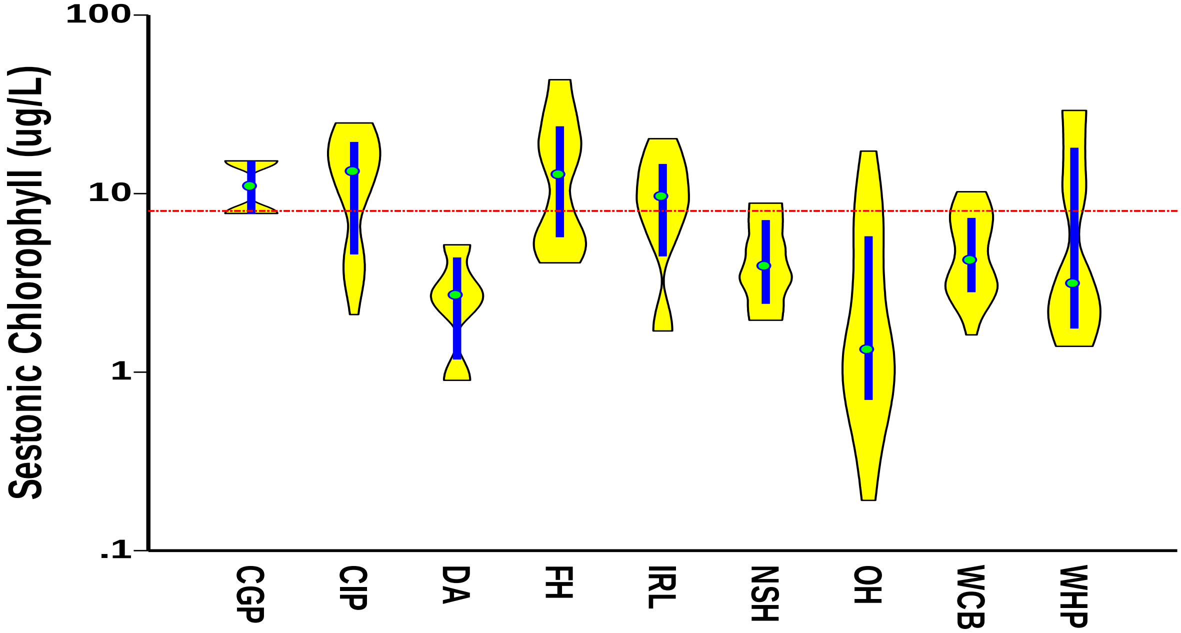


Figure 21. Violin plots of TN (mg/L) for all streams (a) and a priori reference streams (b) within ecoregions. The benchmark value for TN (Section 7) of 0.9 mg/L is represented by the dashed line.



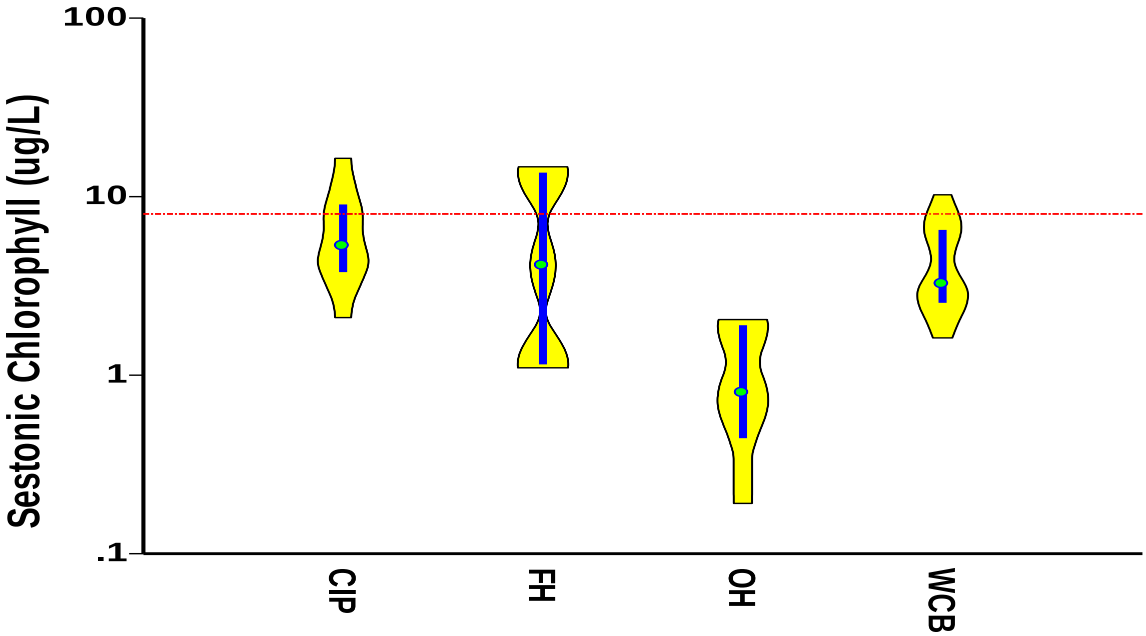


Figure 22. Violin plots of seston Chl*a* (μg/L) for all streams (a) and a priori reference streams (b) within ecoregions. The benchmark value for seston Chl*a* (Section 7) of 8 μg/L is represented by the dashed line.

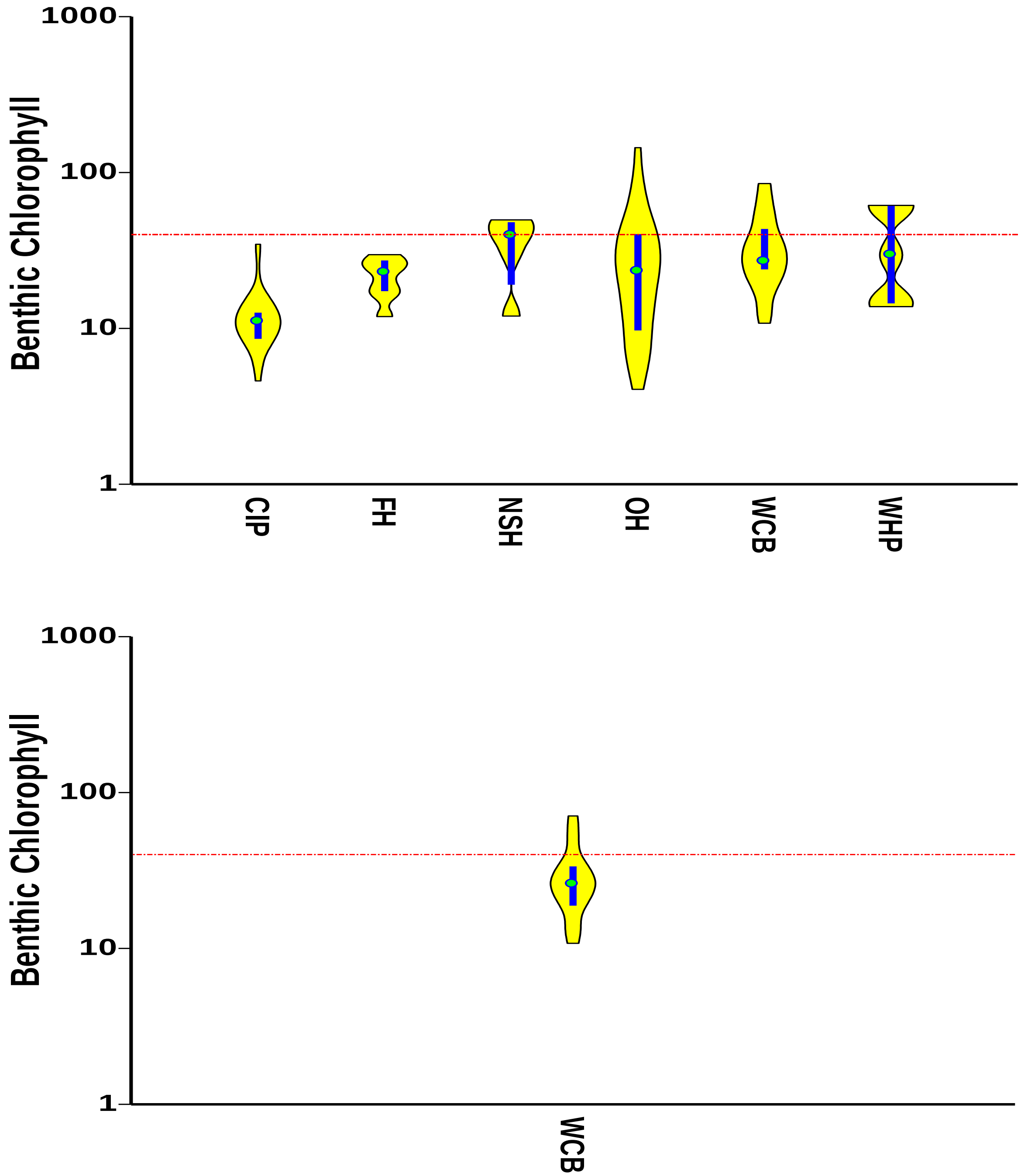


Figure 23. Violin plots of benthic Chl*a* (mg/M^2) for all streams (a) and a priori reference streams (b) within ecoregions. The benchmark value for benthic chlorophyll-*a* (Section 7) of 40 mg/M^2 is represented by the dashed line.

It appears that about half to one-third of the ecoregions have stream populations that had median or 25th quartile values that were at or below the TP and TN benchmark threshold levels (Figure 21 and 22). All but two of the seven ecoregions that had enough reference site data to plot had median TP values below the benchmark concentration of 75 µg/L. In the case of Chl*a* response variables all or nearly all ecoregions that had sufficient data to construct violin plots had median and 25 quartile levels well below the sestonic and benthic chlorophyll benchmark values of 8 and 4 µg/L, respectively. Only the stream populations (i.e., all streams) of the Central Great Plains, Central Irregular Plains, Flint Hills and Interior River Lowlands ecoregions had median sestonic Chl*a* levels above the 8 µg/L benchmark concentration. These comparisons help illustrate the general applicability of these regional benchmark and point out the possible need for more geographically refined benchmark values in some areas of USEPA Region 7.

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9.0 LITERATURE CITED

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