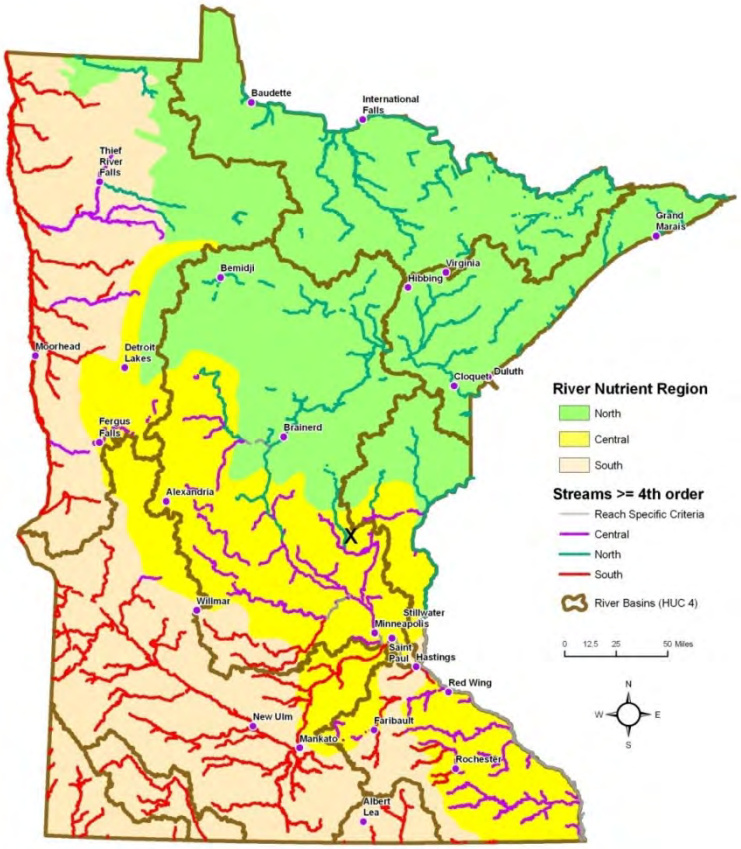
Minnesota Nutrient Criteria Development for Rivers

(Update of November 2010 Report)



Minnesota Pollution Control Agency

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DRAFT

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Dr. Lester Yuan and Dr. Susan Cormier were consulted on use of quantile regression analyses

Sampling

Field sampling for this project spanned several summers from 1999-2008 and involved various MPCA staff from Environmental Outcomes and Analysis Division including Lakes and Streams Unit, Biological Units and Flow and Groundwater Unit.

Foreword

This technical support document for Minnesota's proposed river eutrophication criteria has undergone several revisions as a result of internal and external review, refinements in data analysis, and related factors. This revision of the 2010 draft document is a product of comments from and discussion with USEPA reviewers, Minnesota Center for Environmental Advocacy, and other reviewers. Minor modifications are included to provide greater clarity in descriptions of data sets, statistical analyses, and justifications for the proposed criteria. Slight adjustments to the proposed criteria have been made as a part of this revision. Criteria, as proposed in this revision, will be used to develop rule language and further supported in the Statement of Need and Reasonableness (SONAR) that is developed in support of the rulemaking.

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Acronyms, abbreviations, & commonly used terms in this report

ALUS	Aquatic Life Use Standard
AQRS	Additive quantile regression smoothing
ARUS	Aquatic Recreational Use Standard
AUID	Assessment Unit Identification
benthic algae	algae that live attached to substrates in stream; also referred to as periphyton
BOD_5	5-day biochemical oxygen demand
CDF	cumulative distribution function (also referred to as frequency distribution)
CHF	North Central Hardwood Forests ecoregion
Chl-a	chlorophyll-a, corrected for pheophytin
Chl-T	total chlorophyll-a, which includes chlorophyll-a +pheophytin
CI	confidence interval
CP	Changepoint
CWA	Clean Water Act
DA	Driftless Area ecoregion
diel	A chronological day (24 hours)
DO	dissolved oxygen
DO flux	magnitude of change in DO over the course of a day; as used here reflects daily maximum DO minus daily minimum DO; also referred to as diel flux
EPT	Ephemeroptera, Plecoptera, Trichoptera
HUC	hydrologic unit code
IBI	Index of biotic Integrity
ISS	Inorganic suspended solids
LAP	Lake Agassiz Plain, another name for RRV ecoregion
LOESS	Locally weighted scatterplot smoothing
max	maximum
MDH	Minnesota Department of Health
med	median
metric	used to refer to a biological measurement or class of organisms
mg/L, mgL^{-1}	milligrams per liter; equivalent to parts per million
mg/m^2	milligram per meter squared; an areal-based measure commonly used to express periphyton biomass or chlorophyll-a
min	minimum
MPCA	Minnesota Pollution Control Agency
MSHA	Minnesota Stream Habitat Assessment
NGP	Northern Glaciated Plains ecoregion
NLF	Northern Lakes and Forests ecoregion
NMW	Northern Minnesota Wetlands ecoregion
NTU	Nephelometric turbidity units
PI	prediction interval
QHEI	Qualitative Habitat Index
quantile	Division of ordered data into equally sized portions. For example, quartiles are the division of data into 4 equal portions and percentiles are the division of data into 100 equally sized portions
quartile	a distribution that subdivides population into four equal portions, whereby first quartile represents lowest 25% of population…fourth quartile represents upper 25%
R	a free statistical package
r^2	r squared, correlation coefficient

RNR	River Nutrient Region
RRV	Red River Valley ecoregion
RTAG	Regional Technical Assistance Group
sestonic algae	algae suspended in the water; also referred to as phytoplankton
STORET	USEPA's data system - STOrage and RETrieval
TALU	Tiered Aquatic Life Use
TKN	total Kjeldahl nitrogen
TMDL	Total Maximum Daily Load
TN	total nitrogen; equivalent to sum of TKN +nitrate-N
TP	total phosphorus
TSS	Total suspended solids
µg/L, μgL^{-1}	micrograms per liter, equivalent to parts per billion
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WCP	Western Corn Belt Plains ecoregion
WWTP	Wastewater Treatment Plant

EXECUTIVE SUMMARY

The Minnesota Pollution Control Agency (MPCA) has developed draft eutrophication criteria for rivers protective of Minnesota's aquatic life use. A multiple lines of evidence approach was used to develop the criteria. Conceptual models provide an overview of the focus of our research and the linkages we sought to establish. Several studies and data collection efforts provide the body of information used to develop the criteria. These data sources included the River Nutrient study which consisted of a series of efforts to assess and document relationships and patterns among nutrients, water quality and biological communities. In addition, data from MPCA's biological monitoring program and USEPA's data system, STOrage and RETrieval (STORET), were used to develop the draft criteria. These studies have demonstrated significant and predictable relationships among summer nutrients, sestonic chlorophyll-*a*, and biochemical oxygen demand (BOD_5) in several medium to large Minnesota rivers (Heiskary & Markus 2001, 2003). In addition, diel dissolved oxygen (DO) flux (based on submersible data recorders) also was strongly positively correlated to total phosphorus (TP) and chlorophyll-*a* concentrations. Our findings demonstrate significant relationships among several sensitive macroinvertebrate and fish metrics and TP, TN, chlorophyll-a, and DO flux. Biological thresholds for nutrients and associated stressors were determined using quantile regression and changepoint analyses using macroinvertebrate and fish data. The major steps or approaches that were used to develop draft river eutrophication criteria are summarized below.

- Regressions described basic interrelationships among TP, TKN, sestonic chlorophyll, and DO flux based on the River Nutrient study datasets. Most relationships exhibited high r^2 values and were highly significant.
- Spearman correlation analysis, using the River Nutrient study data, provided an initial basis for identifying relationships among TP, TN, chlorophyll, and DO flux and fish and macroinvertebrate metrics. This provided a basis for identifying responsive metrics for each of these variables and helped to focus subsequent analyses.
- Scatterplots helped visualize relationships among the more responsive metrics and the stressors and begin threshold identification. Statewide interquartile ranges for the biological metrics were used to place metric values in perspective and help discern where an important shift in the metric may be occurring relative to the stressor gradient.
- More advanced statistical techniques including quantile regression and changepoint (regression tree) analyses were employed. These analyses are well-suited to the often wedge-shaped plots that are common with field-collected biological data. Based on the Spearman correlation analysis emphasis was placed on the more responsive biological metrics. These techniques were applied to both the river nutrient dataset and the larger biomonitoring and STORET datasets. Threshold concentrations were produced for statewide, wadeable vs. nonwadeable, and on a region-specific basis.
- Relationships among nutrients, stressor variables, and the biology was further assessed by determining the levels of chlorophyll-a and total phosphorus associated with the BOD_5 threshold concentrations;
- A comprehensive review of the literature was conducted and literature-based thresholds provided further perspective on this issue.
- Consistent with EPA guidance, data and relationships were analyzed in a regional context. Threshold concentrations ranges were placed in context with ecoregion-based frequency distributions compiled by MPCA for representative, minimally-impacted streams (McCollor & Heiskary 1993), a more recent compilation of stream TP data from STORET (period from 1996-2012), and IQ ranges from USEPA criteria manuals (USEPA 2000b, a, 2001). These data distributions reflect distinct regional differences in stream TP, BOD_5, and other variables. This work combined with previous analysis of Minnesota's ecoregional patterns resulted in defining three "River Nutrient Regions (RNR)" for criteria development.
- All of the above was used to move from broad ranges of criteria to region-specific criteria (Table 1).

In addition to these ecoregion-based criteria, we have proposed a numeric translator to address the impact of nuisance levels of periphyton that can limit aquatic life and aquatic recreational uses of Minnesota streams. This numeric translator is as follows: "Rivers shall have an algal biomass not to exceed 150 mg Chl *a* m^{-2} to avoid nuisance algal biomasses that interfere with important aquatic recreation designated uses." This level is well supported in the literature (*e.g.,* Welch *et al.* 1988, Dodds *et al.* 1997, Dodds & Welch 2000, Suplee *et al.* 2008b) and provides a good basis for defining impairment from excess periphyton.

The river eutrophication criteria will be applied in a fashion similar to the previously promulgated lake eutrophication criteria. Prior to 303(d) assessment of a stream reach, water quality samples will be collected 6-8 times per summer for a minimum of two summers. These data will be combined with all available data for the most

recent 10-year assessment period. Means will be calculated and compared to the criteria. For a stream reach to be listed as impaired it must exceed the causative variable: TP and one or more of the response (stressor) variables: sestonic chlorophyll, BOD_5, DO flux, and/or pH. Stream reaches listed as impaired will be subject to development of a TMDL. The TMDL considers all upstream sources that contribute to the excess nutrients and the impairment.

USEPA recommends that downstream protection is considered when developing nutrient criteria. This means that criteria need to be protective of both the assessed water (streams in this case), as well as downstream waters. In the case of river criteria, the downstream waters of concern would typically be lakes, reservoirs, or mainstem pools on major rivers. Based on a long history of lake restoration and watershed projects the proposed stream TP criteria are in the range of stream inflow values proposed as a part of restoration projects. One basis for this argument is comparing the stream criteria to the stream TP values used in the MINLEAP model. The MINLEAP model (Wilson & Walker 1989) has long been used as a basis for predicting in-lake TP for minimally-impacted lakes on an ecoregion basis. The model was regionally-calibrated and has long been used to help define in-lake goals for lake and watershed restoration projects. The corresponding regionally-calibrated NLF and NCHF ecoregion stream TP values used in the model are 52 µg/L and 148 µg/L, which are either equal to or higher than the proposed criteria for the North and Central RNRs (50 and 100 µg/L respectively). These stream values were deemed typical of representative, minimally-impacted watersheds for the two regions. This comparison suggests that the North and Central stream TP criteria will likely be protective of downstream resources. For perspective, about 50% of Northern RNR streams have TP <50 µg/L and 35% of Central RNR <100 µg/L. A similar MINLEAP-based comparison for the South RNR could not be made as the steam inflow TP used in the model was highly calibrated to account for extreme storm event loading and internal recycling within the lakes. However, the South RNR proposed criteria of 150 µg/L ranks near the 25th percentile for South RNR stream sites and should prove to be protective of downstream lakes and reservoirs in most instances.

Ultimately, lake and reservoir nutrient TMDLs will determine the appropriate stream inflow TP required to meet water quality standards. The detailed data analysis and development of allocations assures this and would take precedent over existing stream eutrophication standards. We have demonstrated that the proposed river eutrophication criteria are protective of downstream waters in the case of Lake Pepin on the Mississippi River, where modeling has demonstrated meeting the river criteria should allow for attainment of Lake Pepin site specific criteria.

The eutrophication criteria can also serve as a basis for protecting stream reaches that are currently better than the criteria via the implementation of nondegradation. As with other water quality standards there is an expectation that these are not "degrade down to" standards; rather waters that are currently meeting standards would be expected to continue to do so. The combination of the eutrophication standards and nondegradation language should assure that this is the case.

These criteria represent a first step in a larger process. As Tiered Aquatic Life Use (TALU) standards are developed in future rulemakings there will be refinements to these criteria that reflect the more specific needs of the various tiered uses. One example is coldwater streams that will be addressed more specifically. However, in the interim, the region-based eutrophication criteria provide a basis for assessing the condition of Minnesota streams relative to excess nutrients. In turn, this allows for the development of strategies and policies to protect the condition of streams and to minimize and reverse the impact of excess nutrients on stream ecosystems.

Table 1. Draft river eutrophication criteria by River Nutrient Region for Minnesota.

	Nutrient		Stressor	
Region	**TP µg/L**	**Chl-a µg/L**	**DO flux mg/L**	**BOD_5 mg/L**
North	**≤50**	**≤7**	**≤3.0**	**≤1.5**
Central	**≤100**	**≤18**	**≤3.5**	**≤2.0**
South	**≤150**	**≤35**	**≤4.5**	**≤3.0**

I. INTRODUCTION

Nutrients are naturally a part of aquatic ecosystem function, but excessive loading of nutrients can lead to detrimental effects on aquatic biota (Miltner & Rankin 1998, Wang *et al.* 2007, Evans-White *et al.* 2009). Nutrients originate from a variety of sources including natural and anthropogenic sources (agricultural, forestry, mining, commercial, and residential practices). These human activities include point and nonpoint nutrient sources such as animal wastes, fertilizers, landfills, stormwater, waste water treatment facility (WWTF) effluents, and industrial effluents (Carpenter *et al.* 1998). In addition, activities in a watershed can increase transport of naturally occurring or anthropogenically created nutrients into streams and rivers (*e.g.,* agricultural activities, development of impervious surfaces, and removal of vegetation). Although a number of nutrients are required for plant growth (*e.g.,* sulfur, iron, silicon), phosphorus and nitrogen are generally given the most attention as research identifies these as limiting nutrients in aquatic systems (Dodds 2006, Dodds & Cole 2007).

Some water quality problems due to enrichment can be the direct result of toxicity to biota by some forms of nutrients (*e.g.,* ammonium hydroxide, nitrate, and nitrite; Miltner & Rankin 1998, Dodds 2002). Most other water quality problems arising from eutrophication are less direct and are the result of modifications to aquatic food webs generally caused by increases in the productivity of aquatic plants (*e.g.,* algae, macrophytes) and microbes. Eutrophication leads to increases in the metabolic activity of autotrophic and heterotrophic organisms although the relative impact to autotrophs and heterotrophs depends on the characteristics of the system (Dodds 2006, 2007, Dodds & Cole 2007). Typically, an increase in nutrients to an aquatic system can result in increased growth of algae and microbes (Figure 1 and Figure 2; Smith *et al.* 1999). Increased amounts of algae and microbes in turn result in an increase in respiration and a decrease in dissolved oxygen (DO). The increased productivity of these organisms can also alter food resources and habitat structures. As a result, a system with increased phosphorus loading will shift to a biological community with more generalist taxa, which are more tolerant of low DO. In general, these taxa tend to be less desirable (*e.g.,* poorer ecosystem function, reduced fishery value). Biological communities in enriched habitats may also experience greater occurrence of disease. These modifications can also open these habitats to invasion by nonnative species. Eutrophication can also increase the cyanobacteria growth which can lower DO, change food resources, and produce algal toxins which are harmful to aquatic organisms and can even be dangerous for terrestrial organisms (Dodds & Welch 2000).

The impact of nutrients on aquatic ecosystems and biota through food web alterations depend on a number of factors. For example, turbidity, shading, and water body depth can decrease the impact of nutrients on aquatic systems. In addition, different segments of food webs (*e.g.,* benthos versus seston) may be affected in different habitat types. As a result, the type of habitat (*e.g.,* large versus small rivers) has an impact on how nutrients impact water quality and biological condition in Minnesota's rivers. In large to medium sized rivers, nutrient loading is more likely to result in increased production of phytoplankton (measured as sestonic chlorophyll) and microbes. Three important factors that can limit or promote algal growth in medium to large rivers are nutrients, temperature, and light. In these systems, impact of nutrients is moderated by light and residence time (Figure 1). The amount of light reaching aquatic plants can be decreased by shading, turbidity, and depth (Smith *et al.* 1999) with turbidity probably having a larger impact in large to medium rivers. Vertical mixing may also have an impact on light availability, particularly behind impoundments, by moving algae from deeper portions into the euphotic zone. Residence time or flushing rate also affects sestonic chlorophyll where low residence time will cause algae to be transported downstream at a higher rate (Van Nieuwenhuyse & Jones 1996). Provided with sufficient light, temperature, and residence time, nutrient loading can cause changes in the food web base by altering growth rate and composition of the planktonic algal community. However, even if these factors are not sufficient to create problematic algal blooms, nutrient enrichment can result in increased microbial production and/or the transport of nutrients downstream to river reaches where conditions exists for unwanted algal blooms to occur.

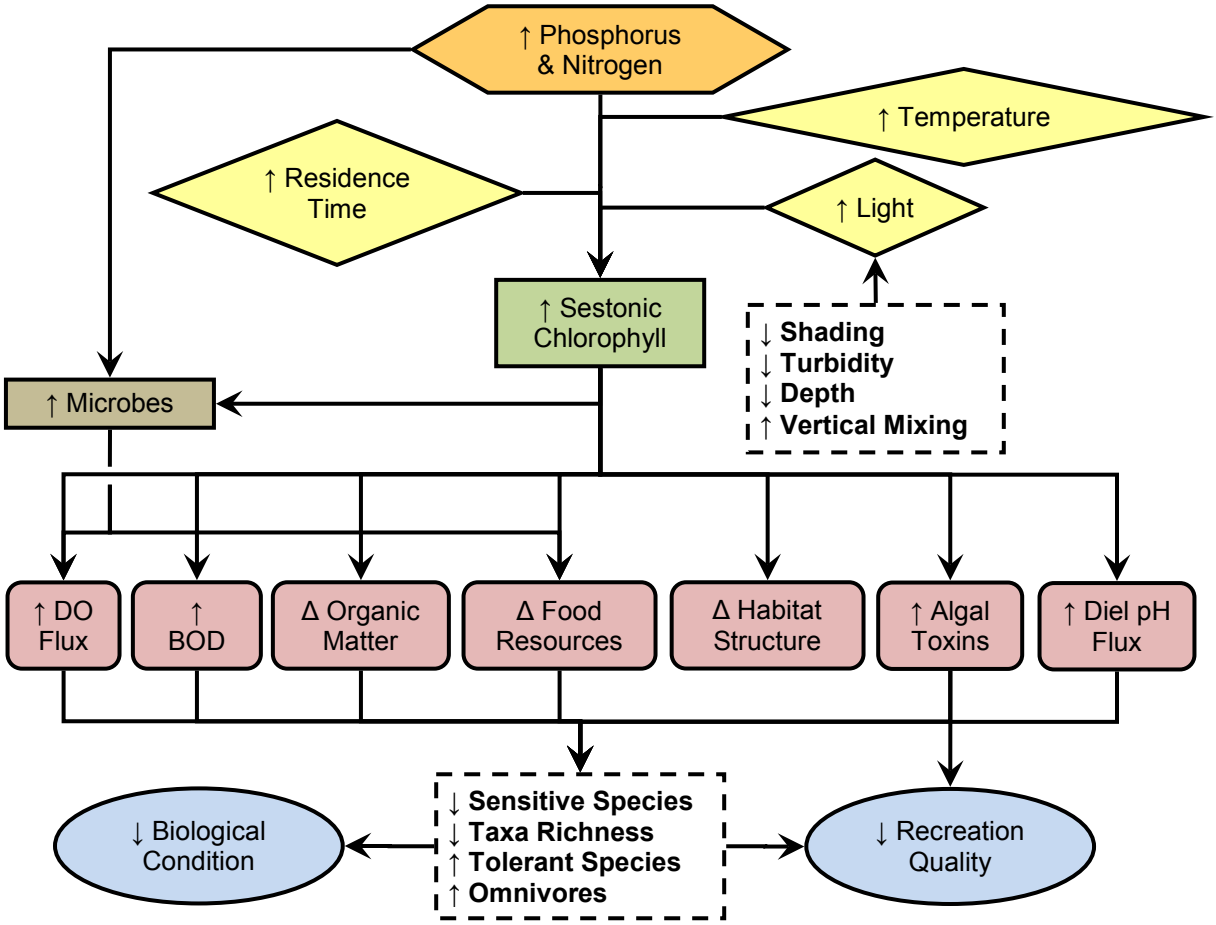


Figure 1. Conceptual model of the impact of nutrient enrichment on biological condition and recreational quality for medium to large rivers.

In small rivers, nutrient loading is more likely to result in an increase in benthic algae or periphyton (measured as benthic chlorophyll). As in large and medium rivers, temperature and light are important determinants of algal growth with turbidity and shading also moderating the effect of light (see Figure 2; Lowe *et al.* 1986, Smith *et al.* 1999). Benthic algal production can also be affected by scouring (Lohman *et al.* 1992) and substrate. Sufficient substrate is needed for growth of benthic algae. For example, coarse substrates such as bedrock, cobble, and large woody debris provide a stable substrate, which allows attachment of some forms of benthic algae. In contrast, fine substrates such as silt and sand are more easily transported which will mobilize benthic algae and reduce benthic chlorophyll. High flows can scour benthic algae from both large and fine substrates and reduce the standing crop of algae (Dodds 2006, Stevenson *et al.* 2006). As with large and medium rivers, in small rivers if the conditions do not exist for increased algal growth (*e.g.*, heavy shading, high scouring), there is the potential for nutrients to be transported downstream to areas where optimal conditions do exist for high algal productivity.

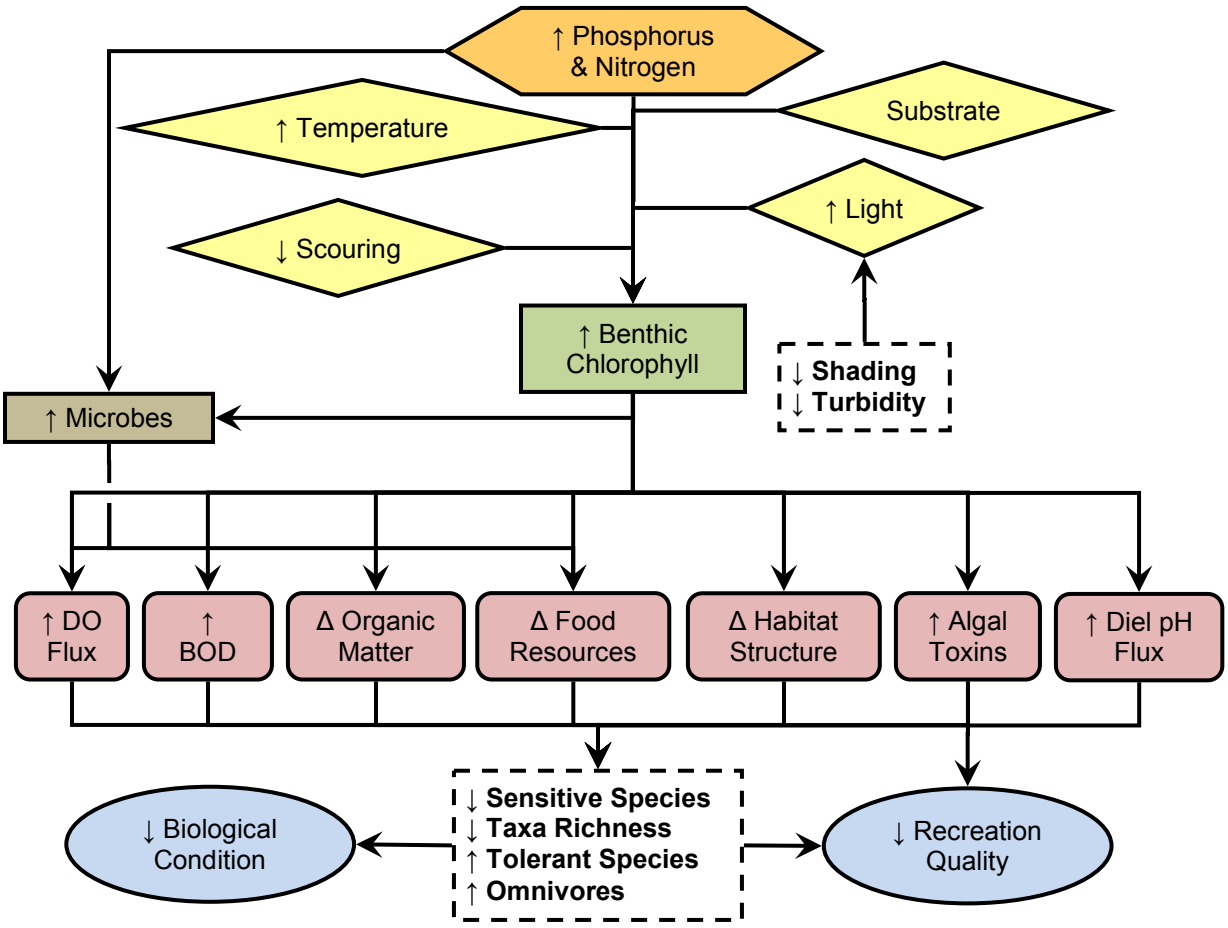


Figure 2. Conceptual model of the impact of nutrient enrichment on biological condition and recreational quality for small rivers.

Regardless of the size of river, once increased algal (sestonic or benthic) and microbial growth occurs, a number of stressors can adversely impact biological condition and recreation quality (Figure 1 and Figure 2). A common and severe stress resulting from nutrient enrichment is low levels of DO, which can reduce or eliminate populations of aquatic species that are not tolerant to low DO. However, DO levels are often more complicated as enrichment tends to also increase the flux in DO (USEPA 2000c). Enrichment increases the amount of primary productivity in a system, which can result in greater levels of DO during daylight hours when photosynthesis is occurring. Conversely, increased nutrients also increase respiration by microbes, plants, and animals due to greater biomass in the system, which has a greater biochemical oxygen demand. This reduction is exacerbated at night when photosynthesis is not occurring and respiration by plants, animals, and microbes is occurring (Hynes 1960). The increased diel range can cause very high DO during the day and very low levels of DO during the night (i.e., DO flux). In some cases, large diel fluctuations are not associated with daily minimums below goals may during measurement periods. Although short deployments of DO loggers (e.g., 1-2 weeks) may not measure unacceptable levels of DO, the large DO swings are probably an indicator that low DO conditions occur in the system at some point during the year. DO levels can become very low during high temperatures, low flow conditions, or during the fall when algae and other plants begin to senesce. Not only do low levels of DO cause stress, but the wide diel fluctuation in DO can also stress aquatic organisms. This may be due to the physiological stress resulting from swings in DO, but high levels of DO or supersaturation can also be a stressor by causing gas bubble disease in fish. In addition to causing direct stress to aquatic organism, low levels of DO can cause the release of toxic metals form sediments and increased availability of toxic substances such as ammonia and hydrogen sulfide (USEPA 2000c). High DO swings can also cause fluctuations in pH which could lead to increases in ammonium hydroxide or toxic metals (Brick & Moore 1996, USEPA 2000c).

Increased productivity of plants and microbes can also modify habitat and food resources (type, quantity, and quality) in aquatic systems (Carpenter *et al.* 1998, Smith *et al.* 2006, Evans-White *et al.* 2009). Food resources can be modified by a change in the plant community and increases in particulate organic matter. For example, there can

be increases in cyanobacteria (*i.e.,* blue green algae) which tend to be less palatable than green algae and diatoms (Carpenter *et al.* 1998). In fact, cyanobacteria can produce toxins which are harmful to aquatic life and can even be harmful to terrestrial organisms (*e.g.,* humans, dogs, waterfowl, and livestock; USEPA 2000c). Large blooms of phytoplankton, especially blue-greens, can be particularly large and problematic during periods of low flows (Dodds 2006). Thick growth of the green alga *Cladophora* is also associated with enrichment and it can change the structure of the biological community by creating a habitat favored by some taxa and by reducing the amount of other more edible food resources (Dodds & Gudder 1992). Eutrophication can result in a decline or increase in macrophytes which affects both food resources and habitat structure. For example, increases in sestonic algae can decrease water clarity causing reductions in macrophytes thereby impacting benthic organisms and organisms that depend on macrophytes (USEPA 2000c). This can cause a shift in the biological community to more generalist feeders. Nutrient enrichment can also have effects on detritus-based systems (*i.e.,* systems based on allochthonous carbon rather than autochthonous carbon) such as wooded streams. Enrichment can induce greater microbial activity and result in shifts in food resources, which can cause in changes to the biological community (Elwood *et al.* 1981, Bärlocher & Corkum 2003, Stelzer *et al.* 2003, Cross *et al.* 2005, Cross *et al.* 2006).

Increases in stressors (*e.g.,* low DO, algal toxins) and shifts in food resources and habitat can have a number of negative impacts on aquatic life and recreation. Excess nutrients usually result in a loss in species richness and diversity (Carpenter *et al.* 1998, Correll 1998). Reductions in DO or increases in DO flux can lead to a shift in a community to organisms less dependent on DO to those which utilize atmospheric oxygen. In general, this leads to a reduction or loss of stoneflies (Plecoptera), mayflies (Ephemeroptera), caddisflies (Trichoptera) and trout and other sensitive fish species. In turn there is often an increase in flies (Diptera), true bugs (Hemiptera), and beetles (Coleoptera) including a number of less desirable forms of aquatic life which can include aquatic worms (some Oligochaeta), fly larvae (*e.g.,* some Chironomidae [midges], Culicidae [mosquitoes], Psychodidae [moth flies]), snails, bullheads, and carp. Broadly these shifts can lead to losses of sensitive, carnivorous, and insectivorous species and an increase in tolerant and generalist (*e.g.,* omnivorous) species (Miltner & Rankin 1998). Low DO can also result in fish kills (Correll 1998). Impacts to food resources and habitats through nutrient loading can also cause shifts in aquatic communities, typically by increasing the proportion of generalist feeders. There is also a positive relationship between nutrient enrichment, bacterial growth, and macroinvertebrate mortality (Lemly 2000). This suggests that increased microbial production could increase infection and disease in fish and macroinvertebrates. Nutrient loading and large amounts of algae or macrophytes can also impair recreation quality in rivers (*e.g.,* swimming, water sports, and fishing; Dodds & Gudder 1992, USEPA 2000c). For example, fishing may be harmed by reduced/altered fisheries (*e.g.,* fish kills, reduced numbers of top carnivores) or by fouling of lines by heavy algal growth (Carpenter *et al.* 1998).

Recent studies and research from around North America (including Minnesota) document linkages between phosphorus and in-stream chlorophyll-a. Our previous studies extended this linkage to BOD_5 and further began to establish linkages with diel DO flux and biological metrics (Heiskary & Markus 2001, 2003). Based on the work at ten stream sites, diel DO flux (from submersible data recorders over a period of three to six days) was found to be positively correlated to summer-mean TP and seston chlorophyll-a; however, no correlation was found relative to periphyton chlorophyll-a or other periphyton-related metrics. It is important to note that DO values were consistently above water quality standards (>5 mg/L) at 11 of 12 sites in the 2000 study and the lowest recorded DO was 4.5 mg/L (Heiskary & Markus 2003). DO flux exhibited inverse relationships with various biotic metrics. For example, inverse relationships were found between fish Index of Biotic Integrity (IBI) scores and the water quality variables TP, nitrate-N, chlorophyll-a, and diel DO flux based on data for ten sites. Macroinvertebrate index values (EPT) from six sites exhibited similar relationships in most cases. The current report builds upon the results of these previous studies by incorporating new data and applying new analyses to determine relationships between nutrients, water chemistry, and the biological constituents in Minnesota rivers. The results of this research are to identify concentrations of nutrients that will protect Minnesota's aquatic life and recreation uses.

Differences have been documented in the background nutrient concentrations between regions in the United States (Rohm *et al.* 2002, Smith *et al.* 2003, Wickham *et al.* 2005). Therefore it is sensible to expect that different nutrient concentrations would be needed as part of criteria to protect aquatic life use goals while minimizing the adoption overly or under protective criteria. The focus on the development of nutrient concentrations relevant to the protection of aquatic life is in line with current efforts to develop tiered aquatic life uses (TALUs) in Minnesota. Development of biological criteria and TALUs requires that natural differences in the biological, chemical, and physical attributes of different stream types be considered when developing water quality standards. Regionalization of nutrient criteria is one way to develop standards that are appropriate to the streams and the biological communities they are meant to protect. In addition, the development of different use tiers will create various levels of protection, which depend on what is attainable in these systems. This will likely include tiers for

exceptional rivers and modified rivers (*e.g.,* channelized streams). Exceptional streams are those that have demonstrated an ability to be much better than the standard and are therefore afforded additional protection to maintain this status. In contrast, modified streams could include waterbodies such as ditches that do not have the ability to meet biological standards due to the nature of these systems. In the case of modified use streams, an attainable standard would therefore be applied. As a result of different biological criteria for these tiers, in the future it may be necessary to develop more or less stringent nutrient criteria to protect exceptional and modified use rivers, respectively.

II. STUDY DESIGN OVERVIEW

The MPCA utilized a multiple lines of evidence approach, also referred to as a "weight of evidence" approach (USEPA 2000c), to develop river nutrient criteria that are protective of Minnesota's aquatic life and recreation goals. Early in this process it was determined that two different approaches would need to be used for the two main pathways of indirect stress from nutrient enrichment: sestonic algae versus benthic algae. To develop criteria to protect aquatic life from excessive benthic algal growth, a translator to the existing narrative criteria was developed using a literature-based analysis. The approach used for the development of criteria that would largely address impacts resulting through excessive sestonic algal growth in nonwadeable rivers was deployed in a hierarchical fashion. This approach first identified relationships between nutrients and biological condition and then used a variety of statistical methods to move from broad to narrow ranges of nutrient concentrations that will be protective of aquatic life. The first analysis used correlation to identify relationships between variables recognized as important in factors or pathways that lead to the loss of biological condition through enrichment. Relationships among correlated variables were further assessed using regression analyses (*e.g.,* phosphorus, chlorophyll, BOD_5) in order to empirically support relationships within the conceptual models (Figures 1 and 2). With the relationships between nutrients and other water quality variables established, an examination of their impact on biological condition could be undertaken. By examining scatter plots, preliminary thresholds could be identified which corresponded to levels of nutrients or other stressors causing in an undesirable decline in biological condition. To further refine the ranges of nutrient concentrations protective of biological condition, two relatively novel methods of statistical analysis were employed: quantile regression and changepoint (regression tree) analysis. These analyses were used to identify nutrient concentrations that corresponded to a biological response threshold that was protective of biological goals. In recognition of distinct regional patterns in stream water quality thresholds were assessed in a regional context. An extensive literature review was also undertaken to identify nutrient thresholds, which have been developed by other states and agencies to compare the nutrient criteria work in Minnesota to other similar regions.

Several studies, often funded by USEPA nutrient criteria grants, were conducted from 1999-2008. The overall purpose of the studies was to generate data (chemical, physical, and biological) that could contribute to our understanding of nutrient impacts in medium to large rivers in Minnesota. In turn, this data combined with existing data from other monitoring programs (e.g., biological monitoring), STORET data, and information from the literature would be used to develop river eutrophication criteria for Minnesota. For this purpose, four somewhat distinct studies were developed and carried out from 1999-2008. The studies did not use a statistically-based design, rather medium to large rivers, representative of the various ecoregion were included. Various considerations were used in site selection, among these were coordination with ongoing biological sample collection, presence of nearby USGS flow gauging stations, and site accessibility to name a few [note - study budgets did not allow for independent biological collections, hence coordination with fish and macroinvertebrate biomonitoring was essential.]. Descriptions of sites included in these studies are found in Tables 2-4. The methods section will provide further description; however, specific details on site selection and study focus may be found in the specific reports for each study (Heiskary & Markus 2001, 2003, Heiskary 2008).

Table 2. River Nutrient study sites for 1999-2006. Site ID numbers and study years noted.

Basin / River	Report ID	MPCA site ID	MPCA Bio site ID	STORET S code	Contributing Ecoregions	Study Year(s)
Rainy						
Big Fork	BF-46	BF-Site 46	05RN081	S002-856	NLF/ NMW	2006
Little Fork	LF-21	LF-21	05RN086	S002-556	NLF/NMW	2006
Red						
Red	RE-536	RE-536	-		RRV / NGP/ CHF	2000
Red	RE-452	RE-452	-		RRV / NGP/ CHF	2000
Red	RE-403	RE-403	-		RRV / NMW / CHF	2000
Red	RE-298	RE-298	-		RRV / NMW /CHF	2000
Red Lake	RL-1	1st St. Bridge	05RD121	S002-076	RRV / NMW	2006
Red Lake	RL-75	75	05RD129	S002-077	RRV / NMW	2006
Wild Rice	WR-1	WR-1	05RD112	S002-102	RRV / CHF	2006
Wild Rice	WR-200	RD200	05RD115	S001-155	RRV / CHF	2006
Buffalo	BUFF-10	HAWUS1	05RD110	S003-154	RRV / CHF	2006
Buffalo	BUFF-01	Buff001	05RD120	S002-708	RRV / CHF	2006
Otter Tail	OT-1	OT-1	05RD109	S000-006	RRV / CHF	2006
Minnesota						
Blue Earth	BE-100		00MN001		WCP	2000
Blue Earth	BE-94		00MN005		WCP	1999, 2000
Blue Earth	BE-73		00MN004		WCP	1999, 2000
Blue Earth	BE-54		00MN003		WCP	1999, 2000, 2001
Blue Earth	BE-18		00MN002		WCP	1999, 2000
Upper Miss.						
Crow Wing	CWR-72		00UM026		NLF	1999, 2000, 2001
Crow Wing	CWR-35		00UM024		NLF/CHF	1999, 2000
Mississippi	UM-1056		00UM087		NLF	2000
Mississippi	UM-1029				NLF	2000
Mississippi	UM-1004				NLF	1999
Mississippi	UM-965				NLF / CHF	1999
Mississippi	UM-953		00UM091		CHF / NLF	1999, 2000
Mississippi	UM-895		00UM092		CHF / NLF	1999, 2000
Mississippi	UM-872	UM-872	00UM098	S000-025	CHF	1999, 2000, 2001, 2006
Rum	RUM-18	RUM-18	00UM066	S000-066	CHF	1999, 2000, 2001, 2006
Rum	RUM-34		00UM044		CHF	1999, 2000
Crow	CR-0.2					1999, 2000
Crow	CR-23	CR-23	00UM080	S000-050	CHF	1999, 2000, 2006
North Fork	CRN-2.33	CRN-2.33		S001-256	CHF	1999, 2000, 2001, 2006
South Fork	CR-44	CR-44	99UM010	S000-165	WCP	2001, 2006

Table 3. Watershed area and flow characteristics for River Nutrient study 1999-2006 sites. Area based on sampling site. Nearest gauge to sampling site used to characterize flow. Flow statistics derived from USGS records.

Basin / River	Report ID	Water-shed km^2	Width[3] m	Depth m	Strahler Stream Order	Annual Mean Flow cfs	Summer Mean Flow cfs	USGS Site ID
Rainy								
Big Fork	BF-46	3,833	50	0.7	4	734	703	5132000
Little Fork	LF-21	4,351	32	0.8	5	1,051	1,010	5131500
Red								
Red	RE-536	10,490	--	1.3		678	671	5051522
Red	RE-452	17,612	--	1.5	6	936	689	5054000
Red	RE-403	56,462	--	2.7	7	2,122	1,978	
Red	RE-298	65,916	--	3.1	7	4,047	2,779	5082500
Red Lake [1]	RL-1	8,936			5			5066500
Red Lake	RL-75	5,957			5	1,214	1,245	5075000
Wild Rice	WR-1	4,217	18	0.4	5	345	339	5064000
Wild Rice	WR-200	2,419			5	210	222	5062500
Buffalo	BUFF-10	842	13	0.6	4	85	84	5061000
Buffalo [2]	BUFF-01	2,525	15	0.5	5	158	152	5062000
Otter Tail	OT-1	4,698			4	370	412	5046000
Minnesota								
Blue Earth	BE-100	811	20	0.6	3			
Blue Earth	BE-94	2,082	30	0.7	4			
Blue Earth	BE-73	3,541	40	0.6	4			
Blue Earth	BE-54	3,603	46	--	4			
Blue Earth	BE-18	3,955	48	1.5	4	1,070	1,183	532000
Mississippi								
Crow Wing	CWR-72	2,668	40	1.0	4			5244000
Crow Wing	CWR-35	5,517	65	1.0	5	484	440	5247500
Mississippi	UM-1056	15,242	35	2.2	5	2,966	2,860	5227500
Mississippi	UM-1029	15,768	35	--	5			
Mississippi	UM-1004	18,959	75	2.3	5	3,673	3,461	5243000
Mississippi	UM-965	30,044			6			
Mississippi	UM-953	34,076	130	2.6	6	4,767	4,662	5267000
Mississippi	UM-872	44,289	300	3.3	7	8,274	8,225	5288500
Rum	RUM-34	3,294	55	--				
Rum	RUM-18	3,546	60	1.1	5	610	624	5286000
Crow	CR-23	6,527	1.3	2.3	6	803	888	5280000
Crow	CR-0.2		--	--	6			
North Fork	CRN-2.33	3,828			5			
South Fork	CR-44	3,307			5			5279000

1. gauge @ Crookston; 2. gauge @ Orwell Dam; 3. mean wetted cross-section

Table 4. River Nutrient study sites for 2008.

Basin/ River	Bio Field #	STORET Site #	Width m	Depth m	Wshed Area km^2	Strahler Stream order	Annual Mean Flow cfs	Summer Mean Flow cfs	USGS site ID (nearest)
Lower Miss.									
N. Branch Root	08LM012	S004-825	>14.5	1.0	598	4			
S. Branch Root	08LM002	S004-829	>14.5	1.0	471	4			
Bear Creek	08LM014	S004-827	>14.5	0.8	254	4			
Wells Creek	08LM127	S001-384	13.0	0.6		4			
Vermillion River	08LM114	S000-896	7.5	1.0		4			5345000
Minnesota									
Maple River	08MN003	S002-427	~18.0	0.3	880	5			
Rice Creek	08MN004	S002-431	9.0	0.7	210	4			
Le Sueur River	08MN035	S003-860	14.0	0.8	1,162	4	70	63	5315000
Big Cobb	08MN005	S003-446	~19.0	0.7	793	4			
Upper Miss.									
Getchell Creek	00UM039	S003-289	8.0	0.5	117	3			
Sauk River	08UM025	S000-284	>14.0	0.8	1,557	5			5270500

III. METHODS OF DATA COLLECTION AND ANALYSIS

A. River Nutrient Study Data Collection

1. River Nutrient Study: Overview of Data

The River Nutrient study refers to a series of projects that the MPCA initiated to understand the relationships among eutrophication-related water quality parameters and biological measures to support the development of eutrophication standards for Minnesota streams. Field and laboratory data were stored in STORET (STOrage and RETrieval), USEPA's national water quality data bank, according to MPCA protocol.

The initial study of 1999-2000 laid the foundation for much of what was to follow. In this study, several medium to large rivers distributed across three ecoregions (Table 2) were sampled to help establish relationships among nutrients, phytoplankton abundance, and biochemical oxygen demand (BOD_5). In 2000, USGS was contracted to conduct periphyton collections and make diel water quality measurements with submersible data recorders on 12 of the sites. As a further complement to these efforts MPCA biological staff conducted macroinvertebrate and fish studies at 10 of 12 sites. Relationships were established and related insights were obtained, which are more fully described in Heiskary and Markus (Heiskary & Markus 2001, 2003). In summer 2001, approximately 21 stream sites across several basins were sampled for the standard suite of water quality variables. These data were used primarily to corroborate the various water chemistry and algal interrelationships developed based on the 1999 and 2000 study (Heiskary and Markus 2003). Since that time, we enhanced these findings with additional data collection and analysis of some pre-existing data–both of which contribute to the identification of quantifiable thresholds of impairment for rivers. The 2006 and 2008 studies build directly on previous efforts that took place from 1999-2001. In 2006, 14 stream sites in the Red, Rainy and Upper Mississippi basins were sampled six - seven times between late June through mid-September, and had water quality sondes deployed for a two week period in late July and early August (Tables 2 and 3). Whenever possible, water quality sites were paired with sites where recent biological data were available or where collections were to be made. This allowed the pairing of water chemistry and diel DO flux data with fish and macroinvertebrate data. This expanded our analysis of relationships, which were originally developed in the 1999-2000 study and described in more detail in Heiskary (2008).

In the 1999-2000 study, Red River main-stem sites exhibited little or no relationship among TP, chlorophyll-a or BOD_5 (in contrast to the other rivers in that study). This lack of a relationship was likely because of light limitation that results from very high TSS concentrations in the Red River. In the 2006 study, representative Red River

tributaries were included in order to determine their response to nutrients (in terms of chlorophyll-a, diel DO flux, and fish and macroinvertebrate metrics) and expand the geographic scope of sample sites. As with previous site selections, rivers where biological collections were made, presence of a USGS gauge on the river and stream order were all important considerations. Many of the Minnesota Red River tributaries drain from either the Northern Minnesota Wetlands (NMW) or North Central Hardwoods Forests (CHF) ecoregions toward the more nutrient and sediment rich Red River Valley (RRV; also referred to as the Lake Agassiz Plain - LAP) ecoregion. Two sites (upstream and downstream) were monitored on three of the tributaries, which exhibited an ecoregion transition (e.g., Red Lake River; Figure 5). This allowed us to determine if there was a change in response to nutrients as a river transitions from a nutrient-poor to a more nutrient-and sediment-rich watershed, where light limitation may be an issue (as is the case with the main stem of the Red River). Three Upper Mississippi Basin sites were included in the 1999-2001 monitoring efforts. By repeating measurements (chemistry and diel DO flux) at these sites, insight is gained as to how variable concentrations and interrelationships among nutrients, chlorophyll-a, and DO flux may be for a given river (site) across various summers. For example, given similar TP and chlorophyll-a in these rivers can we anticipate a similar magnitude of DO flux at these sites? Since these sites are located near USGS flow gauging locations, the role of differing flow regimes among years could be taken into account.

A second facet of the 2006 study allowed for deployment of an automated sensor in the Little Cobb River for a five-month period by USGS. This site has extensive water chemistry and biological data collection and serves to complement the other river sites in this study and provides a basis for assessing longer-term DO flux as a function of nutrients, algae, and flow. Results from that work and sonde deployment at the 2006 study sites are summarized in Lee (2008a). A third facet of this study allowed for a retrospective analysis of a USGS NAWQA data set. This data set includes water quality data collected monthly over the growing season from thirteen gauged stream sites in the Upper Mississippi River basin. Biological data, including phytoplankton, macroinvertebrates, and fish community composition, are available for each site as well. The data set was assembled and preliminary statistical analyses completed to explore relations between nutrient concentrations and biological response variables in this independent data set. Regression analyses were used to determine relations between nutrient concentrations and biological response variables (Lee 2008b).

In 2008 data sets were expanded by taking advantage of previously scheduled biological monitoring and water chemistry monitoring. This provided data for five new sites in the Lower Mississippi Basin, which was not represented in the previous studies, three sites in the Minnesota Basin, and two sites in the Upper Mississippi Basin (Table 2). In addition, field crews were able to instrument these sites for continuous measurement of DO, temperature, pH, and conductivity for approximately two weeks in August 2008.

In summary, data from 2006 and 2008 are combined with data from the previous studies (1999, 2000, and 2001) to allow for an expanded data set to evaluate interrelationships among nutrients, chlorophyll-a, DO flux and biological metrics in a cross-section of medium to high order Minnesota rivers. Data collected at these sites (Tables 2 and 4) are referred to as the "River Nutrient" data set. In addition we were able to integrate a portion of the USGS retrospective data set with the MPCA data sets, described herein, to yield a more robust data set that should enhance our ability to identify thresholds that should aid in the development of river nutrient criteria for Minnesota.

In addition to data collected as part of the "River Nutrient" study, extensive datasets collected by the MPCA biomonitoring units were available to further assess the relationships between nutrients and the biological communities (*i.e.,* fish and macroinvertebrates). This data included chemical, habitat, and biological data sampled from hundreds of river sites across Minnesota. This larger dataset allows examination of relationships between nutrient and biological communities on a regional basis and permits a more robust determination of nutrient concentration levels necessary to protect aquatic life and recreation uses.

2. River Nutrient Study: Study Area and Ecoregion Descriptions

The initial river nutrient study in 1999 and 2000 focused on medium to large rivers that are typical of several Minnesota ecoregions (Heiskary & Markus 2003). Between-region differences in land use, soil characteristics, and geomorphology influence water runoff, nutrient loading, and processing of nutrients in rivers (USEPA 2000c). For lakes, the MPCA clearly established regional differences in lake water quality, morphometry, lake user perceptions, and pre-European phosphorus concentrations (Heiskary & Wilson 2008). These differences allowed for the development of ecoregion-based lake eutrophication standards as defined in the 2008 rulemaking effort (Minn. Rule 7050 2008). The MPCA has previously described ecoregion-based differences in stream water quality based on

representative, minimally-impacted streams (McCollor & Heiskary 1993). This study demonstrated distinct regional differences in stream water quality based on representative streams that were not influenced by an upstream point source discharge. A summary of values from that study (Table I - 1) will be used as one basis for comparison to data and proposed thresholds from the current study. Likewise, USEPA has compiled distributions of water quality variables by ecoregion as a part of their "Ambient Water Quality Criteria Recommendations" that were compiled for the various nutrient ecoregions (*e.g.,* USEPA 2000b) and results are summarized in Table I - 2. Smith et al. (2003) further re-affirm regional patterns in their work where they estimate natural background concentrations of nutrients in U.S. streams and rivers. In this work they estimate background TP for the 14 aggregated nutrient ecoregions that characterize the conterminous U.S. Median background TP for the three regions that characterize Minnesota: Glaciated Upper Midwest and NE, Mostly Glaciated Dairy Region, and Corn Belt and Northern Great Plains Region are as follows: 15, 25 and 55 µg/L. Lastly, a more recent assessment of stream phosphorus data from STORET further reinforces the regional patterns (Figure I - 1).

The rivers included in the 2006 study (Table 2, Figures 3-6) drain one or more of the following Level III ecoregions: Northern Lakes and Forests (NLF), Northern Minnesota Wetlands (NMW), North Central Hardwoods Forest (NCHF), or Red River Valley (RRV). These ecoregions correspond to three aggregated Level III (Omernik 1987) Nutrient ecoregions (VIII, VII, and VI, respectively) that characterize Minnesota and much of the Upper Midwest. These sites were selected to complement the previously sampled sites (Table 2, Figures 3-6) and allow for improved coverage in the RRV, NMW, and NLF ecoregions in particular. Watershed areas range from less than 325 mi^2 (842 km^2-Buffalo River at Hawley) to 3,450 mi^2 (8,936 km^2-Red Lake River at Thief River Falls) (Table 3). Most sites drain 1,000 mi^2 (2,590 km^2) or more. Three sites from the previous studies - Crow, Rum, and Mississippi - were included to allow us to assess variability in nutrients, chlorophyll-a, and diel DO fluctuation across a range of flows and among years. Selecting rivers (sites) from each ecoregion and of varying watershed area allowed us to capture a range of responses. In several instances, two sites were sampled on each river to allow upstream and downstream comparisons of nutrients, chlorophyll-a, and diel DO flux. Whenever possible, sites were located at, or near, USGS stream gauge locations. This allowed for accurate estimates of flow for each sample date, with a minimum of one USGS gauge per river (Table 3).

In 2008, 11 sites scheduled for biological collections, had additional water chemistry samples collected and were instrumented for diel monitoring. This provided some coverage in a basin (Lower Mississippi) and an ecoregion (Driftless Area; DA) that was not included in previous efforts (Table 4). It also provided additional coverage in the Minnesota Basin. Many of the 2008 sites had smaller watersheds and were shallower (Table 4) than the previous study sites (Table 3). Also, four of the sites: Bear Creek, Wells Creek, Vermillion River, South Branch Root - are classified as coldwater streams (Scott Niemela, MPCA, personal communication); whereas all previous sites were classified as warmwater streams. North Branch Root, which has some coldwater designated tributaries, is classified as a warmwater stream (John Sandberg, MPCA, personal communication).

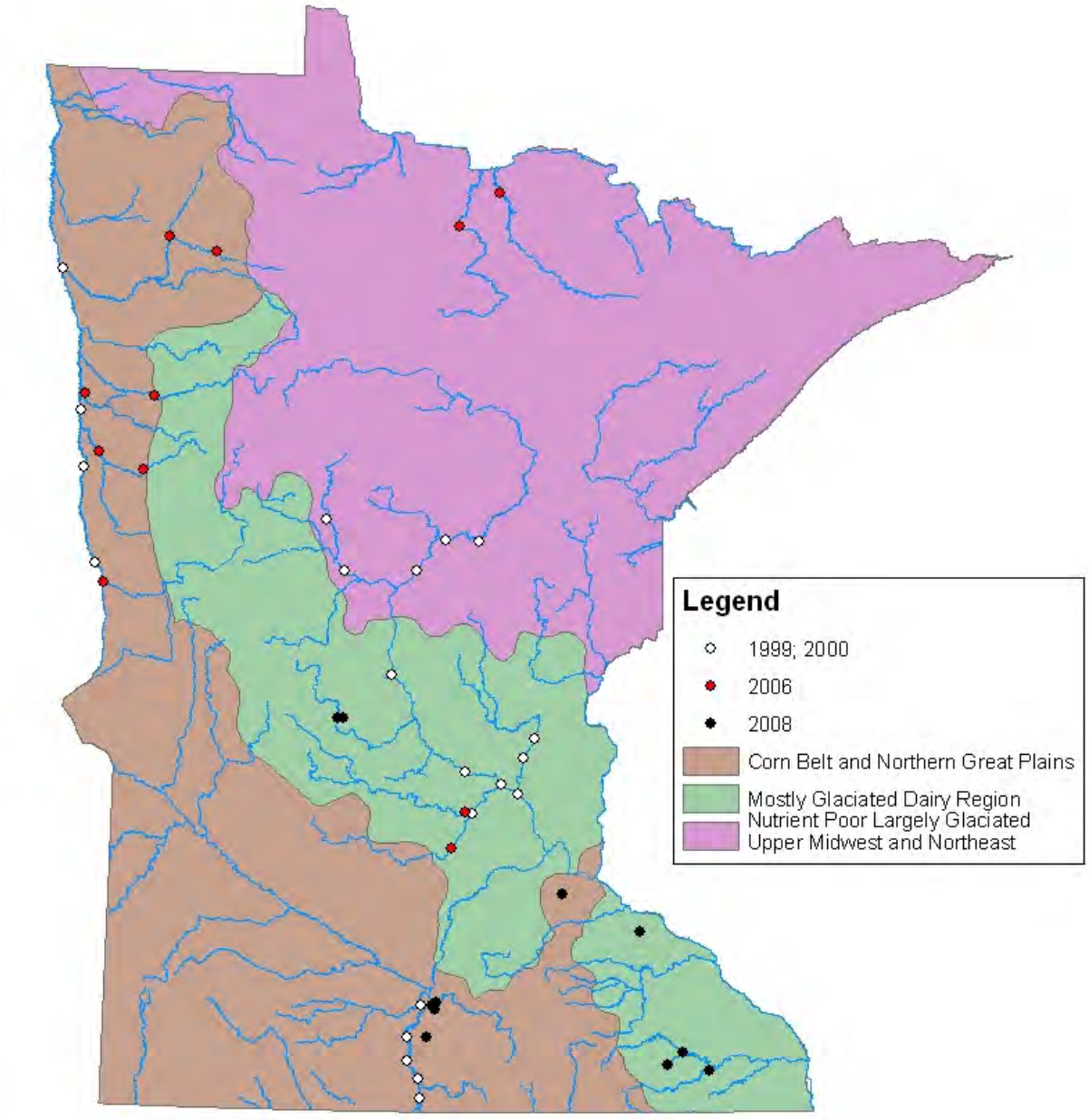
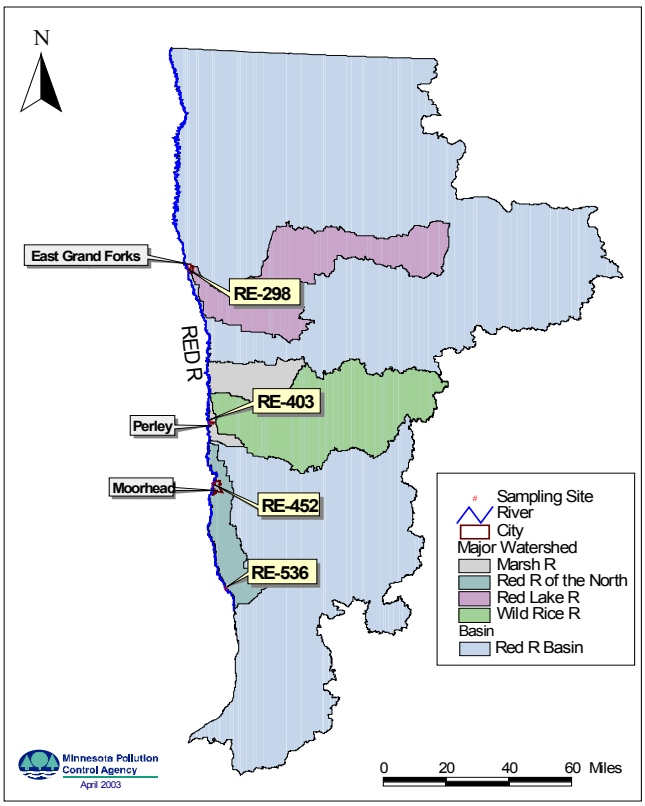
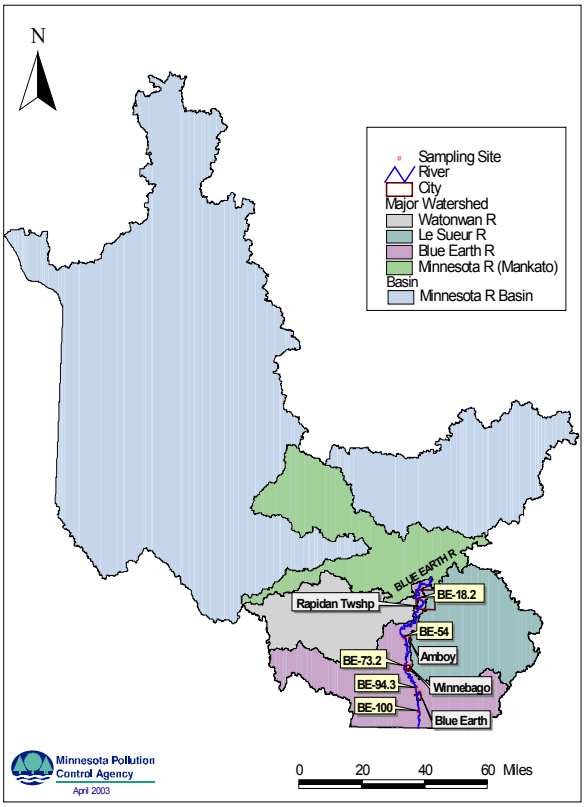


Figure 3. Location of 1999, 2000, 2006 and 2008 river nutrient study sites overlain on USEPA level 3 ecoregions. List of sites noted on Tables 2 and 3. Basin-scale maps of 1999 and 2000 sites (Upper Mississippi, Minnesota, and Red Rivers) follows on next page (from Heiskary and Markus 2003).


N
East Grand Forks
RE-298
RED R
RE-403
Perley
Moorhead
RE-452
RE-536
Sampling Site
River
City
Major Watershed
Marsh R
Red R of the North
Red Lake R
Wild Rice R
Basin
Red R Basin
Minnesota Pollution Control Agency
April 2003
0 20 40 60 Miles


N
Sampling Site
River
City
Major Watershed
Watonwan R
Le Sueur R
Blue Earth R
Minnesota R (Mankato)
Basin
Minnesota R Basin
BLUE EARTH R
Rapidan Twshp
BE-18.2
BE-54
Amboy
BE-73.2
BE-94.3
Winnebago
BE-100
Blue Earth
Minnesota Pollution Control Agency
April 2003
0 20 40 60 Miles

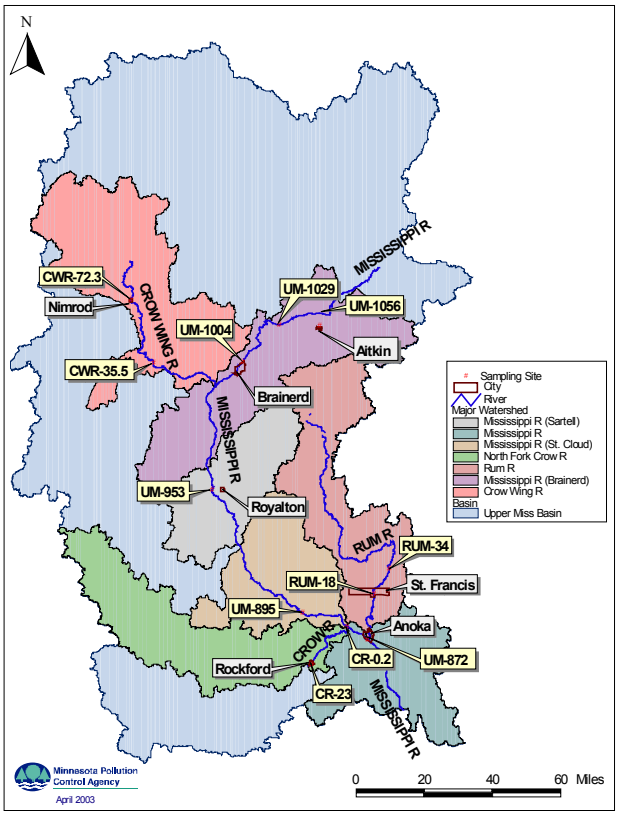

N
MISSISSIPPI R
CWR-72.3
Nimrod
CROW WING R
UM-1029
UM-1056
UM-1004
Aitkin
CWR-35.5
Brainerd
MISSISSIPPI R
UM-953
Royalton
RUM R
RUM-34
RUM-18
St. Francis
UM-895
Anoka
CROW R
CR-0.2
UM-872
Rockford
CR-23
MISSISSIPPI R
Sampling Site
City
River
Major Watershed
Mississippi R (Sartell)
Mississippi R
Mississippi R (St. Cloud)
North Fork Crow R
Rum R
Mississippi R (Brainerd)
Crow Wing R
Basin
Upper Miss Basin
Minnesota Pollution Control Agency
April 2003
0 20 40 60 Miles

Figure 4. Upper Mississippi River (UMR) Basin with 8 digit HUCs overlain on USEPA Level 4 ecoregions. Pictures of representative river nutrient study sites (top to bottom): Crow (CR-23), Rum (RUM-18), and Mississippi (UM-895). Complete list of sites in UMR Basin in Table 2.

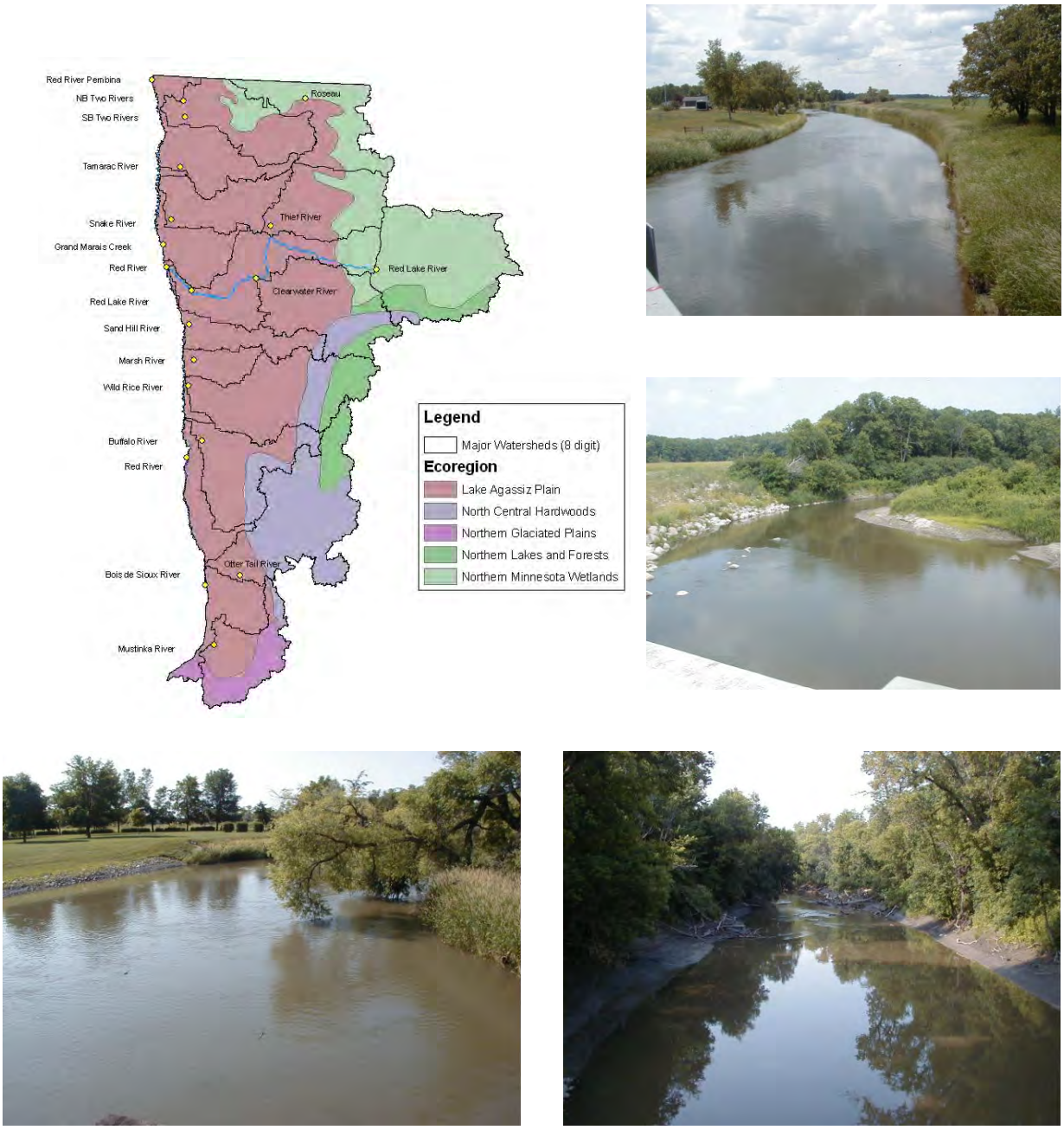


Figure 5. Red River Basin with 8 digit HUCs overlain on USEPA Level 4 ecoregions. Pictures of representative river nutrient study sites (top to bottom clockwise): Red Lake (RL-75), Wild Rice (WR-200), Buffalo (BUFF-01), Otter Tail (OT-1), and Red River at Moorhead (RE-452). Complete list of sites in this basin noted in Table 2.

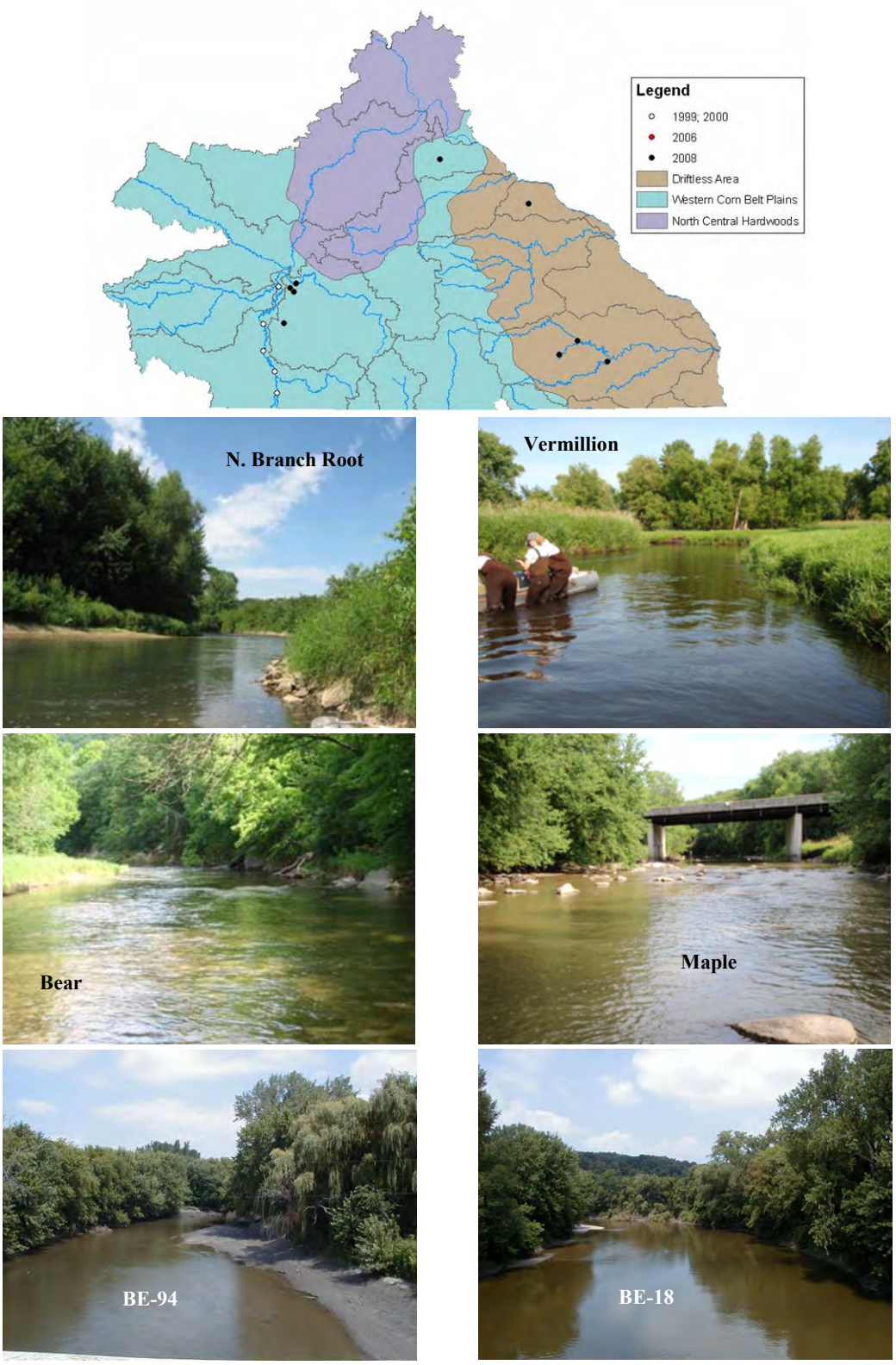


Figure 6. Lower Mississippi (LM) and Minnesota (MN) River Basin sites and level 4 ecoregions noted. Pictures of sites from Blue Earth and Maple in MN Basin and Vermillion, Root and Bear in LM Basin. Complete listing of river nutrient study sites in these basins in Tables 2 and 3.

3. River Nutrient Study: Morphometry, Watershed Area, and Flow Characterization

The watershed area above the site was based on existing MPCA and the USGS data. Stream order (after Strahler 1957) was estimated in the original studies (Heiskary & Markus 2001, 2003) based on 1:190,000 scale quadrangle maps in the Minnesota Atlas and Gazetteer (DeLorme 1994). The Blue Earth River (BE-100) near the Iowa border had the smallest watershed in the 1999 study (811 km^2) and was deemed a third-order stream. The remaining sites on the Blue Earth, Crow Wing at CWR-72, and Rum were deemed fourth-order. The Crow Wing at CWR-35, Crow River, and Mississippi River at UM-1004 to UM-1056 were deemed fifth-order. The remaining sites on the Mississippi (downstream of UM-1004) were deemed sixth order. Red River sites were sixth or seventh- order (Table 3). In the 2006 and 2008 studies, stream order was determined based on information supplied by USEPA Corvallis (personal communication Tony Olsen to Joel Chirhart, 2008). Based on this information, most 2006 sites are considered 5^{th} order, with the exception of the Big Fork, Buffalo (Buff-10), and Otter Tail (OT-1) that are considered 4^{th} order (Table 3). The majority of the 2008 sites were 4^{th} order with the exception of Maple and Sauk (5^{th}) and Getchell (3^{rd}) (Table 4).

4. River Nutrient Study: Instrumented Diel Monitoring

Three separate, but related, diel monitoring studies measured diel fluctuation of DO, temperature, pH, and specific conductivity at select river nutrient study sites. In two of the previously described studies (2000 and 2006), YSI sondes were deployed by USGS staff. The techniques employed in 2006 were quite comparable to those used in the 2000 study with the exception of a longer measurement period (12-15 days vs. 5-8 days in 2000). Details on methods and results are included in Heiskary and Markus (2003), Lee (2002, 2008a), and Heiskary (2008). Similar techniques were employed in 2008; however, sonde deployment was made by MPCA staff and deployments were typically 4-9 days.

Sonde deployment and maintenance. Photos courtesy of USGS

5. River Nutrient Study: Water Quality Sample Collection

Water samples were collected at mid-channel on five to eight occasions from late-June to mid–September during most of the study years. Samples were collected from bridges at each site by means of a bucket on a rope. The bucket was rinsed twice with ambient water prior to sample collection. For quality assurance purposes, duplicate samples were collected on about ten percent of the visits. Though, the collection method was consistent among sites and over the study period we cannot assume that the mid-channel collections are well-mixed and water quality (chemistry) may vary across the stream cross-section (Lee, personal communication).

Nutrient samples were acidified upon collection with H_2SO_4. Chlorophyll-a samples were field filtered on the day of collection and the volume filtered was noted. The filter was folded and placed in a petri-dish and wrapped in foil. Samples were frozen prior to shipment to laboratory and analyzed for chlorophyll-a and pheophytin-a. Reference to chlorophyll-a (Chl-a) in this report implies Chl-a corrected for pheophytin, while reference to total chlorophyll (Chl-T) implies Chl-a plus pheophytin. Based on a comparison of summer-means from 31 river-sites in 1999 and 2000 Chl-a accounted for 71% of Chl-T on average and as expected, the values are highly correlated (R^2=0.99, N=31). Other samples such as total suspended solids and BOD_5 were not preserved, but chilled to 4ºC prior to shipment to the laboratory.

All water chemistry samples were analyzed at Minnesota Department of Health (MDH). Precision estimates were derived from the analysis of ten duplicate samples taken during our study (Table 5). The mean and percentage difference for these duplicates samples were equivalent to or better than routinely reported results of MDH laboratory duplicates.

DO, pH, temperature, and conductivity were measured in the field with a multi-parameter probe during collection of water samples. Most transparency tube measurements were made with a 60 cm long, 3.8 cm diameter clear plastic tube, with the exception of some very clear streams where a 100 cm tube was used. A well-mixed sample was poured into the tube. While looking down into the tube, water was released from a valve at the bottom until the black and white (Secchi) symbol at the bottom of the tube was visible. The depth of the water when the symbol becomes visible was recorded. Typically, two separate readings are averaged to yield the recorded measurement.

Table 5. Laboratory methods and precision estimates for Minnesota river-nutrient study.

Parameter	Reporting Limit and Units	EPA method number	Precision: [1] mean difference	Diff. as percent of observed
Total Phosphorus	10.0 µg/L	365.2	4.8 µg/L	2.7%
Total Kjeldahl N	0.1 mg/L	351.2	0.05 mg/L	2.8%
$NO_2 + NO_3$	0.01 mg/L	353.1		--
Total Suspended Solids	0.5 mg/L	160.2	2.8 mg/L	9.6%
Total Suspended Volatile Solids	0.5 mg/L	160.4	--	--
Turbidity	0.2 NTU	180.1	--	--
BOD_5	0.5 mg/L	405.1	0.15 mg/L	6.6%
Chlorophyll-a	0.16 µg/L	446.0	1.70 µg/L	7.4%
Pheophytin	0.27 µg/L	446.0	--	--

[1] Average of individual means of 10 duplicates and expressed as a % of measured concentrations.

6. River Nutrient Study: Benthic Algae Collection [drawn from Lee 2002]

During the 2000 study, benthic algae (periphyton) were collected from each site during the period of diel water-quality measurements. Site conditions were characterized at the time of benthic algae sample collection (Lee 2002). Benthic algae were collected from both wood (epidendric) and rock (epilithic) substrate at each site and processed separately. Benthic algae samples were collected in accordance with the USGS National Water Quality-Assessment Program (NAWQA) algal sampling protocols (Moulton *et al.* 2002).

Epidendric samples were collected from submerged woody debris that was in the euphotic zone of the stream. Epidendric samples were collected from 10 locations in each stream reach. Snags were gently removed from the water to minimize disturbance of the algal community; a 3–4 inch cylindrical section was cut from each snag with

lopping shears; and the snag sections were retained in a plastic bag prior to processing. After algae were removed from the snag sections, the length and diameter of each section was measured, and the surface area of each snag segment was calculated.

Epilithic samples were collected from submerged rocks located in the euphotic zone. Approximately 10 different rocks, which were carefully removed and placed in a container with benthic algal growth facing up. After algae were removed from each rock, a foil template was created to cover the section of the rock covered with algae. This foil template was retained and measured to determine surface area.

Table 6. USGS Region V 2007 wadeable river study sites for Minnesota.

			Water		Substrate			Channel	
Site	Stream name	Date of site visit	Clarity	Color	Dominant	Secondary	Stream discharge (cfs)	Mid depth (ft)	Wetted width (ft)
MN-1	Clearwater River	9/19/2007	Clear	Clear	Gravel	Sand	3.0	1.3	22
MN-2	Rum River	9/21/2007	Clear	Clear	Sand	Cobble	3.5	0.5	34
MN-3	Shell River	9/19/2007	Clear	Clear	Sand	Gravel	5.6	0.6	30
MN-4	West Fork Beaver Creek	9/20/2007	Slightly turbid	Brown	Sand	Gravel	5.3	0.1	22
MN-5	South Branch Rush River	9/20/2007	Clear	Clear	Sand	Cobble	6.4	0.9	12
MN-6	Little Cobb River	9/20/2007	Slightly turbid	Brown	Gravel	Sand	7.2	1.1	38

Samples were processed similarly as described below. Algae were removed from each snag section or rock using a stiff-bristled brush and de-ionized water from a rinse bottle. The algal suspension from each sample (epilithic and epidendric samples were processed separately) was washed into a small, plastic processing pan. Samples were processed until about 50 to 100 mL of water had accumulated in the processing pan. The combined algal-water suspension was homogenized for approximately 30 seconds. The homogenate was split into subsamples for determinations of chlorophyll-*a* (5 mL), and identification (60 mL). The homogenate from one sample (Mississippi River near Anoka, Minnesota) was split into three portions to determine variability in algal samples.

USGS collected periphyton, water chemistry, and made diel DO measurements at six wadeable river sites in Minnesota as part of a multi-state Region V study that was conducted in 2007. Water chemistry samples were collected three times during the summer (June, July, and September). Site characteristics are summarized in Table 6. Unless otherwise noted we assume sample techniques were consistent with the USGS method noted above. A map of the sites and summary water quality data are provided in Figure III - 2.

B. Biological Monitoring Dataset

Since the 1990s, the MPCA has supported a robust biological monitoring program which is focused on two assemblages: fish and macroinvertebrates. This biological data is collected to support a number of MPCA activities, but it is primarily used to assess attainment of aquatic life use goals. Biological communities are integral to this activity as they integrate the impacts of multiple stressors over time and provide a direct measure of those goals. A major impact of eutrophication is the degradation of biological communities so biological data were an important element of the process to develop eutrophication standards. Biological monitoring resulted in the calculation of fish community metrics, macroinvertebrate metrics, and habitat assessments for each site.

1. Habitat Assessment and Chemistry Sampling

A habitat assessment was performed at each site to characterize the in-stream and riparian features of the stream. In wadeable streams, a modified version of Wisconsin's quantitative habitat assessment procedure (Simonson *et al.* 1994) was used. The habitat assessment included characterization of streambed substrate (*e.g.,* boulders, cobble, silt), in-stream cover (woody debris, macrophytes), and riparian land use at 13 evenly-spaced transects. Channel morphology (riffles, runs, pools) throughout the reach was noted. In non-wadeable streams a modification of the Ohio Qualitative Habitat Evaluation Index (QHEI) (Rankin 1989) was used and became known as the Minnesota Stream Habitat Assessment (MSHA). The MSHA rates habitat based on substrate quality, in-stream cover, riparian zone quality and bank erosion, and pool/glide and riffle/run quality. MSHA data were compiled for all wadeable streams in this study based on results from the quantitative habitat assessment to allow for greater ease in comparing specific habitat and stream features among sites (Table II - 2). The MSHA has now become the standard habitat

assessment technique for both wadeable and nonwadeable streams and was calculated for most River Nutrient study sites.

Water chemistry samples routinely collected by fish biomonitoring crews included total phosphorus, total suspended solids (TSS), ammonia nitrogen (NH3+NH4), and nitrite-nitrate (NO2+NO3). In addition, temperature, conductivity, dissolved oxygen, turbidity, pH, flow, transparency, and water level were also measured at sampling sites. Samples were taken at a point that was judged to represent the water quality of the total instantaneous flow at the cross-section. Sampling avoided areas that were poorly mixed, contained springs, or were upstream of or immediately adjacent to tributaries within the sampling reach. Water chemistry measurements and water samples were taken at an intermediate depth in the water column without disturbing substrate materials or collecting floating materials and constituents from the water surface. Sample bottles were lowered mouth down to an intermediate depth and then turned upstream to collect the sample. Immediately after sample collection, 5 ml of 10% sulfuric acid preservative solution is added to the nutrients sample. Sample bottles were stored at 4°C and shipped to the Minnesota Department of Health Water Laboratory within minimum holding times. Detailed methods for biomonitoring habitat and chemistry sampling can be found on the MPCA website (http://www.pca.state.mn.us/index.php/view-document.html?gid=6089).

2. Fish and Macroinvertebrate Sampling

Fish and macroinvertebrate collections were made during daylight hours between mid-June and September. Fish were collected using electro-fishing techniques following procedures described in Niemela and Feist (2002). Depending on stream size and type, a towed stream electro-fisher, mini-boom electro-fisher, or boom electro-fisher was used to sample the fish community. Macroinvertebrate samples were collected at ten Rainy or Red River Basin sites in September 2005. Multi-habitat samples were taken by means of standard protocols similar to the methods in Barbour *et al*. (1999) and USEPA (1997). As with the Mid-Atlantic Coastal Streams Workgroup protocols, soft bottom substrates were not sampled. A 500 micron mesh, d-frame dipnet was used to collected samples. Complete samples were sub-sampled to a minimum of 300 organisms followed by a large and rare pick to supplement taxa richness. Identifications were made to the genus level or higher (*e.g.,* family) depending on the maturity and condition of the specimens. Chironomidae were identified to genus.

C. STORET Dataset

STORET (STOrage and RETrieval) is the EPA's environmental data system (http://www.epa.gov/storet/). The STORET data comes from a variety of sources including agencies and individuals and includes both probabilistic and targeting sampling efforts. The STORET dataset included TP, chlorophyll-a, and BOD_5 data. Data from the index period (i.e., June, July, August, and September) only was included in datasets. Biological analyses and the cumulative distribution analysis for TP used index period STORET data from 1996 through 2009. The reference condition analyses and determination of water quality relationships used index period STORET data from 1990 through 2012. STORET data were downloaded and linked to AUIDs for use with in the reference condition, biological threshold concentration, and water quality relationship analyses.

IV. DATA ANALYSES

A. Definition of River Nutrient Regions

As with lakes there are some relatively distinct differences in river water quality in Minnesota among the various ecoregions. An early effort by McCollor and Heiskary (1993) examined distributions for various water quality variables based on typical and minimally-impacted river sites in each ecoregion. USEPA (2000b, a, 2001) provided distributions for various nutrient ecoregions as a part of guidance on developing river nutrient criteria. A summary of both analyses is provided in Appendix I.

Determining which ecoregion a lake is located in (for purposes of applying appropriate criteria) is relatively straightforward. However, designating which ecoregion a river should be associated with is more complicated as rivers may originate in one region but eventually flow through and receive drainage from multiple ecoregions. The Mississippi River is a good example as it originates in the NLF and weaves its way through central Minnesota where

drainage from CHF (*e.g.,* Sauk, Rum, and Elk Rivers) and even WCP ecoregions (*e.g.,* South Fork Crow) enter before it reaches the Twin Cities Metro area and merges with the St. Croix and Minnesota Rivers (Figure 4). In view of the regional water quality patterns (Table I - 1) and monitoring and data analysis conducted to-date in development of river nutrient criteria (Heiskary & Markus 2001, 2003, Heiskary 2008), criteria are needed for three river nutrient regions (RNR): North, Central, and South. These regions correspond loosely to the USEPA aggregated Level III Nutrient ecoregions (Figure 3) with aggregations as follows:

- North – NLF and NMW ecoregions;
- Central – CHF and DA ecoregions and
- South – WCP, NGP, and LAP ecoregions.

River-watersheds at the eight digit HUC level were selected as a primary basis to develop our regional framework. These 81 watersheds, as derived from MDNR's major watershed (DNR Catchments) layer, are also a focus of MPCA's "pour-point" monitoring program and are most similar (and include) to rivers from our river nutrient studies (Table 2). When an 8 digit HUC is located completely within an RNR or where a vast majority of the watershed is within a single RNR the assignment to that RNR is rather straightforward, (*e.g.,* North Fork and South Fork of the Crow River; Figure 4). However, as is evident in Figures 4-6, a single river or major watershed often drains more than one ecoregion. This was particularly true for the various tributaries to the Red River, whereby several rivers (Buffalo, Wild Rice, and Otter Tail) originate in the CHF but drains through the RRV ecoregion en-route to the Red River (Figure 4). The Red Lake River has its origin in Red Lake in the NMW ecoregion but transitions into the RRV ecoregion near Thief River Falls. The Big Fork originates in the NLF ecoregion but flows through the NMW ecoregion as well. In this case, land uses are not substantially different between the two ecoregions (both are characterized by forest and wetlands). However for others, such as the Mississippi River, there is a transition from a watershed primarily characterized by forested, lake, and wetland dominated land use (NLF; Heiskary & Wilson 2008) in the upper reaches to the increasingly agricultural and urbanized land uses that characterize the CHF ecoregion (Figure 3). These differences in land use, soil characteristics, and geomorphology influence water runoff and pollutant loading to the river. Therefore, when an 8 digit HUC is characterized by multiple ecoregions the appropriate designation may be less apparent (*e.g.,* Wild Rice, Buffalo, and Red Lake Rivers; Figure 5). In these cases closer inspection was required and 11 digit HUCs (Watershed 99 HUC 11 layer) were incorporated into the mapping coverage to allow for refinement of boundaries. In a few instances where two 8 digit HUCs meet prior to entering the major mainstem river (e.g. North Fork and South Fork Crow Rivers) a "blended" or reach-specific criteria may be required and these reaches are noted on the RNR map (Figure 7). Heiskary and Parson (2010) provide further details on the mapping approach.

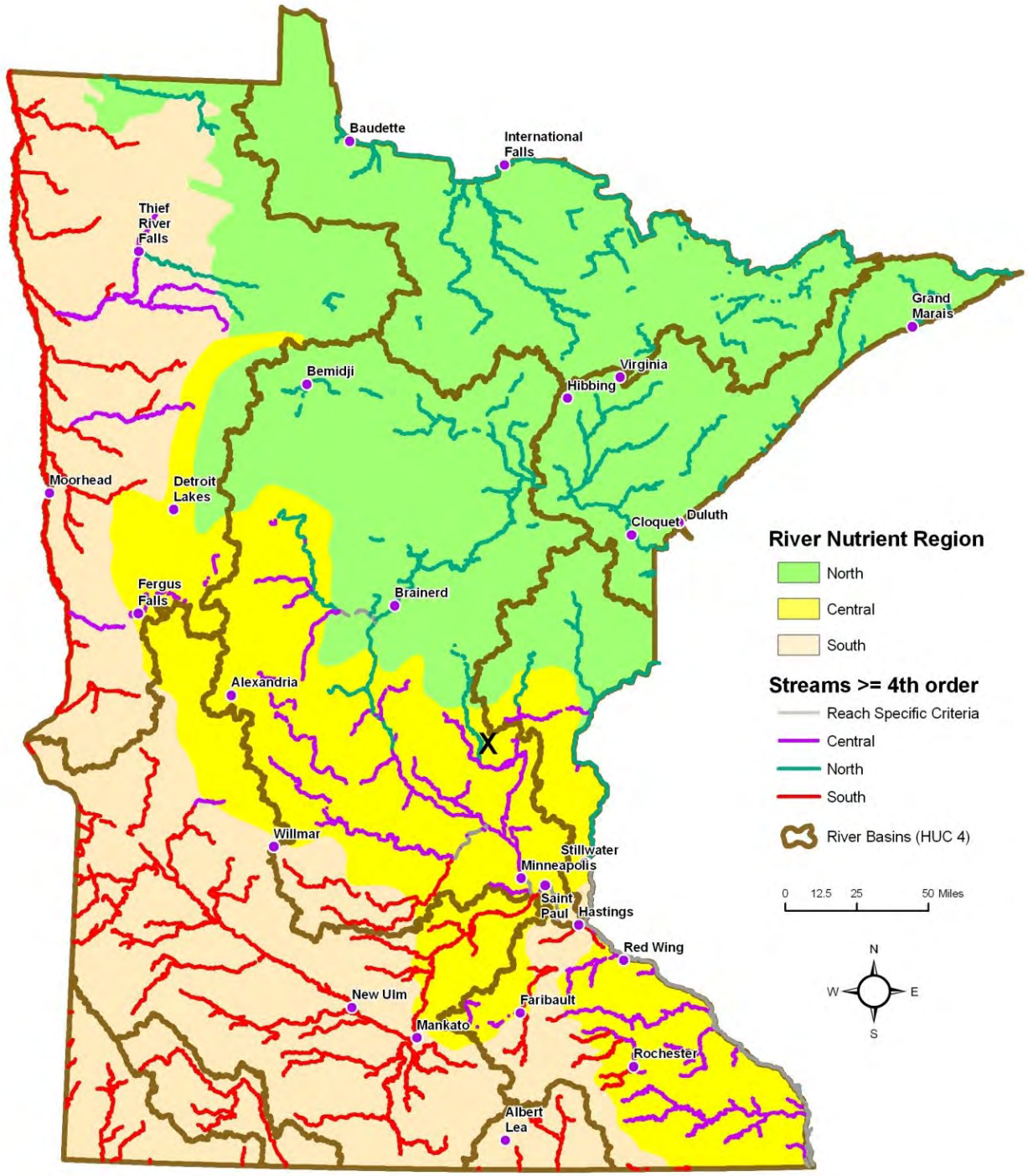


Figure 7. River Nutrient Regions (RNR). Classification developed at the 8 digit and 11 digit HUC level as needed. 4th order and larger rivers coded with their respective RNR. Further details provided in Heiskary and Parson (2010).

B. River Nutrient Study Analyses

1. Water Quality Relationships

A variety of techniques were used to examine the data. Initial data analysis was conducted primarily by EXCEL spreadsheet ver. 2002 (Microsoft Corporation 2002), R ver. 2.10.0 (R Development Core Team 2009), and SYSTAT ver. 12 (Systat Software 2007). Spearman correlation, linear regression, quantile regression, and related techniques were used to describe relationships between variables and guide further detailed assessment of the data. Much of the earlier work is described in more detail in Heiskary and Markus (2001 and 2003) and Heiskary (2008).

Predictive models for TP and Chl-a, BOD_5, and DO Flux using STORET and River Nutrient datasets were developed by fitting 50^{th} and 75^{th} percentiles using nonparametric quantile regression with regression splines ("rq" in "quantreg" package; Koenker 2009 and "bs" in "splines" package; R Development Core Team 2009) in the program R ver. 2.10.0 (R Development Core Team 2009). By interpolating stressor concentrations using draft criteria from these fits the 50% and 75% probabilities of meeting these goals could be assessed. From these predictions, attainment of the total phosphorus criteria will for most parameters and regions result in attainment of the stressor criteria.

2. Exploratory Analysis of Nutrient-Biology Relationships

As a further complement to the water chemistry, plankton, and diel monitoring collected through the River Nutrient study, fish, macroinvertebrate, and habitat studies were conducted by MPCA staff at most of the sites noted in Tables 2 and 3 (sites with a Bio ID #). Common metrics for fish were calculated and are described in Appendix II (Tables II-1 and -2). These are metrics that are commonly responsive to stressors and can be used to identify water quality problems which impact biological condition. A subset of these metrics was also used in IBIs to assess the structural and functional condition of the biological communities. An IBI score was calculated for each sampling event using an IBI developed specifically for streams in each respective basin. The IBI uses multiple attributes of the fish community (termed metrics) to characterize the biological integrity of the stream reach (Karr *et al.* 1986). For the Blue Earth River, the Minnesota River Basin IBI developed by Bailey et al. (1993) was used with one exception: metric classifications for fish species were updated following Niemela and Feist (2000). For sites in the Upper Mississippi River Basin, the Upper Mississippi IBI was used (Niemela & Feist 2002). For sites in the Red River Basin (*e.g.,* Buffalo River), IBI scores were calculated using the IBI developed by USEPA (1998). Fish metric data and IBI scores for all 2006 sites are included in Appendix II (Table II - 7). Because of the differing range in scores between the three IBIs [The total score range for the Minnesota River and Red River Basin IBI's is from 12 to 60; for the Upper Mississippi River Basin IBI, overall scores range is from 0 to 100], narrative descriptions were used to rate the biotic integrity of each site and to allow for the interpretation of overall IBI scores and comparison between river basins (Table II - 3). A variety of macroinvertebrate metrics were also used in this analysis. One example is a metric based on the Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddis flies). This metric, referred to as EPT, provides a relative measure of the presence and diversity of pollution-sensitive macroinvertebrate groups. These taxa (and this metric) are felt to be strong water quality indicators (Barbour *et al.* 1992). Details on other metrics are provided in Appendix II (Table II - 7).

Table 7. Interquartile range for fish and macroinvertebrate metrics and habitat scores based on statewide collections for river sites with watersheds greater than 500 mi^2 (1,295 km^2) (metric descriptions in Appendix II; # = number of taxa, % = percent of individuals in the total sample).

Metric (fish)	25^{th}	50^{th}	75^{th}	# of sites
QHEI	55	62	67	53
MSHA	50	58	68	155
Fish IBI	50	67	78	146
# Taxa	17	21	26	290
# Darter, Sculpin, and *Noturus*	2	3	4	290
# Intolerant Taxa	1	2	3	290
# Sensitive Taxa	2	4	6	290
# Insectivore taxa	9	11	13	290
# Benthic Invertivore Taxa	4	6	8	290
# Omnivore Taxa	2	3	4	290
# Piscivore Taxa	3	5	6	290

% Tolerant Taxa	4	9	20	290
% Sensitive Taxa	3	9	22	290
% Omnivore Taxa	2	6	14	290
% Piscivore Taxa	4	9	17	290
% Simple Lithophilic Taxa	26	42	57	290
% Fish DELT	0.0	0.1	0.5	290
Metric (invertebrate)	**25th**	**50th**	**75th**	**# of sites**
# EPT Taxa	9	12	15	150
# Total Invert Taxa	30	36	46	150
# Sensitive (Intolerant) Taxa	3	6	8	150
# Very Tolerant Taxa	4	8	11	150
# Clinger Taxa	10	13	17	150
# Gatherer Taxa	9	12	16	150
% EPT Taxa	24	48	63	150
% Tolerant Taxa	27	46	68	150
% Sensitive (Intolerant) Taxa	2	5	16	150
% Very Tolerant Taxa	4	12	27	150

Cumulative distribution functions (CDF) were calculated for many of the fish and macroinvertebrate metrics based on data collected in the biomonitoring program, with an emphasis on streams with watershed areas greater than 400 mi^2 (medium to large streams) and CDF graphs were included in Heiskary (2008). A summary of the interquartile range of values and the number of sites is noted in Table 7 for sites with watershed areas greater than 500 mi^2. This provides a framework for describing the typical range (25^{th} to 75^{th} percentile) of the various metrics, with values either above or below this range indicative of either higher or lower quality biological communities (dependent on the metric). For example, sites with more than 26 fish taxa or over 46 macroinvertebrate taxa would be considered high quality sites (at or above 75^{th} percentile). In contrast, sites with less than 17 fish taxa or less than 30 macroinvertebrate taxa would be considered low quality based on this approach (at or below 25^{th} percentile). This provides a means for placing shifts of the various metrics, relative to chemical and physical factors, in perspective to the larger population of sites that have been monitored statewide.

C. Reference Condition Analysis

Central to the reference condition analysis is the identification of stream sites that are least or minimally disturbed using an *a priori* measure of condition independent of the water quality parameters of interest. These models should not be based on water quality or biological parameters, but rather should employ land use and other measures of human activity in a watershed or stream reach. Minnesota has developed an index to measure the degree of human activity in a watershed upstream of stream monitoring site and within the stream monitoring reach called the Human Disturbance Score (HDS). The HDS includes both watershed and reach level measures of human disturbance which receive a score of 0-10. Additional adjustments are made for watershed and reach-level factors which can negatively impact waterbody condition. These metrics and adjustments together have a maximum score of 81 (Table 8). Reference sites for streams were identified as those with an HDS score of 61 or greater (i.e., the upper 25% of the HDS distribution). Once sites were selected based on their HDS score, several additional filters were applied to remove sites disparately influenced by nearby stressors. All sites in close proximity to urban areas (site within or adjacent to urban area), feedlots (feedlot at or immediately upstream of site [only streams >50 mi^2]), or point sources (continuous point source <5 mi upstream of site) were removed. In addition, sites determined to be on channelized reaches (>50% of reach channelized) were not included in the reference site dataset. Sites meeting these criteria were considered to be minimally or least disturbed and therefore representative of attainment of Minnesota's aquatic life use goals.

TP, chlorophyll-a, and BOD_5 from the summer index period (i.e., June-September) and from 1990-2012 were queried from STORET. Average values of these measures were determined for Assessment Units (AUIDs) and associated with HDSs. HDSs scores were calculated for biological monitoring stations so for AUIDs with multiple biological stations the HDS scores were averaged. Using the reference AUID criteria described above, the AUIDs

were divided into reference and non-reference reaches. Only sites with natural channels (i.e., >50% of channel natural or not channelized) were included in these datasets. Samples sizes for these datasets are provided in Table 9. Quartiles for TP, chlorophyll-a, and BOD_5 were calculated for North, Central, and South for both the reference and non-reference reaches.

Table 8. Metrics and scoring for Minnesota's Human Disturbance Score.

Human Disturbance Score Metric	Scale	Primary Metric or Adjustment	Maximum Score
Number of animal units per sq km	watershed	primary	10
Percent agricultural land use	watershed	primary	10
Number of point sources per square km	watershed	primary	10
Percent impervious surface	watershed	primary	10
Percent channelized stream per stream km	watershed	primary	10
Degree channelized at site	reach	primary	10
Percent disturbed riparian habitat	watershed	primary	10
Condition of riparian zone	reach	primary	10
Number of feedlots per km^2	watershed	adjustment	-1
Percent agricultural land use on >3% slope	watershed	adjustment	-1
Number of road crossings per km^2	watershed	adjustment	-1 or +1
Percent agricultural land use in 100m buffer	watershed	adjustment	-1
Feedlot adjacent to site	reach (proximity)	adjustment	-1
Point source adjacent to site	reach (proximity)	adjustment	-1
Urban land use adjacent to site	reach (proximity)	adjustment	-1
		Maximum	**81**

Table 9. Numbers of AUIDs in each dataset used in the reference condition analysis.

Data Source	Region	Stream Size	WQ Variable	Reference	Non-reference
STORET	North	All	BOD	51	15
STORET	Central	All	BOD	12	49
STORET	South	All	BOD	1	58
STORET	North	All	Chlorophyll-a	63	21
STORET	Central	All	Chlorophyll-a	22	91
STORET	South	All	Chlorophyll-a	4	111
STORET	North	All	Total Phosphorus	156	53
STORET	Central	All	Total Phosphorus	69	167
STORET	South	All	Total Phosphorus	6	198

D. Identification of Nutrient Threshold Concentrations

The use of field-collected biological data in developing chemical criteria is often difficult due to complex relationships among chemical and physical measures and the biota. A relatively new analysis method, called quantile regression, has been used as a tool to identify threshold concentrations and to develop criteria to protect aquatic life. Quantile regression is well suited for the wedge-shaped plots (caused by heterogeneous variance; *i.e.,* heteroscedasticity) that are common with biological monitoring data (Terrell *et al.* 1996, Koenker & Hallock 2001, Cade & Noon 2003, Bryce *et al.* 2008; see Figure 8). These wedge-shaped plots are the result of the limitation of biological attributes (*e.g.,* taxa richness) by the variable of interest on the outer or upper edge of the wedge (Bryce *et al.* 2008; see Figure 8). Limitations to biological measures inside the wedge are caused by other unmeasured variables (Figure 8). In the case of this work, nutrients can lower biological condition through alteration of DO levels or shifts in food resources or habitat. However, there are also a number of other factors (*e.g.,* sediment, habitat) that can also limit biological condition in Minnesota streams and rivers. As a result of these different factors reducing biological measures, there is unequal variation of the response variable at different levels of the predictor variable. This unequal variation often makes field-derived data (*e.g.,* biomonitoring data) less suitable for the more traditional least squares regression. Quantile regression differs from least squares regression in that it estimates the median (*i.e.,* 50^{th} quantile) or other quantiles whereas least squares regression estimates the mean. Another advantage of quantile regression is that extreme outliers do not impact regression quantile estimates (Terrell *et al.* 1996).

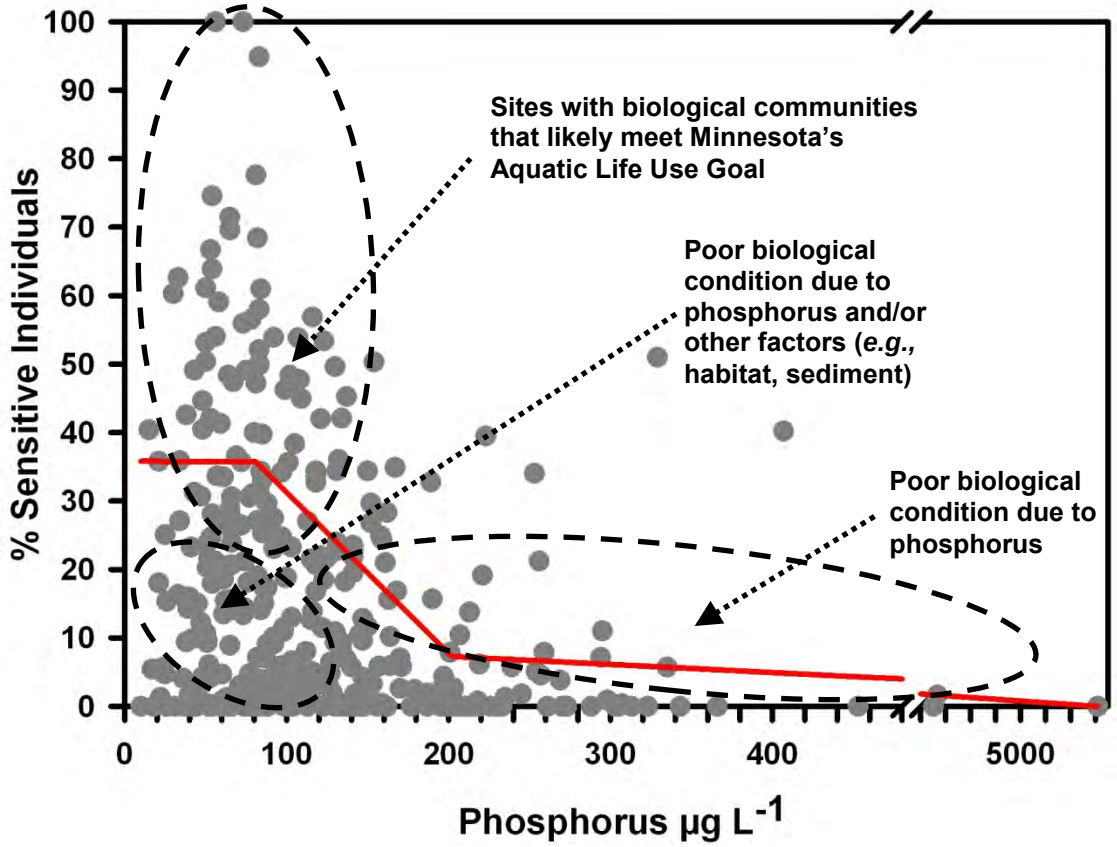


Figure 8. Relationship between phosphorus and the percent of sensitive fish for central streams with additive quantile regression smoothing line (red line). This is an example of the typical wedge-shaped data to which quantile regression is suited.

If we examine habitat and sediment in relation to total phosphorus, there is a relationship between these variables with a positive correlation between total phosphorus and TSS and a negative relationship between total phosphorus and the MSHA (Figure 9). However, with both of these relationships there are still a large number of sites with relatively high total phosphorus but with either low TSS concentrations or high MSHA scores. Therefore the sites with high TSS concentrations or low MSHA scores are more likely to be those on the inside of a wedge-shaped plot of total phosphorus and a biological measure. However, there are sufficient sites with low TSS concentrations and high MSHA scores with high total phosphorus to assess the relationship between total phosphorus and biology by focusing on the on the outside of the wedge.

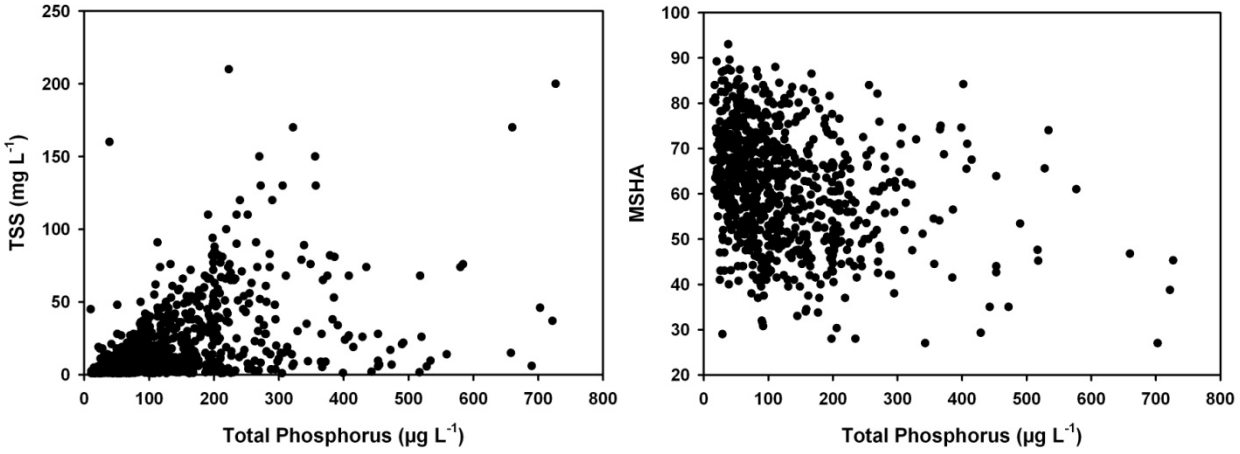


Figure 9. Relationships between total phosphorus and TSS and the Minnesota Stream Habitat Assessment (MSHA). Data from habitat assessment and grab samples collected as part of biological monitoring.

Regression tree or changepoint analysis is another technique that can be used to identify thresholds where biological condition declines in heteroscedastic data. This analysis splits that data into groups where the sites within that group are more homogeneous (De'ath & Fabricius 2000). For example, groups may have different mean values of the response variable. The location of the splits or nodes indicates a change between groups which may suggest that a threshold has been crossed.

The relationships between different water quality variables and biological measures were assessed. These water quality variables included nutrients (*e.g.,* phosphorus) and proximate stressors (*e.g.,* chlorophyll-a, BOD_5 and DO flux). Proximate stressors provide a more direct determination of the impact of these variables on biological condition as they have a direct influence on the composition and health of biological communities. The impact of nontoxic levels of nutrients has an indirect impact on the biology so the causal association between biological health and phosphorus levels may be less clear. However, the use of methods including quantile regression and changepoint analysis allow the assessment of these causal associations. In addition, an understanding of how phosphorus influences proximate stressors allowed the determination of phosphorus concentration thresholds. In this analysis we used quantile regression and changepoint analysis to identify biological threshold concentrations for various water quality variables. These values were used in conjunction with water quality relationships to determine phosphorus levels that will be protective of aquatic life goals.

1. Quantile Regression and Changepoint Datasets

Several different water chemistry datasets were used to develop nutrient criteria from biological information (Table 10). The three sets of data were used to develop water quality threshold concentrations from fish and macroinvertebrate data are called the River Nutrient study, STORET, and Biomonitoring datasets. The names for these datasets refer to the source of the water quality data. Depending on characteristics of each dataset some were useful for examination of different patterns between regions in the state, stream size, or comparing among different data sources. Patterns among northern, central, and southern regions were assessed to determine if different criteria should be proposed for these areas of the state. Differences between streams sizes were also assessed to determine if different criteria were justified for these stream classes. This is important because differences in the presence or effect of the sestonic chlorophyll could result in different responses by biological communities. Different sources of nutrient data were also examined to determine if a similar relationship was observed between nutrient enrichment and the response of the biological community. Similar threshold concentrations developed from these many datasets also provide greater confidence in the final criteria proposed. Descriptions on these datasets are as follows:

- **River Nutrient Study:** The River Nutrient dataset resulted from a study that specifically assessed the impact of nutrients on Minnesota streams (see Section III.A). This dataset included multiple parameters collected concurrently and included multiple water quality measurements during the summer season which made it useful for understanding the relationships between these parameters. The River Nutrient dataset included measurements of total phosphorus, total nitrogen, chlorophyll-a, BOD, DO flux, fish, and macroinvertebrates. The River Nutrient data consisted of both wadeable and nonwadeable streams although this dataset consisted largely of nonwadeable streams. The River Nutrient sites were located throughout the state of Minnesota and included sites from different ecoregions. Due to the relatively small size of the dataset, it could not be analyzed regionally or by stream size. Relationships between these measures and biological metrics were determined although these relationships were not used in criteria development because they could not be divided into regional datasets. The River Nutrient dataset was most useful for modeling stressor thresholds that would be protective of biological communities (see Section IV.D.5, p. 34). For identifying biological thresholds, the River Nutrient dataset was also useful as a screening tool to identify sensitive biological metrics and to begin to understand the effects of eutrophication on the biology.
- **Biomonitoring:** The biomonitoring dataset included data that were not collected specifically to support development of nutrient criteria. However, this was a large dataset of total phosphorus data that was collected concurrently with fish and macroinvertebrate data. As a result biomonitoring phosphorus concentrations were well associated with the biological data. The biomonitoring chemistry data were aggregated with the biological data by station because these water quality measures were collected by the biomonitoring crew at the biological stations during collection of fish data. However, this dataset was

generally limited to single measurements of total phosphorus taken during fish sampling. In contrast, the River Nutrient dataset included summer-mean values that consisted of multiple measurements. As a result of the large size of the dataset and the good association with the biological data, the biomonitoring dataset was most useful for identifying biological thresholds for total phosphorus for all three nutrients regions and for different stream sizes.

- **STORET:** The STORET dataset came from the EPA's environmental data system called STORET (STOrage and RETrieval). The STORET dataset included total phosphorus, chlorophyll-a, and BOD_5 data, but only BOD_5 was analyzed with biology. Nutrient data from STORET were downloaded from EPA's STORET site (http://www.epa.gov/storet/) and linked to AUIDs. Water quality data was only used if:
  - § Measurements made from June to September
  - § Appropriate sampling and lab techniques were used
  - § Water quality measurements made within 5 years of biomonitoring sampling

  The STORET dataset included data that were not collected specifically to support development of nutrient criteria. The biological data were associated with STORET water chemistry data using AUIDs, which allowed determination of relationships between water chemistry and biological communities. The STORET dataset was larger than the River Nutrient dataset and it included many of the same water chemistry measures. However, the STORET dataset include data that were not collected concurrently and data that were not collected systematically during the summer season. These characteristics could result in error, which gives less confidence in the STORET data despite the larger sample size. The STORET dataset was used to regionally determine relationships between the biology and BOD_5. STORET TP data were not analyzed with biological data because it was redundant with the biomonitoring phosphorus dataset. STORET Chl-a data were not analyzed with biological data because the sample size was not large enough to perform the changepoint or quantile regression analyses. The STORET dataset was also useful for modeling stressor thresholds that would be protective of biological communities (see Section 5, p. 34).

For all three water chemistry datasets, the biological data used in analyses came from data collected as part of the MPCA biomonitoring program. Some additional screening was performed to reduce the effects of habitat modification. Sites identified as channelized (*i.e.,* >50% of reach channelized) during biological sampling were excluded from analyses. To avoid anomalous biological samples, sites that were sampled for biology during high flows were not included in analyses. Coldwater streams were also removed from all datasets due to the small number of these streams in the biomonitoring dataset.

The STORET and Biomonitoring datasets were divided by region (North, Central, and South) and the biomonitoring dataset was further divided by stream size (wadeable, nonwadeable). Stream size class was determined by watershed area with streams with drainages <500 mi^2 considered **"wadeable"** whereas those >500 mi^2 were considered **"nonwadeable"**. The regional classification for the biomonitoring dataset was based on level III ecoregions (see Figure 7; **Northern Region:** Northern Minnesota Wetlands, Northern Lakes and Forests; **Central Region:** North Central Hardwoods, Driftless Area; **Southern Region:** Northern Glaciated Plains; Western Corn Belt Plains, Lake Agassiz Plain).

Table 10. Numbers of collections in each dataset used assess relationships between water quality and biological measures (* Most sites are nonwadeable [drainage area >500 mi^2]).

Data Source	Region	Stream Size	WQ Variable	Fish	Invertebrates
STORET	North	All	BOD	25	10
STORET	Central	All	BOD	33	26
STORET	South	All	BOD	53	38
River Nutrient	Statewide	All*	BOD	22	16
River Nutrient	Statewide	All*	DO Flux	25	20
River Nutrient	Statewide	All*	Chlorophyll-a	31	25
River Nutrient	Statewide	All*	Total Phosphorus	31	25
Biomonitoring	North	Wadeable	Total Phosphorus	346	277
Biomonitoring	North	Nonwadeable	Total Phosphorus	81	49
Biomonitoring	North	All	Total Phosphorus	427	326
Biomonitoring	Central	Wadeable	Total Phosphorus	315	247
Biomonitoring	Central	Nonwadeable	Total Phosphorus	53	32
Biomonitoring	Central	All	Total Phosphorus	368	279
Biomonitoring	South	Wadeable	Total Phosphorus	230	161

Biomonitoring	South	Nonwadeable	Total Phosphorus	49	29
Biomonitoring	South	All	Total Phosphorus	280	190

2. Metric Selection

Before quantile regression and changepoint analyses were performed, it was necessary to select appropriate response measures or biological metrics. The selection of a subset of metrics was made using several methods. Spearman rank correlations were examined using the River Nutrient dataset to identify metrics with a strong relationship between the total phosphorus and biological metrics (see Table 16). Some of the metrics that were significantly correlated were eliminated due to the redundancy of metrics and the relevance of the metrics to nutrient enrichment (*i.e.,* can a mechanism between nutrient enrichment and the response in that metric be identified). Eight metrics were selected for fish and six metrics for macroinvertebrates (Table 11). At the time of this work, the MPCA was still in the process of developing new IBIs so this index was not included in the development of concentration thresholds.

Table 11. Fish and macroinvertebrate metrics used to develop concentration thresholds.

Fish Metrics	Invertebrate Metrics
% Sensitive	Total Taxa Richness
% Darter	Collector-filterer Taxa Richness
% Simple Lithophils	Collector-gatherer Taxa Richness
% Tolerant	EPT Taxa Richness
% Insect	Intolerant Taxa Richness
% Piscivore	% Tolerant
Taxa Richness	
% Intolerant	

3. Biological Threshold Analyses

A number of patterns can be observed between nutrients and the biological metrics (Brenden *et al.* 2008) although the relationship between biology and nutrients is often wedge shaped (Wang *et al.* 2007). In the Minnesota datasets used for this study, a distinct wedge with breakpoint(s) (Figures 10a, b and c) was most commonly observed. The "upper plateau" (see Figures 10a and c) occurred at generally low levels of nutrients or stressors and was characterized by high variability in the biological metric. The steep portion of the wedge occurred at moderate levels of the nutrient or stressor and indicated that a threshold had been crossed and that biological condition was declining. At higher levels of nutrients or stressors there was often a lower breakpoint that corresponded to low biological metric scores indicating that the response variable had largely reached bottom and was not declining or declining at a much slower rate (see Figures 10a and b). Additive quantile regression smoothing and changepoint analyses were both effective with this type of dataset. The fit of the quantile regression and the ability of the changepoint analysis to identify thresholds were assessed and analyses with a poor fit or those not identifying relevant thresholds were omitted. For some datasets, no analysis was appropriate as a gradient sufficient for these analyses was not evident in the available datasets (see Figure 10d). For example, some metrics in the southern region had too few sites with good biological communities and did not show a good relationship between the nutrient or stressor and the biological metrics (Figure 10d). This suggests that many streams in this region are enriched and that additional data is needed from less enriched streams in the region. Although threshold analyses were more difficult in the southern region, there were still a sufficient number of good quality sites (i.e., sites that meet biological goals) to derive some thresholds.

The development of river eutrophication criteria is intended to support attainment of the CWA interim goal. This goal is defined in the CWA as:

> *"wherever attainable, an interim goal of water quality which provides for the protection and propagation of fish, shellfish, and wildlife and provides for recreation in and on the water" (U.S. Code title 33, section 1251 [a] [2])*

The interim goal of the CWA does not require that all waters must meet goals equivalent to natural or pristine conditions. Rather a goal of restoring waters to the natural condition is more consistent with the definition of the CWA objective *("restore and maintain the chemical, physical, and biological integrity of the Nation's waters"*; U.S. Code title 33, section 1251 [a]). The statistical methods used in this line of evidence are focused on setting minimum goals that support attainment of the CWA interim goal. This is accomplished by the use of metrics that are sensitive to eutrophication and by identifying thresholds that are consistent with attainment of the CWA interim goal. The quantile regression and changepoint analyses identify thresholds that generally correspond to the upper breakpoint or the midpoint of the steep portion of the curve (Figures 10a b, and c). These relationships and the location of thresholds determined using Minnesota data closely correspond to the location of defensible thresholds derived from stressor-response response relationships in Stevenson et al. (2008) (see Figure 2 in Stevenson et al. [2008]). These thresholds are consistent with the protection of "fishable/swimmable" goals as defined by the interim goal of the CWA and therefore support Minnesota's aquatic life use goals. As a result, the threshold concentrations from each dataset are not intended to represent protection of the natural condition. Additionally, these do not represent pollute-down-to goals and waters that perform better than these goals should be protected.

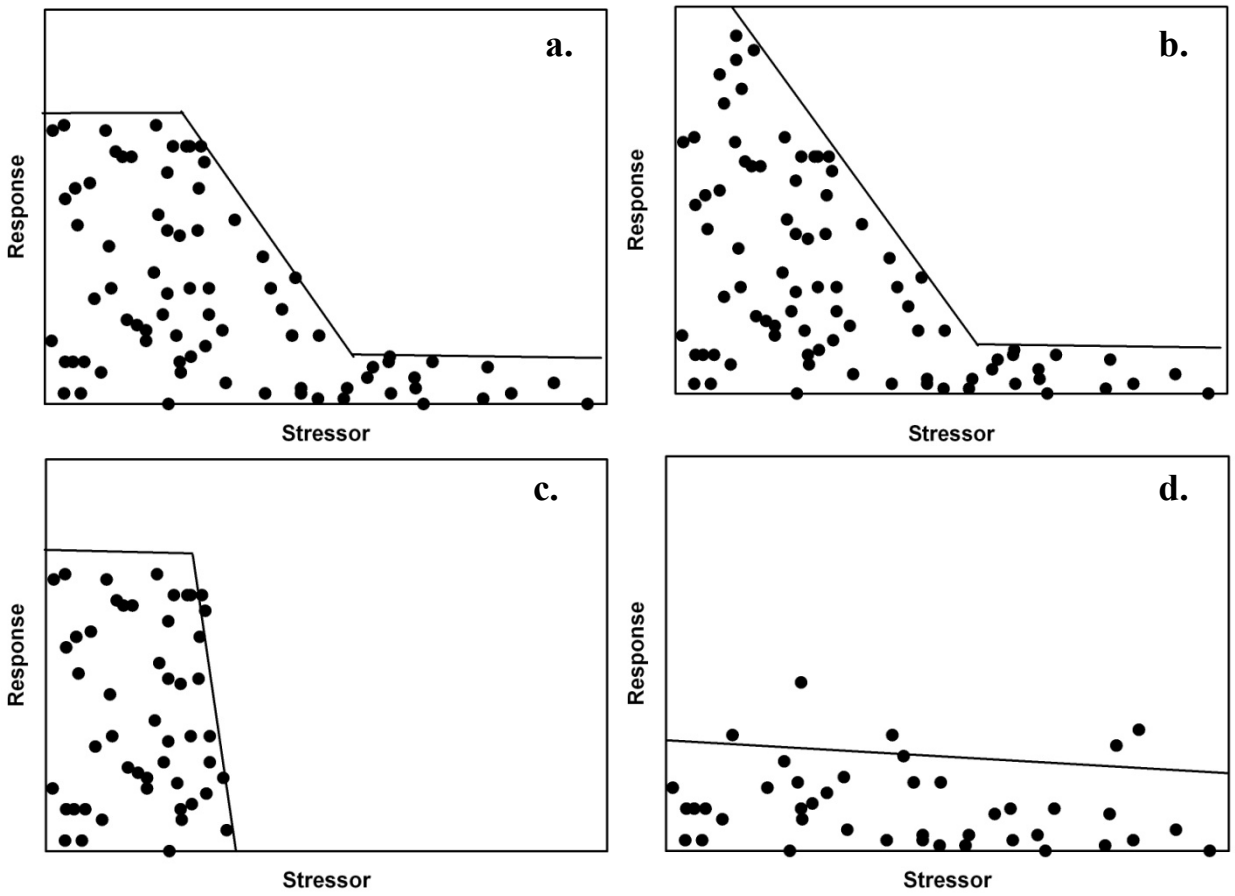


Figure 10. Illustration of response patterns to stress resulting from nutrients and other stressors observed in field-collected data.

4. Statistical Methods

Additive Quantile Regression Smoothing. Additive quantile regression smoothing ("rqss" in "quantreg" package; Koenker 2009) was performed in the program R ver. 2.10.0 (R Development Core Team 2009). This method is similar to linear quantile regression, but instead of fitting a single line to the data, this approach fits a regression line to subsets of the data (see Figure 11). As a result, additive quantile regression smoothing (AQRS) can also be used to identify changepoints in addition to fitting the outside of the data wedge. The 75th percentile ($\tau = 0.75$) was used with additive quantile regression smoothing to minimize the effect of outliers. This was important because there is a tendency for increasing variation in the estimates as τ approaches 1 in some datasets (Cade & Noon 2003). In

addition some of the smaller datasets could not be effectively fit with τ much greater than 0.75. The additive quantile regression smoothing approach required the selection of a lambda (λ) value which determines the amount of smoothing. Values of λ were selected by eye on how well the line fit the outside of the curve and was not affected by single values. Fits were selected by how well they fit the outside of the wedge while minimizing the number of breakpoints. Identification of 3 or 2 breakpoints was optimal. An F-test was used to determine if the regression fit reduced model deviance. 90% confidence bands were also determined to examine regression fits. Following the selection of a good, parsimonious fit, the relationship was examined to determine if it would be used for threshold concentration determination. Metrics were eliminated if the F-test was not significant at the $\alpha = 0.05$ level. In addition, if the metric responded in a manner contrary to the predicted response or had no response it was not included in further analyses.

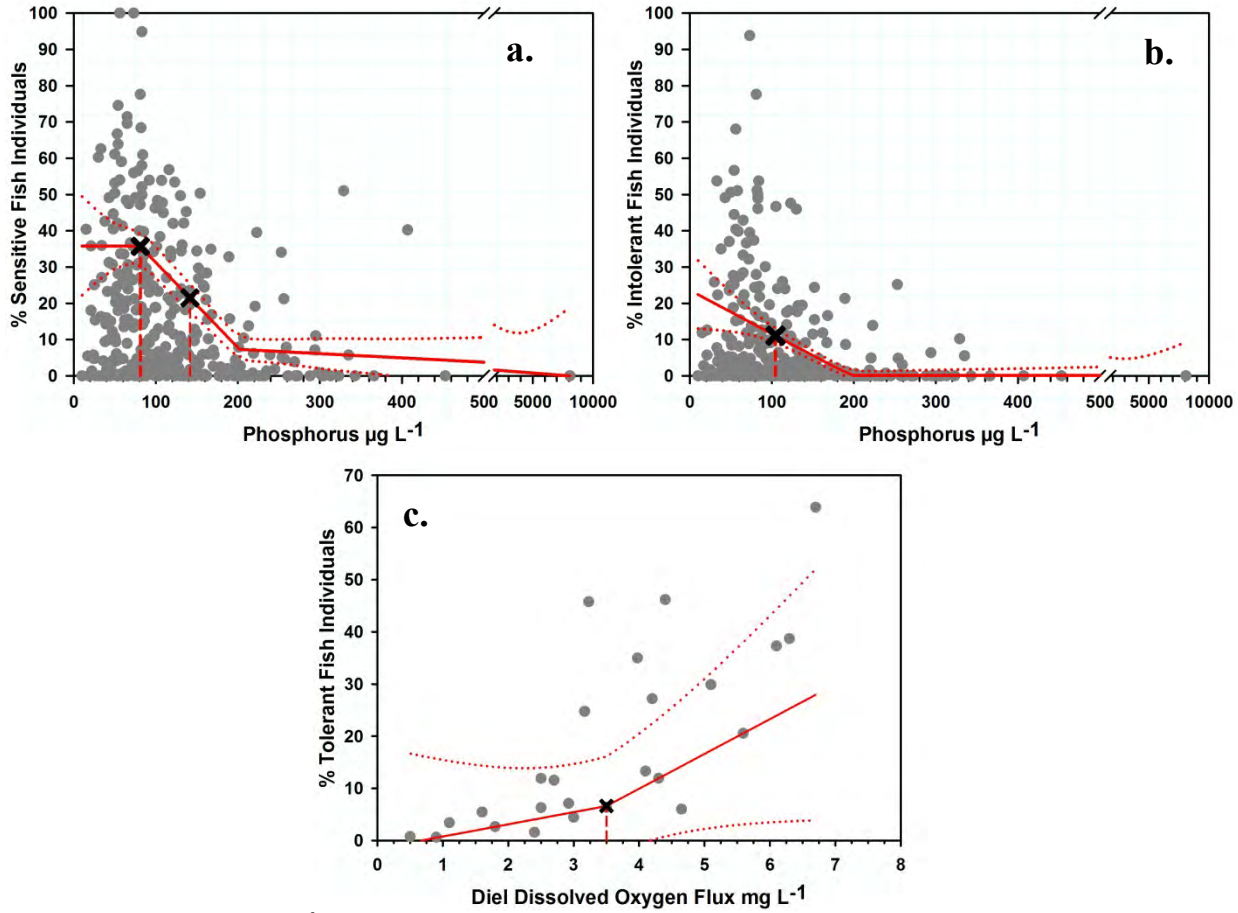


Figure 11. Examples of 75th percentile additive quantile regression smoothing showing examples with a) upper and midpoint thresholds (percent of sensitive fish individuals for the central region using biomonitoring data) and b) midpoint threshold only (percent of sensitive fish individuals for the central region using biomonitoring data), c) upper breakpoint only (percent of tolerant fish individuals for River Nutrient streams) (solid line = AQRS fit; dotted lines = 90% confidence bands).

Once the 75th percentile quantile regression was fitted, threshold concentrations were determined using the fits. In datasets where both upper and lower breakpoints were present, concentrations for the midpoint between the breakpoints and upper breakpoint were determined (see Figure 11a). If no upper breakpoint was present then the midpoint between the lower breakpoint and the lowest stressor value was used (see Figure 11b). If an upper breakpoint was present, but no lower breakpoint was present (see Figure 11c) then the threshold concentration was determined using the upper breakpoint. A chi-squared test was performed in Sigma Plot ver. 11 (Systat Software 2008) to determine if there was a significant difference in the biological metric scores above and below the threshold concentration determined by AQRS. In cases where any of the treatments within the contingency table had fewer than five observations, a Fisher Exact Test was performed in SigmaPlot ver. 11 (Systat Software 2008). Threshold

concentrations that were not significant were not used in further analyses. In cases where both the upper breakpoint and midpoint threshold concentration could be identified, the upper breakpoint was used if it was significant. If the upper breakpoint was not significant, then the midpoint breakpoint was used if it was significant. The process for testing and selecting threshold concentrations is provided in Figure 12.

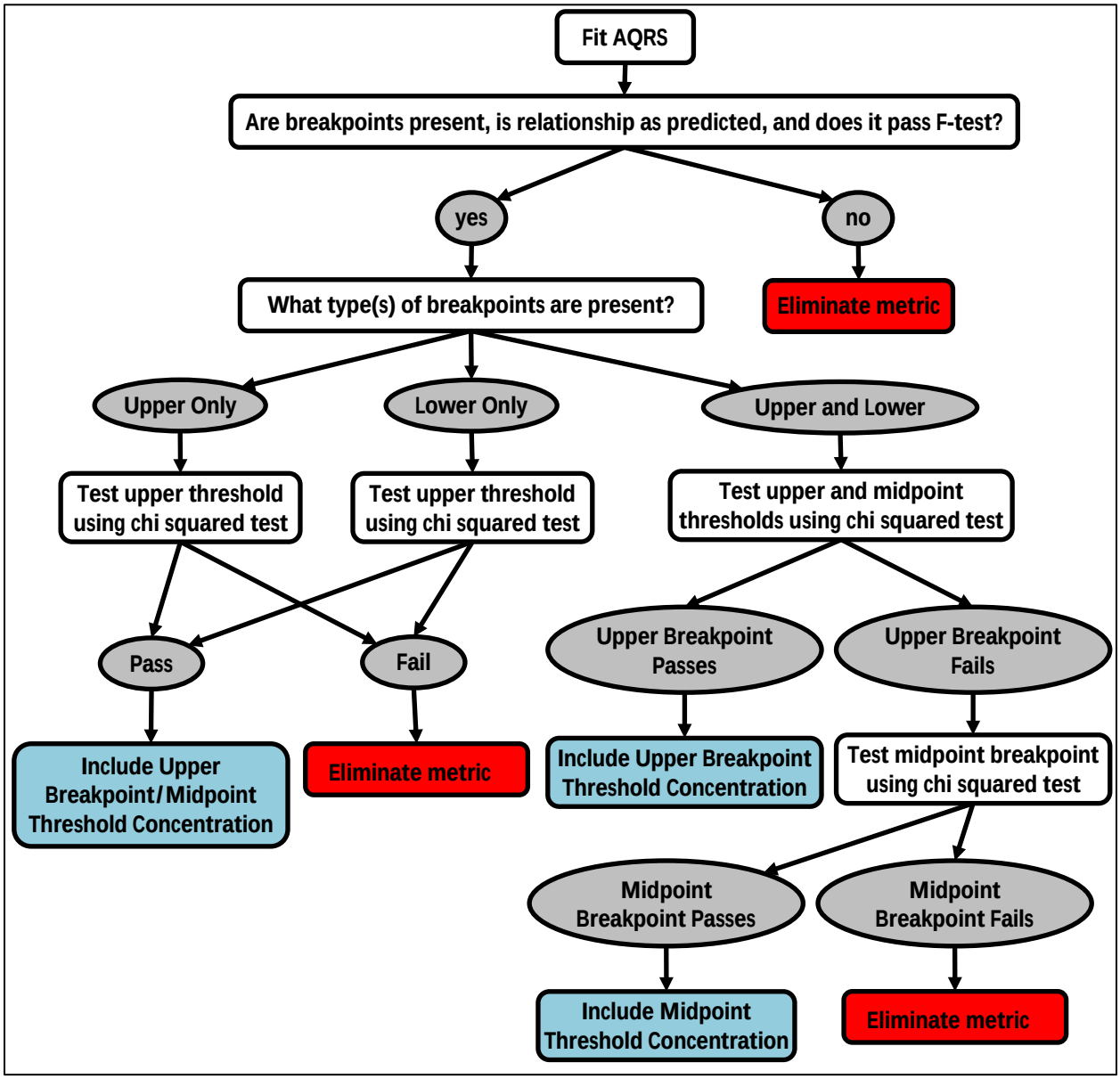


Figure 12. Process for testing threshold concentrations determined using additive quantile regression smoothing (AQRS).

Changepoint Analysis. Changepoint analysis was performed in the program R ver. 2.10.0 (R Development Core Team 2009) using the regression tree analysis ("rpart" in the "rpart" package; Therneau & Atkinson 2008). This method identifies thresholds by dividing samples into two groups based on differences in both their mean and variance (Qian *et al.* 2003). Trees were constrained to a single split with a bucket size of 5 samples or 10% of the sample depending on which was larger (*e.g.*, Figure 13). 90% confidence bands were determined using a bootstrap analysis which resampled 1000 times. Bootstrap analysis was performed in the program R ver. 2.10.0 (R Development Core Team 2009) using the bootstrap function ("boot" in the "boot" package; Canty & Ripley 2009). Since regression tree analysis will identify a changepoint in any dataset, a significance test was applied to determine if the changepoint was significant at the $\alpha = 0.05$ level. A chi-squared test was performed in Sigma Plot ver. 11

(Systat Software 2008) to determine if there was a significant difference in the biological metric scores above and below the threshold concentration determined by regression tree analysis. In cases where any of the treatments within the contingency table had fewer than five observations, a Fisher Exact Test was performed in SigmaPlot ver. 11 (Systat Software 2008). Threshold concentrations identified from non-significant changepoints were not used in further analyses.

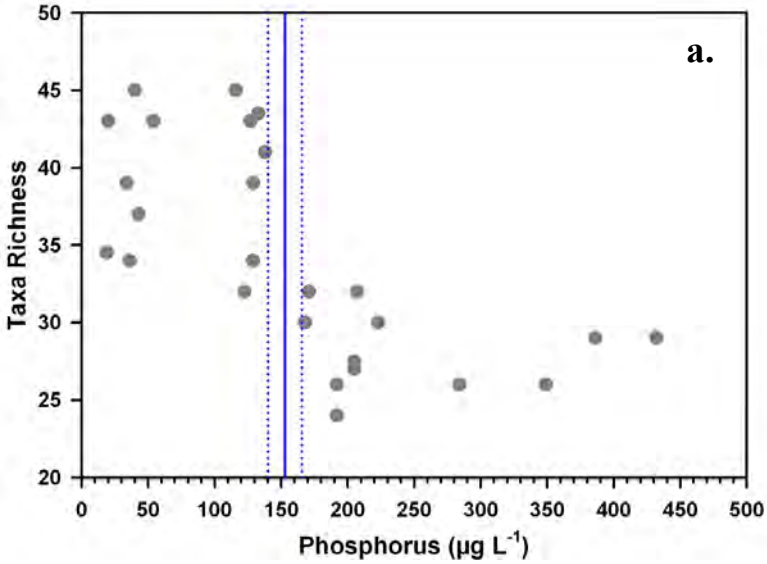


Figure 13. Example of changepoint analyses using macroinvertebrate taxa richness from the River Nutrient Study.

5. Linking Nutrients to Biological Condition.

Relationships among nutrients, stressor variables, and the biology was further assessed by determining the levels of chlorophyll-a and total phosphorus associated with the BOD_5 threshold concentrations. Values of chlorophyll-a and total phosphorus were determined using 75th quantile regression fits derived from the River Nutrient Study and STORET datasets. Regression fits were based on nonparametric quantile regression using regression splines ("rq" in "quantreg" package; Koenker 2009 and "bs" in "splines" package; R Development Core Team 2009) which was performed in the program R ver. 2.10.0 (R Development Core Team 2009). Using the 25th percentile of the threshold concentrations for BOD_5 determined from AQRS and changepoint analyses, concentrations of chlorophyll-a and total phosphorus protective of aquatic life goals could be interpolated using the above regressions. In addition, these quantile regression models were used to predict stressor values for chlorophyll-a, BOD_5, and DO Flux based on the draft total phosphorus criteria.

V. RESULTS

The following results and discussion are drawn, in part, from previous MPCA reports (Heiskary & Markus 2001, 2003, Heiskary 2008, and USGS reports (*e.g.,* Lee 2002, 2008a) compiled as a part of the overall studies conducted in support of river eutrophication criteria development. These reports can be referred to for further details. In addition, recent data from the 2008 study have been included and databases have been adjusted accordingly to include this data. In some instances this has allowed for a refinement of previously reported findings and serves to advance the development of river nutrient criteria.

The results reflect the multiple lines of evidence approach that we used to derive the river nutrient criteria. The generalized steps are as follows:

- Gather basic chemical, physical, and biological data on a range of Minnesota rivers with an emphasis on medium to high order systems.
- Establish basic interrelationships among nutrients, algae, dissolved oxygen, flow, and related factors. Describe these relationships and variability in the relationships with basic statistical and graphing approaches.

- Explore relationships among nutrients, algae, dissolved oxygen and fish and macroinvertebrate communities via basic correlation analysis and scatterplots. Use this analysis to identify sensitive metrics for more detailed analysis.
- Employ changepoint and quantile regression techniques to help define nutrient and stressor thresholds that are protective of aquatic life uses.
- Evaluate range of thresholds relative ecoregional patterns and information from the literature and select specific values to serve as criteria.

A. Diel Water Quality Monitoring Results: River Nutrient Study

1. Diel Patterns of Dissolved Oxygen, Temperature, pH, and Specific Conductivity

The measurement of diel fluctuation of DO, temperature, pH, and specific conductivity at select river nutrient study sites was an integral part of our approach for understanding how nutrients, sestonic algae, and related factors may affect stream metabolism and overall stream health. Measurements were targeted toward mid-late summer when river flow is often stable and water temperature reaches its peak for the year. Lower flow allows for longer water residence time which, when combined with warm temperatures, favors sestonic algal growth. Warm temperatures also limit oxygen solubility and the combined effects of large DO diel swings (because of algal photosynthesis and respiration), warm temperatures, and related factors serve to stress stream biota.

River flow during mid-late summer is often low and somewhat more stable as compared to spring and early summer flows (*e.g.*, Figure 14). The length of sonde deployment varied among the study-years: 5 to 8 days (mode = 6) in 2000; 12 to 15 days (mode = 15) in 2006; and 4-9 days (mode = 8) in 2008 (Table 12). In general, the summers of 2000 and 2006 were characterized by lower flows as compared to long-term norms (Figure 15). Summer 2006 flows were particularly low and stream flows during the actual time of sampling were below long-term daily means for most of the basins sampled (Figure 14). There was also a precipitation event at most sites during the two-week sonde deployment.

Table 12. Diel monitoring sites for 2000, 2006, and 2008 studies. Summary of dissolved oxygen (DO), pH, temperature, specific conductivity, and sonde deployment dates. Flux is based on daily max-daily min and is averaged based on days of deployment.

River/site		DO	mg/L		pH	SU		Temp.		C	Cond.	umhos	
2000	Diurnal dates	Min DO	Max DO	Mean Flux	Min pH	Max pH	Mean flux	Min.	Max.	Med.	Min.	Max.	Med.
CWR-70	8/16 - 8/22	5.8	10.5	4.3	8.0	8.8		18	24	20	290	302	298
CWR-35	8/16 - 8/22	6.5	9.5	2.5				17	23	20	374	394	389
UM-1056	8/10 - 8/15	6.2	7.5	0.5	8.1	8.3		22	25	24	285	295	290
UM-872	8/10 - 8/15	4.5	10.0	3.5	8.3	8.8		26	29	27	380	400	389
RUM-34	8/8 - 8/14	6.3	12.0	4.2	8.1	8.6		22	26	25	297	338	323
RUM-18	8/9 - 8/14	6.0	12.8	4.1	8.4	9.3		22	27	25	260	360	332
CR-23	8/9 - 8/14	5.5	13.0	5.1	8.1	8.8		23	28	26	590	680	651
CR-03	8/9 - 8/14	5.5	13.5	6.1	8.4	8.8		23	28	26	510	620	575
BE-73	8/3 - 8/7	6.5	16.0	6.7	8.0	8.5		22	25	23	530	630	590
BE-54	8/3 - 8/7	6.5	15.0	6.3	7.9	8.5		22	25	24	555	630	588
RE-536	8/15 - 8/22	7.0	9.0	1.4	8.2	8.4		19	26	22	400	650	547
RE-452	8/15 - 8/22	6.5	7.7	0.5	8.2	8.3		21	26	22	500	580	548
2006													
BF-46	7/26 - 8/9	6.1	10.4	2.4	8.2	8.8	0.2	21	29	24	264	297	277
LF-21	7/26 - 8/9	6.4	9.1	0.9	8.0	8.3	0.2	21	28	24	310	342	320
RL-1	7/25 - 8/8	5.1	8.2	1.1	7.9	8.4	0.2	23	27	25	284	297	289
RL-75	7/25 - 8/8	5.0	10.0	1.8	7.7	8.2	0.2	21	28	24	284	294	288
WI-3	7/25 - 8/8	6.3	9.1	1.6	8.3	8.5	0.1	22	32	26	546	612	590
WR-200	7/25 - 8/8	5.1	9.8	2.7	8.1	8.4	0.2	20	32	25	491	573	559
Buff-10	7/26 - 8/7	4.9	11.4	4.4	7.7	8.3	0.3	17	28	22	402	689	626
Buff-01	7/26 - 8/8	5.3	10.2	3.0	8.3	8.7	0.2	22	29	26	528	666	615
OT-1	7/26 - 8/7	6.2	10.9	2.5	8.3	8.8	0.2	23	32	27	408	467	428
UM-872	7/26 - 8/10	5.8	18.2	6.8	8.3	9.1	0.3	25	32	28	173	468	394
RUM-18	7/26 - 8/10	5.5	12.9	4.3	8.2	9.4	0.4	23	31	26	260	371	332
CR-23	7/27 - 8/9	4.0	16.4	6.5	7.9	8.9	0.5	24	32	28	493	685	612
2008													
S. Branch Root	8/21 - 8/28	8.4	13.8	3.8	7.7	8.2	0.2	14	21	17	587	608	599
N. Branch Root	8/21 - 8/28	7.7	13.4	4.1	7.7	8.1	0.2	16	23	19	482	586	575
Bear Creek	8/5 - 8/14	6.7	12.2	3.9	7.7	8.1	0.3	17	24	21	527	567	555
Vermillion River	8/11 - 8/20	7.2	10.6	2.5	7.9	8.3	0.2	15	25	19	520	597	587
Wells Creek	8/21 - 8/26	8.7	10.7	1.0	8.2	8.3	0.1	12	22	17	452	578	477
Maple River	8/5 - 8/13	6.7	13.5	4.7	8.2	8.8	0.4	20	28	24	436	547	502
Rice Creek	8/5 - 8/13	4.8	12.9	5.6	8.2	8.9	0.4	18	28	23	488	588	503
Big Cobb	8/5 - 8/13	6.4	11.7	4.0	7.9	8.6	0.3	20	29	24	489	512	523
Le Sueur	8/5 - 8/13	6.5	12.5	2.9	6.0	8.6	0.7	17	32	24	355	1010	515
Sauk	8/11 - 8/14	6.6	11.3	3.2	8.0	8.5	0.4	20	23	22	443	574	546
Getchell	8/11 - 8/14	2.2	7.2	3.2	7.7	8.2	0.3	20	23	22	595	631	611
Wells (repeat)	8/11 - 8/14	9.5	11.3	1.2	8.0	8.3	0.1						
Minimum	**2 days**	**2.2**	**7.2**	**0.5**	**6.0**	**8.1**	**0.1**	**12**	**21**	**17**	**173**	**294**	**277**
Maximum	**15 days**	**8.7**	**18.2**	**6.8**	**8.4**	**9.4**	**0.7**	**26**	**32**	**28**	**595**	**1010**	**651**
Median	**8 days**	**6.2**	**11.3**	**3.5**	**8.1**	**8.5**	**0.2**	**21**	**28**	**24**	**443**	**574**	**523**
25th %	**6 days**	**5.4**	**9.9**	**2.4**	**7.9**	**8.3**	**0.2**	**18**	**25**	**22**	**304**	**383**	**361**
75th %	**13 days**	**6.5**	**13.0**	**4.3**	**8.2**	**8.8**	**0.3**	**22**	**29**	**26**	**515**	**625**	**588**
Count	**35**	**35**	**35**	**35**	**34**	**34**	**23**	**35**	**35**	**35**	**35**	**35**	**35**

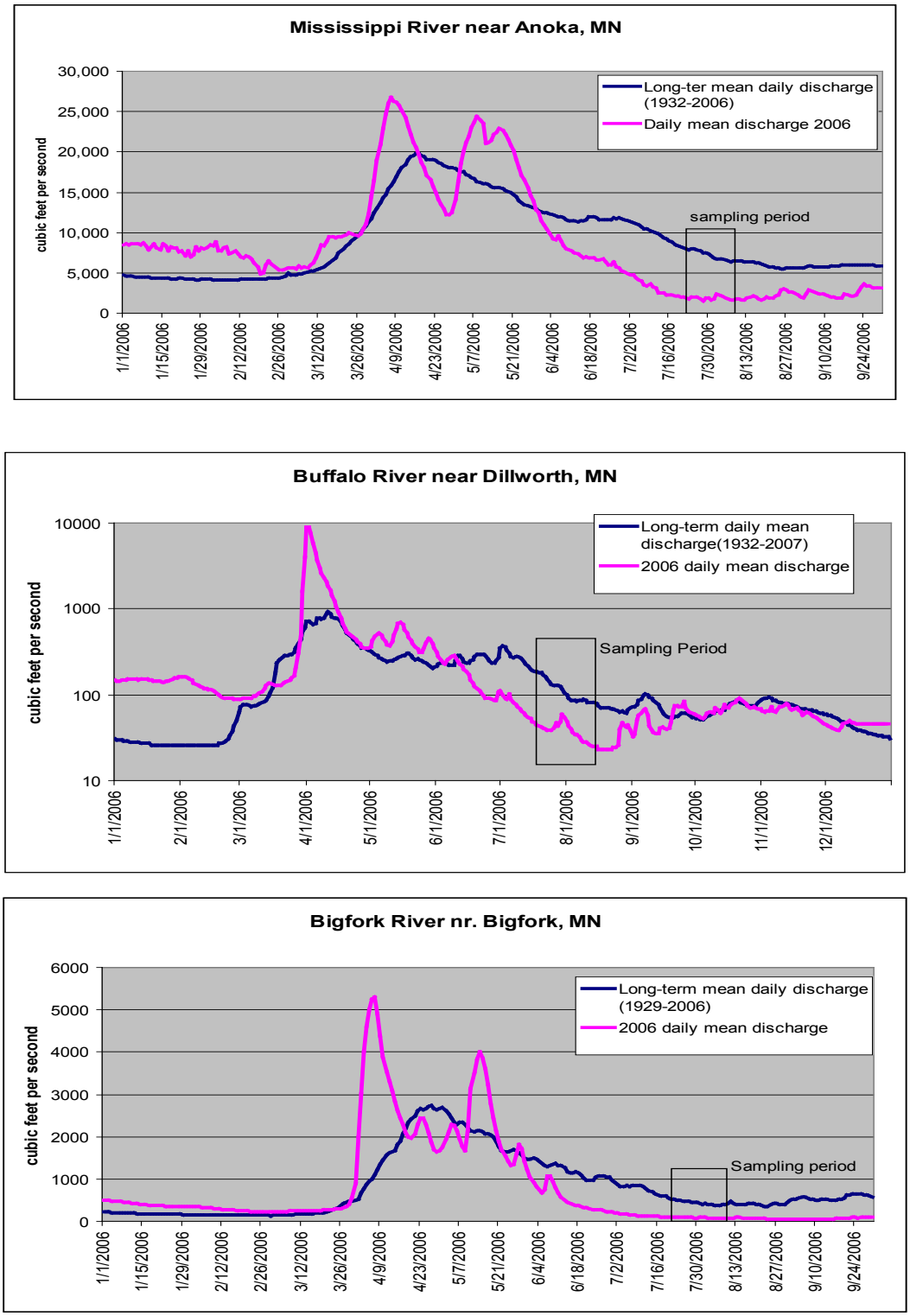


Figure 14. Comparison of long-term daily mean discharge and mean daily discharge during 2006 (from Lee 2008a).

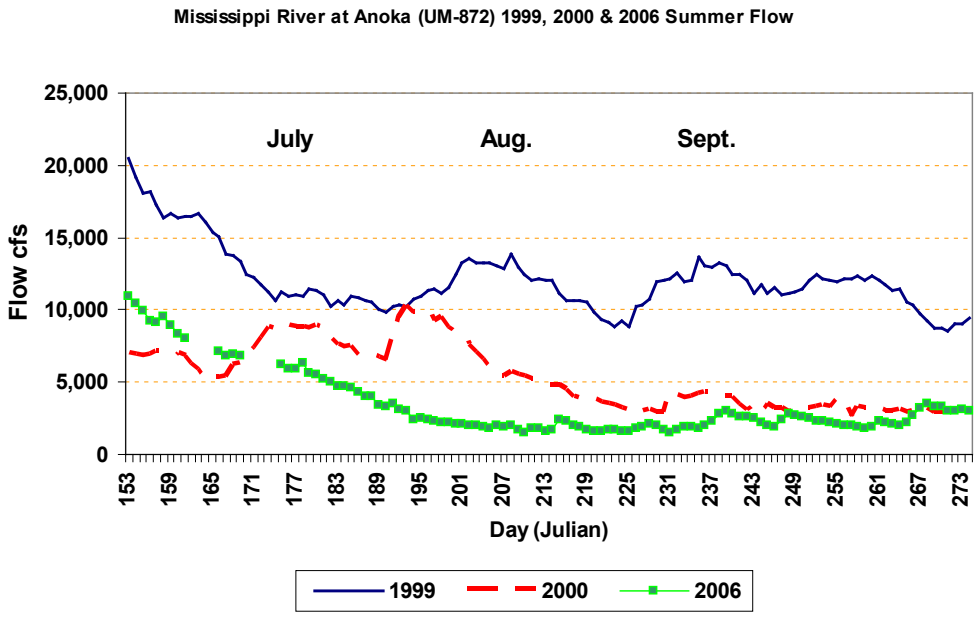


Figure 15. Comparison of summer discharge for 1999, 2000, and 2006 for Mississippi River at Anoka.

Diel variation of DO and pH typically show an increase during the day and a decrease during the night, having a sinusoidal pattern (Lee 2008a; Figure 16). This pattern is because of photosynthesis and respiration. The diel pattern for temperature is similar to DO and pH - it increases during the day and decreases at night. However, the seasonal effect of temperature on DO is different because temperature inversely controls the solubility of oxygen in water. When water temperature over the course of several days increases, DO decreases because it is less soluble. Although the deployment period was only two - three weeks in 2006, 9 of 12 sites showed a decrease in temperature over several days with a corresponding increase in DO. DO also has a direct relation with atmospheric pressure - as the pressure increases because of weather (or elevation) changes, oxygen solubility increases and therefore the DO concentration in the water increases.

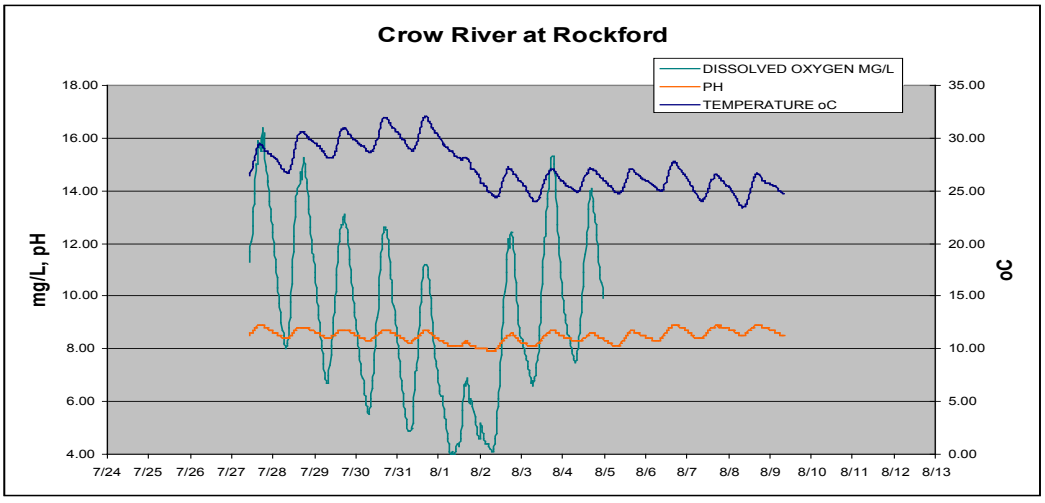


Figure 16. Variability in dissolved oxygen, pH, and temperature at the Crow River at Rockford, July 27 through August 9, 2006 (from Lee 2008a).

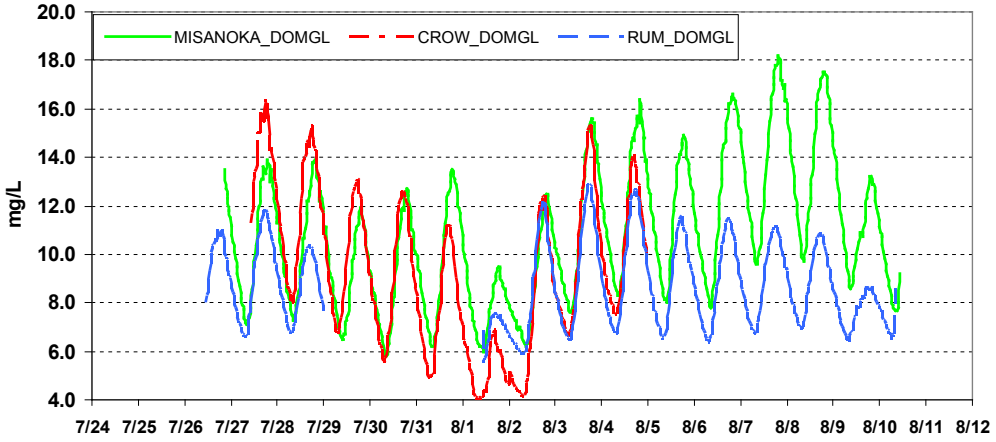
Differences among sites may also be attributed to variable stream size, reduced light availability (because of high turbidity or canopy cover), water temperature, residence time, nutrient inputs, and algal productivity. Physical characteristics, such as stream structure, may be partially responsible for the differences in DO. Summary data from the 35 sites instrumented in 2000, 2006, or 2008 provide a basis for characterizing the range of measurements and fluxes among this diverse set of streams (Table 12). In most instances, minimum DO remained above the 5 mg/L water quality standard; however five sites did exhibit minima below 5 mg/L – Mississippi (Anoka - UM-872), Buffalo (Buff-10), Crow (CR-23), Rice Creek and Getchell Creek. Getchell Creek was the lowest at 2.2 mg/L. The Crow River minima occurred on two consecutive days in August (Figure 17). Typical (defined as interquartile range – 25^{th}-75^{th} percentiles) minimums ranged from 5.4-6.5 mg/L. The maximum DO recorded was 18.2 at UM-872 and typical range was 9.9-13.0 mg/L. Mean daily flux (defined as daily maximum minus minimum, averaged over the deployment period) ranged from 0.5 (RE-452, Red River) up to 6.8 mg/L (UM-872). The typical range was 2.4 – 4.3 mg/L which is consistent with the range (~2-4 mg/L) reported by Ohio EPA (1996) for warmwater Ohio streams in mid-summer. They go on to note that variations greater than this range likely signify increased nutrient enrichment.

pH values generally remained in the circumneutral range for these well-buffered streams. The lowest pH recorded was 6.0 in the Le Sueur, which was a bit of an anomaly as the next lowest value was 7.7. Only two measures exceeded the water quality standard of 9.0 and these were on the Rum (RUM-18, pH=9.4) and Mississippi (UM-872, pH=9.1). The typical range for minima, maxima, and pH flux were 7.9-8.2, 8.3-8.8, and 0.2-0.3.

The typical range for minimum and maximum temperature was 18-22 and 25-29 ºC, respectively. The lowest temperature measured was 12 ºC on Wells Creek and the highest was 32 on the Le Sueur. Median stream temperatures were typically in the 22-26 ºC range with the exception of some of the coolwater streams, *e.g.*, South and North Branch Root, Wells, and Vermillion with medians ranging from 17-19 ºC. Specific conductance values generally ranged between about 300-600 µmhos/cm on most of the streams.

2. Annual Variation in Diel Dissolved Oxygen Flux in the Crow, Rum and Mississippi Rivers

The Mississippi, Crow, and Rum sites provide an opportunity to assess DO flux variability among rivers and among summers (2000 and 2006) to further understand how this measurement varies over space and time. Some similar patterns were evident among the three rivers in 2006 (Figure 17), though the relative magnitude of DO flux varied. The Crow and Mississippi were the most variable with daily DO flux ranging from about 2-3 mg/L up to 9 mg/l. In contrast, the Rum was less variable ranging from 2–6.5 mg/L (Figure 17).



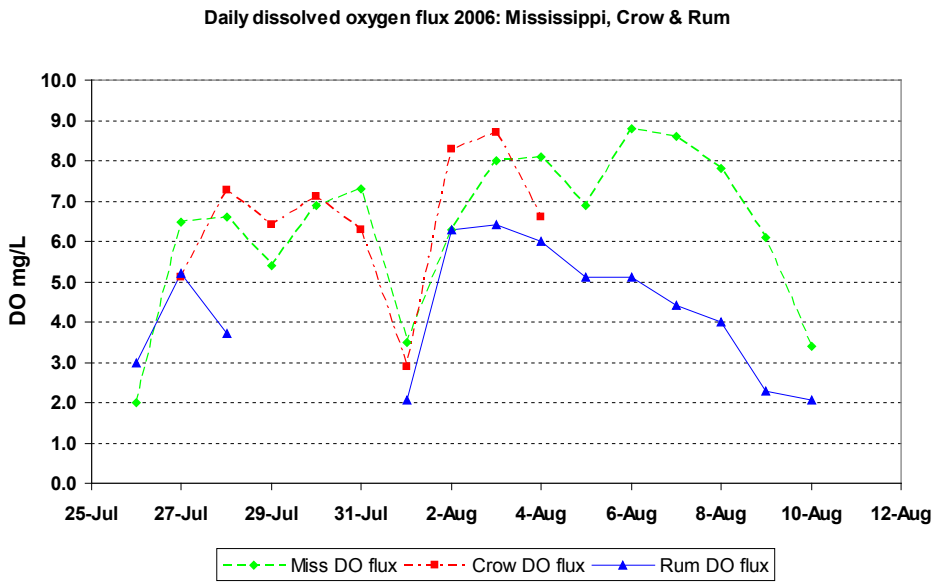


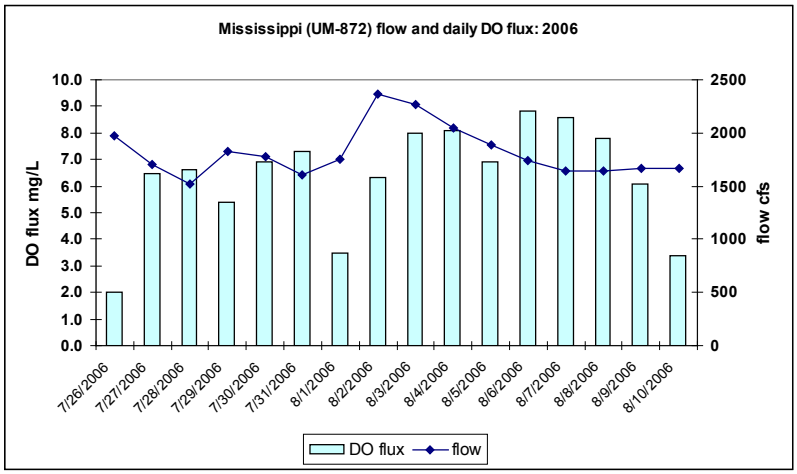
Figure 17. Daily DO and DO flux (max-min) for the Mississippi, Crow, and Rum Rivers: 2006

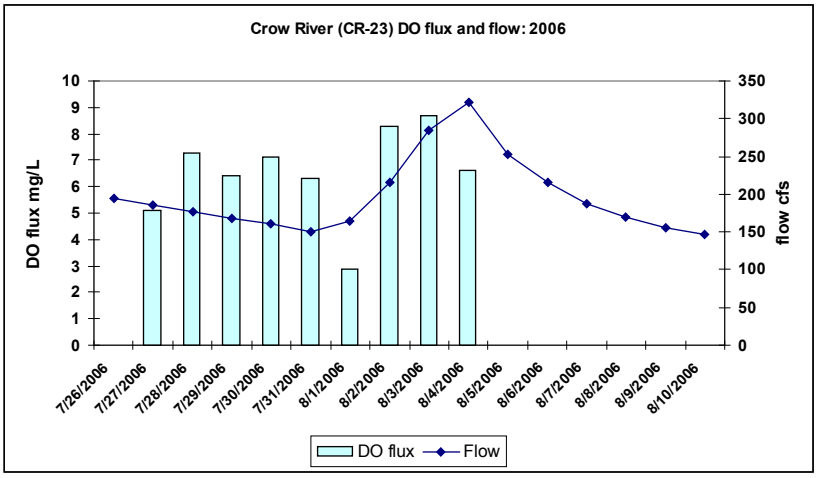
All three rivers exhibited a distinct decline in DO flux from July 31 to August 1, followed by a marked increase (Figure 17). For the Rum and Crow, this corresponded to a decline in flow in late July, followed by a marked increase in flow from about August 1-3 in response to rain events during the July 31–August 2 timeframe (Figure 18). An increase in flow was evident in the Mississippi as well over this period. The increased flows corresponded to reported rainfall of 1.2 inches or more at various locations in these three watersheds.

While DO flux can vary among sites, it may also vary among years at the same site (Table 13). Daily mean flux was slightly higher in 2006 as compared to 2000 for the three sites assessed. The overall range was quite variable with 2006 values for the Mississippi and Crow being much higher than the range for 2000. Based on 2006 data, average daily flux did not change substantially if a consistent four-day (96 hour) record was used for all three sites as compared to the entire record for the 2006 measurement period. These results suggest the need for a common calculation method when the term "DO flux" is determined. In the course of our studies and data analysis, DO flux is determined based on the average of all daily flux measures for each site, rather than simply the overall range (maximum-minimum) of all DO measurements for the measurement timeframe.

Table 13. Comparison of DO flux and range for Mississippi, Crow, and Rum River sites for 2000 and 2006. Includes average of daily mean flux and overall range of flux for measurement period. For 2006, average daily mean based on four days when all three sites measured (see Figure 11).

Year	DO flux measure (mg/L)	Mississippi	Crow	Rum
2000	Daily mean	3.5	5.1	4.1
	Overall range	5.5	7.5	6.8
2006	Daily mean	6.4	6.5	4.3
	Overall range	12.4	12.4	7.4
	Daily mean (4-day)	6.5	6.6	5.2





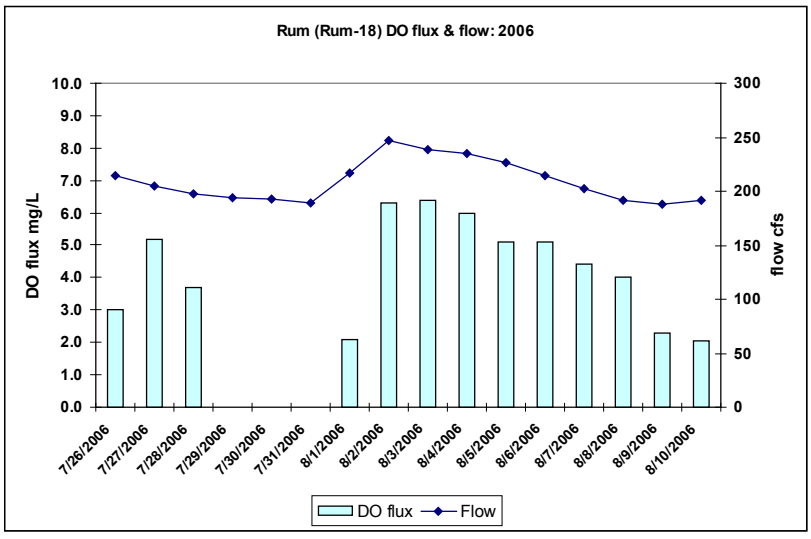


Figure 18. Daily DO flux and flow for Mississippi, Crow, and Rum Rivers: 2006

B. Water Quality Patterns and Relationships: River Nutrient Study

Previous reports have clearly established the relationships among nutrients, chlorophyll-a and flow (Heiskary & Markus 2001, 2003, Heiskary 2008). The majority of this analysis has been based on monitoring conducted in 1999, 2000, and 2006 at what we refer to as the river nutrient study sites. These data were augmented by water quality collections in 2001 at several independent sites (Heiskary & Markus 2003), a retrospective analysis of previously collected USGS data to provide additional data to consider in this analysis (summarized in Lee [2008b]), and most recently by monitoring in summer 2008. Summer-mean water quality data for the 1999, 2000, 2001, 2006, and 2008 studies is summarized in Table 14. Details on sample collection, number of samples, and related information is included in the previous publications on those studies (noted above). In general, we sought 6-8 samples from each station per summer. A summary of sample collection, by year for the sites in Table 14 follows: 1999 5-7 samples, 2000 7-8 samples, 2001 7-8 at most sites with a minimum of 3, 2006 6-7 samples, and 2008 quite variable with 2-18 samples (only 2 samples at S000-284 Sauk and S003-289 Getchell).

Table 14. Summer-mean data from Minnesota river-nutrient study for: 1999, 2000, 2001, 2006, and 2008.

STATION	Year	TP	TKN	NO3	TN	Chl-a	Pheo	ChlT	BOD	TSS	TSV	TSIN	Turb	T-tube
		ug/L	mg/L			ug/L			mg/L				NTU	cm
CWR-72.3	1999	32	0.58	0.21	0.80	3.1	1.4	4.5	1.0	4	1	2	3	60
CWR35.5	1999	59	0.77	0.22	0.99	2.4	1.9	4.2	1.0	6	3	3	4	60
UM-1004	1999	71	0.81	0.09	0.90	4.5	3.8	8.3	1.1	23	3	20	18	25
UM-965.4	1999	63	0.75	0.14	0.89	4.0	3.9	7.8	1.2	15	3	13	12	40
UM-953.7	1999	62	0.72	0.15	0.15	4.4	2.9	7.2	1.0	13	3	10	12	37
UM-895	1999	67	0.76	0.20	0.97	5.2	4.5	9.7	1.2	13	3	10	9	48
UM-872	1999	92	0.88	0.38	1.26	15.6	6.6	22.2	1.5	19	5	14	12	37
RUM-34	1999	137	1.14	0.27	1.41	13.3	6.6	19.9	1.6	17	4	13	10	46
RUM-18	1999	131	1.11	0.26	1.37	18.8	7.9	26.7	1.8	16	5	11	8	49
CR-23	1999	359	2.06	2.11	4.17	83.4	21.2	104.6	4.5	73	18	55	53	12
CR-0.2	1999	329	1.92	1.86	3.79	74.1	19.6	96.8	4.0	75	17	58	49	15
BE-94.3	1999	247	1.18	7.64	8.82	29.1	12.0	41.1	2.1	125	18	107	59	15
BE-73.2	1999	243	1.55	6.28	7.84	47.7	14.7	62.3	3.6	110	18	92	57	13
BE-54	1999	248	1.47	6.41	7.88	64.4	17.0	81.3	3.4	126	20	106	68	10
BE-18.2	1999	240	1.44	6.61	8.06	57.6	16.1	73.7	3.4	135	21	114	68	13
CWR-72.3	2000	34	0.78	0.12	0.90	3.4	1.5	4.9	1.2	3	2	1	3	>60
CWR-35.5	2000	49	1.19	0.23	1.41	3.7	1.7	5.4	1.2	6	3	3	3	>60
UM-1056	2000	59	0.72	0.07	0.79	4.7	2.1	6.9	1.1	19	3	17	12	53
UM-1029	2000	60	0.74	0.08	0.82	5.1	2.7	7.8	1.0	22	3	19	14	42
UM-953.7	2000	54	0.72	0.11	0.83	7.6	4.8	12.4	1.3	9	2	7	8	53
UM-895	2000	77	0.82	0.19	1.01	11.0	6.2	17.1	1.6	11	3	8	7	53
UM-872	2000	84	0.93	0.23	1.16	22.7	6.4	29.2	2.1	16	5	11	9	47
RUM-34	2000	143	0.85	0.28	1.13	20.5	7.2	28.7	1.8	11	4	7	6	56
RUM-18	2000	133	0.97	0.18	1.16	31.4	10.7	43.9	2.3	11	5	6	6	52
CR-23	2000	349	1.94	1.69	3.63	120.3	25.5	142.6	6.6	75	18	57	41	13
CR-0.2	2000	284	1.98	1.56	3.53	112.4	26.6	135.7	7.0	64	18	46	32	15
BE-100	2000	116	0.57	7.18	7.75	6.4	4.8	10.6	1.1	35	5	30	16	42
BE-94.3	2000	192	1.14	6.11	7.24	41.8	6.7	47.6	2.7	61	11	50	31	23
BE-73.2	2000	205	1.63	5.55	7.18	87.4	15.4	100.9	5.1	74	15	59	41	17
BE-54	2000	207	1.60	5.35	6.95	86.7	11.5	96.8	6.3	91	18	73	46	15
BE-18.2	2000	223	1.63	5.37	7.00	73.1	10.2	82.0	5.3	108	19	89	57	15
RE-536	2000	208	1.18	0.25	1.43	18.9	8.3	27.2	2.8	55	9	46	27	22
RE-452	2000	312	1.48	0.25	1.73	23.2	14.5	37.7	2.1	144	19	125	69	14
RE-403	2000	602	2.84	0.59	3.43	16.4	21.5	37.9	4.7	374	46	329	151	7
RE-298	2000	502	1.73	0.60	2.33	10.4	15.1	25.5	2.9	324	33	292	148	7

Table 14 (continued). Summer-mean data from Minnesota river-nutrient study for: 1999, 2000, 2001, 2006, and 2008.

STATION	Year	TP	TKN	NO3	TN	Chl-a	Pheo	ChlT	BOD	TSS	TSV	TSIN	Turb	T-tube
		ug/L	mg/L			ug/L			mg/L				NTU	cm
BE-0	2001	229	1.29	6.69	7.98	50.3	5.9	56.1	3.5	98	14	84	50	16
BE-54	2001	393	1.55	4.42	5.97	69.9			4.2	82	16	66	47	15
BR-3	2001	245	1.22	0.30	1.52	11.4	4.7	16.0	2.4	112	16	96	73	12
CA-13	2001	155		4.38	4.38	14.3	5.5	19.8	2.0	13	3	10	6	55
CO-0.5	2001	205	1.26	6.07	7.33	56.9	10.8	67.7	3.9	90	16	74	43	18
CR-23	2001	296	1.61	1.04	2.65	58.8	17.6	76.4	4.3	53	13	40	29	17
CR-44	2001	436	2.05	2.68	4.73	79.2	22.7	101.9	6.2	54	15	38	30	18
CRN-2.33	2001	262	1.44	0.38	1.82	53.0	10.9	63.9	3.8	61	13	48	34	17
CWR-72.3	2001	33	0.58	0.24	0.82	2.2		2.2		3	2	2		60
EDM-6	2001	201		9.17	9.17	32.3	8.6	40.9	3.2	58	15	43	57	30
MI-212	2001	206	1.50	0.55	2.06	36.5	10.2	46.7	3.0	52	10	42	23	26
MU-0	2001	355	1.62	0.41	2.04	17.4	7.0	24.5	3.0	27	6	21	24	23
OT-49	2001	75	0.97	0.05	1.02	18.6	4.7	23.3	2.4	10	4	6	7	72
RUM-18	2001	105	0.95	0.21	1.16	9.9	3.9	13.8	1.5	9	3	6	6	55
RWR-1	2001	253	1.09	3.42	4.51	41.2	12.9	54.2	2.3	40	9	31	27	21
SL-21	2001	27	0.64	0.06	0.70	4.3	1.7	6.0	1.0	6	2	4	5	89
ST-18	2001	382		6.90	6.90	8.7	2.7	11.4	1.2	19	4	14	11	45
UM-872	2001	76	0.87	0.32	1.18	15.6	4.4	20.0	1.7	14	4	10	8	47
WA-6	2001	373	1.27	7.98	9.25	35.0	3.5	38.5	2.9	55	10	45	26	27
WDM-3	2001	235		3.25	3.25	154.4	19.0	173.4	9.3	55	19	36	35	19
WI-3	2001	166	1.05	0.09	1.14	9.4	1.9	11.3	1.7	94	11	83	56	14
BF-46	2006	19	0.63	0.01	0.64	1.1	0.4	1.4		1	1	0	2	
LF-21	2006	20	0.52	0.01	0.53	1.1	0.5	1.3		2	0	2	5	98
OT-1	2006	129	1.01	0.06	1.07	20.4	6.3	26.7	1.2	69	9	61	36	20
WR-200	2006	43	0.55	0.02	0.57	2.3	1.9	3.7	0.9	13	3	10	9	52
WR-1	2006	123	0.72	0.02	0.74	11.9	3.8	15.7	1.1	72	7	69	62	13
RL-1	2006	40	0.81	0.01	0.82	2.8	0.9	3.5	0.7	5	2	4	5	64
RL-75	2006	36	0.78	0.01	0.79	2.2	1.0	3.1	0.7	5	2	4	4	68
BUFF-01	2006	205	1.42	0.02	1.43	50.0	8.0	58.0	2.3	67	16	59	56	13
BUFF-10	2006	127	1.11	0.20	1.31	22.7	5.1	27.8	1.5	13	6	8	14	46
UM-872	2006	101	1.05	0.28	1.33	38.6	13.5	52.1		14	6	8	9	47
CR-23	2006	258	2.04	0.26	2.29	148.1	51.4	199.5		76	21	55	38	16
CR-44	2006	386	2.17	1.46	3.63	121.1	33.3	154.5		63	19	44	31	19
CRN-6	2006	218	1.84	0.10	1.94	128.3	35.9	164.2		78	20	58	43	13
RUM-18	2006	133	0.92	0.25	1.17	32.6	14.8	47.5		11	5	6	8	51
S. Branch Root	2008	97	0.48	7.63	7.15	2.0	3.2	5.2		35	4	32		59
N. Branch Root	2008	102	0.56	5.89	6.45	2.5	2.9	3.9		32	5	26		47
Vermillion	2008	100	0.58	3.68	3.10	10.0	3.2	13.2		12	4	8		82
Bear Creek	2008	59	0.48	8.01	7.53	3.1	2.0	5.1		15	2	13		56
Maple	2008	192	1.50	10.17	8.66	4.4	3.6	8.0		110	12	98		24
Rice Creek	2008	138	0.87	4.81	3.94	4.1	3.6	7.7		30	5	25		27
Big Cobb	2008	168	1.14	8.82	7.68	35.9	3.4	39.3		95	10	84		20
Le Sueur	2008	171	1.13	8.92	7.79	29.6	3.2	32.8		123	12	111		26
Sauk	2008	129	1.91	3.48	1.56	30.1	8.8	38.9		14				50
Getchell	2008	432	2.52	3.29	0.78	25.9	9.9	35.9		9				83

1. Relationships among Nutrients, Chlorophyll, and DO Flux

This section of the report describes relationships in various variables across the entire set of rivers, sites, and years. It includes not only relationships among nutrients and chlorophyll but also linkages among biotic and physical data. These interrelationships provide a foundation upon which nutrient criteria for rivers can be developed. In most instances, relationships (*e.g.,* based on linear regression) are defined based on the initial 1999 and 2000 study data. Data collected in studies since that time (*i.e.,* 2001, 2006, and 2008) provide a basis for "testing" the applicability of these relationships to independent data sets. This provides a sense as to how well the regressions perform and the range of river-types where these relationships may be applied. In general, relationships between water quality variables (*e.g.*, phosphorus, chlorophyll, etc.) should be similar across Minnesota although other factors may differentially influence these relationships in different stream classes. For example, northern Minnesota streams are more likely to be shaded which can reduce the amount of chlorophyll produced. However, if there is limited shading northern streams would be expected to produce algal blooms leading to degradation of biological condition. Similarly, smaller streams are more likely to be shaded and therefore less likely to have a decline in biological condition resulting from eutrophication.

The TP and Chl-a relationship for medium to large Minnesota rivers is highly significant but is different from the previously established relationship for lakes (Figure 19a). While both exhibit a high R^2 the lake relationship indicates that lakes produce greater Chl-a per unit TP than do rivers. For example, at a TP of 100 µg/L the predicted Chl-a for lakes is ~50 µg/L, whereas for rivers it is ~25 µg/L. The 95% confidence interval (CI) for lakes is slightly smaller than that for rivers. However, in terms of the 95% prediction interval (PI) the lake and river equations are relatively similar (Figure 19b).

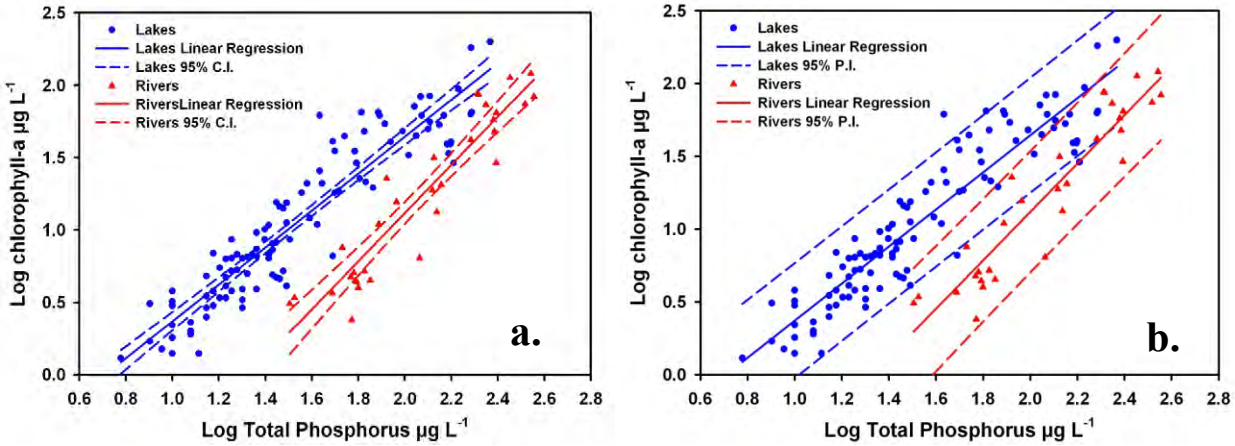


Figure 19. Total phosphorus and chlorophyll-a relationships for Minnesota rivers as compared to lakes. (Confidence interval (C.I.) and 95% prediction interval (PI) noted for each regression equation; lake equation from Heiskary and Wilson (2008); lakes: n=108, rivers: n=31).

Previous studies (Heiskary & Markus 2001, 2003) demonstrated significant (F-test < 0.001), consistent and positive relationships between TP and sestonic chlorophyll in Minnesota rivers based on sites monitored in 1999 and 2000 (excluding the Red River). The slope for the 2000 data appeared steeper, as compared to the 1999 regression, but was not significantly different (95% confidence level). The significant relationship between TP and chlorophyll is consistent with a worldwide study conducted by Van Nieuwenhuyse and Jones (1996) and a Canadian study by Basu and Pick (1996). In each of these studies linear regressions (log-log) of TP and total chlorophyll exhibited significant R^2 values of 0.72 and 0.76, respectively. These studies also prompted our initial emphasis on total chlorophyll (Chl-T) rather than Chl-a (e.g. Heiskary & Markus 2001). However, in all instances Chl-a and pheophytin were both measured, which allowed us to use either Chl-a or Chl-T in our analyses. As noted in subsequent discussion, we did shift emphasis to Chl-a and graphics and statistics were re-run as necessary to support criteria development.

The regression equations summarized below were derived based on MPCA's 1999 and 2000 river nutrient studies (Heiskary & Markus 2001). Given the geographic spread of these sites (Figure 3), their representation of targeted stream size (essentially 4^{th} – 7^{th} order), and range in flow years (Figure 14) these equations were felt to provide a

sound basis for predicting relationships among nutrients, chlorophyll, and BOD_5 for medium to large Minnesota streams.

Regression equations:
Chl-T (µg/L) = 0.39 • TP (µg/L) – 14.84 R^2**=0.84 (Eq. 1)**
BOD_5 (mg/L)= 0.042 • Chl-T (µg/L) + 0.74 R^2**=0.94 (Eq. 2)**

The expansion of our datasets since the original studies of 1999 and 2000 and some of the observed variability led us to include data from all summers where river nutrient-related monitoring had been conducted in support of this effort including: 1999, 2000, 2001, 2006 and 2008 (Table 14), in an attempt to develop a more robust relationship between TP and chlorophyll (Figure 20). Data were log-transformed because the data were not normal and had unequal variance. Linear regression and prediction intervals were developed based on log transformed data for nonwadeable rivers (Figure 20), which were the primary focus for river nutrient criteria development. With this analysis (Figure 20) and all subsequent analyses, we shifted emphasis to Chl-a as the parameter to be used for data analysis and criteria development rather than Chl-T. Chl-a is a measure of the viable or living fraction of the chlorophyll and is more routinely used in eutrophication assessment, which allowed for more consistent linkage with previous work on lakes, e.g. Figure 19 and Minnesota's promulgated lake standards.

Chl-a µg/L = $10^{\wedge((1.47 \cdot \log(\text{TP ug/L}) - 1.82)}$ R^2**=0.81 (Eq. 3)**

The resulting regression (Eq. 3) is somewhat problematic as when it is back transformed as it predicts that Chl-a continues to increase with increasing total phosphorus. In reality, Chl-a would be expected to plateau at some concentration of phosphorus since there are limitations to the amount of Chl-a that can be produced in a system. A 50th percentile smoothing splines quantile regression fit was used to characterize the relationship between total phosphorus and Chl-a (Figures 21 and 22). In addition, smoothing splines quantile regression was also fit to the data to create a 90% prediction interval. From this prediction interval it is apparent that uncertainty in this relationship increases as total phosphorus increases. This regression provides further basis for understanding factors that contribute to variability in this relationship. Flow, for example, has been demonstrated to have an impact on sestonic Chl-a (Heiskary & Markus 2001). Examination of LOESS regression lines from different years indicates an effect of discharge (Figure 23). These five summers break out as follows relative to flow: 1999 and 2001 high flow (near 75th percentile for Mississippi at Prescott); 2000 and 2008 average to low flow (25th-30th percentile for Mississippi at Prescott); and 2006 very low flow (10th percentile for Mississippi at Prescott). Three other sites, all monitored in 2006, that were quite high included: North Fork Crow (CRN-6), Crow mainstem (CR-23) and Mississippi (UM-872), which is near Anoka and just downstream from the Crow (Figure 23). In this case extremely low flows during summer 2006 (near 5th percentile for Mississippi at Anoka) allowed for increased residence time in these river reaches, which favors sestonic algal production (Figures 15 and 23). The amount of sestonic chlorophyll tends to be greater in drier years or years with lower flows. In some cases, higher discharge appears to be responsible for relatively low levels of total chlorophyll. Some nonwadeable sites monitored in 2001 exhibited very low Chl-a relative to TP including Watonwan (WA-6), Mustinka (MU-0), Buffalo (BR-3), and Wild Rice (WI-3). A combination of high flow and high TSS (Wild Rice 94 mg/L and Buffalo 112 mg/L) contribute to the low Chl-a observed at these sites. Regardless of discharge, most sites with TP greater than 150 µg L^{-1} have sestonic chlorophyll levels above 40 µg L^{-1} indicating that annual variation in discharge only has a moderate effect on the levels of total chlorophyll in nonwadeable rivers. In any case, the moderating effects would only occur in high flow years and violations of standards would still be expected to occur in average and low flow years.

Other factors that cause variation in the relationship between phosphorus and chlorophyll include turbidity, stream size, and anomalous features (*e.g.,* impoundments). One observation from Figure 20 is that all wadeable sites fall on or below the regression line. For those below the regression line this implies they produce less sestonic Chl-a per unit TP as compared to nonwadeable (larger, higher order) sites. As noted previously, algal production in these shallow, low order sites is in the form of periphyton rather than seston. Several wadeable sites *e.g.* Maple, Straight (ST-18), and Getchell Creek exhibited Chl-a below the prediction interval (Figure 21) indicting very low Chl-a per unit TP. At the upper end of the prediction interval there were a few sites that exhibited extremely high Chl-a per unit TP. One site with extremely high chlorophyll-a (175 µg/L, Figure 21) relative to TP was on the West Des Moines River (WDM-3). This site had a small but highly eutrophic reservoir upstream of it, which may have contributed to the high chlorophyll-a concentration (exported algal downstream). Other effects on chlorophyll noted

previously by Heiskary and Markus (2003) include the extremely turbidity. For example the very nutrient-rich Red River main-stem sites often do not have high levels of sestonic chlorophyll due to the high turbidity.

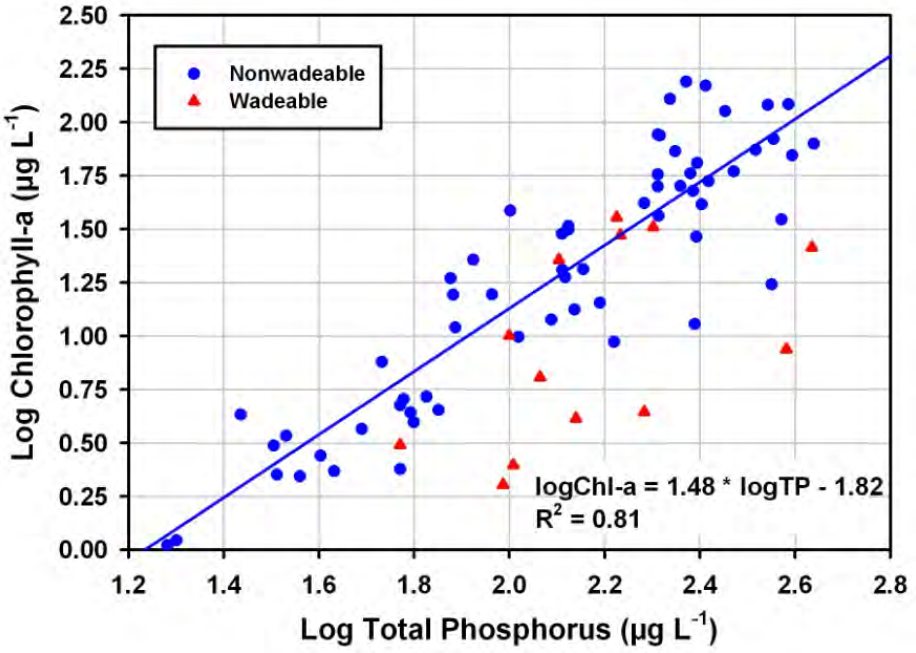


Figure 20. Relationship between log transformed TP and chlorophyll-a for River Nutrient Study data. (least squares regression line based on nonwadeable river sites; nonwadeable streams: n=63; wadeable streams: n=13).

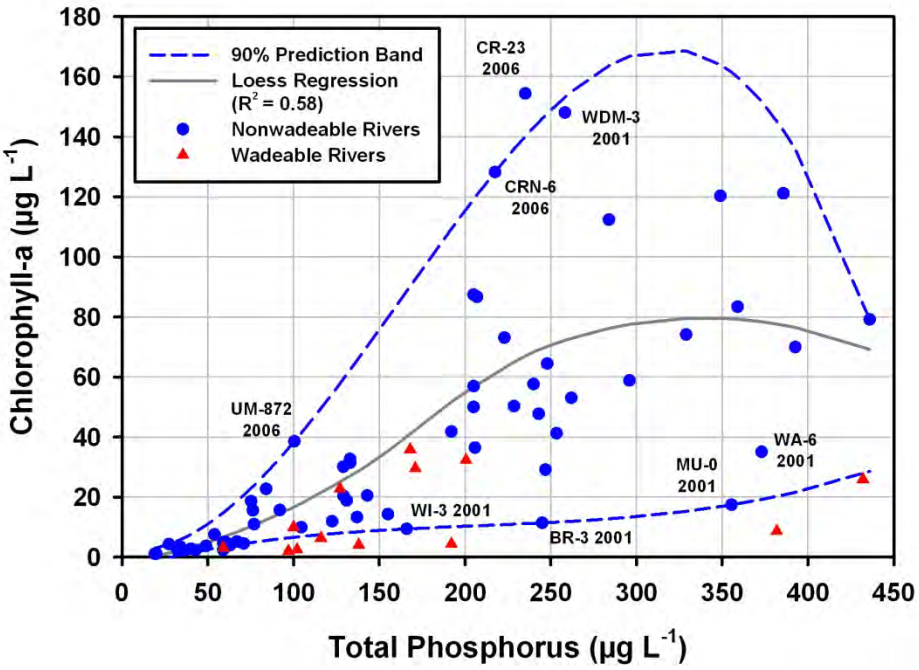


Figure 21. Relationship between TP and chlorophyll-a for River Nutrient Study data. The regression fits were based nonwadeable streams only using a LOESS regression and the 90% prediction interval was estimated using the 95th and 5th quantile smoothing splines regression lines (nonwadeable streams: n=63; wadeable streams: n=13).

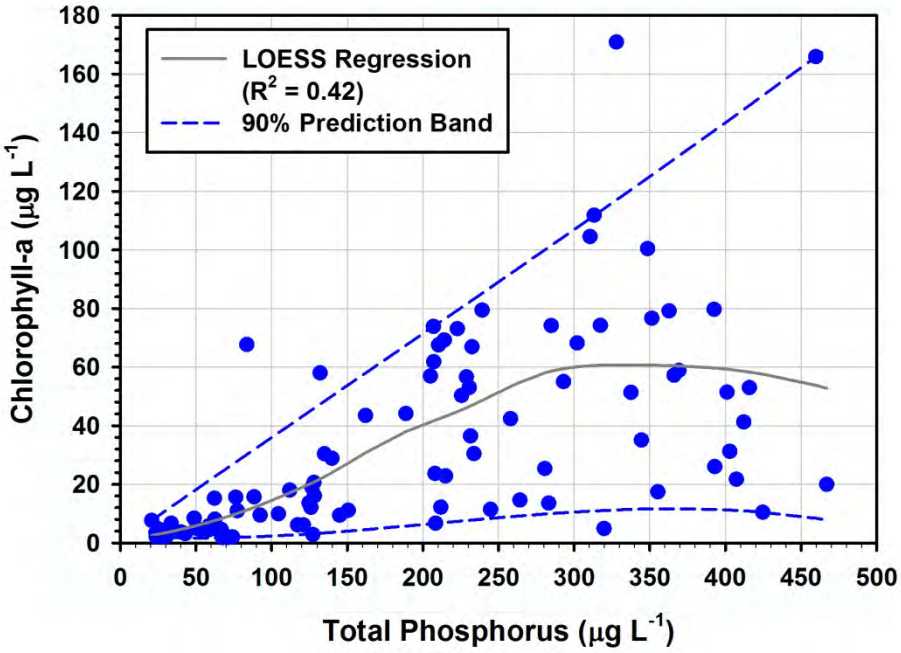


Figure 22. Relationship between TP and chlorophyll-a for STORET data (1990-2012). The regression fits were based nonwadeable and wadeable streams using a LOESS regression and the 90% prediction interval was estimated using the 95th and 5th quantile smoothing splines regression lines.

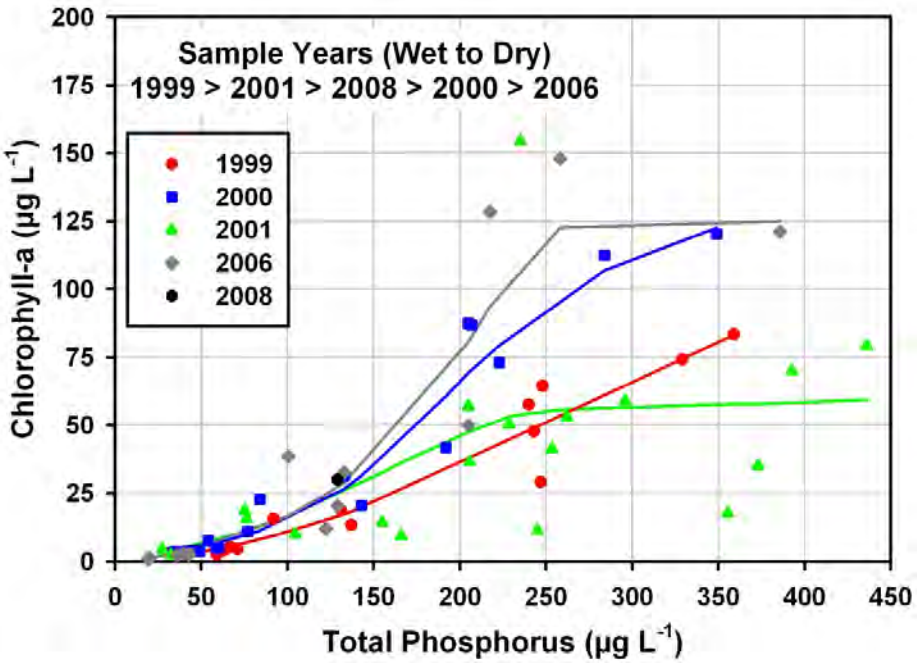


Figure 23. Relationship between total phosphorus and chlorophyll-a in nonwadeable streams for 1999, 2000, 2006, and 2008 River Nutrient Study data with loess regression (R^2: 1999 = 0.93 [n=15], 2000 = 0.95 [n=15], 2001 = 0.33 [n=19], 2006 = 0.91 [n=13]).

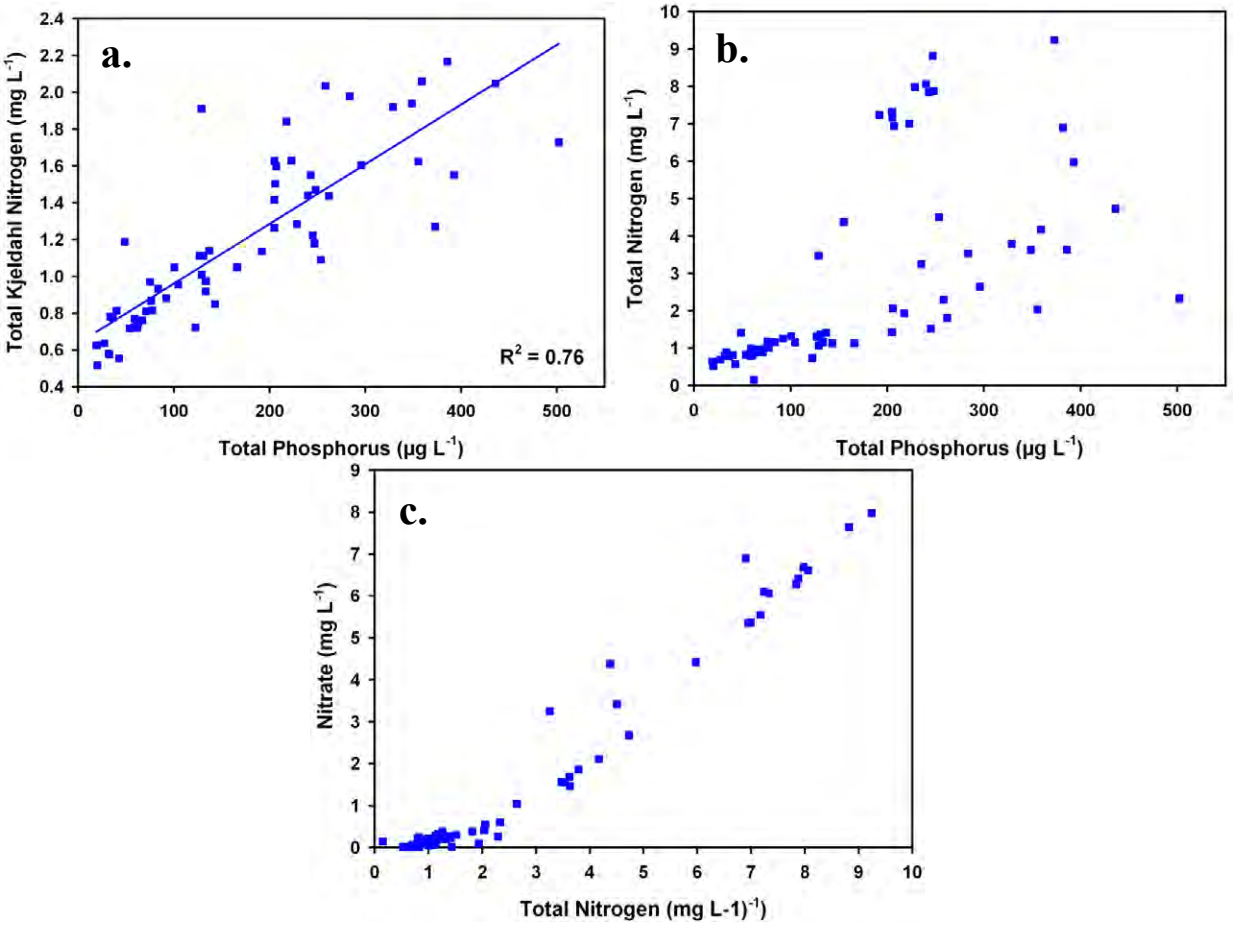


Figure 24. Relationship among a) TP and TK, b) TP and TN, and c) nitrate-N and TN based on River Nutrient Study data (watersheds > 500 mi2, Red River sites removed; a) n = 63, b) n= 65, c) n=66).

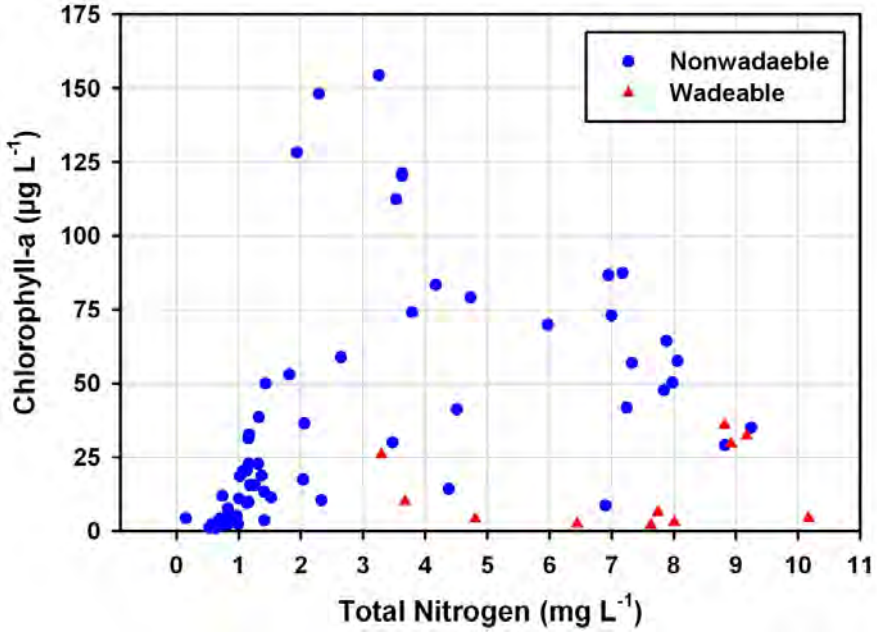


Figure 25. Relationship between total nitrogen and total chlorophyll-a using data collected as part of the River Nutrient Study (nonwadeable streams: n=66; wadeable streams: n=11).

TP and TKN are highly correlated based on the River Nutrient data (Figure 24a). This was anticipated since sestonic algae comprise much of the organic N in the TKN measurement. TP and TN are not highly correlated (Figure 24b) however, because nitrate N accounts for much of the TN as TN exceeds 2-3 mg/L (Figure 24c). A significant linear relationship between TKN and Chl-a was previously noted based on the 1999 and 2000 River Nutrient data (Heiskary & Markus 2001). There was no linear relationship between TN and Chl-a based on the combined 1999, 2000, 2006 and 2008 data (Figure 25). As noted in previous reports (Heiskary & Markus 2001, 2003) this is primarily because of elevated nitrate-N, which contributes to the elevated TN (Figure 24c). In general, based on the sites in Table 14 and Figure 24c, TKN is the majority of TN at concentrations less than about 1.5-2.0 mg/L. As TN increases above 2.0 mg/L nitrate-N becomes an important contributor to TN and often exceeds TKN concentration when TN exceeds ~3-4 mg/L. The lack of relationship between TN and chlorophyll is particularly evident in wadeable streams and is a function of low sestonic chlorophyll and lush supplies of dissolved nitrate-N in these systems. In general, elevated nitrate-N is found primarily in the highly drained watersheds of the WCBP ecoregion (Table I - 1), which would include the Blue Earth, Maple, Straight and East Des Moines Rivers in our River Nutrient dataset (Table 14).

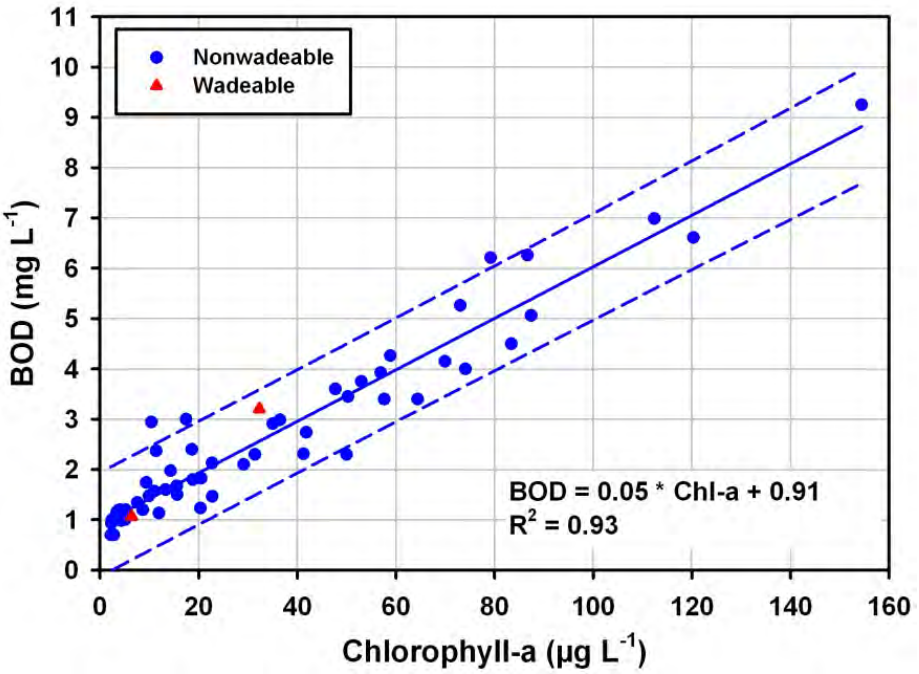


Figure 26. Relationship between chlorophyll-a and BOD_5 using data collected as part of the River Nutrient Study (least squares regression lines based on nonwadeable river sites with 95% prediction intervals; nonwadeable streams: n=57; wadeable streams: n=2).

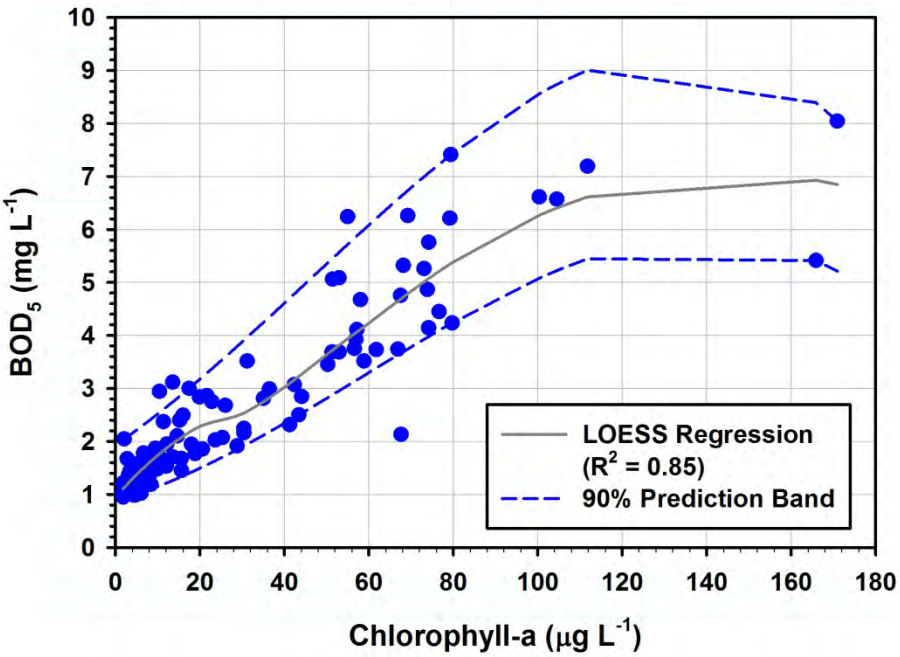


Figure 27. Relationship between chlorophyll-a and BOD_5 using STORET data (1990-2012). The regression fits were based nonwadeable and wadeable streams using a LOESS regression and the 90% prediction interval was estimated using the 95th and 5th quantile smoothing splines regression lines.

The relationship between BOD_5 and chlorophyll-a was highly significant (Figures 26 and 27). The relationship in Figure 26 is characterized by the following equation:

BOD_5 (mg L^{-1}) = 0.05 • Chl-a (µg/L) + 0.91 R^2=0.93 (Eq. 4)

Data from wadeable sites was not used in the model but they are shown in Figure 26. However, due to the small sample size and the narrow range of measurement, few conclusions can be made regarding this relationship in wadeable streams. This strong relationship is due to increased productivity in these systems which make more organic matter available to heterotrophs (*e.g.*, bacteria). For example increased algal growth resulting from phosphorus inputs creates greater organic matter available to bacteria as the algae dies. A strong relationship is also apparent between total phosphorus and BOD_5 (Figures 28 and 29) although this relationship is weaker than the chlorophyll-a-BOD_5 relationship. This weaker relationship is in part due the variability in the TP-chlorophyll-a relationship discussed above. However, the TP-BOD_5 is important because BOD_5 is not driven solely by sestonic chlorophyll. Microbes (e.g., fungi and bacteria) and benthic algae can also be important sources of BOD_5. This is more important in wadeable stream and helps to explain why the wadeable streams in Figure 26 respond similarly to the nonwadeable streams despite producing less sestonic chlorophyll (see Figure 21).

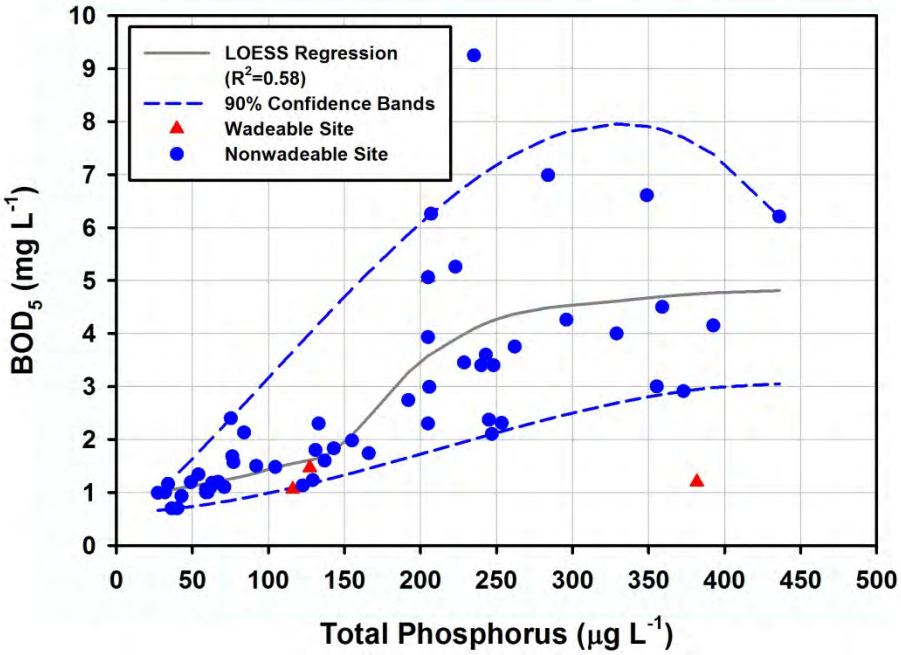


Figure 28. Relationship between total phosphorus and BOD_5 using data collected as part of the River Nutrient Study. The regression fits were based nonwadeable streams (n=54) only using a LOESS regression and the 90% prediction interval was estimated using the 95th and 5th quantile regression lines.

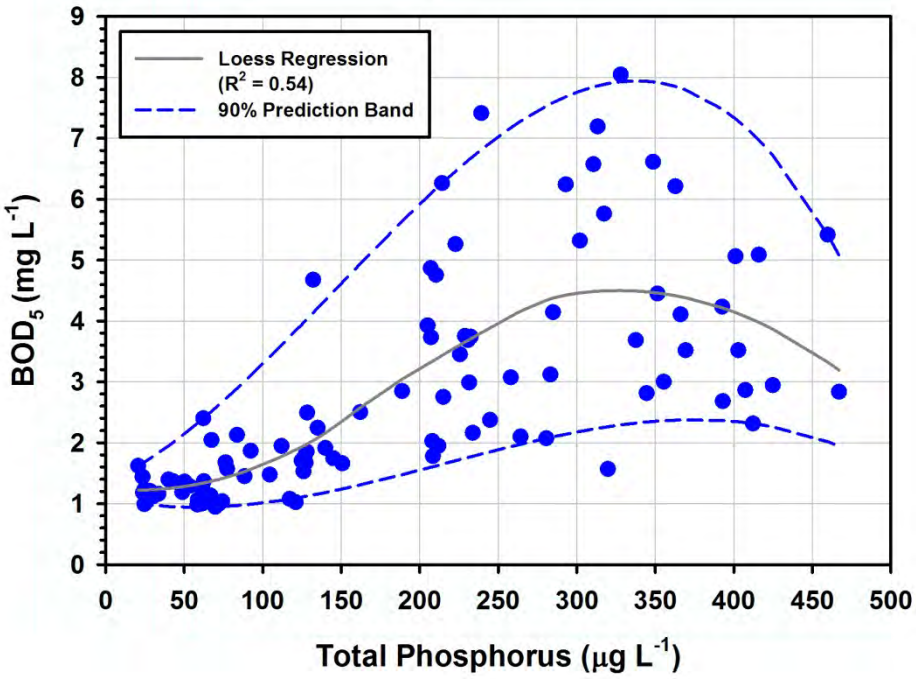


Figure 29. Relationship between total phosphorus and BOD_5 using STORET data (1990-2012). The regression fits were based nonwadeable and wadeable streams using a LOESS regression and the 90% prediction interval was estimated using the 95th and 5th quantile regression lines.

Although the dataset was not as large for diel DO flux, significant relationships between DO flux and TP and Chl-a were observed. Based on data from nonwadeable rivers, 52% of the variation in DO flux can be explained based on TP (Figure 30a).

DO Flux (mg L^{-1}) = 0.01 • TP (µg L^{-1}) + 1.60 R^2= 0.52 (Eq. 5)

The wadeable river data generally corresponded to the relationship and range of values from nonwadeable rivers with the exception of Getchell Creek, a nutrient-rich, but shallow, low order stream (Table 4). Chl-a exhibits a stronger relationship with DO flux (Figure 30b) with 66% of the variation explained in nonwadeable rivers.

DO Flux (mg L^{-1}) = 0.01 • Chl-a (µg L^{-1}) + 1.60 R^2= 0.66 (Eq. 6)

In general, 9 of 11 wadeable sites fall within the range and relationship based on the nonwadeable river data; however two sites - Maple River and Rice Creek - both exhibit a rather high DO flux relative to their Chl-a values (Figure 30b). Both streams have moderate to high nutrient concentrations and the Maple River has very high TSS (Table 14). While excessive benthic algae (periphyton) could be a possible explanation, biological unit field crews noted little or no periphyton at either site in 2008. The relatively low DO flux of the Red River sites for 2000 is evident as well and these sites appear as outliers based on the regression lines in Figure 30a, b.

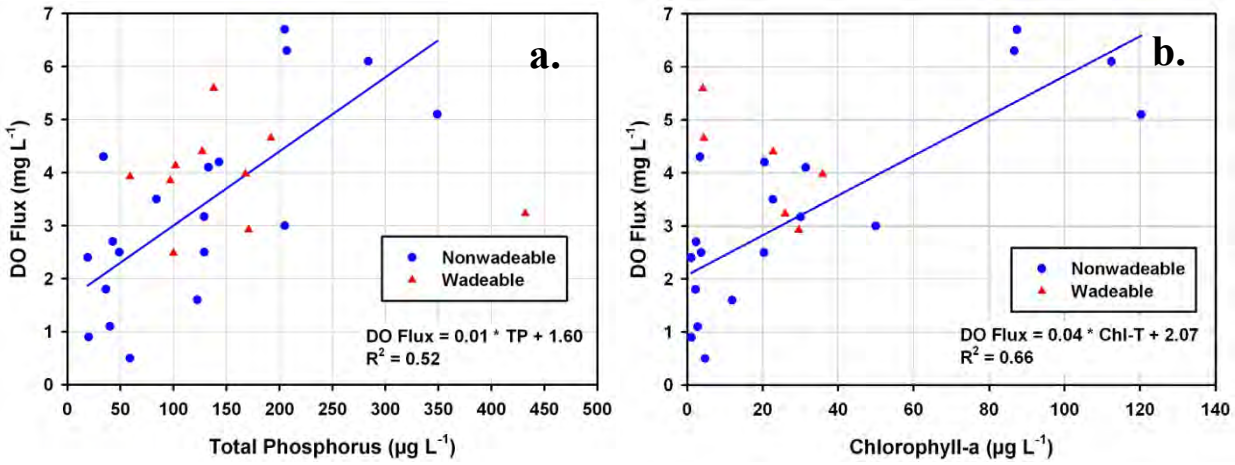


Figure 30. Diel DO flux compared to a) TP and b) seston chlorophyll-a using data collected as part of the River Nutrient Study (regression lines based on nonwadeable river sites; a. nonwadeable streams: n=19, wadeable streams: n=10; b. nonwadeable streams: n=19, wadeable streams: n=6).

As previously demonstrated (Heiskary & Markus 2003) there is no relationship among Chl-a and minimum DO (Figure 31a) based on the sites monitored in 2000, 2006 and 2008 (Table 12). A relationship similar to that observed with DO flux was noted among Chl-a and maximum DO (Figure 31b). This is consistent with our previous work as well.

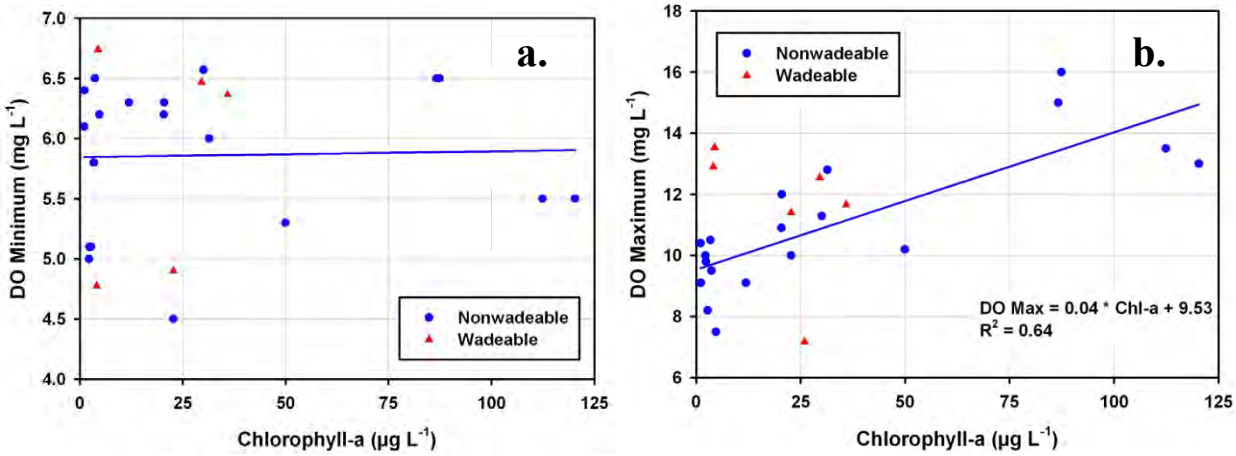
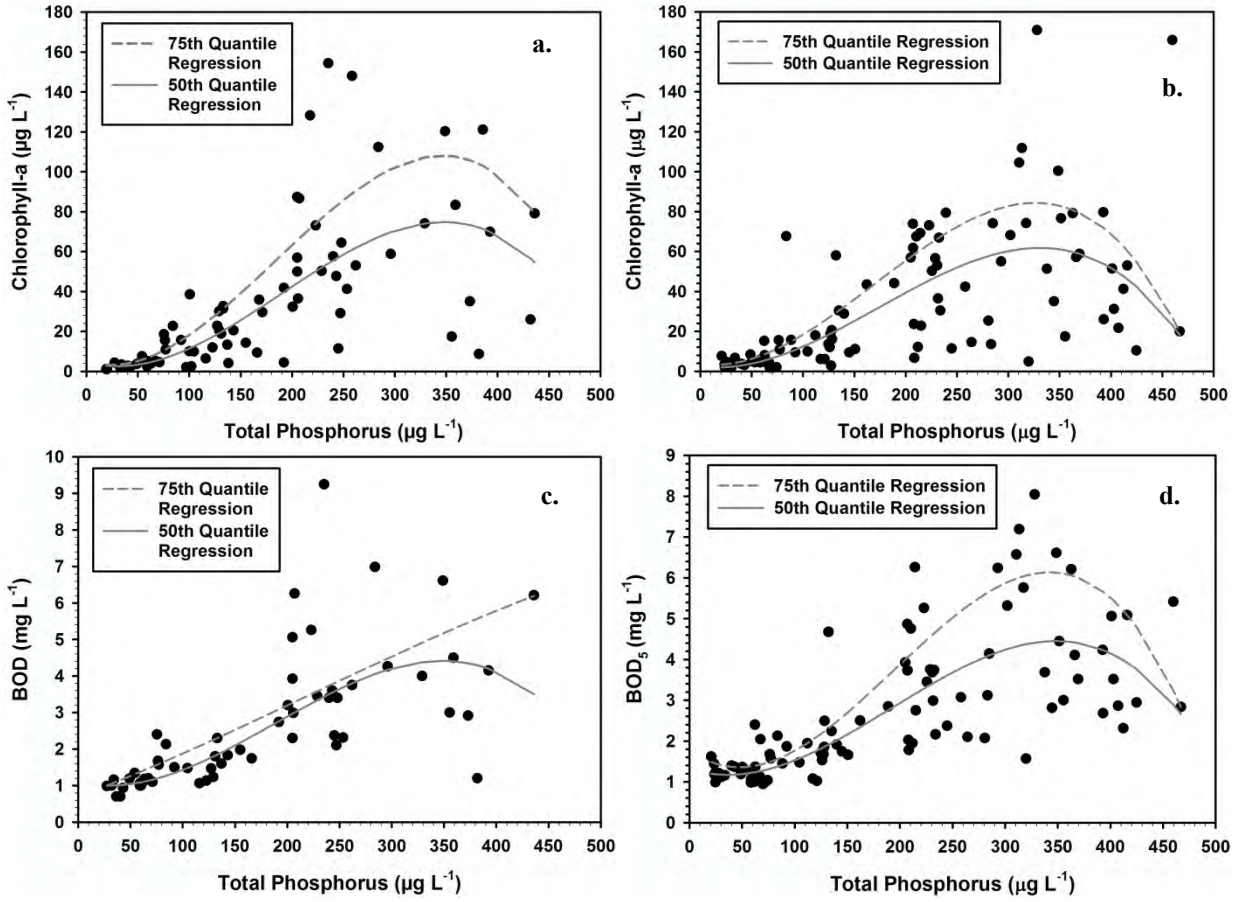


Figure 31. Diel DO a. minimum and b. maximum compared to sestonic chlorophyll-a using data collected as part of the River Nutrient Study (regression lines based on nonwadeable river sites; nonwadeable streams: n=19; wadeable streams: n=6).

75th and 50th percentile quantile regressions were fit to TP and Chl-a, BOD_5, and DO Flux datasets to provide predictions on the attainment of stressor parameters given different concentrations of TP (Figure 32). Both River Nutrient and STORET datasets were used and the resulting models resulted in similar predictions for Chl-a and BOD_5 (Table 15). No STORET data were available for DO Flux so no model is available for this parameter using this dataset. These models can be used to predict the probability of meeting stressor levels for different TP concentrations. By using the 75th and 50th percentile quantile regressions, the predicted amount of a stressor at different TP concentrations has a 75% and 50% probability of being at or below the predicted concentration. These predictions are important to understand projected outcomes if the recommended TP criteria are met. In addition, these models are useful in criteria setting for parameters where the datasets were not large enough to determine regional threshold concentrations (i.e., Chl-a and DO flux).



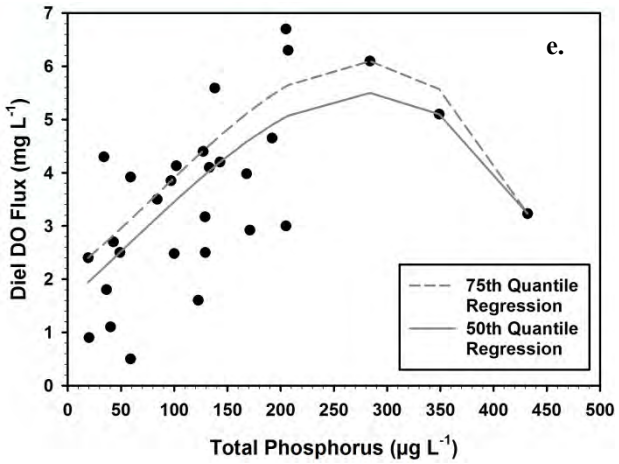


Figure 32. Relationship between TP and chlorophyll-a (a. River Nutrient & b. STORET), biochemical oxygen demand (c. River Nutrient & d. STORET), and diel dissolved oxygen flux (e. River Nutrient) for River Nutrient Study and STORET datasets. The regression lines were based on nonwadeable and wadeable streams using 50th and 75th percentile quantile regression spline fits (a. n =76, b. n = 96, c. n=58, d. n = 96, e. n=29).

Table 15. Predicted values of chlorophyll-a (Chl-a), biochemical oxygen demand (BOD_5), and Diel DO flux based on a range of total phosphorus (TP) values. Predicted values are based on interpolation of 50th and 75th percentile quantile regression spline fits using nonwadeable and wadeable streams.

TP	Chl-a (RN) (TP → Chl-a)		Chl-a (STOR) (TP → Chl-a)		BOD_5 (RN) (TP → BOD_5)		BOD_5 (STOR) (TP → BOD_5)		DO Flux (RN) (TP → DO Flux)	
	50th	75th	50th	75th	50th	75th	50th	75th	50th	75th
50	3.3	5.2	3.8	6.2	1.0	1.3	1.2	1.4	2.5	3.0
100	11.4	18.2	12.4	18.4	1.4	1.9	1.5	1.8	3.5	3.9
150	25.6	39.1	25.2	36.1	2.1	2.5	2.2	2.7	4.3	4.8
200	42.4	63.2	39.3	55.3	2.9	3.2	2.9	3.9	5.0	5.6
250	58.5	85.8	51.9	72.2	3.6	3.9	3.7	5.0	5.3	5.9
300	70.3	102.1	60.1	82.8	4.2	4.5	4.2	5.9	5.4	6.0
350	74.8	108.1	61.1	82.9	4.4	5.2	4.5	6.1	5.1	5.5
400	67.4	97.4	51.7	68.6	4.1	5.8	4.1	5.5	4.0	4.1

Flow can have a distinct but variable effect on stream seston chlorophyll concentrations and can also influence DO concentration and DO flux as well (Figure 33). An examination of patterns in chlorophyll and flow can provide some indication of the origin of the seston (phytoplankton) in the stream (Figure 33). In low to medium order streams, with low nutrients, sestonic Chl-a often declines as flow declines (Figure 33). Increases in Chl-a may occur as flow increases and benthic algae are scoured from the bottom of the stream. Medium to high order streams with moderate nutrients may exhibit stable to increasing Chl-a under stable to declining flows reflecting sustained seston in the water column (Figure 34). However, as flows drop to low levels and the stream becomes quite shallow (*e.g.,* Rum; Figure 34) sestonic Chl-a may decline. At medium to high order sites, with high nutrients, very high sestonic Chl-a may be sustained over a range of flows (Figure 35). In all instances there is an interplay between benthic and sestonic algae but there is a general tendency for sestonic algae to become more prominent in nutrient-rich, medium to high order streams as compared to nutrient poor, low to medium order streams where benthic algae likely represent the majority of the algal production for the stream.

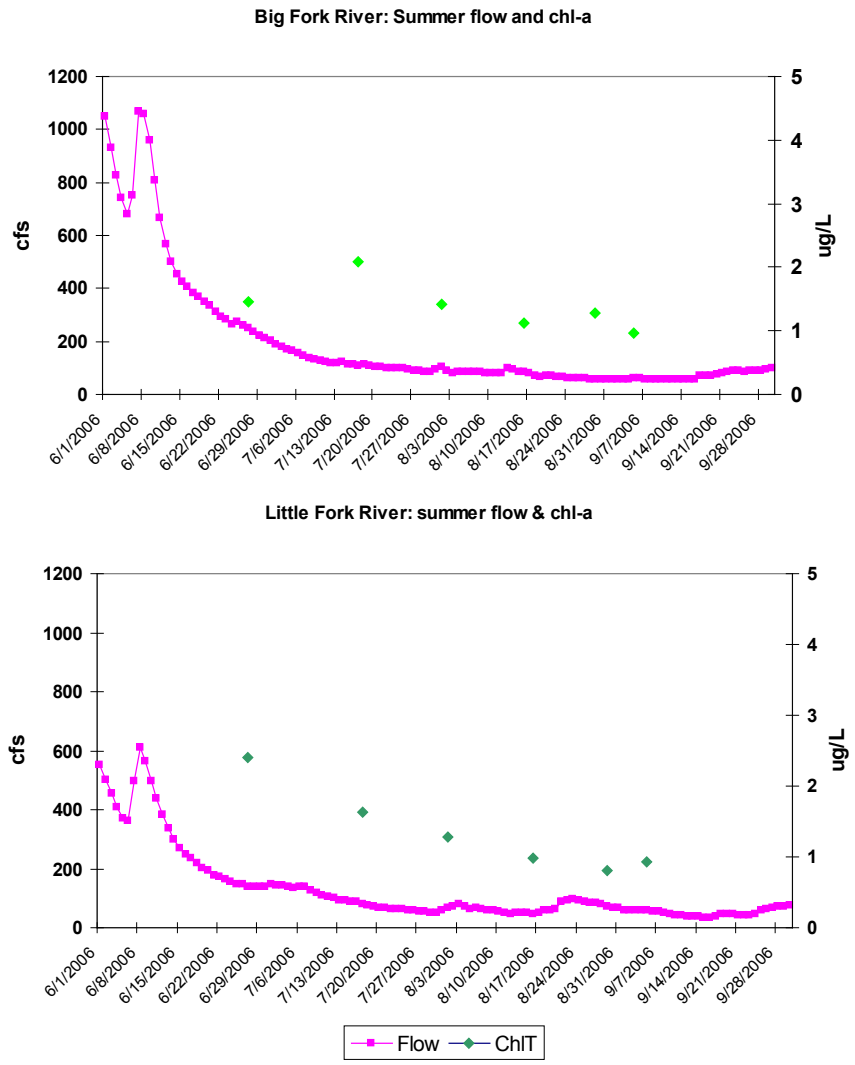


Figure 33. Comparison of chlorophyll (Chl-T) and flow: low to medium order sites with low nutrients.

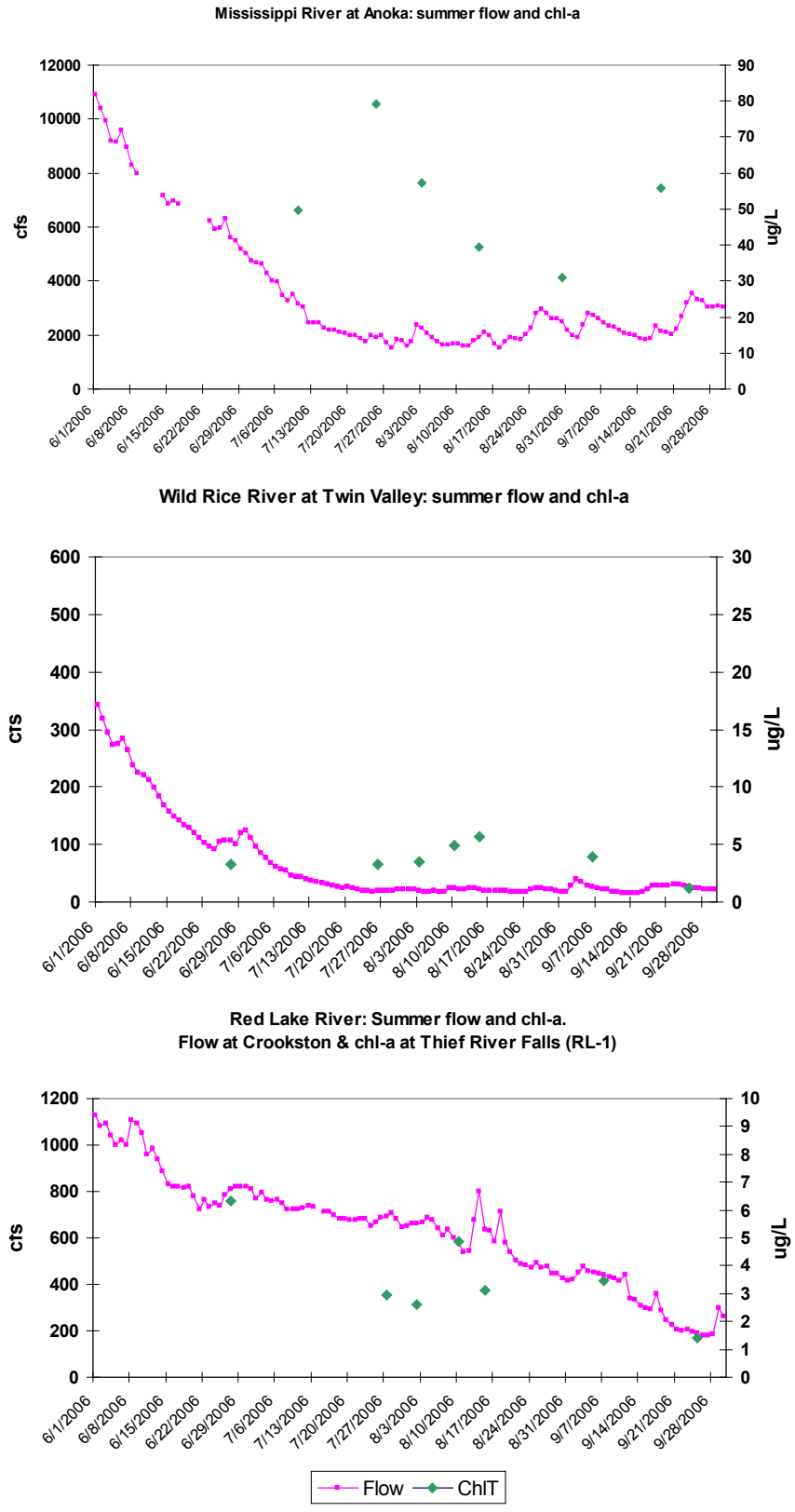


Figure 33 (continued). Comparison of chlorophyll (Chl-T) and flow: low to medium order sites with low nutrients.

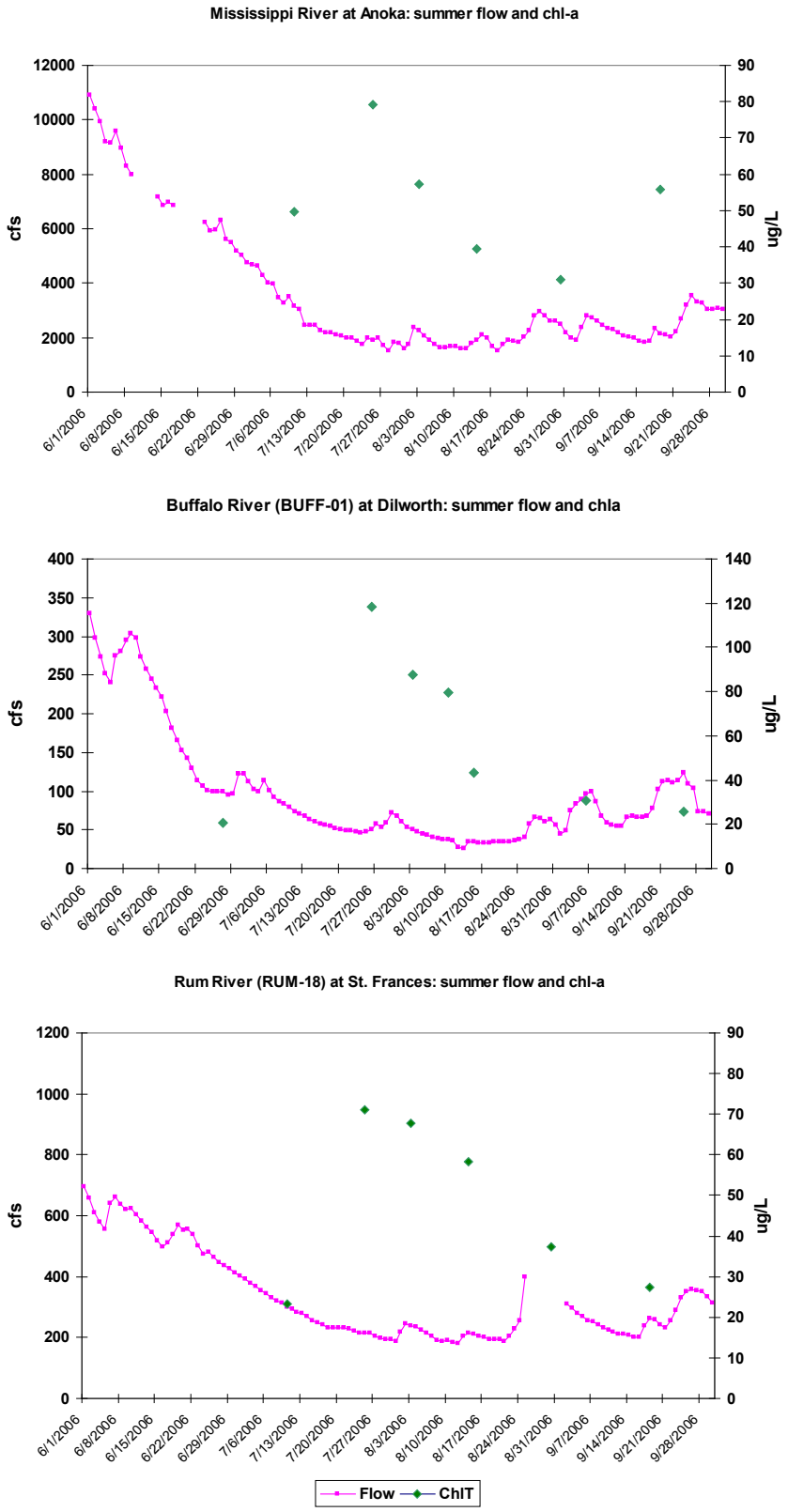


Figure 34. Comparison of chlorophyll (Chl-T) and flow: medium to high order sites with moderate nutrients.

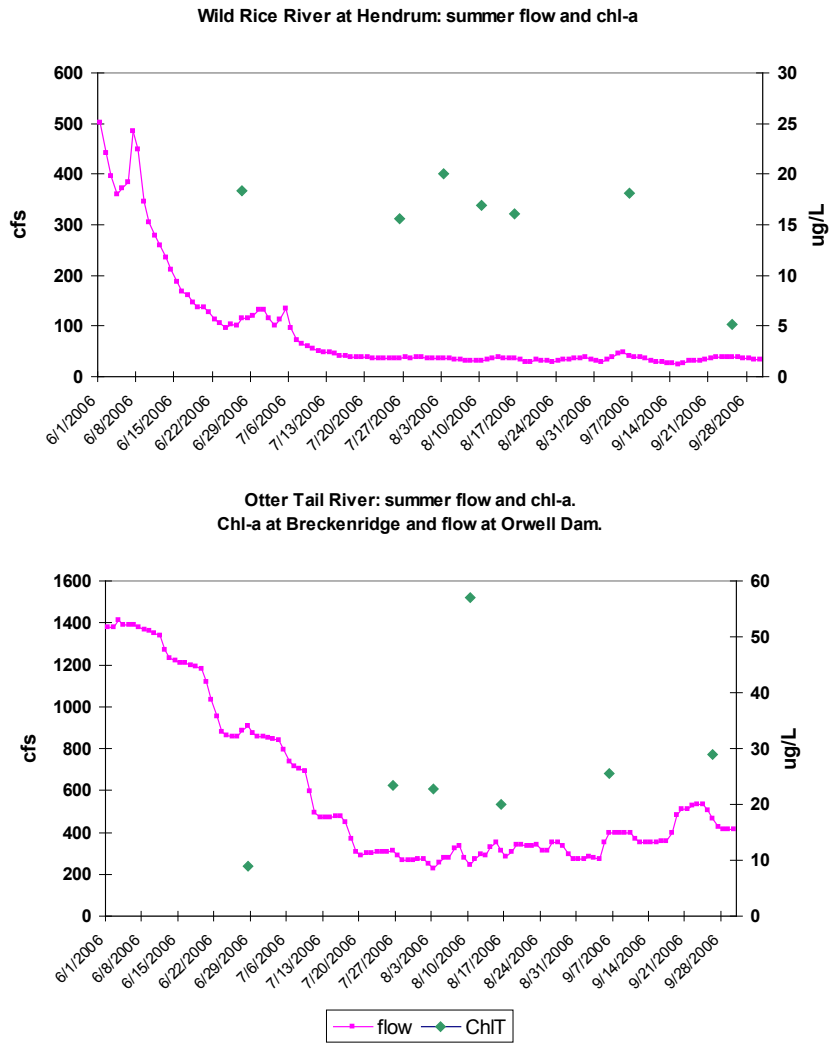


Figure 34 (continued). Comparison of chlorophyll (Chl-T) and flow: medium to high order sites with moderate nutrients.

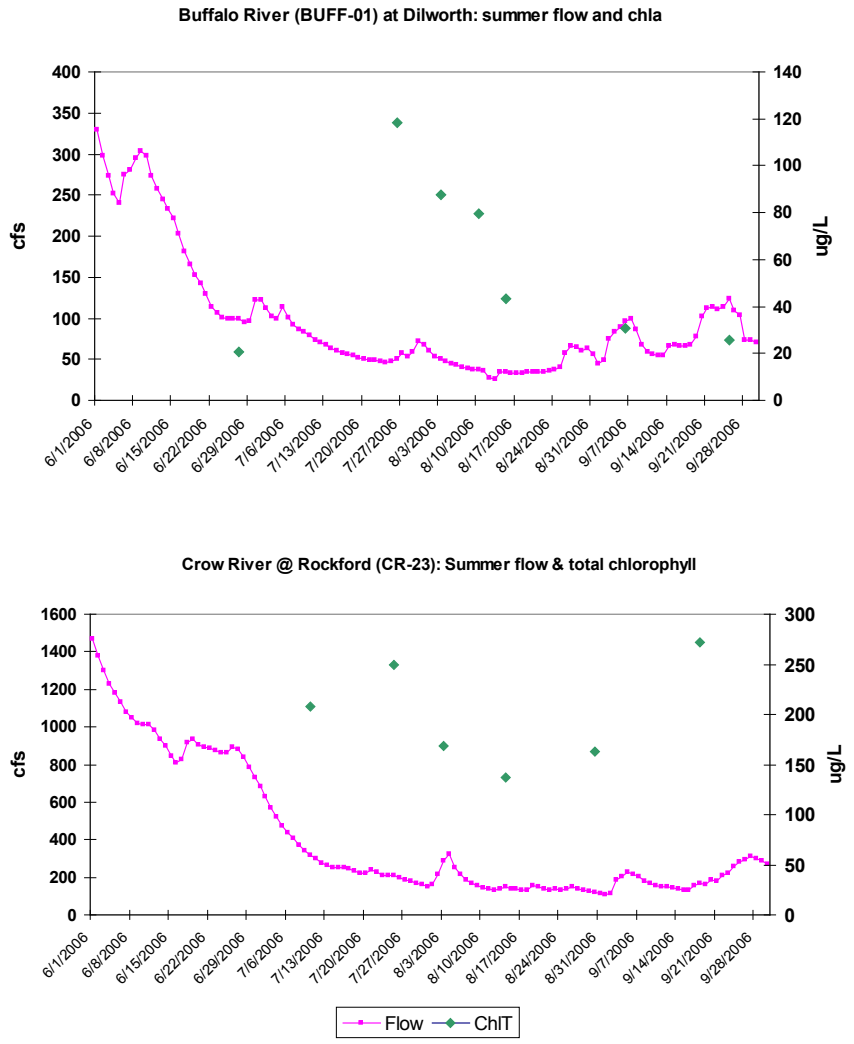


Figure 35. Comparison of chlorophyll (Chl-T) and flow: medium to high order sites with high nutrients.

2. Annual Variability of Total Phosphorus and Total Chlorophyll

The Mississippi, Rum, and Crow data afford an opportunity to characterize nutrient and chlorophyll-a variability over time (four summers) and a range in flow conditions. This type of variability is important to consider when developing nutrient criteria and comparisons will be made between previous data (1999, 2000, and 2001) and 2006 when available.

The Rum River has its headwaters in Lake Mille Lacs in the NLF ecoregion but the majority of its watershed is in the CHF ecoregion (Figure 4). It has moderate nutrient concentrations for a CHF river. Chl-T was rather variable in 2006, ranging from 20-70 µg/L, and tended to decline from mid-July through September–perhaps in response to increased flow later in the summer (Figure 34). The Chl-T and flow relationship in 2006 was somewhat similar to that noted in 1999 (Heiskary & Markus 2003). Summer-mean TP and Chl-T are not highly variable in the Rum based on data from 1999, 2000, 2001 and 2006 (Figure 36); however Chl-T is higher during summers of lower flow (2000 and 2006) as compared to higher flow (1999 and 2001). Thus river flow (residence time) can explain some of the variability in the TP and Chl-T relationship (Figure 23).

The Crow River is an extremely nutrient-rich stream that drains from the CHF ecoregion (North Fork) and from a combination of the NCHF and WCBP ecoregions (South Fork). Flow declined steadily from June to July and the river remained at base-flow from July through September (Figure 36). Chl-T concentrations were very high at all

three sites on the Crow (Table 14). Chl-T was often the highest under lower, stable flows with concentrations typically in the 100–200 µg/L range. Based on flows for summer 2006 the South Fork contributes about 50% of the flow to the main-stem of the Crow. TP concentrations have declined slightly from 1999 to 2006 on the main-stem of the Crow (Figure 37). This is in part a function of differing flow regimes as 1999 was a very high flow summer and 2006 was very low flow (Figure 36). The North and South Forks had slightly lower TP in 2006 as compared to 2001. Chl-T, however exhibited no such trend as concentrations were at their highest in 2006 -- likely a direct result of the extremely high nutrient concentrations and the low flow that provided adequate residence time and stable conditions for excessive algal growth in the stream. As with the Rum, Chl-T is highest during summers of lower flow.

The Mississippi River at Anoka is a reflection of the CHF and NLF ecoregions (Figure 4). Nutrient concentrations were moderate in 2006. The patterns in flow were similar to that observed in the Rum and Crow Rivers. The range in chlorophyll was similar to that observed in the Rum (Figure 36). The highest Chl-T concentrations occurred during low flow periods suggesting phytoplankton were a significant component of the seston at this site (Figure 36). Based on Chl-T data for all four years no distinct temporal trends were evident; however Chl-T was higher in years of lower flow (2000 and 2006) as compared to years of higher flow (1999) (Figure 36).

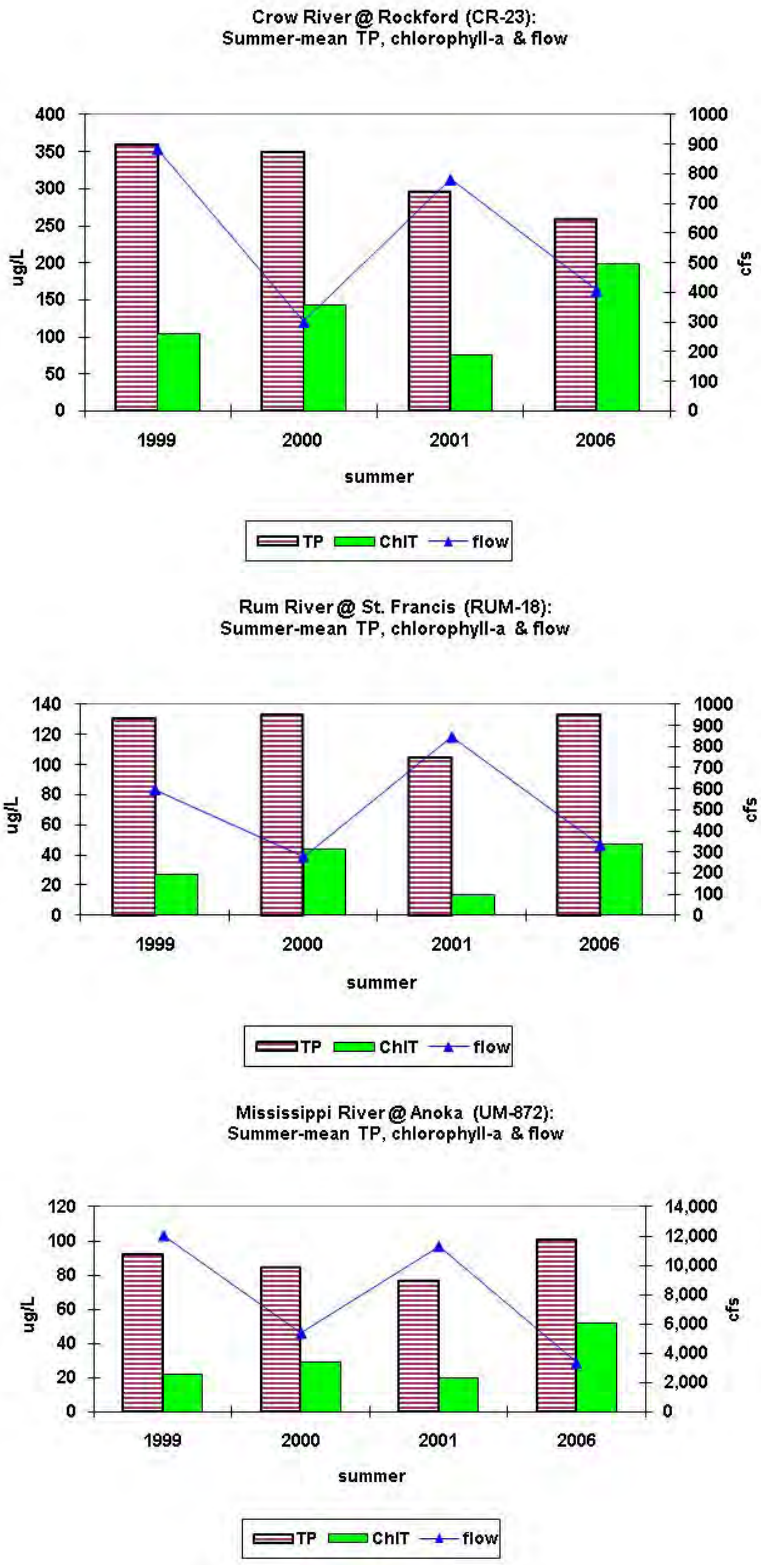
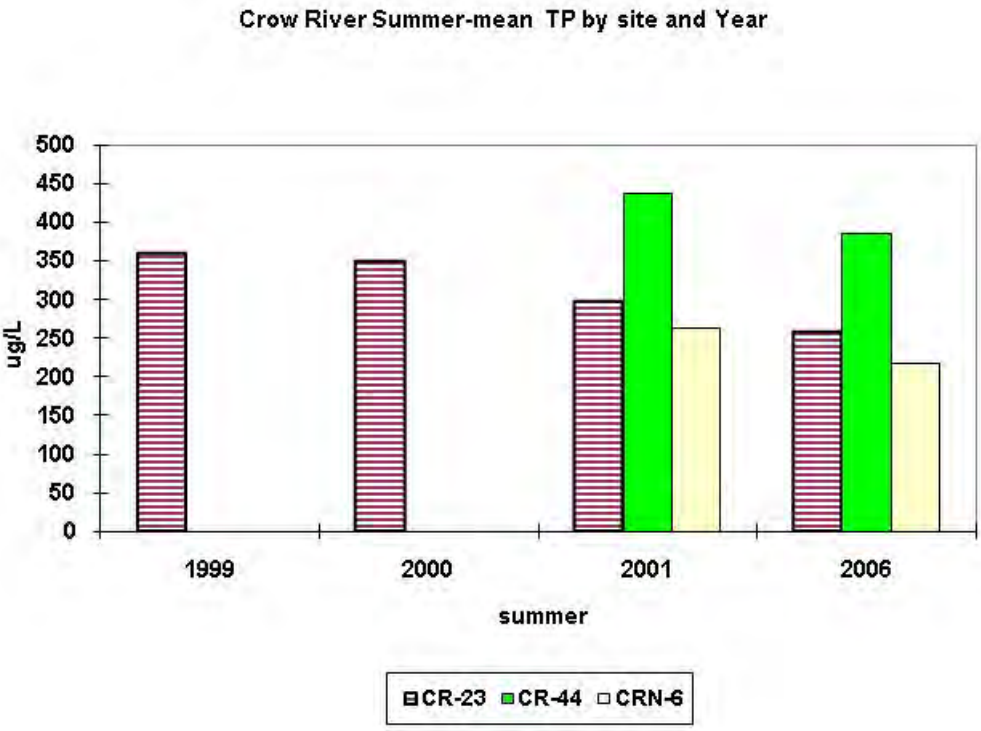


Figure 36. Comparison of summer-mean flow, TP and chlorophyll-a (total) for Upper Mississippi River basin sites. TP and Chl-T collected from late-June-mid-September. Summer-mean flow based on June-September flow.



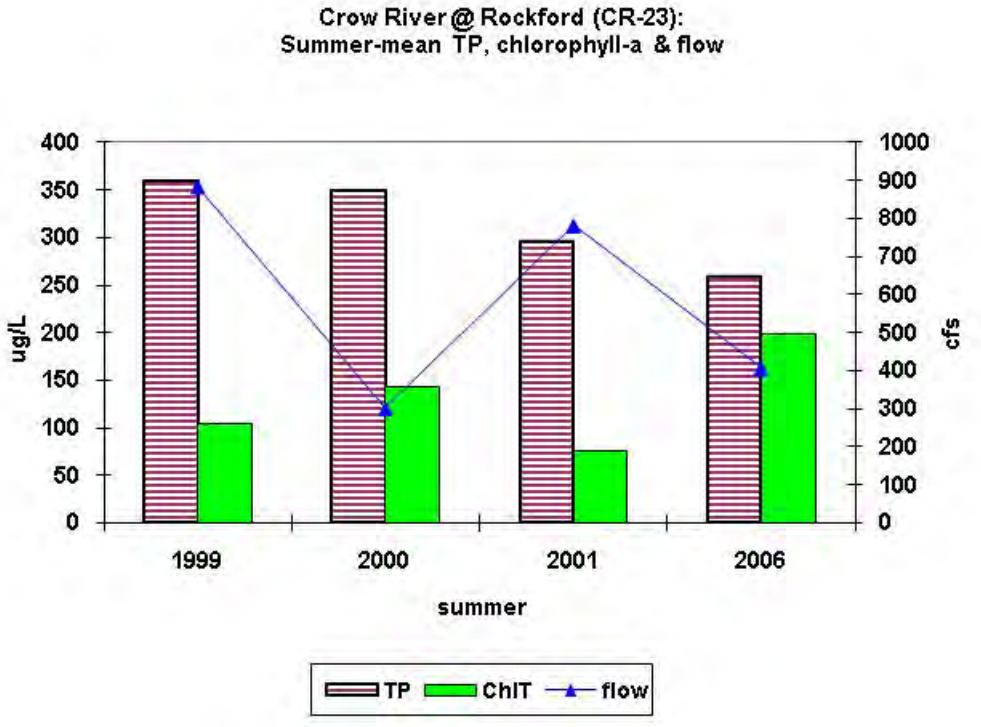


Figure 37. Inter-year and among-site comparisons for the Crow River.

3. Relationships among Nutrients, Water Chemistry and Biological Indicators: Exploratory Analyses

Correlations among TP, TN, chlorophyll, and DO flux (this document will refer to these as the four primary variables) have been firmly established based on the current study and previous work (*e.g.,* Heiskary & Markus 2001, 2003). An additional emphasis of the 2000, 2006, and 2008 studies was to explore how various biological metrics (*e.g.,* fish and macroinvertebrate metrics) correlated with TP, TN, sestonic Chl-T and DO flux. Table 16 provides an overall summary for the four primary variables and how they relate to a variety of chemical, physical, and biological measures. The various chemical and physical measures were derived from the previous monitoring in 1999, 2000, 2006 and 2008 monitoring (Tables 12 and 14). The corresponding biological data was collected over a similar time period and that data is summarized in Appendix II along with descriptions of the various metrics.

Strong correlations are evident for many of the biological metrics relative to the four primary variables based on data from 1999, 2000 and 2006 studies. The majority of the biological metrics exhibit inverse (negative) correlations with nutrients, Chl-T, and DO flux. In some instances the correlation coefficients (R_s) of the biological variables are higher than many of the chemical and physical variables relative to the four primary variables (Table 16). Among the more prominent biological measures, as shown by high R_s, are as follows: number of macroinvertebrate taxa, number of EPT taxa, fish IBI, # of sensitive fish taxa, percent sensitive fish, simple lithophils (both as # of taxa and as a percent of overall fish community), and relative abundance of amphipods. In addition to the biological metrics, MSHA exhibits a negative correlation with each of the four variables. This suggests that the role of habitat quality must be considered as well as the four primary variables when a closer examination of relationships is conducted.

Some fish and macroinvertebrate metrics exhibit strong positive relationships with nutrients, Chl-T, and/or DO flux; among the more prominent metrics are: # tolerant macroinvertebrate taxa, omnivorous fish (# of taxa and % of community), dominant two macroinvertebrate taxa (% of community), and Hilsenhoff Biotic Index (HBI). Where positive relationships are observed, there is a less consistent response among the four variables, in contrast to the negative (inverse) relationships. For example, the number of macroinvertebrate taxa exhibits a strong negative correlation with all four variables, whereas HBI exhibited a strong positive correlation for only TN and DO flux (Table 16). Certain macroinvertebrate feeding and functional groups also exhibit strong correlations; however these vary from negative to positive dependent on the primary variable they are associated with, and include number of clinger taxa, number of collector / gatherer taxa and to a lesser degree number of collector / filterer taxa. Habitat is an important driver relative to the presence or absence of taxa from these functional and feeding groups and may be difficult to separate from the influence of the four primary variables (Table 16).

The relationships in Table 16 provide a basis for a more detailed examination of select biological metrics relative to the four primary variables. For this purpose scatter plots are used to examine the relative relationship among various biological metrics and TP, TN, Chl-T, and DO flux. Distribution statistics for the various metrics (Table 7) are used as a basis to suggest where important shifts in the various metrics may be occurring, with an emphasis on those values that fall into the lower and upper quartiles for the respective metric.

Table 16. Spearman Rank correlations (list-wise method) for TP, TN, Chl-T and DO flux relative to water quality, physical measures and fish and macroinvertebrate metrics. Based on river nutrient data set (1999, 2000, & 2006) and includes all measures exhibiting Rs ≥ 0.40. See notes below and Appendix II for metric definitions.

Total P		Total N		Chl-T		DO Flux	
Metric	Rs	metric	Rs	Metric	Rs	Metric	Rs
Invert Taxa W Ch #	-0.87	Sensitive F %	-0.97	Invert Taxa Ch #	-0.93	Sensitive F %	-0.97
Amphipoda #	-0.78	Fish IBI #	-0.94	Invert Taxa #	-0.90	Fish IBI #	-0.94
Invert Taxa #	-0.75	MSHA #	-0.94	Sensitive F #	-0.83	MSHA#	-0.94
Plecoptera #	-0.72	Clinger Ch #	-0.94	Collect Gath Ch #	-0.81	S Lithop F #	-0.93
Sensitive F #	-0.66	Darter F %	-0.89	Amphipoda #	-0.78	Clinger Ch I #	-0.88
Collect Gather Ch #	-0.64	S Lithop F %	-0.89	EPT #	-0.77	Amphipoda #	-0.85
EPT #	-0.60	Piscivore F %	-0.83	Intolerant Ch #	-0.77	Darter F %	-0.83
Intolerant Ch #	-0.60	Sensitive F #	-0.83	Clinger Ch #	-0.70	Piscivore F %	-0.83
Temp	-0.60	Invert Taxa #	-0.78	Darter F %	-0.66	S Lithop F %	-0.83
Clinger Ch #	-0.58	Amphipoda #	-0.78	S Lithop F %	-0.66	Piscivore F #	-0.81
Darter F %	-0.54	S Lithop F #	-0.77	Temp	-0.66	FISH Taxa #	-0.79
S Lithop F %	-0.54	EPT #	-0.77	Plecop #	-0.52	Collector Filter Ch #	-0.78
Sensitive F %	-0.44	Intolerant Ch #	-0.77	Sensitive F %	-0.50	Darter F #	-0.77
DO Min	-0.44	Insect F %	-0.71	Fish IBI #	-0.49	EPT #	-0.77
Fish IBI #	-0.43	Piscivore F #	-0.64	QHEI	-0.49	Intolerant Ch #	-0.77
MSHA#	-0.43	Fish Taxa #	-0.59	Tanytarsini Ch #	-0.46	Insect F %	-0.77
Tolerant I #	0.43	Collector Filter Ch #	-0.55	DO Min	-0.44	Insect tolerant F #	-0.75
pH	0.43	Insect tolerant F #	-0.52	DO Flux	0.43	Sensitive #	-0.71
DO Min	0.43	EPT #	-0.49	Tolerant I #	0.49	Invert Taxa #	-0.64
Omnivore F #	0.46	Invert Taxa W Ch #	-0.46	pH	0.49	Invert Taxa W Ch #	-0.52
NO3	0.49	Darter F #	-0.46	DO Min	0.49	Trichop #	-0.49
TN	0.49	pH	-0.64	Collector Filterer #	0.54	Minnows tolerant	-0.48
Omnivore F %	0.60	Chironomidae Tax #	-0.59	NO3	0.54	pH	-0.43
Turbidity	0.64	BOD	-0.55	TN	0.54	Cond.	0.43
TSS	0.71	Collector Gatherer I#	-0.52	Turbidity	0.55	BOD	0.43
Collector Filterer I #	0.77	Drain Sq mi	0.49	TSS	0.60	TKN	0.43
Drain Sq mi	0.89	TP	0.49	Omnivore F #	0.62	Chl - T Mean	0.43
Pheo	0.89	Pheo	0.49	Omnivore F %	0.71	DO Min	0.44
TSV	0.90	Chl - T Max	0.49	TSV	0.75	Drain Sq mi	0.49
Cond.	0.94	DO Min	0.50	BOD	0.89	Dom Two I %	0.49
BOD	0.94	Cond.	0.54	Drain Sq mi	0.94	Pheo	0.49
TKN	0.94	TKN	0.54	TP	0.94	TSV	0.49
Chl - T Mean	0.94	Chl - T Mean	0.54	Pheo	0.94	Odonata I #	0.54
Chl - T Max	1.00	Omnivore F #	0.62	Chl - T Max	0.94	Ephem I #	0.54
		TSV	0.64	Cond.	1.00	Omnivore F #	0.65
Notes		HBI	0.71	TKN	1.00	TSS	0.66
F=Fish		Dom Two I %	0.77			Collector Gatherer I#	0.71
I=Invertebrate		TSS	0.83			Turbidity	0.75
Ch=chironomids		DO Flux	0.89			HBI	0.77
W=with		Turbidity	0.90			Omnivore F %	0.77
#=number of taxa		Omnivore %	0.94			NO3	0.89
%=% individuals in		Tolerant I #	0.94			TN	0.89
sample		DO Min	0.94			Tolerant F #	0.94
		NO3	1.00			DO Min	0.94

Total macroinvertebrate taxa exhibited a strong correlation with TP (Table 16 and Figure 38a). However, the shift in the distribution of this metric, relative to changes in TP, may be more useful for defining the relationship between these two variables (Figure 38a). Total taxa remain above the 25th percentile for this metric over a range in TP from about 20–130 µg/L. As TP increases from ~130 µg/L to ~170 µg/L there seems to be a distinct change in the

distribution of this metric; whereby all metric values at or below the 25th percentile (as drawn from Table 7) when TP>~170 µg/L. Insects that cling to substrates (clingers) also exhibited a strong negative relationship to TP. At TP of 120 µg/L or less, the number of clinger taxa remains above the 25th percentile (Figure 27b). As TP increases from ~120-170 µg/L there is a shift in the distribution of the metric and all metric values above TP of ~170 µg/L are at or below the 25th percentile. Good habitat, as reflected by high MSHA or QHEI, may account for differences in the number of total and clinger taxa for sites with similar TP concentrations. For example, the Rum River with a high habitat metric score (QHEI =78 and TP=133 µg/L) exhibits a higher number of total and clinger taxa as compared to the Wild Rice (WR-1) (TP=123 µg/L) with a lower QHEI (49, Figure 39). In general, MSHA declines as TP increases above ~120-130 µg/L, though there are some exceptions such as the two nutrient-rich Crow River sites that maintain relatively high QHEI values (60 and 68; Figure 39). However, the number of total taxa at each of these two sites is below the 25th percentile (Figure 38a).

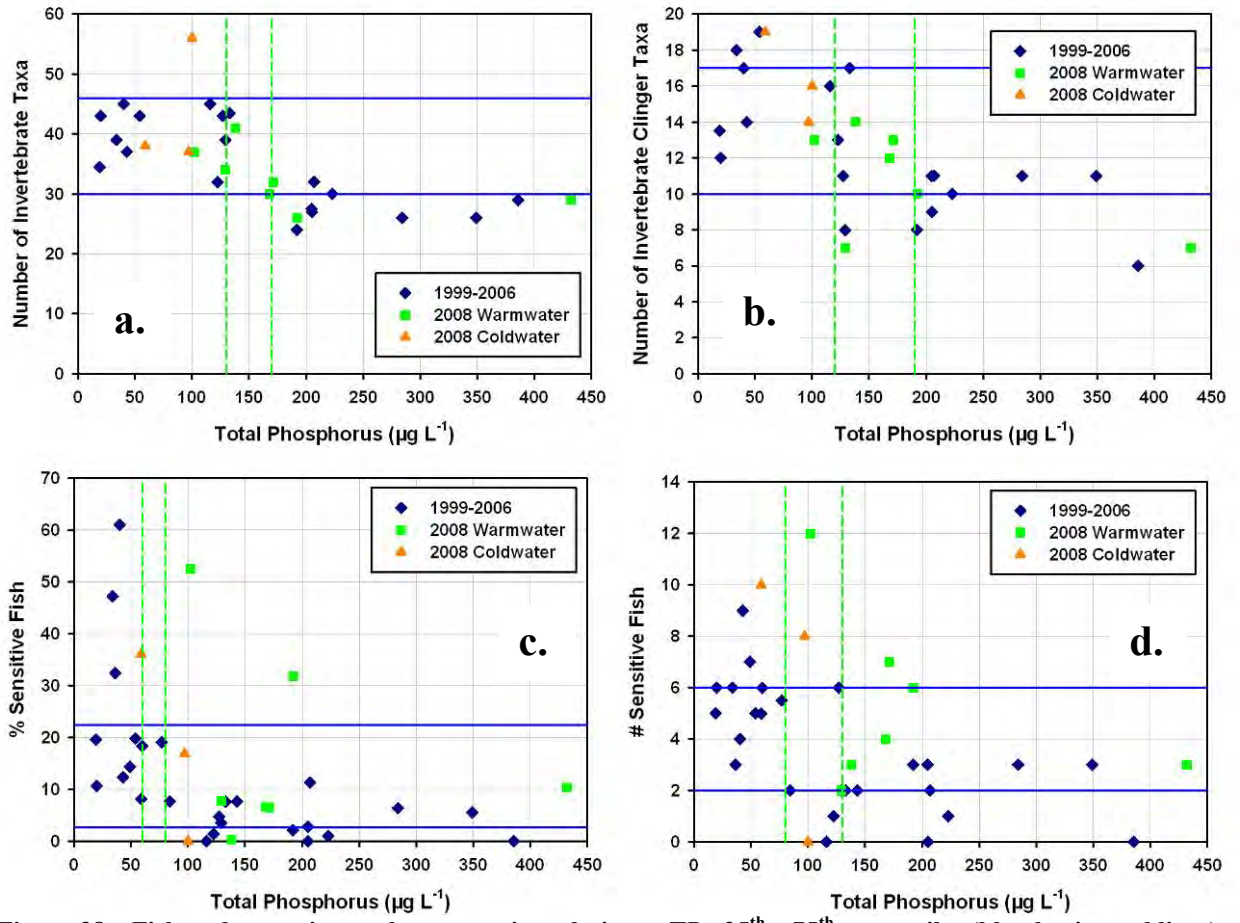


Figure 38. Fish and macroinvertebrate metrics relative to TP. 25th – 75th percentiles (blue horizontal lines) for nonwadeable rivers noted. Green vertical bar represents a shift in metric distribution.

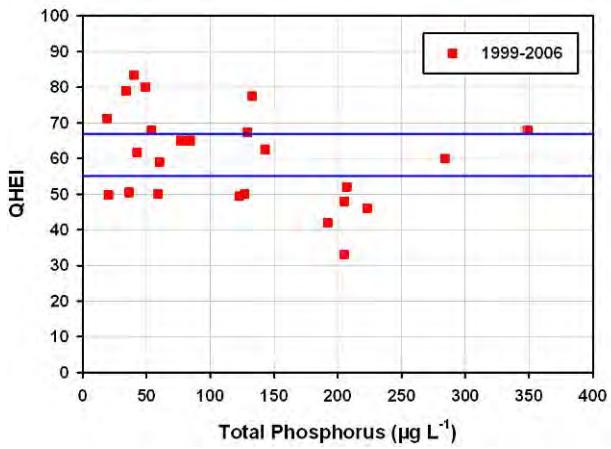


Figure 39. Relationship between total phosphorus and QHEI (habitat) for nonwadeable rivers. 25th – 75th percentiles (blue horizontal lines) for nonwadeable rivers noted.

Percent sensitive fish and number of sensitive fish taxa exhibited a strong response to increasing TP as well (Figure 38c, d). Percent sensitive fish taxa declined markedly as TP increased above 60-80 µg/L. At TP of 100 µg/L or more, percent sensitive fish comprised 10 percent or less of the catch. In general, the 2008 sites exhibited good correspondence with the 1999 - 2006 datasets with the exception of two warmwater sites (North Branch Root and Maple). The North Branch Root, while classified as warmwater, does have coldwater tributaries and a few trout were present in the collection at that site (John Sandberg, personal communication). Based on the 1999 - 2006 data, the number of sensitive fish taxa declined markedly as TP increased above ~80 µg/L and, at TP > 130 µg/L, there were generally three or fewer sensitive taxa (Figure 38d). Several of the 2008 sites (coldwater in particular) maintained higher number of sensitive taxa at higher TP concentrations as compared to the 1999 - 2006 sites.

Total macroinvertebrate taxa and several fish metrics exhibited strong relationships with TN (Table 16), with percent sensitive fish and number of sensitive fish taxa being the most prominent. Based on the 99-06 data the number of macroinvertebrate taxa declined markedly at TN >1.5 mg/L and most metric values were at or below the 25th percentile above this concentration (Figure 40a). An exception was BE-100, a shallow, third order site on the Blue Earth River (Table 3). The 2008 data were not quite as consistent in this regard. Based on the 99-06 data the percent sensitive fish fall to 10 percent or less as TN exceeds ~1.0-1.5 mg/L (Figure 40b). Five of seven 2008 warm water sites exhibit a similar pattern; however two (North Branch Root and Maple) and one coldwater site (Bear Creek) maintain percentages of 30 percent or more at elevated TN (Figure 40b). The pattern for number of sensitive taxa is similar; however several 2008 cold and warm water sites exhibit values in excess of the 75th percentile at elevated TN (Figure 40c). Simple lithophils exhibited a strong negative correlation with TN (Table 16). Based on the 99-06 data, simple lithophils fell at or below the 25th percentile at TN of 1.5 mg/L or more. Also, four of six USGS sites exhibited the same pattern. Two exceptions were the pooled, high order sites: Minnesota River at Jordan and Mississippi at Red Wing sites (Figure 40d). Four of seven 2008 warm water sites followed this pattern as well with the exception of North Branch Root, Getchell Creek, and Maple River. The coldwater sites varied from this pattern as well (Figure 40d). In general, the discontinuous nature of the TN data and the shift from organic N to nitrate-N as the predominant form of TN (Figure 24) makes it difficult to clearly define thresholds. While it appears that metric values are often depressed (*e.g.*, below 25th percentile) when TN>1.5 mg/L there are several exceptions – particularly with the 2008 sites and high order USGS sites.

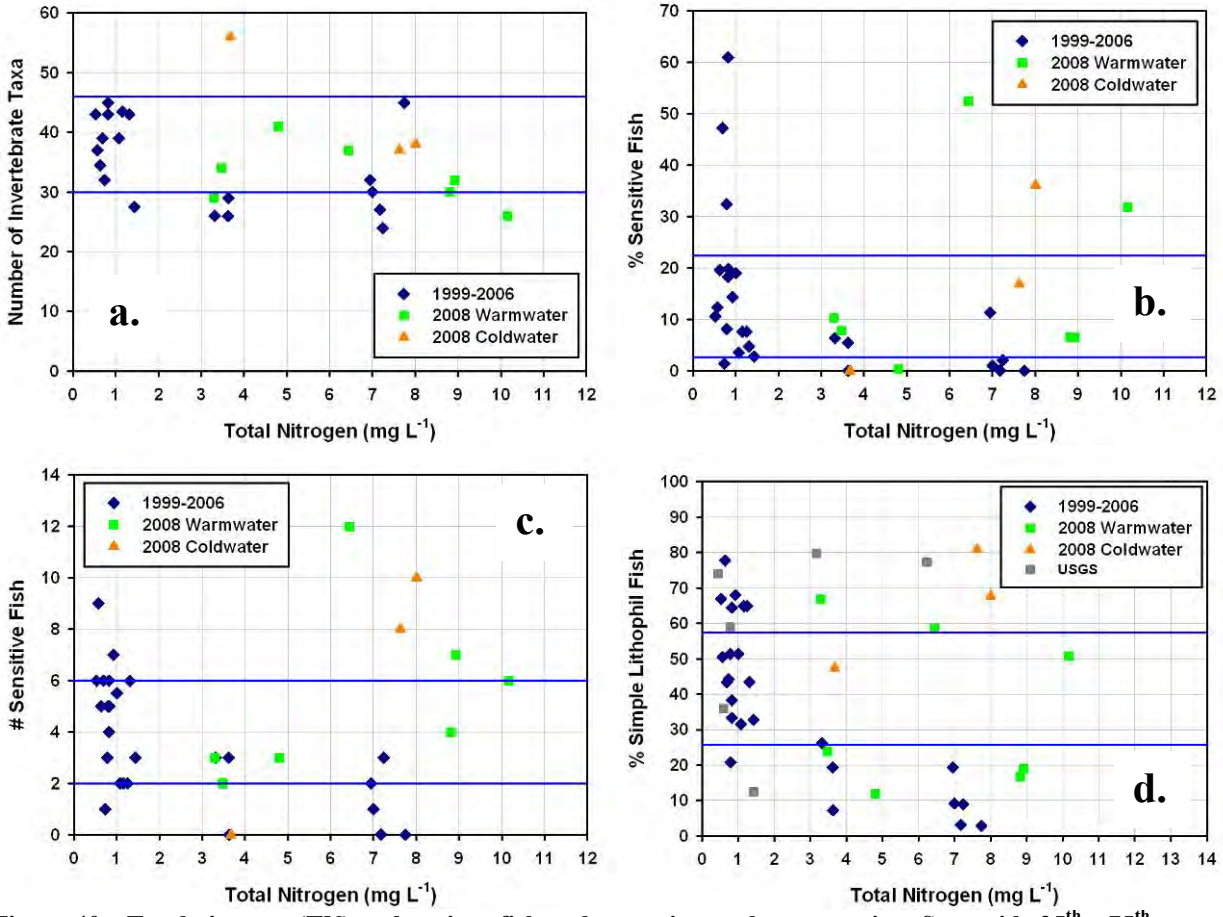


Figure 40. Total nitrogen (TN) and various fish and macroinvertebrate metrics. Statewide 25th – 75th percentile values (blue horizontal lines) for nonwadeable rivers noted.

Several macroinvertebrate metrics exhibit strong negative relationships with chlorophyll (Table 16). Among the stronger relationships are number of total macroinvertebrate taxa, collector-gatherers, relative abundance of amphipods, and EPT. Total macroinvertebrate taxa exhibits a high R^2 (0.60) relative to chlorophyll. In general, based on the 1999-2006 data total taxa remain between (or above) the 25th–75th percentile when Chl-a is less than ~25 µg/L (Figure 41a). However, as Chl-a increases above 33-40µg/L (with exception of RUM-18 at 43 µg/L), total taxa values fall below 32 (Figure 41a). The 2008 data correspond similarly with the exception of the Maple River, which has low Chl-a and low number of taxa (Figure 41a). EPT is not quite as sensitive to change in Chl-a; however values begin to fall below the 25th percentile as Chl-T increases above ~25-40 µg/L (Figure 41b). At higher Chl-a, the number of EPT taxa fall between 10 and 15 and no high values are noted.

Number of sensitive fish taxa exhibited the highest inverse relationship for the fish metrics, while percent omnivore fish was among the highest positive relationships relative to chlorophyll (Table 16). As Chl-a increases above 20-30 µg/L, percent sensitive fish comprise 20 percent or less of the catch (Figure 41c). Data from 2008 coldwater and warm water streams were consistent with the 99-06 data. The number of sensitive taxa exhibited a strong relationship as well. Sensitive taxa remain at or above the 25th-75th percentiles, at most sites, when Chl-a is at or below ~25-30 µg/L. One exception is the 3rd order Blue Earth River site (BE-100), with a low QHEI, no sensitive taxa and four omnivore taxa (Figure 41d). As Chl-a increases above ~30-40 µg/L, the number of sensitive taxa falls below the 75th percentile for this metric.

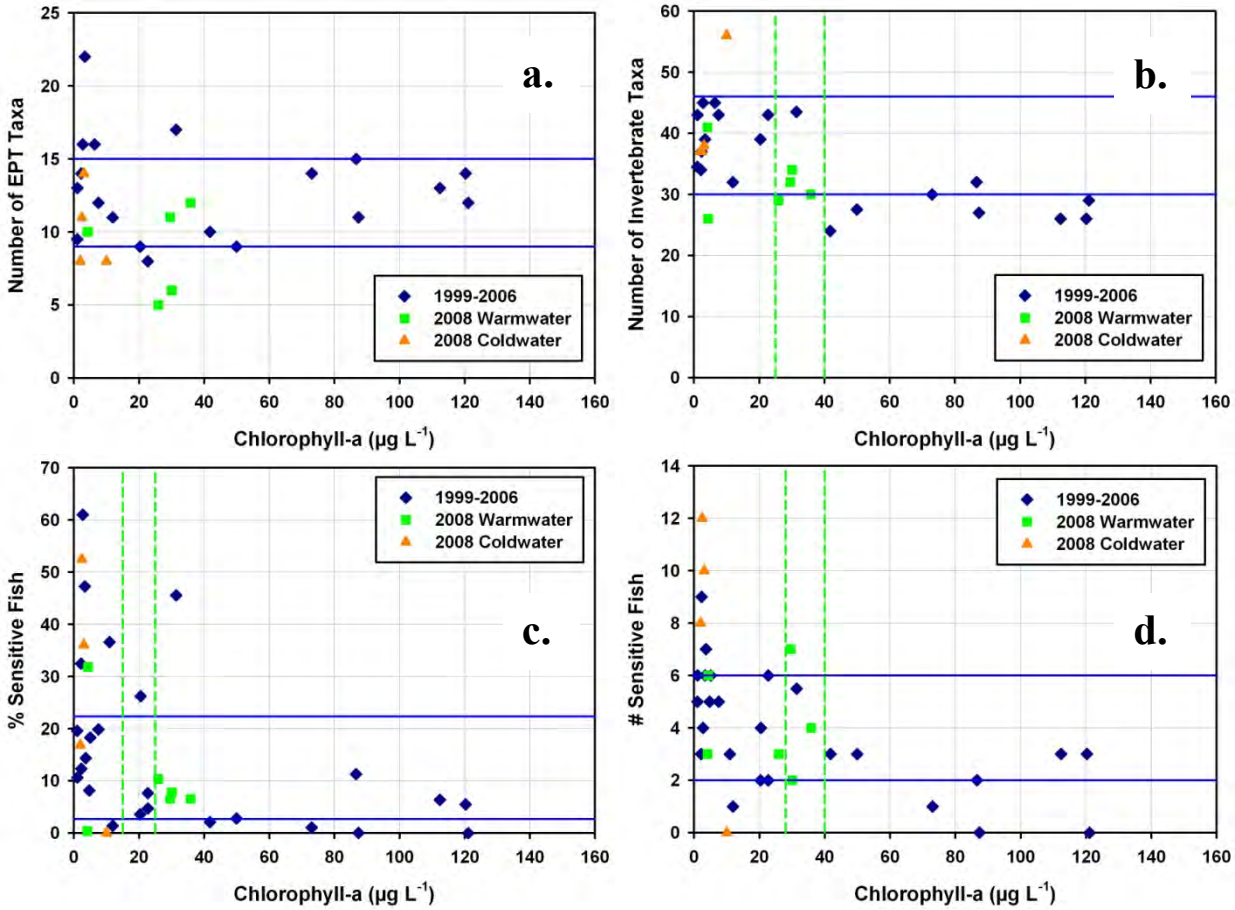


Figure 41. Chlorophyll-a and fish and macroinvertebrate metrics. Green vertical bars represent a shift in distribution of metric values. 25th– 75th percentile statewide values for nonwadeable rivers noted (blue horizontal lines).

Several fish metrics (*e.g.*, % sensitive fish and number of sensitive taxa) and a few macroinvertebrate metrics exhibited strong negative relationships with DO flux (Table 16). Total macroinvertebrate taxa, number of EPT taxa, and number of clinger taxa were among the highest ranking macroinvertebrate metrics. Total number of macroinvertebrate taxa generally remain between the 25th-75th percentiles at DO flux <4.5 mg/L; however, above this range values are at or below the 25th percentile (Figure 42a). Sensitive fish (% and number of taxa) exhibit a wide range of values at DO flux less than about 4 mg/L; however, as DO flux increases above ~4.5 mg/L, sensitive fish decline to 10 percent or less of the sampled population (Figure 42b, c). The 2008 streams generally correspond to this pattern as well. Strong positive relationships noted for tolerant fish species and omnivores (Table 16). At DO flux of 4.5 mg/L or less, tolerant fish species were generally a small (10 percent or less) percent of the total population (Figure 42d). As DO flux increased above 4.5 mg/L, tolerant species increased as a portion of the total and values were above the 75th percentile for this metric. The 2008 data are more variable with respect to this metric and two of the coldwater streams - Wells Creek and Vermilion River - exhibit high percentages of tolerant species at low DO flux concentrations (Figure 42d), which suggests other factors likely drive the relative distribution of tolerant versus sensitive species in these coldwater streams.

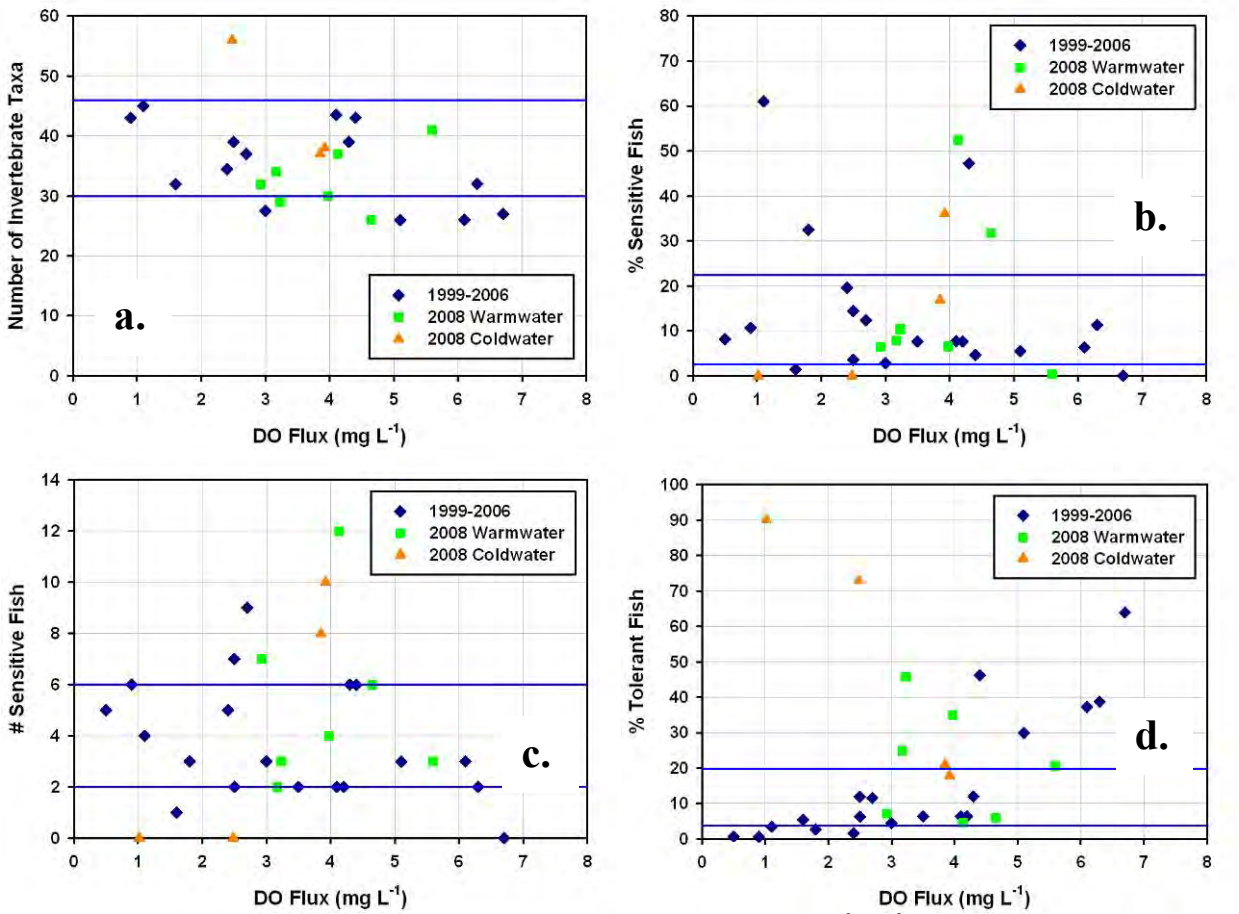


Figure 42. DO flux and fish and macroinvertebrate metrics. Statewide 25th-75th percentiles for nonwadeable rivers noted (blue horizontal lines).

BOD_5 is an important measure of the potential stress on a biological community as there is a well-documented relationship between BOD_5 and biological condition. There is a strong relationship between sestonic chlorophyll and BOD_5 (Figure 26) presumably due in part to the increase in organic matter available to heterotrophs as a result of algal death. The increase in BOD_5 can lead to lower DO levels and may also indicate a shift in the food resources in the system. Both of these responses lead to declines in biological condition and data from Minnesota indicates that there is a strong response of biological metrics to increases in the BOD_5. Many biological metrics indicated a negative shift in biological condition at ~2-3 mg/L for BOD_5 (Figure 43). This was particularly apparent in with macroinvertebrate taxa richness where most sites with a BOD_5 above 2 mg/L was below the statewide 25th percentile for this metric (Figure 43c). All sites with available BOD_5 data were warmwater and all but one site had a drainage area greater than 500 mi^2. As a result, no analysis of the impact of stream size and thermal regime on BOD_5 can be provided.

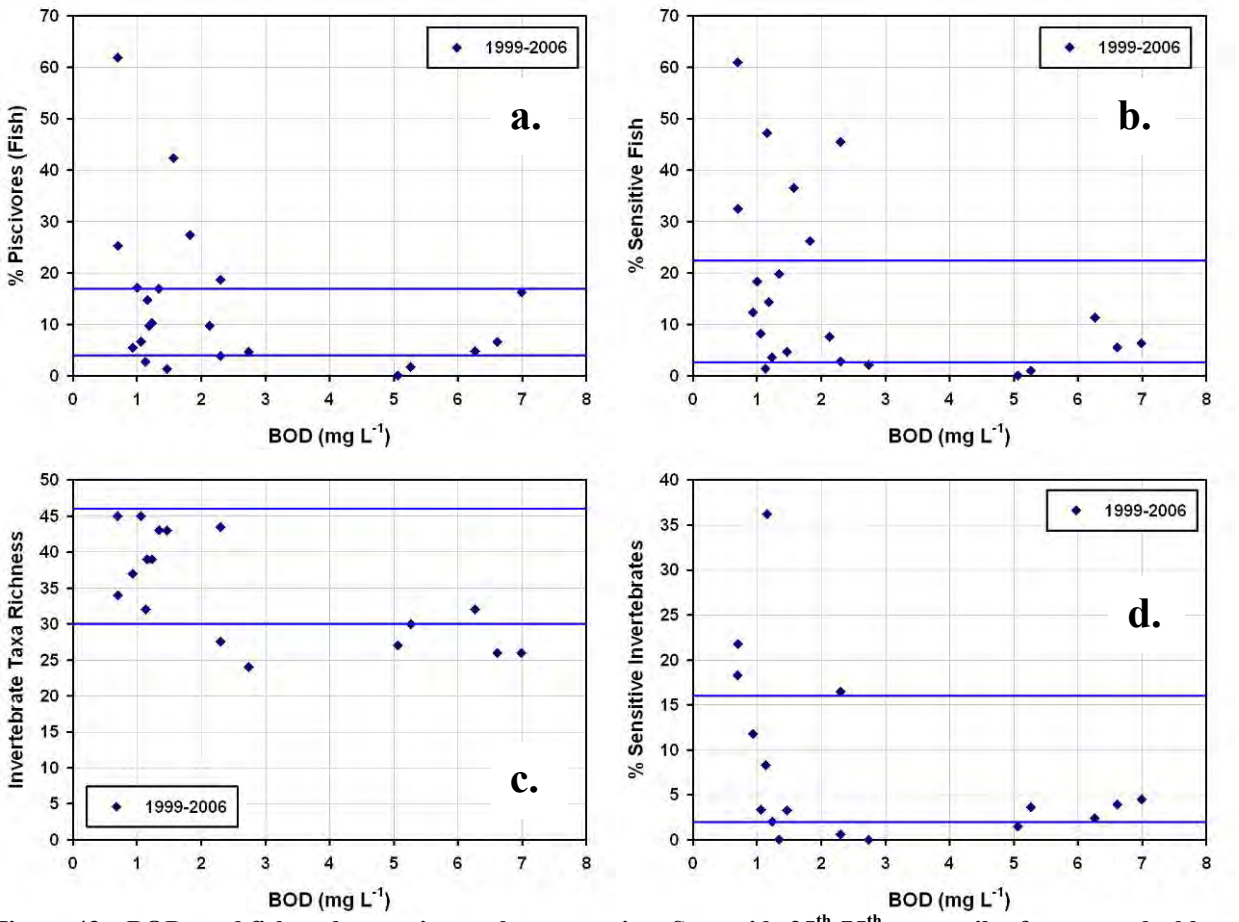


Figure 43. BOD_5 and fish and macroinvertebrate metrics. Statewide 25th-75th percentiles for nonwadeable rivers noted (blue horizontal lines).

C. Reference Condition Analysis Results

Sample sizes for reference condition AUIDs using STORET data were sufficient to calculate quantiles for many of the datasets although for all three water quality measures, the south region had very few reference AUIDs (Table 17). In addition, the number of reaches in for nonwadeable streams was limited for some regions. As a result, any conclusions drawn from this analysis for this region and this stream size must be treated with caution. A comparison of reference and non-reference distributions indicated that the reference AUID selection process was generally effective in identifying higher quality reaches (Figures 44-46). Following the methods of USEPA (2000c), the 3rd quartile (i.e., 75th percentile) of the reference reaches is most relevant to the criteria development process (highlighted in Table 17). The most useful values from this analysis are the 75th percentiles for the north and central regions for BOD_5 and TP. As mentioned previously, the small sample sizes for the south region limit the conclusions that can be drawn from these results. In addition, the results from chlorophyll-a analyses are not useful due the nature of this measure. Most of the reaches in this dataset are considered wadeable (watershed <500 mi^2). These waters generally do not produce large blooms of sestonic algae and as a result the distribution of chlorophyll-a values is relatively low in the STORET dataset. Although this analysis is informative, the nature of the STORET dataset limits the application of these values. The STORET database includes data collected for a variety of reasons including probabilistic and targeted sampling. As a result, these data may contain biases and should be treated cautiously.

Table 17. Quantiles and sample sizes for reference AUID datasets.

Data Source	Region	Stream Size	WQ Variable	25th	50th	75th	n
STORET	North	All	BOD_5 (mg L^{-1})	1.1	1.2	2.0	51
STORET	Central	All	BOD_5 (mg L^{-1})	1.0	1.6	2.0	12
STORET	South	All	BOD_5 (mg L^{-1})	-	-	-	1
STORET	North	Wadeable	BOD_5 (mg L^{-1})	1.0	1.3	2.0	31
STORET	Central	Wadeable	BOD_5 (mg L^{-1})	0.9	1.6	2.0	10
STORET	South	Wadeable	BOD_5 (mg L^{-1})	-	-	-	1
STORET	North	Nonwadeable	BOD_5 (mg L^{-1})	1.1	1.1	1.8	20
STORET	Central	Nonwadeable	BOD_5 (mg L^{-1})	-	-	-	2
STORET	South	Nonwadeable	BOD_5 (mg L^{-1})	-	-	-	0
STORET	North	All	Chlorophyll-a (µg L^{-1})	1	2	3	63
STORET	Central	All	Chlorophyll-a (µg L^{-1})	2	3	5	22
STORET	South	All	Chlorophyll-a (µg L^{-1})	3	11	19	4
STORET	North	Wadeable	Chlorophyll-a (µg L^{-1})	1	2	3	40
STORET	Central	Wadeable	Chlorophyll-a (µg L^{-1})	1	2	4	17
STORET	South	Wadeable	Chlorophyll-a (µg L^{-1})	3	11	19	4
STORET	North	Nonwadeable	Chlorophyll-a (µg L^{-1})	2	3	4	23
STORET	Central	Nonwadeable	Chlorophyll-a (µg L^{-1})	4	5	16	5
STORET	South	Nonwadeable	Chlorophyll-a (µg L^{-1})	-	-	-	0
STORET	North	All	Total Phosphorus (µg L^{-1})	29	42	61	156
STORET	Central	All	Total Phosphorus (µg L^{-1})	61	90	139	69
STORET	South	All	Total Phosphorus (µg L^{-1})	65	125	302	6
STORET	North	Wadeable	Total Phosphorus (µg L^{-1})	27	42	65	124
STORET	Central	Wadeable	Total Phosphorus (µg L^{-1})	61	98	157	58
STORET	South	Wadeable	Total Phosphorus (µg L^{-1})	64	134	325	5
STORET	North	Nonwadeable	Total Phosphorus (µg L^{-1})	66	69	74	32
STORET	Central	Nonwadeable	Total Phosphorus (µg L^{-1})	63	63	66	11
STORET	South	Nonwadeable	Total Phosphorus (µg L^{-1})	-	-	-	1

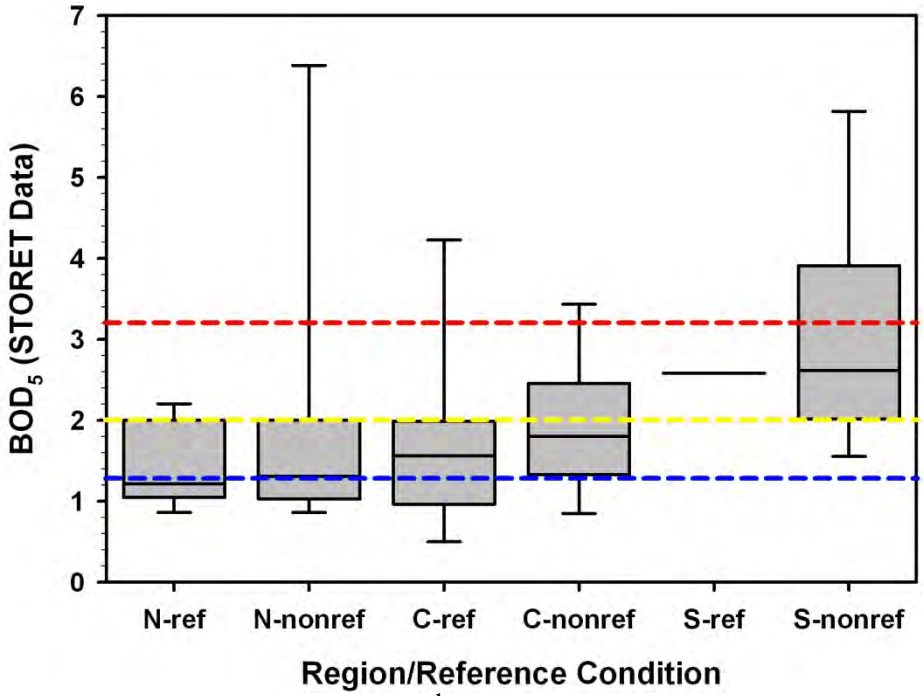


Figure 44. Box plots of BOD_5 concentrations (mg L^{-1}) by region for reference and non-reference AUIDs (description of box plots: solid line = median, upper and lower bounds = 75th and 25th percentiles, whisker caps = 10th and 90th percentiles; blue dashed line = north region draft criterion, yellow dashed line = central region draft criterion, red dashed line = south region draft criterion).

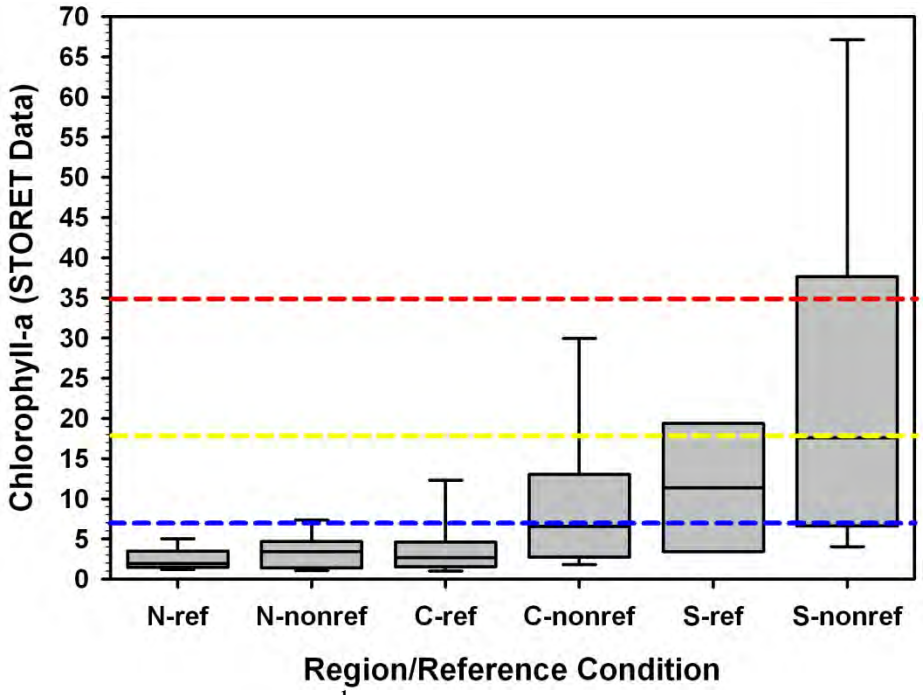


Figure 45. Box plots of chlorophyll-a (µg L^{-1}) concentrations by region for reference and non-reference AUIDs (description of box plots: solid line = median, upper and lower bounds = 75th and 25th percentiles, whisker caps = 10th and 90th percentiles; blue dashed line = north region draft criterion, yellow dashed line = central region draft criterion, red dashed line = south region draft criterion).

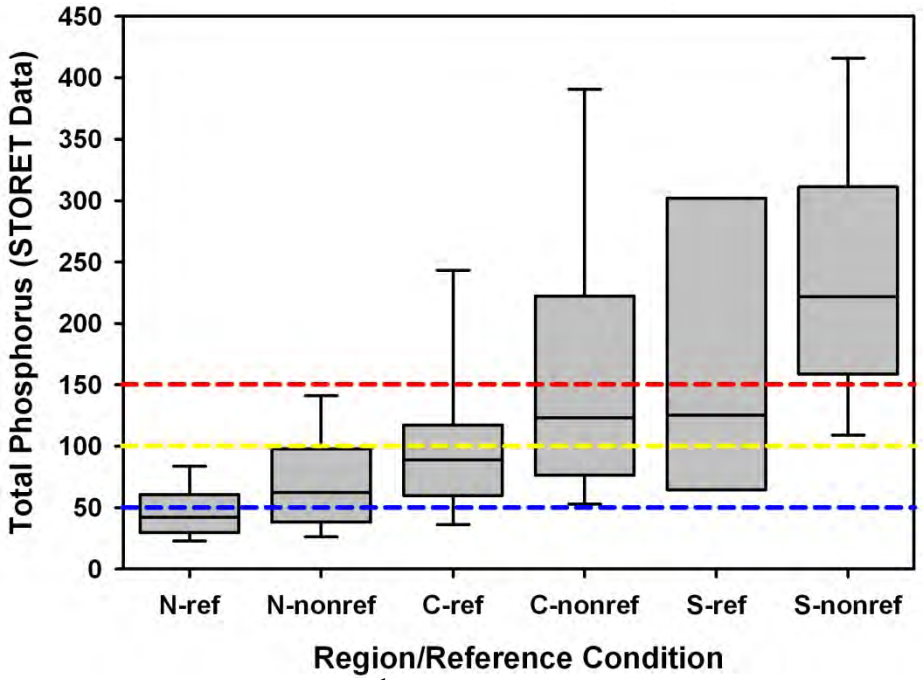


Figure 46. Box plots of total phosphorus (µg L^{-1}) concentrations by region for reference and non-reference AUIDs (description of box plots: solid line = median, upper and lower bounds = 75th and 25th percentiles, whisker caps = 10th and 90th percentiles; blue dashed line = north region draft criterion, yellow dashed line = central region draft criterion, red dashed line = south region draft criterion).

D. Threshold Concentration Development: Results from Quantile Regression and Changepoint Analyses

Threshold concentration values were identified for BOD_5, diel DO flux, chlorophyll-a, total phosphorus, and total nitrogen using the two analysis methods (*i.e.*, additive quantile regression smoothing and changepoint) from the available datasets. Scatter plot figures showing the analyses used to develop threshold concentration for each dataset are included in Appendix IV. A large number of datasets and biological metrics we analyzed using two different statistical methods, but concentration thresholds could not be identified for many of these datasets. Effective use of AQRS and changepoint analyses required datasets of sufficient size with sites across a gradient of enrichment. The analyses were not effective in datasets with overall poor condition (*e.g.*, some southern stream classes) where many streams had low metric scores due to non-nutrient stressors. There were a number of reasons that threshold concentrations could not be determined for these datasets and they include:

- The metric did not respond to the stressor or responded in a manner contrary to the predicted response
- AQRS fit failed F-test
- Threshold concentration failed significance test (chi-squared or Fisher Exact Test)

A summary of statistics for quantile regression- and changepoint-derived ranges for threshold nutrient and stressor concentrations from the various stream classes are presented in Table 18.

Table 18. Summary statistics for threshold concentrations for total water quality variables developed from fish and macroinvertebrate biomonitoring data using quantile regression and changepoint analyses (see Appendix IV for the raw threshold concentration values used to calculate these statistics; # T.C. = number of the threshold concentration values used to calculate statistics, RN = River Nutrient Study Data, STOR = STORET Data, BM = Biomonitoring Data).

Region	Range	Mean	Median	25th %ile	75th %ile	#T.C.
BOD_5 (mg^{-1})						
North (STOR)	-	-	-	-	-	0
Central (STOR)	1.5-4.1	2.8	2.2	2.1	3.8	7
South (STOR)	1.7-5.1	3.8	4.3	3.1	4.5	14
Statewide (RN)	1.9-3.9	2.9	2.5	2.5	3.7	5
DO Flux (mg^{-1})						
Statewide (RN)	3.0-4.9	3.6	3.3	3.1	3.8	4
Total Chlorophyll (μg^{-1})						
Statewide (RN)	11-62	31	31	21	35	11
Total Phosphorus (μg^{-1})						
North (BM)	33-154	72	68	44	91	26
North Nonwadeable (BM)	27-29	28	29	28	29	3
North Wadeable (BM)	33-126	66	64	48	81	22
Central (BM)	81-209	140	142	110	164	24
Central Nonwadeable (BM)	75-144	105	102	86	121	14
Central Wadeable (BM)	81-290	143	148	108	164	23
South (BM)	66-411	258	310	145	373	17
South Nonwadeable (BM)	131-199	165	165	148	182	2
South Wadeable (BM)	50-411	225	273	115	318	18
Statewide (RN)	42-233	135	136	98	168	15
Total Nitrogen (mg^{-1})						
Statewide (RN)	1.4-3.7	2.5	2.5	1.9	3.1	2

Data limitations were apparent for some datasets and as a result threshold concentrations could not be developed or caution should be exercised for those that could be identified. For example, in the nonwadeable river datasets were limited by a low number of sites and some southern datasets had a limited range of nutrient concentrations. In the south this was largely caused by a lack of streams with minimal impairment which is required to identify the response of the biological community to stressors. As a result fewer threshold concentrations could be identified and there was more variability in these values when thresholds could be determined (Figure 48). Other datasets had a small number of sites although analysis could be performed due to the strong response pattern. However, these

smaller datasets required a stronger relationship between the biological metric and the water quality variable for a significant relationship to be present. Some datasets were also not suited to the quantile regression or changepoint analyses although visual inspection did indicate that there was a response by the community to nutrient enrichment (*e.g.*, percent piscivore individuals in central streams using biomonitoring nutrient data).

The threshold concentrations were developed from different biological metrics which were selected because they were most sensitive to eutrophication. However, depending on the metric and biological group they have different responses to nutrients and stressors. As a result, the 25th percentile of these values more relevant to the development of protective aquatic life criteria. A mean or median statistic would be under protective because the concentration threshold would be exceeded for approximately half of the biological metrics. Stevenson et al. (2008) states that: "Setting criteria below thresholds in responses demonstrating assimilative capacity provides a margin of safety to protect valued attributes". This safety factor is incorporated into this line of evidence by using the 25th percentile of threshold concentrations for each dataset. The combination of this more protective statistic and the use of sensitive metrics resulted in a line of evidence that is supportive of the CWA interim goal and Minnesota's aquatic life use goals.

No threshold concentrations could be determined for BOD_5 in the northern streams due to a limited stressor range in this region (Figure 47). There was a significant difference between BOD_5 threshold concentrations for the central and southern stream classes based on Mann-Whitney Rank Sum test (data failed normality test) f (P = 0.0399) (SigmaPlot ver. 11; Systat Software 2008). This suggests that different thresholds are appropriate for these two regions.

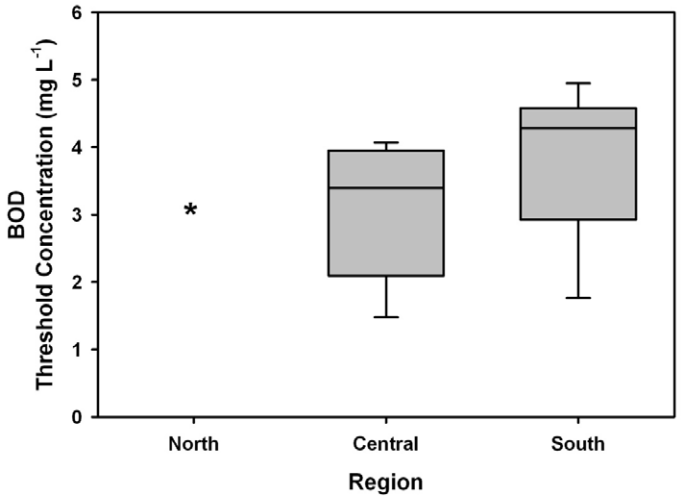


Figure 47. Box plots of BOD_5 threshold concentrations by region using additive quantile regression smoothing and changepoint threshold concentrations (description of box plots: solid line = median, upper and lower bounds = 75th and 25th percentiles, whisker caps = 10th and 90th percentiles; n values: Central = 7, South = 14). See Appendix IV for raw threshold concentration values used to generate box plots.

A Kruskal-Wallis Analysis of Variance (ANOVA) on Ranks was performed due to non-normality to test for differences in the total phosphorus threshold concentrations from different regions and river sizes (SigmaPlot ver. 11; Systat Software 2008). A significant difference (P = <0.0001) between the mean threshold concentrations was identified for the difference regions and river sizes with both datasets (i.e., midpoint additive quantile regression smoothing and changepoint threshold concentration dataset and upper breakpoint or midpoint additive quantile regression smoothing and changepoint threshold concentration dataset). Due to an unequal number of threshold concentrations in the different groups, a Dunn's multiple comparison test was performed to determine where there were significant differences between mean threshold concentrations (SigmaPlot ver. 11; Systat Software 2008). The most obvious differences were among the regional total phosphorus threshold concentrations with criteria values increasing from north to south. The threshold concentrations for both northern river size classes were significantly different from the southern wadeable rivers (Figure 48a). In general, threshold concentrations for nonwadeable rivers were lower than those for wadeable rivers. However, there were no significant differences between the mean total phosphorus concentration thresholds between nonwadeable and wadeable rivers within any of the regions

(Figure 48a). This suggests that different criteria may not be needed for different stream sizes. It is likely that a smaller proportion of wadeable streams will have poor biological condition resulting from eutrophication, but there is no indication that these streams are not affected by eutrophication. As a result, wadeable streams should not be excluded from nutrient standards. The relatively low number of threshold concentrations that could be determined for nonwadeable rivers also increases the importance of the values determined for the wadeable rivers. The low number of threshold concentrations was at least partly driven by the relatively small number of nonwadeable rivers from which data was available. A similar regional pattern appears when regional threshold concentrations are compared for streams of all sizes. The threshold concentrations for the north region was significantly different (P = <0.0001) from the central and southern regions (Figure 48b). This suggests that regionalizing criteria is justified.

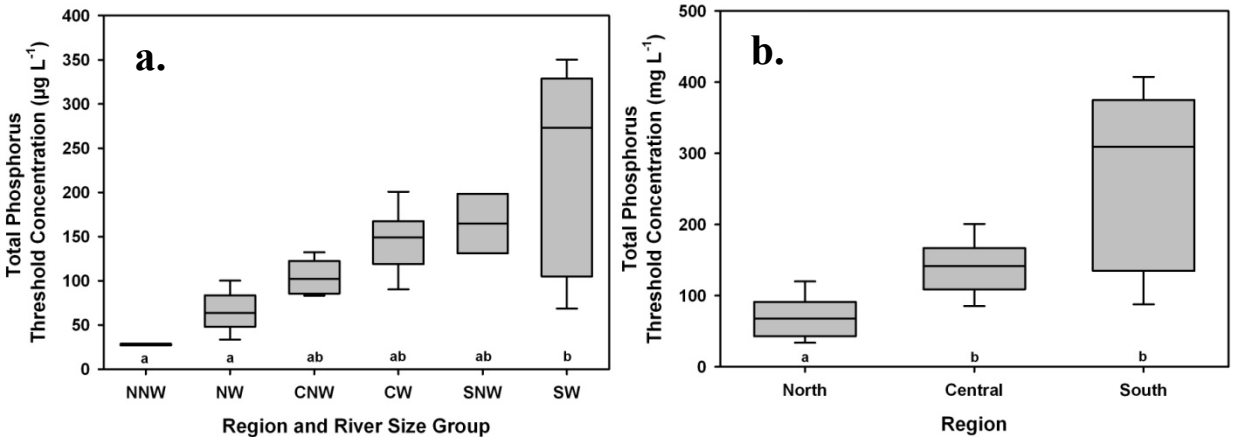
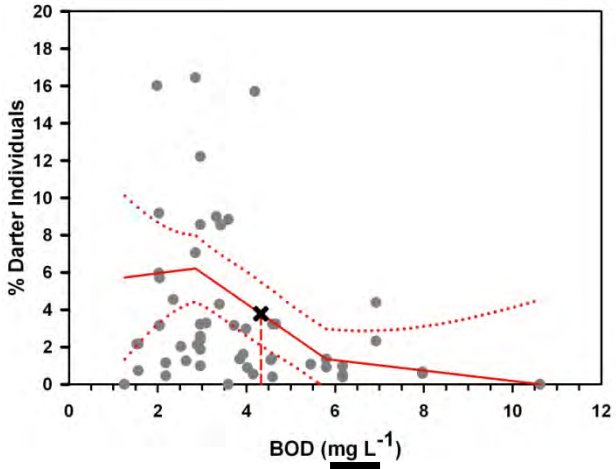


Figure 48. Box plots of phosphorus threshold concentrations for a) the three regions and two river sizes and b) three regions only using additive quantile regression smoothing and changepoint threshold concentrations (description of box plots: solid line = median, upper and lower bounds = 75th and 25th percentiles, whisker caps = 10th and 90th percentiles; n values: North Nonwadeable (NNW) = 3, North Wadeable (NW) = 22, Central Nonwadeable (CNW) = 14, Central Wadeable (CW) = 23, South Nonwadeable (SNW) = 2, Southern Wadeable (SW) = 18), North = 26, Central = 24, South = 17. Region and river size groups with significantly different (p<0.05) mean threshold concentrations are indicated by different letters below each box plot as determined by a Kruskal-Wallis ANOVA on Ranks with Dunn's multiple comparison test. See Appendix IV for raw threshold concentration values used to generate box plots.

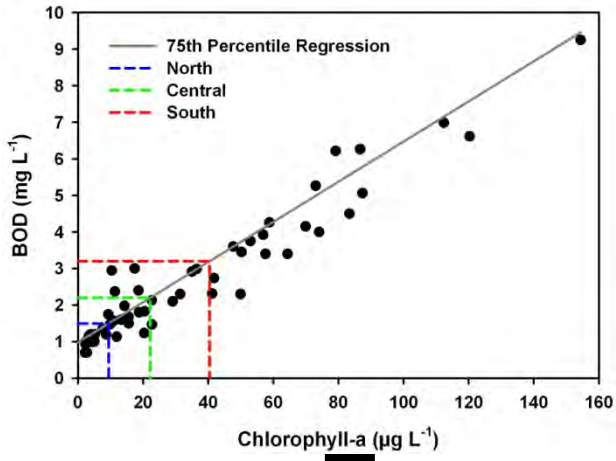
The relationship between biology and total nitrogen was also examined however the relationships were not strong and only a few threshold concentration values could be identified (see Tables IV-17 and 18). These relationships are also be complicated by a covariance with phosphorus (Figure 24b). Additional work is needed to determine if eutrophication-based standards are appropriate for nitrogen. Research has indicated that nitrogen is often a limiting or co-limiting nutrient in freshwater systems (Dodds 2006, Dodds & Cole 2007), which suggests that nitrogen may contribute to eutrophication in Minnesota streams.

Threshold concentrations developed using the causal association between total phosphorus and the decline in biological metrics should be considered cautiously in a "multiple lines of evidence" because they may be under protective of biological condition. There are a number of factors that reduce or mitigate the effect of nutrients on aquatic life in streams (*e.g.*, shading and low residence time). As a result, some streams may support relatively high levels of nutrients with minimal impact to aquatic life. These streams may result in the outer edge of the wedge in the nutrient-biological metric plots to shift to the right. This shift will tend to cause the concentration threshold to increase and may not be reflective of protective nutrient levels for streams without characteristics that mitigate the effects of nutrients on these systems. Therefore, the analyses linking proximate stressors (*e.g.*, BOD_5 and DO flux) to biological condition are a better determination of protective concentrations. These stressor concentrations still need to be linked to nutrient levels as nutrients are a major cause of these stressors (see Figure 28 Figure 29 Figure 30). Nutrient levels can be associated with levels of stressors using a series of regressions. Using BOD_5 threshold developed from the AQRS and changepoint analyses and 75th percentile quantile regressions for water quality variables, nutrient levels to protect aquatic life can be determined (Figures 49-50). Unfortunately, sufficient information for DO flux was not available to determine regional patterns of the impact of this stressor on the biology.



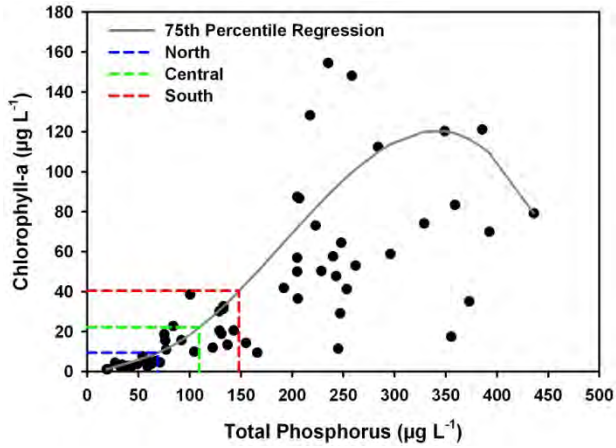
BOD_5 (25th Percentile)

North	Central	South
1.5*	2.1	3.1



Chlorophyll-a

North	Central	South
10(5)	21(13)	39(28)

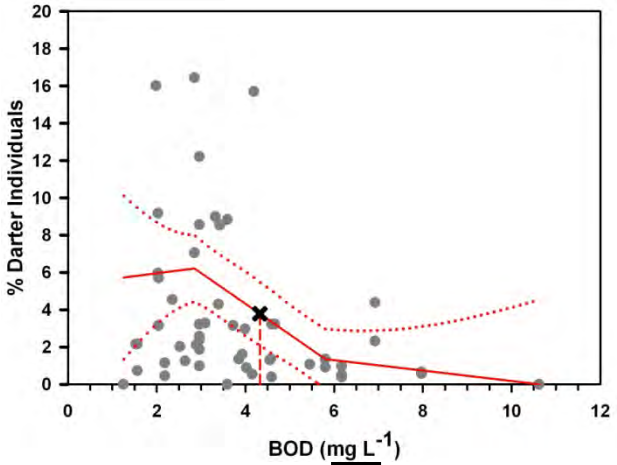


Total Phosphorus

North	Central	South
72(41)	107(83)	149(129)

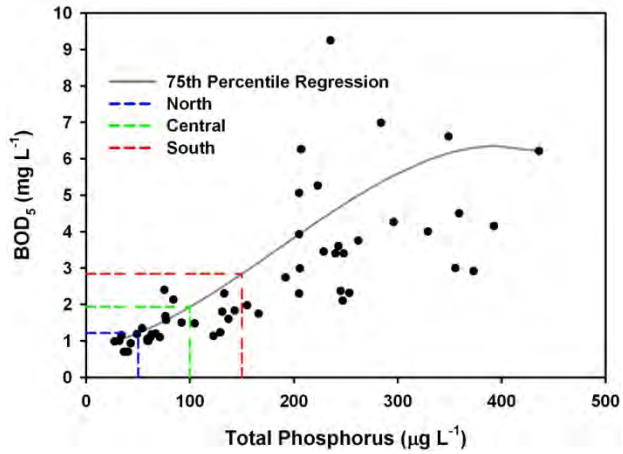
Figure 49. Interpolation of phosphorus levels protective of aquatic life use goals using the relationships between BOD_5, chlorophyll-a, and total phosphorus. BOD_5 thresholds were derived from the 25th percentile of threshold concentration values using biology-BOD_5 relationships (upper breakpoint or midpoint additive quantile regression smoothing and changepoint threshold concentrations) for each region. Regressions for BOD_5 → Chl-a and Chl-a → Total Phosphorus were fit using 75th smoothing splines quantile regressions. The first value was interpolated from the River Nutrient data and the value in parentheses was determined

using the STORET dataset. *The threshold values for BOD_5 were based on the maximum values observed in this region with this the River Nutrient dataset.



BOD_5 (25th Percentile)

North	Central	South
1.5*	2.1	3.1



Total Phosphorus

North	Central	South
70(78)	118(121)	193(168)

Figure 50. Interpolation of phosphorus levels protective of aquatic life use goals using the relationships between BOD_5 and total phosphorus. BOD_5 thresholds were derived from the 25th percentile of threshold concentration values using biology-BOD_5 relationships (additive quantile regression smoothing and changepoint threshold concentrations) for each region. Regressions for BOD_5 → Total Phosphorus were fit using 75th smoothing splines quantile regressions. The first value was interpolated from the River Nutrient data and the value in parentheses was determined using the STORET dataset. *The threshold values for BOD_5 were based on the maximum values observed in this region with this the River Nutrient dataset.

The threshold concentrations for total phosphorus developed using AQRS and changepoint analysis were similar to those derived from the serial regression of BOD_5 → Chlorophyll-a → total phosphorus (Table 18, Figures 49-50). The 25th percentile of values from AQRS and changepoint analysis for the north was 44 µg/L (Table 18). Using the serial regression (BOD_5 → Chlorophyll-a → total phosphorus) an interpolated protective value for TP was 72 µg/L for the River Nutrient dataset and 41 µg/L for the STORET dataset. Using the direct regression of BOD_5 → total phosphorus, an interpolated protective value for TP was 70 µg/L for the River Nutrient dataset and 78 µg/L for the STORET dataset. The lower values for AQRS and changepoint analysis may be caused by the limited disturbance gradient, which resulted lower values from the changepoint analysis. Specifically, the changepoint analysis may be responsive to the initial decrease in the biological metric because there is a limited disturbance gradient. When a more complete disturbance gradient is present, the changepoint often falls in the middle of the area where the metric score is most rapidly declining. In the central region, the biological analyses and the serial regions resulted in similar values. The 25th percentile of concentration values was 110 µg/L for AQRS and changepoint analysis in the central region (Table 18). Using the serial regression (BOD_5 → Chlorophyll-a → total phosphorus) an interpolated

protective value for TP in the central region was 107 µg/L for the River Nutrient dataset and 83 µg/L for the STORET dataset. Using the direct regression of BOD_5 → total phosphorus, an interpolated protective value for TP was 118 µg/L for the River Nutrient dataset and 121 µg/L for the STORET dataset. The southern region values were also similar for the two methods with 145 µg/L determined using the AQRS and changepoint analyses (Table 18). Using the serial regression (BOD_5 → Chlorophyll-a → total phosphorus) an interpolated protective value for TP in the south region was 149 μgL^{-1} for the River Nutrient dataset and 129 µg/L for the STORET dataset. Using the direct regression of BOD_5 → total phosphorus, an interpolated protective value for TP was 193 µg/L for the River Nutrient dataset and 168 µg/L for the STORET dataset. The discrepancies between the River Nutrient and STORET datasets are the result of a greater number of wadeable streams in the STORET dataset. These streams are less likely to grow sestonic algae so models based on the STORET dataset would predict lower Chl-a per unit of TP. However, when a model is developed to directly predict TP from BOD_5, the River Nutrient and STORET datasets predict very similar concentrations. This pattern likely results from the fact that BOD_5 is a more comprehensive measure of productivity as it also captures the impacts of benthic algae, bacteria, fungi, and other organisms on the trophic status of the waterbody. These impacts are more important in wadeable streams and support the need for nutrient criteria in wadeable streams.

The use of different metrics and statistical approaches resulted in a range of concentration thresholds for a given stream class (Table 18). This range represents variability between these datasets and some of the uncertainly around these thresholds. In general, these statistical methods identify areas along a gradient of nutrients where there is a change in the biological community. These thresholds are typically not specific enough for these methods to identify the exact concentration where the community will shift or violate biological goals. Some of this variability comes from sampling variability and others come from natural differences between streams. Even though we controlled some of this natural variability through a stream classification, natural variability still exists. However, these methods do identify relatively consistent concentration ranges within stream classes, which indicate that these methods are effective tools for determining where a negative and unwanted change in the community occurs along a nutrient gradient. As a result the 25th percentile of threshold concentrations are considered as part of the "multiple lines of evidence" approach used for river eutrophication criteria development.

E. Periphyton Analysis: MPCA River Nutrient and USGS Studies

Periphyton (benthic algae) samples were collected from rock and wood substrates at each of twelve separate sites (see Methods) in August 2000. Diatoms, greens, and blue-greens were the predominant algal types found at each site, with diatoms being the most abundant. Based on paired data for 12 sites, periphyton was greater on rock compared to wood at 7 of 12 sites and in terms of density at 9 of 12 sites. The total number of diatom species found at any one site ranged from 25 (wood) at UM-872 to 8 (wood) at BE-54, with 13- 15 species being typical for most sites. Blue-greens were frequently the next most abundant form and ranged from nine (rock) species at UM-872 to two species at several sites, with four to five species typically found at most sites. Greens varied from 12 (wood) species at CR-0.2 to one at UM-1056 and CWR 72.3, with three to four species typically found at most sites. Periphyton and water quality data for 2000 may be found in Heiskary and Markus (2003).

Among the diatoms the most common genera found included *Navicula*, *Nitzschia*, *Rhoicosphenia*, *Cocconeis, Cyclotella*, and *Melosira*. These genera were often represented by three or more species. Some like *Navicula* were represented by 16 species and others like *Rhoicosphenia curvata* were represented by one species. Among the highest densities of diatoms were *Cocconeis placentula* (CWR 35.5) – 202.1E6 µm ^{3}/cm^2, *Cyclotella meneghiniana* (BE-54) - 494.1E6 µm ^{3}/cm^2, *Gyrosigma spencerii* (RE-452) – 157.1E6 µm ^{3}/cm^2 and *Melosira varians* (CWR-35.5) - 237E6 µm ^{3}/cm^2. The most common blue-green algal types were non-motile blue-greens (found at all sites), followed by the filamentous *Lyngbya* and *Oscillatoria*. Among the highest densities of *Oscillatoria* 377.6E6 µm ^{3}/cm^2 (wood) and 122.7E6 µm ^{3}/cm^2 were noted at RE-536 and RE-452 respectively. The highest density of *Lyngbya* was found at CWR-35.5: 146.8E6 µm ^{3}/cm^2. Typical densities of blue-greens at most sites were on the order of 10E6- 20E6 µm ^{3}/cm^2.

Green algae were represented by very few genera. Of the eight noted, only *Chlorococcum* was found at eight sites with densities ranging from 44.45E6 µm ^{3}/cm^2 (rock @ CWR-72.3) to 2.254E6 µm ^{3}/cm^2 (wood @ BE-73.3). Though filamentous greens such as *Cladophora* and *Spirogyra* were found infrequently they did exhibit extremely high densities when present. *Cladophora* accounted for 53 and 99 percent of the bio-volume at RU-34 (rock) and

CWR-72.3 (wood), respectively, with densities of 517.1E6 and 58.02E9 μm ^{3}/cm^2. *Spirogyra*, found only at RU-34, accounted for 87 percent of the bio-volume at the site with a density of 2.494E9 μm^3/cm^2.

Other forms such as Pyrrhophyta, Euglenophyta, Chrysophyta, and Cryptophyta were seldom present in these samples. Only *Euglena*, which comprised 15 percent of bio-volume at BE-73.2 and *Batrachospermum vagum*, a filamentous red alga, which comprised 31 percent of bio-volume at RE-536 were found in any appreciable quantities.

Hynes (1970) lists controlling factors for the presence or absence of benthic algal species, including current, substrate, light, and scour and stated that strength of current is the paramount factor for determining species composition. Once a scour event has occurred, often the initial algal "colonizers" can significantly affect which algae can secondarily attach. If a filamentous green alga can attach as a colonizer, there can be a large bio-volume and biomass present (as was the case for *Cladophora* at RU-34 and CWR-72.3). However, if these algae are not present initially, they may have a difficult time gaining a holdfast, which would result in reduced bio-volume, but may increase biodiversity.

Three rock samples were collected at UM-872, near Anoka, for quality assurance purposes. This provided one basis for assessing precision in characterizing the density, bio-volume, and form of algae at a given site from a consistent substrate type. Some observations from this comparison follow:

		Sample A	**Sample B** (rep. 1)	**Sample C** (rep. 2)
1. # of species accounting for 80% of bio-volume		9	12	12
2. Algal forms contributing	Diatom	8 (17, 59%)	7 (17, 52%)	11 (26, 60%)
to 80% of bio-volume	Green	-- (5, 17%)	2 (7, 21%)	-- (9, 21%)
(# and % total species found)	Blue-green	1 (7, 24%)	3 (9, 27%)	1 (8, 19%)
	Sum	**9 (29)**	**12 (33)**	**12 (43)**
3. Total bio-volume (μm^3/cm^2)		216,515,943	227,948,884	129,906,614
4. Total density (#cells/cm^2)		1,188,846	1,567,368	736,078

Based on this comparison, the three samples provide a similar indication of the number of species that comprise 80 percent or more of the bio-volume. However, there is variability for the total number of species, which ranged from 29-43. Of the species that comprised the upper 80 percent, only four were common to all three samples and only the diatom *Amphora pediculus* was consistently among the dominant species in each sample at 15, 12, and 29 percent respectively. Variability in the estimates of total bio-volume and total density was evident based on the three replicates. For example, total bio-volume of sample C was 40 percent lower than sample A and total density was 38 percent lower, while sample B was five percent and 24 percent higher, respectively. This suggests care must be taken when comparing total bio-volume and/or total density among sites. Comparisons of dominant algal forms were comparable among the replicates.

Benthic chlorophyll-a concentrations ranged from 2.1 to 150 mg/m^2 among all samples (Lee 2002). Individual measurements on wood substrate ranged from 4.0 mg/m^2 at BE-73.2 up to 117 mg/m^2 at UM-872. Individual measurements on rock substrate ranged from 2.1 mg/m^2 at UM-1056 to 150 mg/m^2 at UM-872. There was no consistent relationship between chlorophyll-a concentrations found on wood vs. rock substrate.

There was no consistent relationship between periphyton Chl-a and sestonic Chl-a (Figure 51a). In general, periphyton Chl-a was relatively low at most sites (<50 mg/m^2) and there was no distinct relationship with TP, which is in contrast to sestonic Chl-a (Figure 51a). Based on the 2000 data there was no relationship between periphyton Chl-a and DO flux (Figure 51b). In contrast, as previously noted (Figure 17) DO flux tends to increase as sestonic Chl-a increases. Based on the 2000 data four sites with sestonic Chl-a >75 ug/L had a DO flux >5.0 mg/L (Figure 51b). Corresponding periphyton was relatively low at these four sites. Sestonic Chl-T tends to increase for 3rd-4th order sites, as a function of increased residence time and nutrient enrichment (Heiskary & Markus 2001). However, periphyton concentrations do not exhibit a distinct relationship with watershed size (Figure 51a and Table 3).

Data collected as a part of the 2007 USGS Region V study provide some additional insight into periphyton concentrations and relationships for Minnesota streams. Site locations and data are summarized in Appendix III. The 2007 river sites are lower order sites (in contrast to MPCA 1999-2000 sites) and are generally considered wadeable.

Three of six sites exhibited elevated periphyton Chl-a (>150 mg/m^2) and one site (South Branch Rush River) exhibited elevated periphyton and sestonic Chl-a (Figure 52a). Sestonic Chl-a was low in the remainder of the sites and there was no distinct relationship between periphyton and sestonic Chl-a or TP. DO flux tended to increase as periphyton and/or sestonic Chl-a increased (Figure 52b). Of the three sites with elevated DO flux (>5.0 mg/L; Clearwater, South Branch Rush and West Fork Beaver) all had high periphyton Chl-a and in the South Branch Rush seston Chl-a was high as well.

Fish data, for select metrics, were available for three of these sites based on previous MPCA collections and select metrics (Table 16). The percent sensitive fish for these sites is very low and well below the 25th percentile (Table 7). In contrast, percent tolerant fish is high and is above the 75th percentile in each case (Table 7). The total number of taxa for the South Branch Rush and Little Cobb are at or below the 25th percentile, while West Fork Beaver is between the 25th-50th percentiles (Table 7). These responses are similar to what was demonstrated for the medium to high order sites in Figure 30 through Figure 35.

Table 19. USGS 2007 Minnesota study sites.

River	Site number	Sensitive %	Sensitive #	Simple lithophils %	Simple lithophils #	Tolerant %	Tolerant #	# of taxa	TP µg/L	TN mg/L	Seston Chl-a µg/L
West Beaver	03MN018	1.2%	5	55%	227	25%	102	18	263	5.6	18.0
S. Branch Rush	03MN025	0%	0	6%	57	61%	571	15	162	8.9	44.7
Little Cobb	08MN902	1.2%	6	6%	29	34%	165	7	218	7.6	4.5

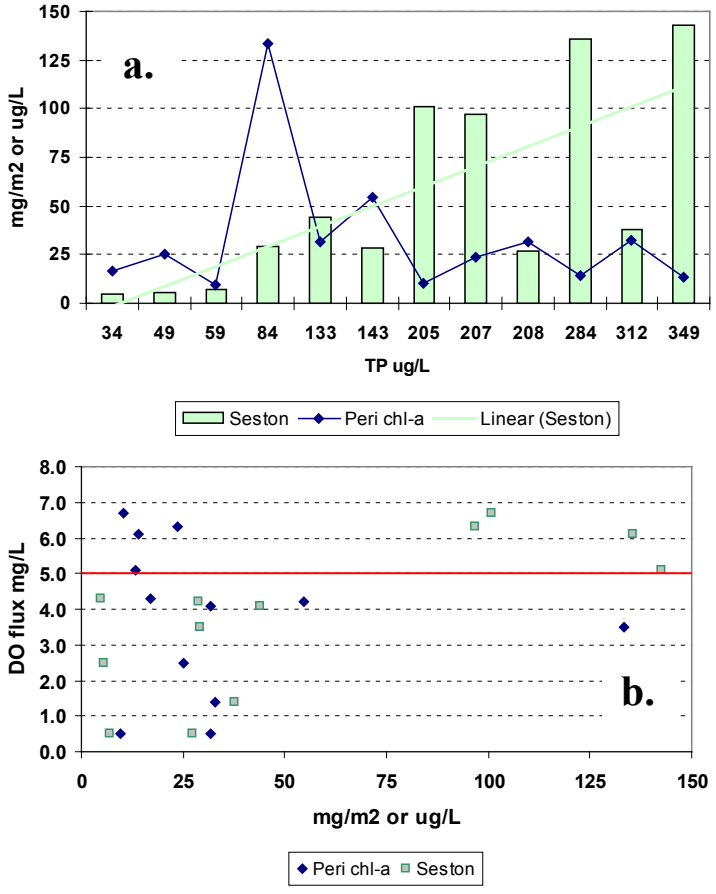
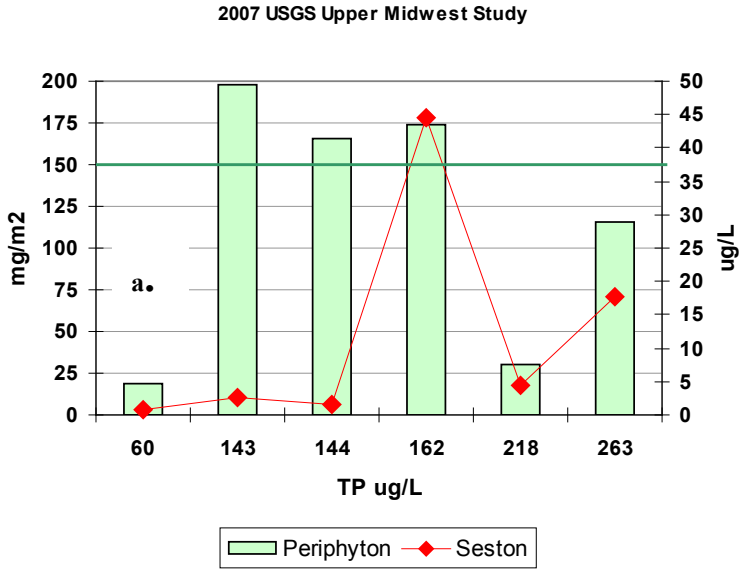
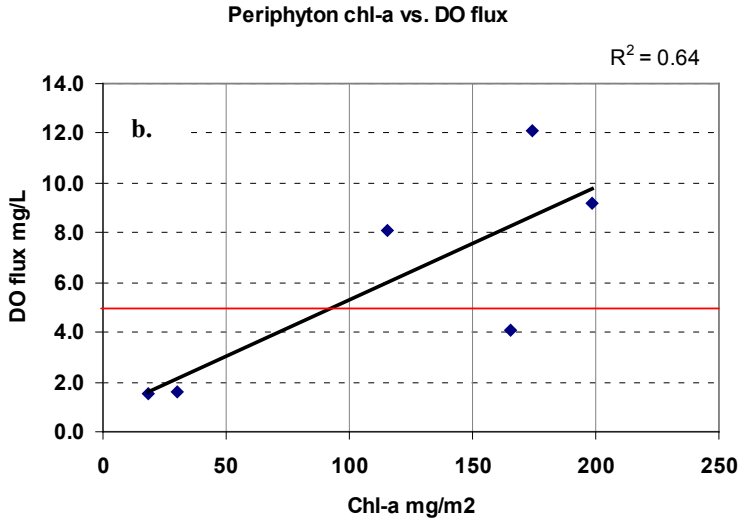


Figure 51. Summer-mean sestonic Chl-a and mean periphyton Chl-a for MPCA-USGS 2000 study sites as related to: a) TP and b) DO flux.





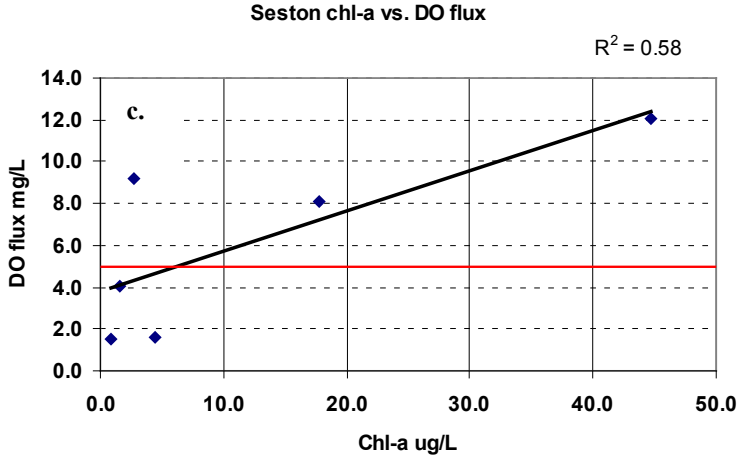


Figure 52. USGS 2007 Region V study sites for Minnesota. Comparison of periphyton and sestonic Chl-a relative to TP (a) and DO flux (b and c).

VI. DISCUSSION

A. RELATIONSHIPS AMONG NUTRIENTS, ALGAE, STREAM CHEMISTRY AND BIOTA

Large rivers are more autotrophic than smaller streams with an increasing fraction of the organic carbon being fixed by primary producers within the stream channel with increasing stream order. In these waters nutrient turnover is rapid (*i.e.,* short spirals), resulting in higher concentrations of readily available forms of nutrients (Rankin *et al.* 1999). In headwater streams that have been either channelized, had riparian vegetation removed, or the habitat otherwise degraded, the nutrient processing mimics that of large rivers in having comparatively short spirals (rapid turnover) and high algal biomass. Modified streams usually support large populations of omnivores, which have been shown to further increase nutrient recycling in streams (Grimm 1988). Lack of retention of inorganic nutrients is further exacerbated in streams with low gradients where a combination of excess sunlight, readily available nutrients, and slow flow velocity and volume result in degraded aquatic communities dominated by undesirable and highly tolerant species.

In our studies strong relationships are evident among in-stream nutrients (TP and TKN in particular) and algae (expressed as chlorophyll-a) for medium to high order streams during the summer growing season, based on studies conducted in 1999, 2000, 2001, 2006 and 2008. These findings are similar to that found in earlier studies (*e.g.,* Basu & Pick 1996, Van Nieuwenhuyse & Jones 1996). The relationship between TN and Chl-a was neither as strong nor as consistent as that for TKN. This is in part because TKN is largely comprised of organic N (*i.e.,* algae). TN, on the other hand, is essentially equal to TKN when concentrations are 1-2 mg/L or less; however as concentrations increase above this range, nitrate-N becomes a more significant portion of the TN and typically accounts for 50% or more of the TN at TN concentrations above ~4.0 mg/L. Our previous work (Heiskary & Markus 2001) also demonstrated that BOD_5 is highly correlated with phosphorus and chlorophyll and that relationship was reaffirmed with the 2006 study. The previous studies also demonstrated that the relationships (*e.g.,* slope) among nutrients, algae and BOD_5 varied (between sites and years) as a function of watershed size, flow and residence time (Heiskary & Markus 2001). In general, many of the relationships were stronger in years of average to low flow (2000 and 2006) in contrast to years with higher flow (1999).

Because eutrophication of rivers can alter biotic community composition and decrease biotic integrity, we have placed our emphasis on making associations among excess nutrients and impacts on stream biota. The generally described mechanism for impact of nutrients on streams is stimulation of excess primary productivity, which can degrade habitat, alter food resources, and deplete DO (Wang *et al.* 2007). Miltner and Rankin (1998), for example, found a deleterious effect on fish communities when TN and TP levels exceeded natural background level in lower order streams but found no affect in higher order streams and, at that time, indicated that not much is known about the response of fish communities in large rivers to the cascade of effects caused by an imbalance of nutrients. Rankin *et al.* (1999) note that while nutrients are essential to the functioning of healthy aquatic ecosystems, they can exert negative effects at much lower concentrations by altering trophic dynamics, increasing algal and macrophyte production (Sharpley *et al.* 1994), increasing turbidity (via increased sestonic algal production), decreasing average DO, and increasing fluctuations in diel DO and pH. Such changes, caused by excessive nutrient concentrations, result in shifts in species composition away from functional assemblages of intolerant species, benthic insectivores and top carnivores (*e.g.,* darters, insectivorous minnows, redhorse, sunfish, and black basses) typical of high quality warmwater streams towards less desirable assemblages of tolerant species, niche generalists, omnivores, and detritivores (*e.g.,* creek chub, bluntnose minnow, white sucker, carp, and green sunfish) typical of degraded warmwater streams.

One area we have emphasized is interrelationships among excess nutrients, algal productivity and diel DO fluctuations (flux). It is widely known that large plant and algal growths caused by excessive nutrient concentrations in streams can cause large diel DO fluctuations (Wilcock *et al.* 1995). Numerous investigators have identified the need for diel monitoring of DO and temperature in streams and establishing the linkages with nutrients and excess algal productivity. Huggins and Anderson (2005) note "By examining continuous measures of DO, it is hypothesized that the relationships between the watershed, water chemistry, nutrients, stream biota, and DO levels will become apparent." Arnwine and Sparks (2003) in their studies on wadeable streams note "DO appeared to be affected by the amount of periphyton present in the streams. While DO remains above criteria, diel fluctuations

were above stream reference condition. Extreme changes in DO levels are believed to have a detrimental effect on aquatic life even when minimum DO criteria are met." Sabater *et al.* (2000) noted diel DO variations were much higher (and reached hypoxia) at a site with higher biomass accumulation. The cyprinid-dominated fish community (which is more tolerant to hypoxia) at that site was attributed in part to the influence of algal biomass accumulation. Further, Sabater *et al*. (2000) cite several authors who note that excessive periphyton growth can affect fish distributions by altering the physical and chemical (DO, pH) characteristics of the river system. In general, algal biomass (either seston or periphyton) has been identified as the cause of large fluctuations in DO in rivers – and several authors note that these variations might affect fish populations.

In streams, large DO fluxes are often accompanied by fluctuations in pH and temperature making it hard to separate the effects of any one of those stressors. McDowell (1990), Boubee *et al.* (1991) and Richardson *et al.* (1994) concluded that the presence of stressful conditions, such as large diel variations in DO, temperature, and pH, may influence the composition of stream fish communities. Murdock and Dodds (2007) suggest that more primary production and increased heterotrophic microbial activity creates greater diel oxygen swings that could lead to areas of hypoxia within the river. Hypoxia typically occurs during periods of very low discharge or in rivers with limited flushing rate. USEPA (1986) notes various studies have focused on maximum temperatures in combination with low DO as being useful indicator of stress on streams. However, overall while there seems to be an acknowledgement that diel flux is a stressor of stream biota, there is a lack of information on dynamics of DO to the presence/absence of organisms (Garvey *et al.* 2007) and in general, there are few studies that effectively isolate DO as the single, most important stress on a fish's physiology.

Stream habitat is an important factor to consider when assessing the impacts of a pollutant (in this case excess nutrients) on stream biota. For example, Heatherly *et al.* (2007), in a study on wadeable streams, note that observed differences in macroinvertebrate populations were deemed a function of habitat and nutrient concentrations. However, they could not support the hypothesis that habitat was the primary factor governing biotic integrity since habitat degradation was generally evident in streams with elevated nutrient concentrations and felt both were of equal importance. High physical habitat and substratum quality can indirectly result in decreased nutrient export through enhanced physical retention, which allows for increased biological uptake and may in some instances allow for better "biology" than might otherwise be anticipated based on nutrient or other pollutant concentrations. Miltner and Rankin (1998) note that habitat scores generally explained the majority of the variance in the fish IBI scores (across all stream sizes and models). They also found that TP concentrations in Ohio were highest where habitat quality is lowest and they cautioned that the decreases observed in ICI and IBI scores along a TP gradient may also be reflecting degraded habitat conditions. In wadeable streams, however, the IBI differences behaved independently of habitat conditions (at least up to the 50th percentile), supporting "a cause and effect relationship between nutrients and biotic integrity" In our work we demonstrate that habitat, as reflected by metrics such as MSHA (QHEI), generally exhibited inverse relationships with TP, TN, chlorophyll, and DO flux (Table 16). However, some sites with moderately high nutrients but relatively good habitat are able to maintain more diverse assemblages of macroinvertebrates and fish than would be anticipated based upon nutrient concentrations alone (***e.g.,*** Rum-18; Figures 33-35).

Numerous fish and macroinvertebrate metrics were reviewed for their relationships with TP, TN, sestonic chlorophyll, and diel DO flux as we sought to identify appropriate metrics for criteria threshold identification (Table 16). Of those tested, several metrics appeared to provide useful and relatively consistent responses including the following: number of macroinvertebrate taxa, number of sensitive fish taxa, percent sensitive fish, and percent tolerant fish (Figures 38-43). Other metrics that also proved useful were percent macroinvertebrate clingers, number of EPT taxa, number of intolerant macroinvertebrate taxa, percent lithophils, and percent omnivore fish. Quantile regression focused on a subset of these variables and serves as a further refinement in our approach. Several of these metrics have been featured elsewhere in efforts to identify thresholds for nutrient criteria development.

Weigel and Robertson (2007) used an approach similar to ours when they made initial associations among variables by means of a Spearman correlation matrix. Several strong relations between biotic measures and nutrient variables were revealed by this technique. They found more nutrient variables were related strongly with fish metrics than macroinvertebrate metrics. Fish IBI, sucker species, intolerant species, riverine species, percent riverine, percent lithophils, percent invertivore, and percent round suckers correlated with multiple nutrient variables consistently in the same direction. They chose IBI and percent round suckers as the best fish measures for additional detailed investigation because correlation analyses suggested that they were the most responsive to nutrients, and appeared to

be representative of other fish measures. For macroinvertebrates, species richness was the only macroinvertebrate measure significantly correlated with our nutrient variables of greatest interest (TP and TN) at the $p < 0.01$ level, in addition to being correlated with the most nutrient variables overall. Arnwine *et al.* (2003) note a strong correlation with percent clingers and TP and number of EPT taxa and TP. Ortiz and Puig (2007) noted that EPT taxa richness was a sensitive indicator in their work. In an upstream – downstream comparison they found taxa richness was between 8 and 18 units higher at the upstream reach as compared to downstream. EPT richness was between 6 and 10 units higher at upstream site. They note further that, as a result of increased nutrient concentrations, sensitive taxa declined while tolerant taxa increased. Robertson *et al.* (2006) in their study of wadeable streams in Wisconsin note strong correlations with nutrients for EPT and HBI metrics and for fish they note IBI, percent intolerant, percent omnivores strongly correlated with sestonic Chl-a and nutrients. Overall we see similarities in the various studies that have been conducted; whereby there is an emphasis on sensitive species and taxa richness (fish and macroinvertebrates).

B. Nutrient Criteria Development

The reasons for development of nutrient criteria for rivers are many. Dodds and Welch (2000) note "Nutrient criteria for streams may be needed to avoid direct toxicity, taste and odor, alterations in biotic integrity, and interference with recreation." Walker *et al.* (2006) summarize a variety of reasons for addressing excess nutrients in streams as well as important factors to consider in the process. Many of their ideas touch on areas we have addressed in our efforts and they bear further mention as follows:

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

1) Impairment of the aquatic life use; whereby
   - Daily fluctuations in oxygen concentrations and pH values may negatively impact aquatic life;
   - Toxicity may result if high ammonia levels (*e.g.,* > 1 mg/L NH3-N) contribute to high nitrogen levels;
   - Blue-green algal blooms may release toxic compounds (*e.g.,* cyanotoxins);
   - A loss of diversity and other changes in the aquatic plant, macroinvertebrate, and fish community structure may result;
   - Extremes in stream pH are stressful and can even be deadly to aquatic organisms. High pH levels increase the toxicity of some substances, such as ammonia, whereas low pH levels can make heavy metals in stream sediment more mobile.
2) Negative impact on the drinking water and industrial water supply use:
   - Methemoglobinemia (blue-baby syndrome) may affect infants if nitrate levels >10 mg/L;
   - Diatoms and filamentous algae can clog intake screens and filters in water treatment plants;
   - Decay of algae may lead to taste and odor problems of drinking water;
   - Potentially carcinogenic disinfection by-products (trihalomethanes (THMs) may form during treatment of drinking water from eutrophic waters;
   - Treatment costs may rise for waters drawn from eutrophic sources by requiring more backwashing, etc.
3) Degradation of the aesthetic and recreational use (Figure 53):
   - Unsightly algal growth is unappealing to many swimmers and other stream users;
   - Slippery streambeds caused by heavy growths of algae on rocks are difficult to walk on;
   - Fishing lures may become tangled in algae and macrophytes and boat propellers may get tangled by aquatic vegetation.

Figure 53. Examples of severe blue-green algae (cyanobacteria) blooms on rivers that contribute to aesthetic, recreational use, and aquatic life impairment. a) Blue Earth River MN July 8, 2002, b) Watonwan River July 25, 2007, c) Pipestone Creek August 5, 2008, d) Minnesota River September 2005.

Weigel and Robertson (2007) summarize the difficulties in understanding relationships between stream biota and nutrients based on the observations of several researchers, as follows: "One of the greatest impediments to understanding biotic–nutrient relations is that biota may not respond to nutrient enrichment in the same way that they react to other stressors (Yoder & Rankin 1995, Karr & Chu 1999). Nutrients can provide a subsidy rather than stress effect on assemblages (Odum *et al.* 1979). Furthermore, environmental variables are often highly correlated, making it difficult to differentiate correlations from cause–effect relations (Miltner & Rankin 1998, Wang *et al.* 2003, Dodds & Oakes 2004). If the effect of the controlling factor is strong, the response should vary little, whereas if the effect of the controlling factor is weak or absent, the response may vary greatly with effects of other controlling actors (Garvey *et al.* 1998)."

Environmental data frequently exhibit a "wedge" distribution of data points between two variables (*e.g.*, Figure 8), with the upper-edge representing a threshold beyond which co-occurrence of the two variables is unlikely. For example, plots of species richness versus stream size or drainage area exhibit this pattern (Karr 1981, Fausch *et al.* 1984). Terrell *et al.* (1996) examined similar wedge-shaped patterns of variation in habitat and fish standing stock relationships. A line fit by eye through the upper 5% of these points along the angle of the upper surface of the wedge represents the maximum number of species expected for a given stream size. Lines drawn through the upper 5% of plots of a biological index versus the concentration of a water chemistry variables is similarly interpreted as the maximum biological index values normally expected to coincide with a given chemical concentration. If a chemical variable exceeds such a value, there is a strong likelihood the aquatic community would be unable to achieve that level of performance (*i.e.*, at least 95% of all observed index values were associated with values below this concentration) (Rankin *et al.* 1999).

There are numerous examples in the literature of associations among nutrients and fish and macroinvertebrate metrics using techniques similar to what we have employed. Miltner and Rankin (1998) found headwater streams with either total inorganic nitrogen (TIN) below 1.37 mg/L or TP below 0.17 mg/L (50th percentiles) to have significantly higher IBI scores than headwaters with higher nutrient concentrations. For wadeable streams, the mean IBI scores were significantly higher the lower the nutrient concentration (25th > 50th > 75th > 90th percentiles), such that the highest IBI scores were for fish communities where TIN concentrations were less than 0.61 mg/L and TP was less than 0.06 mg/L (25th percentiles). In headwaters and wadeable streams with low or intermediate nutrient concentrations (< 50^{th} percentile; headwaters: TIN < 1.37 mg/L, TP < 0.17 mg/L; wadeable: TIN < 1.65 mg/L, TP < 0.12 mg/L), the number of sensitive fish species was significantly higher. Similarly, in nutrient-rich headwaters, wadeable streams, and small rivers, the relative abundance of tolerant and omnivorous fish increased significantly. In large rivers, there were no observed relationships between the fish community and nutrient concentrations, except that top macroinvertebrate carnivores were positively related to higher nutrient levels. A comparison of macroinvertebrate data with fish data suggested that a loss in EPT taxa corresponded with a decrease in the number of sensitive fish. Also, an increase in the number of dipterans and non-insects related to a decrease in insectivorous fish and an increase in omnivorous fish. Robertson *et al.* (2006) in their work on wadeable streams found strong correlations between the following: HBI and TP, DP and TKN, EPT and TP, DP, and ammonia-N. They found further that macroinvertebrate indices responded similarly to changes in nutrients in all areas of the state. Thresholds from their work suggested responses to P~0.090 mg/L; TKN ~0.609-1.106 mg/L; and a fairly broad range for nitrate-N ~1.16 – 3.59 mg/L – whereby, wide fluctuations in metrics were noted below the threshold while generally poor metric values above.

Other studies suggest somewhat similar ranges of concentrations. Rankin *et al.* (1999) reported that macroinvertebrate and fish IBI scores were typically *good* (40 – 49) in waters with TP concentrations between 0.10 and 0.20 mg/L and tended to be *exceptional* (50 – 60) when TP concentrations were below 0.10 mg/L. A set of 18 reference reaches in Virginia without macroinvertebrate impairments had a mean TP concentration of 0.06 mg/L (median = 0.07 mg/L, n = 59), whereas 19 sites with benthic impairments had a mean TP value of 0.28 mg/L (median = 0.10 mg/L, n = 69) (Hill & Devlin 2003).

Weigel and Robertson (2007), using regression tree analyses, found breakpoint values of TP above which biota were consistently impaired ranging from 0.06 to 0.15 mg/L TP. The breakpoint values found for macroinvertebrate species and fish IBI nearly matched, whereas they were similarly low for percent round suckers and mean pollution tolerance value (MPTV). Biologically meaningful breakpoints in P concentrations were typically higher than reported reference concentrations for Wisconsin's streams and rivers. In addition, the breakpoints were consistent with a trophic transition from mesotrophic to eutrophic status. Depending upon the biotic measure, the analyses suggested that the largest changes in biological metrics were at TP concentrations between 0.064 and 0.150 mg/L. The number of macroinvertebrate species had the highest TP breakpoint of the measures tested with regression tree analysis, but they note that species richness did not exceed 40 unless TP <0.06 mg/L. Similarly, all sites had fish IBI scores of ''fair'' (IBI = 60) or better at TP < 0.06 mg/L. A TP concentration of 0.06 mg/l is ~2 to 3 times the reference concentration found in studies of Wisconsin wadeable streams. In the case of MPCA data there were a few sites that maintained 40-45 macroinvertebrate taxa up to a TP of ~120-130 µg/L (Figure 38b); however metrics like percent sensitive fish (Figure 38c) seemed to correspond more closely to the ranges described by Weigel and Robertson (2007).

Breakpoints in the biotic relations with TN were similar to reference conditions for Wisconsin's streams and rivers, and they were mostly consistent with a transition from oligotrophic to mesotrophic status (Weigel & Robertson 2007). For three of the biotic measures, regression tree analyses suggested that the largest changes in biological metrics were at TN = 0.635 mg/l, whereas the breakpoint was ~3 times higher for macroinvertebrate species (1.925 mg/l). Species richness appeared more variable at TN ~ 1.925 mg/l, but sites consistently had high species richness at TN ~ 0.635 mg/l. Reference nutrient concentrations found in this study were within the ranges reported for the nutrient ecoregions, but the breakpoints in the biotic–nutrient relations almost always exceeded reported reference concentrations in national (USEPA 2000b, 2001) and regional studies (*e.g.*, Robertson *et al.* 2006). This study suggests that biologically meaningful shifts may occur with nutrient enrichment slightly above reference concentrations, consistent with values determined through breakpoint analyses. Morgan *et al.* (2007) describes the relationship between nutrients (including nitrate nitrogen) and IBIs for macroinvertebrates and fish in small-order Maryland streams. Employing quantile regression they derived critical values for water quality variables with two biological indices and recommended nitrate-N values above ~0.83-0.86 mg/L as indicative of degraded urban water.

Visual assessment of MPCA fish and macroinvertebrate data (Figure 40) suggest that breakpoints may be in the 1.0-1.5 mg/L range. At values lower than this a wide range of values occur; while at concentrations greater than this metric values for most of the sites remained at low levels. However, there are some notable exceptions in the 2008 data and the USGS data set (Figure 40a-c).

A number of biological metrics for fish and macroinvertebrate were examined in relation to nutrients and other stressors to identify biological thresholds. Many of the datasets examined revealed a wedge-shaped relationship between total phosphorus, BOD, and Chl-a and these metrics. The wedge-shaped pattern indicates that these stressors were a factor that caused the low metric scores found at a stream or river site. As a result of the observed patterns, these datasets were well suited to quantile regression analysis. Relationships among nitrogen and biological metrics were less consistent and few threshold concentrations could be developed for this nutrient. However, many of the datasets were insufficient to develop nitrogen threshold concentrations. Much of the difficulty was a result of small datasets or datasets with a narrow range of nitrogen levels. Because of these limitations, the criteria derived from field-based measures presented here should be treated with caution. Without additional datasets, further discussion of nitrogen criteria developed using quantile regression would not be appropriate.

Examination of the threshold concentrations derived from both fish and macroinvertebrate data reveals a number of apparent patterns. There was a gradient of increasing threshold concentrations from north to south. The north-south criteria gradient may be due to differences in the biological communities between regions and may also reflect differences in land use, soils, and geomorphic patterns across the state (*i.e.,* ecoregions). This suggests that statewide nutrient criteria may not be appropriate due to the range of criteria developed using quantile regression and changepoint analyses across the state (Table 20), and that these criteria should be regionalized. Regional patterns in modern-day water quality (*e.g.,* TP and BOD; Table 20 and Appendix I) and estimated background TP (Smith et al. 2003) further reinforce regional patterns and differences between threshold concentrations from wadeable and nonwadeable streams were not consistent across regions. The causes of this pattern are not clear, but it is possible that natural differences in nutrient concentrations are partially responsible for differences in the native species pools present in these regions. For example, southern fauna are better suited to more enriched conditions than are the northern fauna. Regardless of the cause of the pattern, these results suggest that regionalized nutrient criteria are appropriate. There was little difference between threshold concentrations developed for the two taxonomic groups (*i.e.,* fish and macroinvertebrates), suggesting that both taxonomic groups respond to nutrients and related stressors and can be used together to develop nutrient criteria. Observed thresholds from basic regressions (Figure 37) and ranges for phosphorus criteria developed from quantile regression and changepoint analysis, using fishes and macroinvertebrates, were within or near the range of thresholds reported in the literature (Table 20b).

Table 20. Summary statistics: a) for total phosphorus, chlorophyll-a, and BOD_5 derived from quantile regression and changepoint analyses (summarized from Table 18) (BM = Biomonitoring data, RN = River Nutrient data, STOR = STORET data); b) based on recommended ranges from the literature; c) Minnesota ecoregion-based interquartile ranges based on representative minimally impacted streams, and d) regional reference conditions.

a. Threshold concentrations developed from quantile regression and changepoint analyses for Minnesota rivers (# T.C. = number of the threshold concentration values used to calculate statistics).

Region	25th %ile AQRS and Changepoint	# T.C.
Total Phosphorus (µg L^{-1})		
North (BM, all sizes)	44	26
Central (BM, all sizes)	110	24
South (BM, all sizes)	145	17
Chlorophyll-a (µg L^{-1})		
Statewide (RN, all sizes)	21	12
BOD_5 (mg L^{-1})		
North (STOR, all sizes)	-	0
Central (STOR, all sizes)	2.1	7

South (STOR, all sizes)	3.1	14

b. Literature-based criteria ranges.

TP range	Notes from literature	Source (state)
<170 µg/L (headwater)	significantly higher fish IBI as compared to streams with higher TP"	Miltner & Rankin 1998 (OH)
<120 µg/L (wadeable)	# of sensitive fish sp. was significantly higher than streams with higher TP	Miltner & Rankin 1998 (OH)
~90 µg/L	macroinvertebrate changepoint; generally poor metric values above this TP	Robertson *et al.* 2006 (WI)
<100 µg/L	exceptional IBI	Rankin *et al.* 1999 (OH)
100-200 µg/L	good IBI	
60-150 µg/L	biota impaired above this range	Weigel & Robertson 2007 (WI)
<60 µg/L	fish IBI fair or better and invert. taxa richness >40	Weigel & Robertson 2007 (WI)
70 µg/L	Median TP for streams without macroinvertebrate impairments (mean=60 µg/L)	Hill & Devlin 2003 (VA)
100 µg/L	threshold identified by shift in algal community to cyanobacteria (one study)	Carleton *et al.* 2009 (MN)
100 µg/L	Median TP for streams with macroinvertebrate impairments (mean=280 µg/L)	

c. Typical (interquartile) ranges based on: a. representative, minimally-impacted Minnesota streams (McCollor & Heiskary 1993), b. STORET summary of all stream TP (see Table 25) and c. USEPA ecoregion-based criteria summaries (estimated from Appendix I, Table 2).

Region (basis)	TP (a) µg/L	TP (b) ug/L	TP (c) µg/L	BOD_5 (a) mg/L
North (NLF, NMW)	40-70	33-70	32-70	1.0-1.7
Central (NCHF)	70-170	77-225	40-200	1.6-3.3
South (WCBP, NGP)	185-320	147-308	170-403	2.4-6.1

d. 75th percentile values by nutrient region for reference sites from STORET (see Table 17) (TP = total phosphorus, Chl-a = Chlorophyll-a, BOD_5 = Biochemical Oxygen Demand).

Region (basis)	TP µg/L	Chl-a µg/L	BOD_5 mg/L
North (NLF, NMW)	61	3	2.0
Central (NCHF)	139	5	2.0
South (WCBP, NGP)	302	19	-

C. PERIPHYTON BIOMASS AS A NUMERIC TRANSLATOR FOR "NUISANCE ALGAL GROWTH"

While strong relationships were evident among TP, TKN, BOD_5, and sestonic Chl-T, we do not see the same type of relationship relative to periphyton data. Since periphyton grows on various substrates in the river, the concept of wetted surface area (WSA) is important when discussing periphyton. As streams go from silt/clay sides and bottom to adding rocks to adding macrophytes/brush/trees, the WSA increases exponentially, allowing far more holdfast area for periphyton. In many streams, periphyton is limited by habitat even when there are sufficient nutrient concentrations and light.

The Michaelis-Menton model was originally developed to model enzyme kinetics but has proven to be robust in describing nutrient uptake as a function of concentration at a broad range of scales:

$$U = \frac{U_{max} C}{C + K_m}$$

where U is uptake, C is nutrient concentration, K_m is the half-saturation constant, and U_{max} is maximum uptake.

Maximum uptake and the half-saturation constant (K_m, the concentration at which uptake is one-half of U_{max}) vary widely among organisms and in response to environmental conditions. These metrics are indices of organismal or

system affinity for a nutrient. At low concentrations uptake approximates a linear relationship with increasing nutrient concentration. As nutrient concentration increases to near-saturating concentrations, U approaches U_{max} asymptotically (Earl *et al.* 2006).

Phytoplankton growth and development depend on nutrients like phosphorus and nitrogen and usually grow in biomass in relation to the amount of nutrients present in the water column, up to the point that the water is saturated with one or both of the essential nutrients. Diatoms have special siliceous frustules (*i.e.*, glass cases) in which they grow, so they are also dependent on silicon being available.

Many researchers have studied nutrient – chlorophyll – light availability relationships in periphyton (*e.g.*, Cushing *et al.* 1983, Bothwell 1985, Delong & Brusven 1992, Dodds *et al.* 1997, Biggs & Smith 2002, Davis 2002, Carr *et al.* 2005, Robertson *et al.* 2006, Stevenson *et al.* 2006, Bowes *et al.* 2007). Snelder *et al.* (2004) describe how growth rate is determined primarily by nutrient supply and light and biomass loss is determined by hydrological disturbance and invertebrate grazing. They go on to acknowledge differences among low gradient and high gradient streams and present a model for prediction of benthic algal biomass.

There are important differences in algal growth potential and algal nutrient saturation concentrations between phytoplankton and periphyton. Hill *et al.* (2009) noted that phytoplankton's saturation concentration for soluble reactive phosphorus (~ 3 µg/L) was far lower than that for periphyton (~ 25 µg/L). They noted that the small cells of most phytoplankton were bathed in nutrients while periphyton has diffusive layers that slow the movement of nutrients. The longer filaments of attached green algae can have a competitive advantage over smaller diatom cells.

Hill *et al.* (2009) noted the saturation threshold for phosphorus effects occurred at 25 µg/L of soluble reactive phosphorus for periphyton. Phosphorus enrichment in streams is likely to have its largest effect at concentrations < 25 µg/L soluble reactive phosphorus (SRP) but the effect of enrichment is probably minimized when streambed irradiances are kept below 2 mol photons per meter squared per day by riparian shading or turbidity. Horner *et al.* (1990) noted areal uptake of P by algae increased with SRP concentration, up to about 15 µg/L in overlying water. Sosiak (2002) found similar results.

Suplee *et al.* (2008a) found that periphyton in Montana were already saturated with phosphorus at concentrations of 20 µg/L and that there was a TN breakpoint at about 250 µg/L that resulted in a change in algal composition from diatoms to filamentous green algae. These are very low concentrations for most streams in Minnesota. As such, periphyton in Minnesota streams is probably more limited by lack of habitat or light. As such, setting periphyton goals are not as amenable to the causative-response aspects of nutrient water quality standards as setting phytoplankton goals are (Snyder *et al.* 2002).

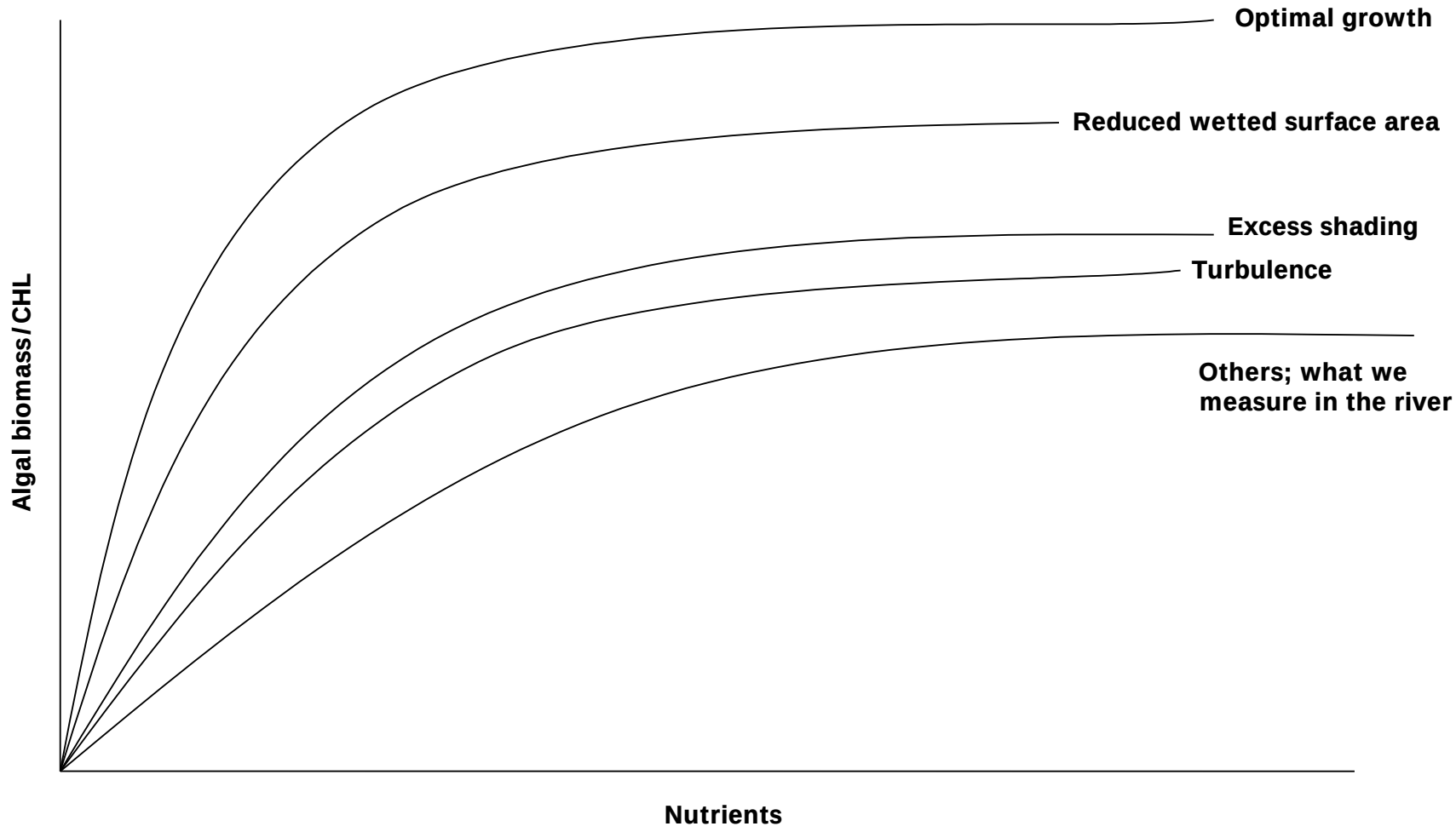
We are proposing a series of nutrient and chlorophyll water quality criteria for the phytoplankton in the water column. It is also appropriate to protect beneficial designated uses of rivers from excess periphyton by setting biomass concentrations, usually in terms of mg chlorophyll per square meter [mg CHL *a*/m^2]. This is consistent with observations of Snelder *et al.* (2004), in their work on New Zealand streams, who note "By focusing on biomass, the analysis is meaningful to stakeholders, which is a key to seeking consensus in environmental planning." There have been several studies in the upper Midwest that have determined what biomass levels are considered excessive and polluting, both from technical and user perception approaches.

Except for the far north and northeast portions of the state, most streams exceed the 20 to 25 µg/L SRP and 250 to 300 µg/L TN needed for benthic algae to have unlimited growth potential based solely on nutrient concentrations and growth rates. The question becomes not "Why is there so much algae?" but "Why is there so little algae?"

Other stressors come into play, such as limited habitat, shading/turbidity, and turbulence. Figure 54 shows this diagrammatically. These stressors limiting optimum growth have a number of implications for setting nutrient water quality criteria and for developing reduction goals for impaired waters.

Typically, we set nutrient reduction goals for the current river condition, but we should recognize that those goals might have to be modified if turbidity decreases through management practices aimed at reducing nutrients, for example. River dynamics, including the actual world of sub-optimal algal growth potential, is very complicated.

Figure 54. Algal optimum growth and the stressors that limit its potential



D. Criteria Recommendations for Nutrients, Seston and Related Factors

The multiple lines of evidence approach we have used to develop eutrophication criteria is well supported in the literature. Stevenson *et al.* (2008), for example, describe how algae and phosphorus relationships, threshold analysis and frequency distributions can guide development of nutrient criteria. In their example they focus on benthic algal growth; however, they acknowledge that this approach could be applied to other stream biota as well. In summary they note – "multiple analytical approaches can and should be used when developing nutrient criteria to provide the diversity of information that justify criteria to stakeholders and increase the probability of successful management actions."

As such, we have used successive levels of data analysis to characterize datasets, interrelationships among variables and supporting information to move from potential ranges for eutrophication criteria to region-specific criteria. Basic steps are summarized as follows, with each step building on previous analyses – allowing for a refinement in the selection of criteria values (*i.e.,* move from general criteria ranges to region-specific criteria):

- Assessed linkages among nutrients, sestonic Chl-a, BOD_5 and diel DO flux (Figures 20, 26, and 30). These provide a basis for describing interrelationships and predicting changes in potential "response variables" (*e.g.,* Chl-a) as a function of changes in causal variables (*e.g.,* TP and TN);
- Demonstrated relationships among these variables and select fish and macroinvertebrate metrics based on the River Nutrient dataset by means of Spearman rank correlation (Table 16), plotting data (*e.g.,* Figure 38), and review of plotted data for thresholds or shifts in distribution of responsive metrics (*e.g.,* macroinvertebrate taxa richness in Figure 38a);
- Expanded the analysis to include biomonitoring data sets and statistical analyses including: quantile regression and changepoint analysis (Table 18). Results from these various techniques allowed us to assemble a range of potential values from which we developed criteria for the causative variable (TP) and several response variables (*e.g.,* BOD_5, sestonic chlorophyll-a);
- Relationships among nutrients, stressor variables, and the biology was further assessed by determining the levels of chlorophyll-a and total phosphorus associated with the BOD_5 threshold concentrations;
- Reviewed thresholds put forth in the literature to provide further perspective on this issue;
- Concentrations ranges were placed in context with ecoregion-based frequency distributions compiled by MPCA for representative, minimally-impacted streams (McCollor & Heiskary 1993), STORET summary of Minnesota streams and IQ ranges from USEPA criteria manuals (USEPA 2000b, a, 2001), which are summarized in Table 20. A more recent CDF for stream TP concentrations (based on data from 1995-2009) is also used to place TP concentrations in perspective for each RNR (Appendix I, Figure I-1).

The multiple lines of evidence, as described above, provide the basis for selection of ecoregion-based criteria. This approach does not rely heavily on the reference condition, a recommended approach in early EPA guidance (e.g. USEPA 2000a-c), as a primary basis for criteria selection. Rather, the datasets and summaries provided in that guidance help place proposed criteria in perspective with the overall distributions for each ecoregion. Our approach emphasized the threshold concentrations developed from the biomonitoring data using quantile regression and changepoint analysis (Table 18). Further, we chose to begin with selection of TP criteria, since TP had the largest number of threshold concentrations developed for each RNR (Table 18). Once selected, we sought protective response variables based on Table 18, the serial regressions (Table 15), and tried to ensure there was good correspondence between TP and the primary response variable Chl-a (Figure 32).

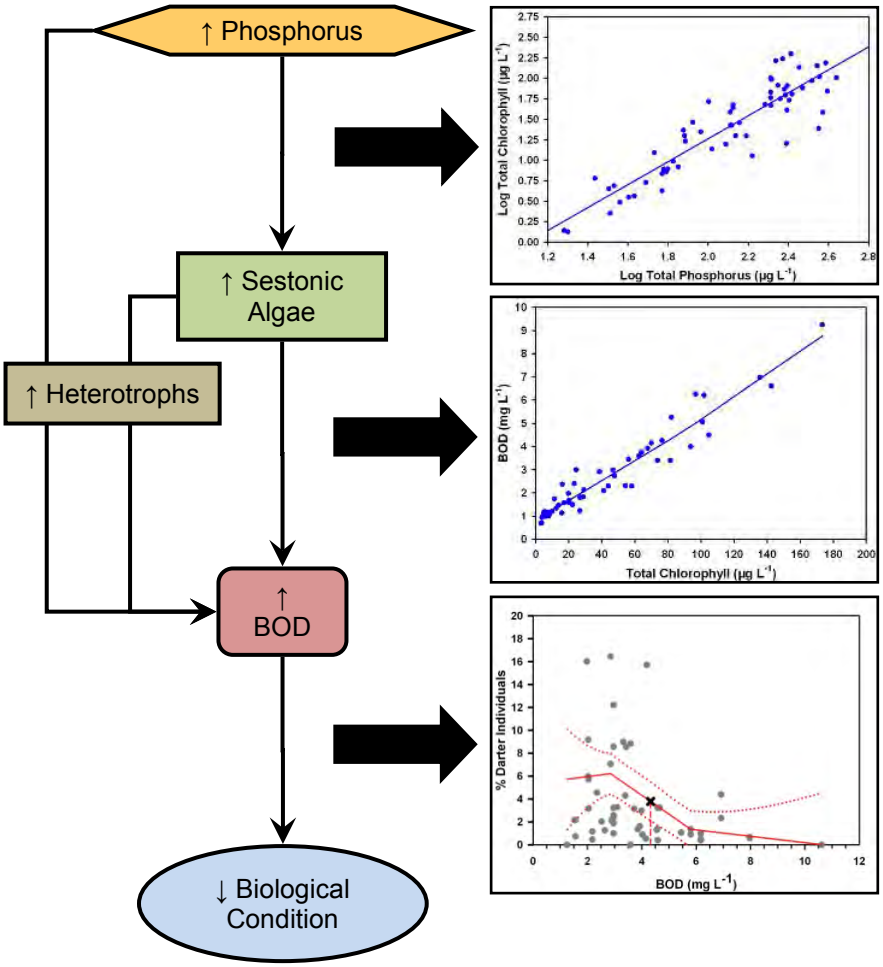


Figure 55. Conceptual model with empirical data that supports the relationships between nutrient enrichment and biological impairment.

1. Northern River Nutrient Region Criteria Development

Northern RNR rivers drain landscapes dominated by forest and wetland land uses (Table I - 3). These rivers, by comparison to their counterparts in the Central and Southern regions, have minimal nutrient-related anthropogenic impacts. Example rivers from the River Nutrient dataset include Big Fork, Little Fork, and upper reaches of the Crow Wing River (Table 2). Nutrient and Chl-a concentrations in these rivers are quite low and are well within the typical range for the Northern RNR (Table 20c). TP threshold concentrations as derived from quantile regression and changepoint analysis averaged 72 µg/L, with an IQ range of 44-91 µg/L (Table 18). The 25th percentile values (implies 75% are higher) for the North overall, nonwadeable, and wadeable are 44, 28, and 48 µg/L, respectively (Table 18). Of these, the overall and wadeable have the highest number of threshold concentrations. In contrast, the nonwadeable had only three threshold concentrations. Interpolation from BOD_5-TP and BOD_5- Chl-a-TP models (Figures 49-50) resulted in concentrations of TP of 41-78 µg/L needed to maintain BOD_5 below threshold levels in the Northern region. The interquartile range based on representative minimally impacted Minnesota streams was 40-70 µg/L and USEPA's criteria summary for the northern ecoregions was 32-70 µg/L (Table 20c). The 75th percentile of TP for reference sites in the Northern RNR was 61 µg/L (Table 20d). Based on the available thresholds and other evidence, a TP criterion of ≤50 µg/L is recommended (Table 21). This criterion is near the median for the North RNR based on Figure I-1 (Appendix I). This TP is also below most reported thresholds from the literature (Table 20b). A criterion of 50 µg/L is also protective of the majority of the metrics tested (Table 18) and will provide protection to aquatic life in this region.

Table 21: Summary of evidence used to develop recommended river eutrophication criteria for the Northern River Nutrient Region (* indicates threshold is based on statewide data; Abbreviations: IQR = Interquartile Range; %ile = Percentile; TP = Total Phosphorus; Chl-a = Chlorophyll-a; BOD_5 = Biochemical Oxygen Demand; DO Flux = Diel Dissolved Oxygen Flux).

Line of Evidence	TP (µg/L)	Chl-a (µg/L)	DO Flux (mg/L)	BOD_5 (mg/L)
25th %ile Threshold Concentrations (Table 18)	44	21*	3.1*	-
IQR for Minimally impacted MN streams (Table 20c)	40-70		-	1.0-1.7
IQR for USEPA Ecoregion Summaries (Table 20c)	32-70	-	-	-
75th %ile for MN Reference Sites (Table 20d)	61	3	-	2.0
Predicted Concentration Using TP-Chla-BOD_5 Threshold Models (Figure 49)	41-72	5-10	-	-
Predicted Concentration Using TP-BOD_5 Threshold Models (Figure 50)	70-78	-	-	-
Predicted Concentration Using 75th %ile water quality models (Table 15)	-	5-6	3.0	1.3-1.4
Recommended Criterion (Table 24)	**50**	**7**	**3.0**	**1.5**

Interpolation from the BOD_5- Chl-a-TP model (Figures 49) resulted in concentrations of Chl-a of 5-10 µg/L needed to maintain BOD_5 below threshold levels. The 75th percentile of Chl-a for references site in the Northern RNR was 3 µg/L (Table 20d), however, this low value was likely due to the dominance of wadeable streams in this dataset. Maintaining Chl-a at 10 µg/L or lower should minimize risk of reduced macroinvertebrate taxa richness and percent and number of sensitive fish species in the Northern region (Figure 41). The interquartile range for BOD_5 based on representative minimally impacted Minnesota streams was 1.0-1.7 mg/L (Table 20c) and the 75th percentile of BOD_5 for references site in the Northern RNR was 2.0 mg/L (Table 20d). Due to the small sample size for DO Flux, a limited number of analyses could be used with this stressor. However, impacts to biological communities were identified for this stressor (Figure 42) so a recommended regional criterion for DO Flux was developed using models to predict DO Flux from TP (Table 15). The recommended response criteria are ≤7 µg/L for Chl-a, 1.5 mg/L for BOD_5 ≤, and ≤3.0 mg/L DO flux ≤3.0 mg/L (Table 21). These values should minimize the risk of reduced macroinvertebrate taxa richness, loss of sensitive fish species, and replacement by tolerant fish species. These levels may also minimize the risk of excessive periphyton accumulations as well. These recommended concentrations are based on concentration threshold analyses, predictive water quality relationships, and the ability to meet these goals given the recommended TP criteria. The stressor criteria should be attainable based on water quality relationship models. Focusing on the 75th percentile values (implies 75% of predicted values are at or below the threshold for the given TP concentration) for the water quality relationships predicts a 75% likelihood of achieving the response criteria when the TP criterion is met. Using the 75th percentile quantile regression models, Chl-a is predicted to be 5-6 µg/L or lower when a TP of 50 µg/L or lower is met (Table 15). Corresponding BOD_5 values at this concentration of TP are predicted to range from 1.3-1.4 mg/L and DO flux is predicted to be 3.0 mg/L (Table 15).

2. Central River Nutrient Region Criteria Development

The Central RNR, which consists of the NCHF and DA ecoregions, is a transitional area between the forest and wetland dominated North RNR and agriculturally dominated South RNR. While land uses have changed toward increased developed land in recent years, the CHF and DA land use percentages are quite different from those of the NLF and NMW ecoregions, which are dominated by forested and wetland (water) landuse. Because of differing soils, landform, and landuse, streams draining the Central RNR landscapes are more nutrient-rich than North RNR streams (Table 20c). TP threshold concentrations, as derived from quantile regression and changepoint analysis averaged 140 µg/L with an IQ range of 110-164 µg/L (Table 18). The 25th percentile TP for Central region for all streams, nonwadeable streams, and wadeable streams were 110, 86, and 108 µg/L, respectively (Table 18). Interpolation from BOD_5-TP and BOD_5- Chl-a-TP models (Figures 49-50) resulted in concentrations of TP of 83-121 µg/L needed to maintain BOD_5 below threshold levels in the Central region. The interquartile range based on representative minimally impacted Minnesota streams was 70-170 µg/L and USEPA's criteria summary for the central ecoregions was 40-200 µg/L (Table 20c). The 75th percentile of TP for reference sites in the Central RNR was 139 µg/L (Table 20c). Based on these thresholds, a TP criterion of ≤100 µg/L is recommended (Table 22). This criterion was protective of the majority of the metrics tested (Table 18). In addition, TP of 100 µg/L or lower should also minimize the risk of dominance by blue-green algae (Figure 17 in Heiskary and Markus 2003), which can negatively affect aquatic recreational uses. This criterion is near the 35th percentile for the Central RNR (Figure I-1, Appendix I).

Table 22: Summary of evidence used to develop recommended river eutrophication criteria for the Central River Nutrient Region (* indicates threshold is based on statewide data; Abbreviations: IQR = Interquartile Range; %ile = Percentile; TP = Total Phosphorus; Chl-a = Chlorophyll-a; BOD_5 = Biochemical Oxygen Demand; DO Flux = Diel Dissolved Oxygen Flux).

Line of Evidence	TP (µg/L)	Chl-a (µg/L)	DO Flux (mg/L)	BOD_5 (mg/L)

25th %ile Threshold Concentrations (Table 18)	110	21*	3.1*	2.1
IQR for Minimally impacted MN streams (Table 20c)	70-170		-	1.6-3.3
IQR for USEPA Ecoregion Summaries (Table 20c)	40-200	-	-	-
75th %ile for MN Reference Sites (Table 20d)	139	5	-	2.0
Predicted Concentration Using TP-Chla-BOD_5 Threshold Models (Figure 49)	83-107	13-21	-	-
Predicted Concentration Using TP-BOD_5 Threshold Models (Figure 50)	118-121	-	-	-
Predicted Concentration Using 75th %ile water quality models (Table 15)	-	18	3.9	1.8-1.9
Recommended Criterion (Table 24)	**100**	**18**	**3.5**	**2.0**

Interpolation from the BOD_5- Chl-a-TP model (Figures 49) resulted in concentrations of Chl-a of 13-21 µg/L needed to maintain BOD_5 below threshold levels. The 75th percentile of Chl-a for references site in the Central RNR was 5 µg/L (Table 20d), however, this relatively low value was likely due to the dominance of wadeable streams in this dataset. The 25th percentile of BOD_5 threshold concentrations for Central region for all streams was 2.1 mg/L (Table 18). The interquartile range for BOD_5 based on representative minimally impacted Minnesota streams was 1.6-3.3 mg/L (Table 20c) and the 75th percentile of BOD_5 for references site in the Central RNR was 2.0 mg/L (Table 20d). Due to the small sample size for DO Flux, a limited number of analyses could be used with this stressor. However, impacts to biological communities were identified for this stressor (Figure 42) so a recommended regional criterion for DO Flux was developed using models to predict DO Flux from TP (Table 15). The recommended response criteria values are ≤18 µg/L for Chl-a, ≤2.0 mg/L for BOD_5, and ≤3.5 mg/L for DO flux. These recommended concentrations are based on concentration threshold analyses, predictive water quality relationships, and the ability to meet these goals given the recommended TP criteria. The stressor criteria should be attainable based on water quality relationship models. The corresponding Chl-a for TP=100 µg/L is 18 µg/L based on 75th percentile quantile regressions (Table 15). Corresponding BOD_5 values at this concentration of TP are predicted to range from 1.8-1.9 mg/L and DO flux is predicted to be 3.9 mg/L.

3. Southern River Nutrient Region Criteria Development

The South RNR, which consists of the WCBP, NGP, and LAP ecoregions, is characterized by agricultural land uses with cultivated landuse being the dominant land use across all three ecoregions. These landuses are an inherent reflection of the soils, landforms, and potential natural vegetation characteristic of these ecoregions, which result in more nutrient-rich streams in this RNR as compared to the North or Central RNRs (Table 20c). TP threshold concentrations, as derived from quantile regression and changepoint analysis, averaged 258 µg/L and an IQ range of 145-373 µg/L (Table 18). The 25th percentile TP values for South overall, nonwadeable, and wadeable were 145, 148, and 115 µg/L, respectively (Table 18). Interpolation from BOD_5-TP and BOD_5- Chl-a-TP models (Figures 49-50) resulted in concentrations of TP of 129-193 µg/L needed to maintain BOD_5 below threshold levels in the Southern region. The interquartile range based on representative minimally impacted Minnesota streams was 185-320 µg/L) and USEPA's criteria summary for the southern ecoregions was 170-403 µg/L (Table 20c). The 75th percentile of references site in the Southern RNR was 302 µg/L (Table 20d). Based on the aforementioned data and predictive relationships a TP value of 150 µg/L is recommended (Table 23). This criterion is protective of the majority of the metrics tested (Table 18) and will provide protection to aquatic life in this region. This criterion is near the 25th percentile for the Southern RNR (Figure I-1, Appendix I).

Table 23: Summary of evidence used to develop recommended river eutrophication criteria for the Southern River Nutrient Region (* indicates threshold is based on statewide data; Abbreviations: IQR = Interquartile Range; %ile = Percentile; TP = Total Phosphorus; Chl-a = Chlorophyll-a; BOD_5 = Biochemical Oxygen Demand; DO Flux = Diel Dissolved Oxygen Flux).

Line of Evidence	TP (µg/L)	Chl-a (µg/L)	DO Flux (mg/L)	BOD_5 (mg/L)
25th %ile Threshold Concentrations (Table 18)	145	21*	3.1*	3.1
IQR for Minimally impacted MN streams (Table 20c)	185-320		-	2.4-6.1
IQR for USEPA Ecoregion Summaries (Table 20c)	170-403	-	-	-
75th %ile for MN Reference Sites (Table 20d)	302	19	-	-
Predicted Concentration Using TP-Chla-BOD_5 Threshold Models (Figure 49)	129-149	28-39	-	-
Predicted Concentration Using TP-BOD_5 Threshold Models (Figure 50)	168-193	-	-	-
Predicted Concentration Using 75th %ile water quality models (Table 15)	-	36-39	4.8	2.5-2.7
Recommended Criterion (Table 24)	**150**	**35**	**4.5**	**3.0**

Interpolation from the BOD_5- Chl-a-TP model (Figures 49) resulted in concentrations of Chl-a of 28-39 µg/L needed to maintain BOD_5 below threshold levels. The 75th percentile of Chl-a for references site in the Southern RNR was 19 µg/L (Table 20d), however, this relatively low value was likely due to the dominance of wadeable streams in this dataset. The 25th percentile of BOD_5 threshold concentrations for Southern region for all streams was 3.1 mg/L (Table 18). The interquartile range for BOD_5 based on representative minimally impacted Minnesota streams was 2.4-6.1 mg/L (Table 20c). Due to the small sample size for DO Flux, a limited number of analyses could be used with this stressor. However, impacts to biological communities were identified for this stressor (Figure 42) so a recommended regional criterion for DO Flux was developed using models to predict DO Flux from TP (Table 15). The recommended response criteria values are ≤35 µg/L for Chl-a, ≤3.0 mg/L for BOD_5, and ≤4.5 mg/L for DO flux (Table 21). These recommended concentrations are based on concentration threshold analyses, predictive water quality relationships, and the ability to meet these goals given the recommended TP criteria. The stressor criteria should be attainable based on water quality relationship models. Using the 75th percentile quantile regression models, Chl-a is predicted to be 36-39 µg/L or lower when a TP of 150 µg/L or lower is met (Table 15). Corresponding BOD_5 values at this concentration of TP are predicted to range from 2.5-27 mg/L and DO flux is predicted to be 4.8 mg/L (Table 15).

While the South RNR TP criterion is relatively "high" compared to literature values (Table 20b), it is consistent with the regional differences exhibited by modern-day water quality as demonstrated by MCPA and EPA data summaries and estimates of background stream TP (Smith *et al.* 2003). Smith et al. (2003) estimate background stream TP for the North, Central and Southern regions of Minnesota at 15, 25 and 55 µg/L, which translates to about a three-fold difference between the North and South. The criteria (Table 24) exhibit a similar relative difference. Also, this three-fold difference between the North and South is similar to the difference in lake TP criteria for the NLF ecoregion as compared to the WCBP/NGP ecoregions (Heiskary & Wilson 2008). Lastly, 150 µg/L is at the 25th percentile for the South RNR (Table I-1 Appendix I). Based on a comparison with reference and non-reference South RNR sites, 150 µg/L is near the median for reference and is below the 25th percentile for non-reference sites (Figure 46). The use of the 25th percentile (overall) or 75th percentile (reference), as a basis for establishing criteria, is consistent with early EPA nutrient criteria guidance (USEPA 2000c).

Table 24. Draft river eutrophication criteria ranges by River Nutrient Region for Minnesota.

	Nutrient	Stressor		
Region	**TP µg/L**	**Chl-a µg/L**	**DO flux mg/L**	**BOD_5 mg/L**
North	≤50	≤7	≤3.0	≤1.5
Central	≤100	≤18	≤3.5	≤2.0
South	≤150	≤35	≤4.5	≤3.0

E. Criteria Recommendation for Periphyton Algal Biomass as a Numeric Translator

Rivers shall have an algal biomass not to exceed 150 mg Chl *a* m^{-2} to avoid nuisance algal biomasses that interfere with important aquatic recreation designated uses. Dodds *et al.* (1997), Dodds & Welch (2000), Welch et al. (1988), and Suplee *et al* (2008b) are very illustrative and also provide excellent literature reviews and biomass recommendations. Suplee *et al* (2008b) also provides examples of photographs for excellent quality, diatom-dominated streams, and poor quality green algae [*Cladophora*] - dominated streams. Their study showed a clear demarcation in algal type as biomass concentration increased from 150 mg Chl-a m^{-2} to 200 mg Chl-a m^{-2}, mediated by nitrogen concentrations.

Periphyton can be sampled by using artificial substrates or on naturally occurring substrates (Aloi 1990). There are several national sampling protocols available for assessing the periphyton in wadeable streams (Standards Methods Committee 2001 and the US Geological Survey Open-File Report 02-150). We recommend that the method as described in the USGS National Field Manual be used so there is consistency among results (Hambrook Berkman & Canova 2007).

For assessment purposes, sampling should occur during the algal growing season of June through September and no more than one year in ten should exceed 150 mg CHL *a* m^{-2}. Appropriate sampling areas are those where light penetration reaches the area being sampled.

It is reasonable to ask how a periphyton impairment Total Maximum Daily Load (TMDL) study can be developed. Since there are many factors that go into the determination of periphyton biomass, as has been discussed above, the approach that will work the best is utilizing EPA's Stressor Identification Guidance Document (EPA/822/B-00/025) (Cormier *et al.* 2000) at the following web link: http://www.epa.gov/waterscience/biocriteria/stressors/stressorid.pdf.

This document contains an introduction to the Stressor Identification [SI] process, listing candidate causes, approaches to analyze the evidence, characterization of cause, and iteration options, as well as two examples.

Because the periphyton CHL WQS is a numeric translator of a narrative standard, there is no *a priori* presumption of cause if an impairment determination is made. As such, there will be no linkage assumed between NPDES dischargers and excess periphyton CHL until a Stressor Identification determination is established.

F. SUMMARY

Research across North America (including Minnesota) has documented linkages between phosphorus and in-stream chlorophyll-a. While many states have focused on periphyton in low order, wadeable streams we elected to focus the majority of Minnesota's efforts on medium to high order streams (typically 4th order and higher). Most of the streams included in this work (1999-2008) have watershed areas of 500 mi^2 or greater (most >1,000 mi^2) and are generally considered non-wadeable. Initial studies in 1999 and 2000 focused on several sites on the Crow Wing, Upper Mississippi, Crow, Rum, Blue Earth, and Red Rivers (Table 2). This work provided the basis for identifying significant links between TP, TKN, sestonic Chl-a, BOD_5 and began to establish linkages with diel DO flux, and fish and macroinvertebrate metrics (Heiskary & Markus 2001, 2003). It also provided insights on the role of flow and residence time on algal production; whereby sites monitored in the low flow summer of 2000 tended to exhibit higher sestonic Chl-a per unit TP than did the same sites during the high flow summer of 1999 (Heiskary & Markus 2001). Data from the highly turbid Red River helped demonstrate how light limitation by suspended inorganic solids can reduce the amount of Chl-a per unit TP. In summer 2001 approximately 21 stream sites across several basins were sampled for the standard suite of water quality variables. These data were used primarily as a basis for corroborating the various interrelationships developed based on the 1999 and 2000 studies and in general the relationships were found to be quite robust over a wide range of streams (Heiskary & Markus 2003).

Following that work increased emphasis was placed on collecting diel DO, temperature, pH, and conductivity data to see how these measurements related to nutrients and Chl-a and pair this with fish and macroinvertebrate collections whenever possible. A collaborative project in 2005 and 2006 that involved USGS and MPCA's biological unit staff provided an opportunity to gather this type of data on several streams in the Red and Rainy River Basins (Table 2). Subsequent work in 2008 allowed for similar collections on several streams in ecoregions (*e.g.,* DA) that were under-represented in the previous work and included some cold water streams as well. This led to a comprehensive database on DO flux (Table 12) and water chemistry (Table 13) and biology (Appendix IV) for a wide array of sites from several different ecoregions.

An approach using multiple lines of evidence was used to develop the eutrophication criteria that are protective of Minnesota's aquatic life use goals (Table 24). This type of approach is well supported in the literature, including the USEPA criteria guidance manual for rivers and streams (USEPA 2000c). The previously described interrelationships were combined with statistically derived threshold concentrations, compared to reported literature values (Table 20) and placed in an ecoregion context to produce both stressor (TP) and response criteria (Chl-a, BOD_5 and DO flux) for each of the three defined river nutrient regions (Table 24). These criteria are intended to be protective of aquatic life and aquatic recreational use relative to TP. Developing these criteria in a regional context recognizes the gradient in landuse, landform, soil type, and potential natural vegetation that characterizes Minnesota's heterogeneous landscape and is consistent with USEPA guidance that supports criteria development on an ecoregional basis (*e.g.,* USEPA 2000c, b, a).

The conceptual models (Figure 1, Figure 2) provide an overview of the focus of our research and the linkages we sought to establish. The various steps/procedures employed to derive the criteria were noted in the preceding section. As the various studies that were conducted from 1999-2008 built-upon one another so did the steps used to derive the criteria. The major steps or approaches that were used are summarized below.

- Linear regression described basic interrelationships among TP, TKN, sestonic Chl-a, and DO flux based on the river nutrient datasets. Most relationships exhibited high R^2 values and were highly significant.
- Spearman correlation analysis provided an initial basis for identifying relationships among TP, TN, Chlorophyll and DO flux and fish and macroinvertebrate metrics (Table 16). This provided a basis for identifying responsive metrics for each of these variables and helped to focus subsequent analyses.
- Scatterplots were then used to visualize relationships among the more responsive metrics and the stressors (Figures 37-41) and begin threshold identification. Statewide interquartile ranges (Table 7) for the biological metrics were used to place metric values in perspective and help discern where an important shift in the metric may be occurring relative to the stressor gradient.
- More advanced: statistical techniques quantile regression and changepoint analysis, which are well-suited to the often wedge-shaped plots that are common with field-collected biological data, were employed. Based on the previous analyses emphasis was placed on some of the more responsive metrics for fish and macroinvertebrate taxa. These techniques were applied to both the river nutrient dataset and the much larger biomonitoring datasets. Threshold concentrations were produced for statewide, wadeable vs. nonwadeable, and on a region-specific basis. A series of graphs and summary statistics from this effort were included in Appendix IV. This work is summarized in Table 18, which provides an important basis for selection of criteria.
- A comprehensive review of the literature was conducted and literature-based thresholds were used to provide further perspective on this issue.
- Threshold concentrations ranges were placed in context with ecoregion-based frequency distributions compiled by MPCA for representative, minimally-impact streams (McCollor & Heiskary 1993), a compilation of stream TP data from STORET (period from 1999-2009), and IQ ranges from USEPA criteria manuals (USEPA 2000b, a, 2001) and is summarized in Table 20 and Figure I-1 (Appendix I).
- All of the above was used to move from broad ranges for criteria setting, to region-specific criteria as defined in Table 24.

Data from STORET (Figure 56 and Table 25) and previous MPCA and USEPA ecoregion-based summaries can help place the TP criteria (Table 20 and Appendix I) in perspective for Minnesota. Less than 25 percent of Minnesota's streams have TP <50 µg/L (Figure 56). Based on MPCA's STORET summary for Northern RNR streams about 50 percent have TP <50 µg/L (Figure I - 1 and Table I - 1). These percentages are similar to that reported by USEPA (Table I - 2). Based simply on TP this suggests that ~50% of Northern RNR stream-sites will likely comply with the criteria. However, once the response criteria are considered a higher percentage may meet the criteria.

About 40 percent of stream sites statewide have a TP less than the Central RNR criteria (Table 25 Figure 56). Based on the STORET summary about 65 percent of the Central RNR stream sites exceed 100 µg/L (Figure I - 1). Based on Figure 56 there are streams with mean TP >100 µg/L in each 8 digit HUC in the Central RNR, with some such as the Crow, Snake, Cannon, Root, and Zumbro having a high density of sites >100 µg/L. The STORET summary (Table 25) indicates that about 35 percent of the Central RNR stream sites are <100 µg/L, which suggests that, dependent on a streams response to TP (sestonic Chl-a), many stream-sites (AUIDs) in the Central RNR may be deemed impaired for nutrients.

About 55 percent of the stream sites statewide have TP less than the South RNR criteria (Figure 56). The STORET summary suggests about 25 percent of the South RNR stream sites have TP <150 µg/L (Table 25). Figure 56 indicates that all 8 digit HUCS in the South RNR have one or more stream sites with TP >150 µg/L, which implies that most 8 digit HUCS may have one or more streams (AUIDs) deemed impaired for nutrients, dependent on response variables.

In addition to these ecoregion-based criteria, we have proposed a numeric translator to address the impact of nuisance levels of periphyton that can limit aquatic life and aquatic recreational uses of Minnesota streams. This numeric translator is as follows: "Rivers shall have an algal biomass not to exceed 150 mg Chl *a* m^{-2} to avoid nuisance algal biomasses that interfere with important aquatic recreation designated uses." This level is well supported in the literature (*e.g.,* Welch *et al.* 1988, Dodds *et al.* 1997, Dodds & Welch 2000, Suplee *et al.* 2008b) and provides a good basis for defining impairment from excess periphyton.

The combination of the ecoregion-based criteria and the numeric translator for nuisance levels of periphyton represent Minnesota's eutrophication criteria for rivers. Concentrations at or below the appropriate numeric criteria for a given RNR indicates a river meets its designated uses relative to phosphorus. In contrast, a river reach (or

other assessment unit as appropriate) that exceeds its ecoregion-based TP criteria and one or more of the response criteria is deemed impaired for nutrients. This would result in the need to develop a TMDL for that river reach and would require an assessment of upstream sources and contributions. Also any river reach where periphyton exceeds the numeric translator would be deemed impaired for aquatic recreational use because of excess periphyton. The TMDL in this case would involve Stressor Identification that would help identify the causes of the impairment and iterate options for addressing the impairment.

Table 25. Summary of total phosphorus concentrations from all Minnesota stream sites in STORET. Mean values calculated based on samples collected from 1995-2009 (June-September) for 595 AUIDs sorted by RNR.

TP	North	Central	South
Criteria (µg/L)	**50**	**100**	**150**
25th %ile	33	77	147
median	48	122	218
75th %ile	70	225	308
min	18	14	20
max	234	2100	5460
range	216	2086	5440
# of sites	128	239	206
% of reaches not meeting TP criteria	48%	64%	73%

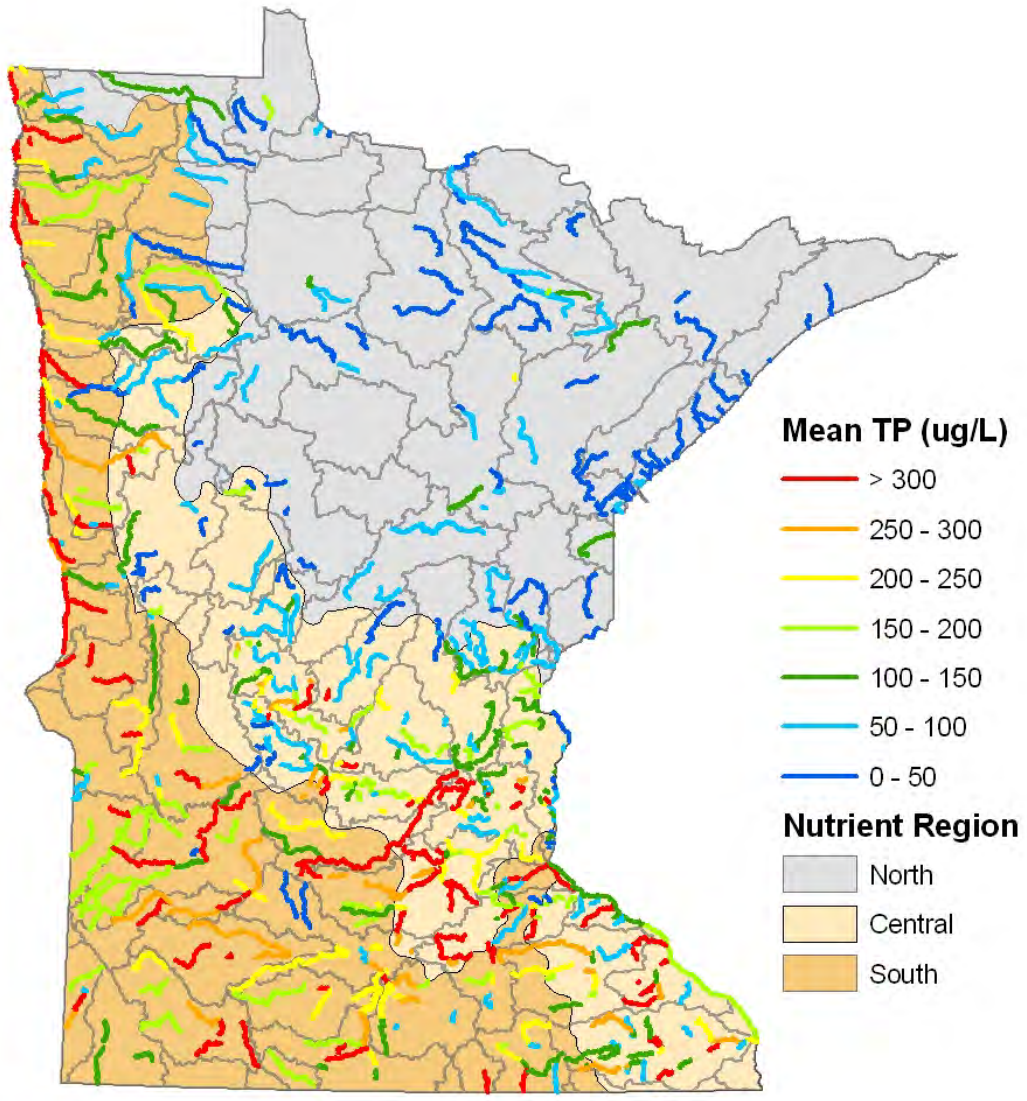


Figure 56. Total phosphorus data from STORET. Based on 27,265 TP measurements from 595 AUIDs. Values represent mean for each AUID based on data collected between Jan. 1, 1995-March 24, 2009.

IMPLEMENTING CRITERIA AND RELATED ISSUES

Implementing these criteria will be somewhat similar to the approach used for assessing lakes for nutrient impairment. River sites subject to assessments will be monitored about 6-8 times each summer for a minimum of two summers. All available data from the most the most recent 10-year period will be used in the assessment. For some rivers the assessment will be based on data from the two years of targeted monitoring while for others there may be multiple years of data available within the 10-year period.TP and sestonic chlorophyll-a data will be averaged for the entire period and compared to the RNR-based criteria. BOD_5, diel DO flux and pH data (when available for the assessment period) may be considered as well in the assessment. Stream sites that exceed the causative variable – TP and one or more of the response (stressor) variables will be deemed impaired and the river reach (AUIDs) will be included on Minnesota's 303(d) list and appropriate steps as described in TMDL guidance would be taken to address this impairment. Absent information otherwise (e.g. upstream tributary or stream reach meets standards), this impairment would likely apply to all river miles upstream from the point that was assessed. The resulting TMDL would focus on achieving the TP criteria for the listed stream reach. It is assumed that achievement of the TP criteria will result in the response variables being met in subsequent assessments. Further details on implementation will be provided in guidance and as appropriate in the SONAR for this rule.

An example assessment is provided in Table I - 4 where recent data from sites in MPCA's pour point monitoring program was summarized. River sites included have a sufficient number of observations and data for the causative variable: TP and one or more of the response (stressor) variables: Chl-a and BOD. Based on this example most North RNR streams, with the possible exception of the Crow Wing at Pillager, meet the criteria and could be considered supportive of ALUS relative to nutrients. Both the Kettle and Rapid Rivers slightly exceed TP but are well below the response criteria. In the Central RNR the Cannon, North Fork of the Crow and Sauk Rivers exceed the draft standards, while the Leaf, Otter Tail, and Red Lake Rivers meet criteria. The Mississippi at Anoka and Rum Rivers are very close to the draft criteria and would likely warrant closer inspection of data and/or continued monitoring. In the South RNR most of the rivers exceed draft criteria including the Minnesota, Blue Earth, Le Sueur, Des Moines, Redwood, South Fork of the Crow and Shell Rock. The Pomme de Terre, Mustinka, and Watonwan Rivers all meet draft criteria for stressor variables – though each exceeds the TP criteria.

An additional analysis was performed to determine how draft nutrient criteria compare to preliminary biological criteria. STORET data for TP, Chl-a, and BOD_5 was obtained for AUIDs and matched to biological monitoring sites where both fish and macroinvertebrates were sampled. A total of 33 AUIDs had sufficient biological and water quality data to perform this analysis (Table I - 5). In general there was good agreement between the biological and nutrient assessment. Overall they were in agreement in 79% of cases with an additional 15% possibly agreeing. In only 6% of cases (2 AUIDs) did the IBIs indicate that biology was meeting designated aquatic life uses, but the nutrient criteria were exceeded. A single AUID in the north region indicated nutrient impairment, but the biological measures were mixed in this AUID. Ten AUIDs in the central region exceeded the draft nutrient criteria and of these 8 AUIDs indicated biological impairment and 2 did not. In the south region 22 AUIDs exceeded the draft nutrient criteria and all indicated biological impairment or possible impairment. Approximately 42% of the 33 AUIDs were wadeable reaches (*i.e.,* <500 mi^2) and included AUIDs with drainage areas as small as 19 mi^2 and several below 100 mi^2. The agreement between nutrient criteria and preliminary biological criteria was similar to proportions determined for streams of all sizes. Further details on this analysis are provided in Appendix I.

Downstream protection is frequently brought up with respect to nutrient criteria. This means that criteria need to be protective of both the water that is being assessed as well as downstream waters. In the case of river criteria, the downstream waters of concern would typically be lakes, reservoirs, or mainstem pools on major rivers. Based on a long history of lake restoration and watershed projects the proposed stream TP criteria are in the range of stream inflow values proposed as a part of restoration projects. One basis for this argument is comparing the stream criteria to the stream TP values used in the MINLEAP model. The MINLEAP model (Wilson & Walker 1989) has long been used as a basis for predicting in-lake TP for minimally-impacted lakes on an ecoregion basis. The model was regionally calibrated and has long been used to help define in-lake goals for lake and watershed restoration projects. The corresponding regionally-calibrated stream TP values used in MINLEAP for the NLF and NCHF ecoregions are respectively 52 µg/L and 148 µg/L, which are either equal to or higher than the proposed criteria for the North and Central RNRs (50 and 100 µg/L respectively). These stream values were deemed typical of representative, minimally-impacted watersheds for the two regions. This comparison suggests that the North and Central stream TP criteria will likely be protective of downstream resources. For perspective, about 50% of Northern RNR streams have TP <50 µg/L (Table 25 and Figure I - 1) and 75% of Central RNR <100 µg/L. A similar MINLEAP-based comparison for the South RNR could not be made as the steam inflow TP used in the model was highly calibrated to account for extreme storm event loading and internal recycling within the lakes – both characteristics that are common in southern WCBP and NGP ecoregion lakes and watersheds.

Ultimately, lake and reservoir TMDLs will dictate the necessary stream TP to meet WQS in nutrient-impaired waterbodies. Erdmann (personal communication, 2012) conducted a review of eight EPA-approved lake nutrient TMDL projects covering 16 lakes in the NCHF ecoregion. This review indicated that required stream inflow TP to meet the TMDL allocation ranged from 41-45 µg/L for several lakes directly on the mainstem of the Clearwater River to 215 µg/L for a small lake with a small watershed. The median stream TP for these 16 lakes was 71 µg/L. It is evident from this brief review that no single river TP could meet the downstream protection needs of all of these lakes; rather, a closer examination through the TMDL process is needed to come up with a protective stream inflow concentration.

Specific examples of how the proposed river eutrophication criteria are protective of downstream needs is addressed in the Mississippi River navigational pools (Heiskary and Wasley 2012) and Lake Pepin (Heiskary and Wasley 2010) technical support documents. In each case, we have demonstrated through modeling that the proposed river criteria are protective of downstream uses in the pools and Lake Pepin. The proposed criteria are also consistent with our neighboring state of Wisconsin, which shares these waters. By extension, meeting these criteria should yield a sufficiently low TP concentration to meet downstream uses in the Mississippi River as it flows out of Minnesota.

Another major concern with criteria is that the criteria are adequately protective of waters that are of higher quality than the proposed criteria. We anticipate this being accomplished by appropriate implementation of nondegradation language. As with other water quality standards there is an expectation that these are not "degrade down to" standards; rather waters that are currently meeting standards would be expected to continue to do so. The combination of the eutrophication standards and nondegradation language should assure that this is the case. Absent the river eutrophication standards there is currently no basis in rule for addressing stream nutrient over enrichment.

These criteria represent a first step in a larger process. As Tiered Aquatic Life Use (TALU) standards are developed in future rulemakings there will be refinements to these criteria that reflect the more specific needs of the various tiered uses. One example is coldwater streams that will be addressed more specifically. However, in the interim, the region-based eutrophication criteria provide a basis for assessing the condition of Minnesota streams relative to excess nutrients. In turn, this allows for the development of strategies and policies to protect the condition of streams and to minimize and hopefully reverse the impact of excess nutrients on stream ecosystems.

Many of these concerns will be addressed in greater detail in the Statement of Need and Reasonableness (SONAR) that is developed as a formal part of the rulemaking process.

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VII. APPENDICES

Appendix I. Ecoregion-based data summaries for streams

Appendix II. Fish and macroinvertebrate data and metric descriptions

Appendix III. Water quality site maps for 1999 and 2000, 2000 periphyton data and 2007 USGS Upper Midwest study sites and data

Appendix IV. Quantile regression and changepoint analysis

A. APPENDIX I. ECOREGION-BASED DATA SUMMARIES FOR STREAMS

Table I - 1. Interquartile range of summer-mean concentrations for minimally impacted streams in Minnesota, by ecoregion. Data from 1970-1992. TP = total phosphorus, TSS = total suspended solids. (McCollor & Heiskary 1993).

	TP (µg/l)			Turbidity (NTU)			TSS (mg/l)		
	25%	50%	75%	25%	50%	75%	25%	50%	75%
NLF	30	40	50	2	2	4	2	4	6
NMW	50	60	90	5	7	12	7	11	20
NCHF	70	100	170	5	7	10	8	10	18
NGP	160	220	290	20	23	37	37	55	89
RRV	140	220	330	13	19	28	28	50	74
WCBP	210	270	350	14	19	27	26	47	76

	Nitrate-N (mg/l)			BOD_5 (mg/L)		
	25%	50%	75%	25%	50%	75%
NLF	0.01	0.01	0.03	0.9	1.2	1.6
NMW	0.01	0.01	0.03	1.2	1.5	1.9
NCHF	0.03	0.06	0.12	1.6	2.2	3.3
NGP	0.01	0.07	0.43	2.6	3.8	5.6
RRV	0.01	0.02	0.10	2.0	2.8	4.5
WCBP	0.89	2.60	6.50	2.2	4.3	6.6

Table I - 2. Summer IQ range from USEPA (2000b, a, 2001) nutrient criteria guidance documents.

	TP (µg/l)			Turbidity (NTU)		
	25%	50%	75%	25%	50%	75%
NLF	15	30	60			
NMW	50	60	80			
NCHF (51)	40	95	200	2.6	3.9	5.8
NGP (46)	210	314	448	-	-	-
RRV (48)	170	230	285			
WCBP (47)	130	240	359	15.0	40.0	55.0

Table I - 3. Ecoregion land use composition as summarized from 1968-69 Planning Information Center interpretations of 40 acre parcels. Summarizations completed on a minor watershed basis and represent percentage of 40 acre parcels with the described characteristic (Fandrei *et al.* 1988).

Ecoregion	Area mi^2	Forest %	Water / Marsh %	Cultivated %	Pasture / open %	Developed %
NLF	26,586	75	11	5	7	2
NMW	8,371	54	30	9	6	1
CHF	16,775	16	8	49	21	6
DA	1,488	37	6	41	14	2
WCBP	15,956	3	2	83	10	2
NGP	6,736	1	3	84	11	1
RRV (LAP)	9,072	6	3	82	9	1

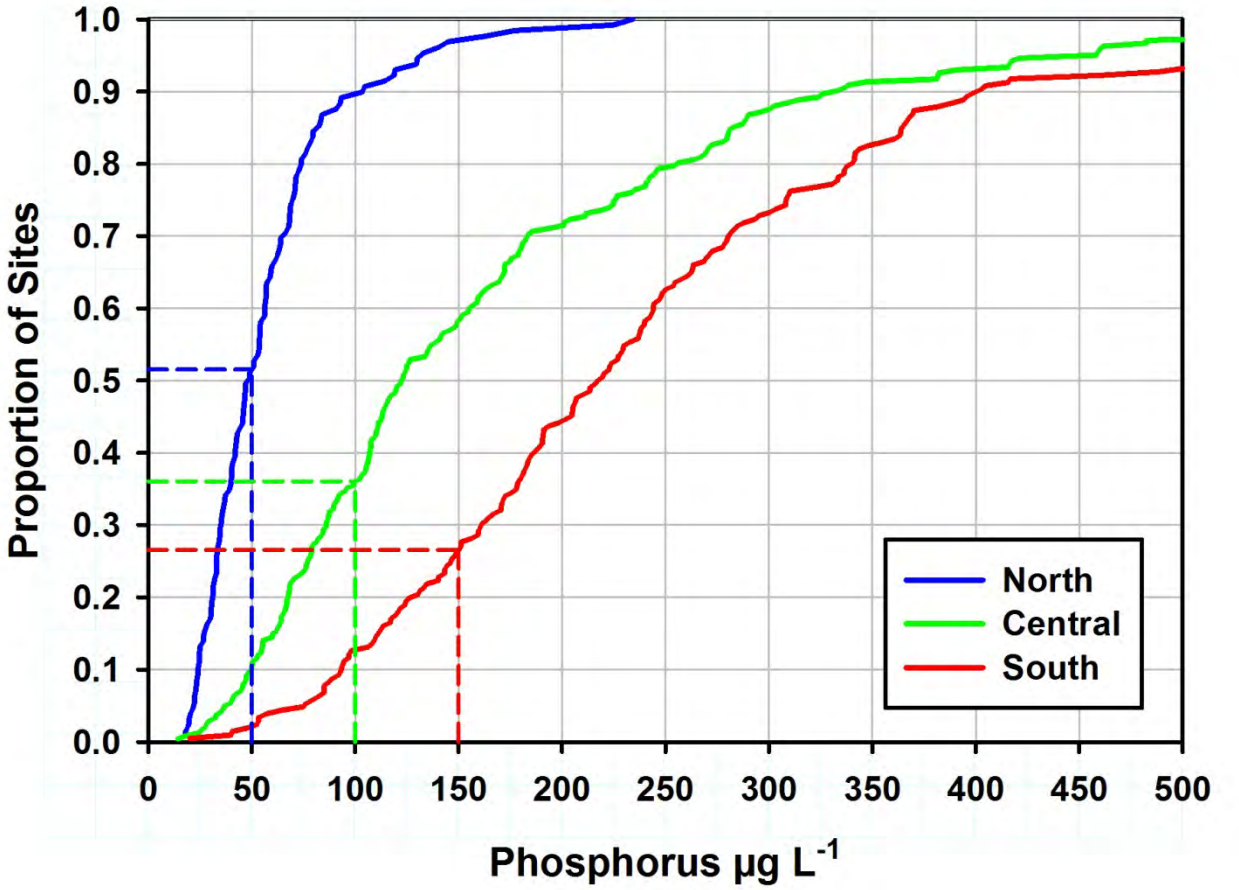


Figure I - 1. Cumulative distribution functions for stream total phosphorus concentrations by RNR. Mean summer (June through September) concentrations for AUIDs from 1995-2009 data drawn from STORET. North= 128 AUIDs, Central=239 AUIDs, and South=206 AUIDs. Dashed lines interpolate the proportion of sites meeting or not meeting the draft total phosphorus criteria for each RNR.

Table I - 4. Summary of summer-mean data based on the most recent ten years (2000-2009). All sites included have 12 or more observations within the assessment period. Data drawn from MPCA pour-point monitoring program. Most sites are located near mouth of 8 digit HUC. A "draft" assessment was made relative to the proposed criteria for each RNR (N=No and Y=Yes with respect to likelihood of 303(d) listing. "?" indicates at least one value exceeds criteria-or- assessment is in question).

Name	TP mg/L	BOD mg/L	Chl-a ug/L	303(d) list
North RNR	**0.055**	**1.5**	**10**	
Crow Wing River nr Pillager, MN [1]	0.064	1.8		N
Kettle River nr Sandstone, MN	0.057	1.0	3	?
Leech Lake River nr Ball Club, CR139	0.028	1.5		N
Pine River nr Mission, CSAH11	0.028	1.0		N
Rapid River at Clementson, MN11	0.057	1.2	2	?
St. Croix River nr Danbury, WI	0.039	1.0	3	N
Mississippi River at Aitkin, MN	0.052	1.2	6	N
Central RNR	**0.100**	**2.0**	**20**	
Cannon River at Welch, MN	0.190	2.6	16	Y
Leaf River nr Staples, CSAH29	0.084	1.2	3	N
Mississippi River at Anoka	0.088	1.8	23	?
North Fork Crow River nr Rockford, Farmington Ave	0.253	3.5	56	Y
Otter Tail River at Breckenridge, CSAH16	0.140		14	N
Red Lake River at Fisher, MN	0.182	1.6	12	N
Sauk River nr St. Cloud, MN	0.172	2.6	25	Y
Rum River at St. Francis	0.125	1.9	19	?
South RNR	**0.150**	**3.5**	**40**	
Blue Earth River nr Rapidan, MN	0.248		59	Y
Le Sueur River nr Rapidan, MN66	0.312		45	Y
Minnesota River at Judson, CSAH42	0.264		82	Y
Minnesota River at Morton, MN	0.229	4.4	64	Y
Minnesota River nr St. Peter, MN99	0.260		81	Y
Mustinka River nr Wheaton, MN	0.403		24	?
Pomme De Terre River at Appleton, MN	0.212	2.6	33	N
Redwood River nr Redwood Falls, MN	0.444	5.1	94	Y
Shell Rock River nr Gordonsville, CSAH1	0.566	6.3	67	Y
South Fork Crow River at Delano, Bridge St	0.395	7.9	102	Y
Watonwan River nr Garden City, CSAH13	0.243	2.9	39	?
West Fork Des Moines River at Jackson, River ST	0.283	8.0	170	Y
[1] *meets "blended" North & Central RNR standards*				

Comparison of draft nutrient criteria to preliminary biological criteria

An additional analysis was performed to determine how draft nutrient criteria compare to preliminary biological criteria. STORET data for TP, Chl-a, and $BOD_5$was obtained for AUIDs and matched to biological monitoring sites where both fish and macroinvertebrates were sampled. To be used water quality data needed to be collected within 5 years of the biological sampling. At least 10 records of TP was required although this was relaxed for Chl-a and BOD_5 in order to increase the number of AUIDs analyzed. AUIDs considered channelized were not included in this analysis. Sites that would be considered impaired based on the draft nutrient criteria (i.e., exceeds TP and either Chl-a or BOD_5). Fish and macroinvertebrates IBIs for each AUID was then compared to preliminary biological criteria and the confidence intervals around the criteria. AUIDs with IBI scores for either biological group below the criterion were identified as impaired. AUIDs with IBIs above the criterion but within the confidence interval were identified as possibly (?) impaired. If IBI score for the AUID was above the confidence interval the sites was identified as not impaired. If a one or both biological groups in an AUID did not meet biological criteria the AUID was determined to be impaired.

A number of cautions and caveats need to be made regarding this analysis. This was a relatively straightforward analysis that does not necessarily reflect the outcomes of a full assessment process. Formal assessment of Minnesota's waterbodies requires a team of experts in both biology and chemistry as well as the input of external stakeholders. As a result the assessments in Table I - 5 may be different from the decisions made by the assessment teams and stakeholders when these waterbodies are formally assessed for the attainment of Minnesota's aquatic life and recreation standard. This analysis did not examine if the nutrient criteria would miss biological impairments. This analysis was not performed because it would require an understanding of the potential stressors in each AUID. For example in cases where the biology is impaired, but the nutrient criteria are met, it is not clear if the nutrient criteria are under protective or if another type of stress is causing the biological impairment.

A total of 33 AUIDs had sufficient biological and water quality data to perform this analysis (Table I - 5). In general there was good agreement between the biological and nutrient assessment. Overall they were in agreement in 79% of cases with an additional 15% possibly agreeing. In only 6% or cases (2 AUIDs) did the IBIs indicate that biology was meeting designated aquatic life uses, but the nutrient criteria were exceeded. A single AUID in the north region indicated nutrient impairment, but the biological measures were mixed in this AUID. Ten AUIDs in the central region exceeded the draft nutrient criteria and of these 8 AUIDs indicated biological impairment and 2 did not. In the south region 22 AUIDs exceeded the draft nutrient criteria and all indicated biological impairment or possible impairment. Approximately 42% of the 33 AUIDs were wadeable reaches (*i.e.*, <500 mi^2) and included AUIDs with drainage areas as small as 19 mi^2 and several below 100 mi^2. The agreement between nutrient criteria and preliminary biological criteria was similar to proportions determined for streams of all sizes.

Table I - 5. Comparison of draft nutrient criteria to preliminary biological criteria using water quality data from STORET (water quality values represent means and the value in parentheses is the number of water quality records; DA = drainage area; Inv = Macroinvertebrates; yes = site impaired for biology; no = site not impaired for biology; ? = above biological criteria but within confidence interval; nd = no data; na = not assessable). ***Note*****: Some AUIDs have too few Chl-a or BOD_5 measurements (<10 records during the index period) for assessment, but were still included in this analysis. Values in red exceed eutrophication criteria.**

AUID	River Name	DA (mi^2)	Chl-a ($\mu g\ L^{-1}$)	BOD_5 ($mg\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	Fish	Inv	Overall
NORTH								
09020314-501	Roseau	1397	**22.95 (3)**	**2.75 (2)**	**126 (51)**	no	?	?
CENTRAL								
07040002-502	Cannon	1296	16.25 (20)	**2.56 (20)**	**190 (37)**	no	yes	yes
07040002-542	Cannon	96	15.57 (6)	**5.00 (20)**	**730 (36)**	?	yes	yes
07010204-502	Crow	2637	**70.87 (40)**	**4.27 (33)**	**309 (90)**	yes	yes	yes
07010204-503	N.F. Crow	1340	**55.11 (24)**	**3.33 (27)**	**248 (61)**	yes	yes	yes
07010206-596	Hardwood Creek	29		**5.44 (2)**	**246 (23)**	yes	yes	yes
07010202-501	Sauk	1038	**27.53 (22)**	**2.49 (7)**	**171 (75)**	no	no	no
07010202-505	Sauk	570	**30.05 (2)**		**158 (62)**	yes	yes	yes
07030004-587	Snake	974	**23.90 (4)**	**2.08 (20)**	**100 (42)**	no	yes	yes
07040004-507	S.F. of Zumbro	312	**24.08 (16)**	**2.24 (15)**	**209 (58)**	no	no	no
07040002-560	Waterville Creek	19		**3.55 (11)**	**278 (21)**	yes	?	yes
SOUTH								
07100001-503	Beaver Creek	170	**70.83 (3)**	2.07 (48)	**186 (87)**	yes	yes	yes
07020009-507	Blue Earth	1539	**67.79 (15)**	**4.55 (15)**	**237 (16)**	?	yes	yes
07020009-515	Blue Earth	1385	**85.88 (35)**	**4.59 (35)**	**306 (35)**	yes	yes	yes
07040002-509	Cannon	952	31.60 (15)	**4.15 (12)**	**371 (43)**	yes	no	yes
07020012-516	Carver Creek	74	66.91 (46)		**352 (86)**	?	no	?
07020009-503	Center Creek	92	34.35 (19)	**5.80 (12)**	**371 (105)**	?	yes	yes
07100001-533	Des Moines	480	**166.00 (2)**	**6.92 (49)**	**280 (50)**	yes	yes	yes
07100001-501	Des Moines	1182	**196.20 (2)**	**7.77 (49)**	**323 (50)**	yes	yes	yes
07020009-502	Elm Creek	191	**57.86 (20)**		**193 (128)**	yes	yes	yes
07100001-527	Heron Lake Outlet	450	**139.78 (1)**	**10.96 (80)**	**388 (101)**	yes	yes	yes
07020011-501	Le Sueur	1109	**41.47 (56)**		**279 (109)**	yes	no	yes
07020011-504	Little Cobb	128	**66.33 (56)**		**257 (73)**	yes	nd	yes
07020004-509	Minnesota	8056	**52.81 (18)**	**4.02 (18)**	**205 (18)**	no	yes	yes
07020007-501	Minnesota	15102	**72.73 (77)**	**4.57 (15)**	**252 (70)**	?	?	?
07020007-505	Minnesota	11280	**69.95 (48)**		**259 (100)**	?	?	?
07020002-501	Pomme de Terre	651	**42.08 (10)**	2.96 (10)	**198 (84)**	yes	yes	yes
07020006-501	Redwood	697	**79.12 (29)**	3.39 (26)	**328 (29)**	no	yes	yes
07020006-509	Redwood	610	**93.70 (12)**	**5.08 (4)**	**449 (83)**	nd	?	?
07020012-521	Rush	402	**42.95 (4)**	3.18 (4)	**230 (74)**	yes	?	yes
07020012-662	Sand Creek	93	**72.18 (88)**	**4.19 (11)**	**345 (53)**	yes	nd	yes
07080202-501	Shell Rock	187	**78.19 (25)**	**6.17 (19)**	**508 (51)**	?	yes	yes
07010205-508	S.F. Crow	1167	**69.84 (24)**	**5.45 (26)**	**407 (64)**	yes	yes	yes

	#	**%**
yes	**26**	**79**
?	**5**	**15**
no	**2**	**6**

B. APPENDIX II. BIOLOGICAL DATA, METRICS, AND METRIC DESCRIPTIONS

Table II - 1. Fish metric descriptions for large rivers.

Metric Abbreviation	Brief Description	Why this metric is used.
# of Species	Number of taxa	The metric was considered to be one of the best for determining stream condition due to correlation between high quality resources and the number of fish species in warmwater assemblages.
Evenness	The distribution of abundance of individuals among species by comparing the observed diversity to a theoretical maximum	Using evenness as a metric provided a measure of the degree that tolerant species dominated a particular environment. Reduced evenness indicated a loss of biotic integrity.
% Large River Individuals	Percentage of individuals that are large river species	This metric was used because certain species were commonly found in large river habitats. A lower proportion of large river taxa suggest a loss of biological integrity in large river habitats.
% Round Bodied Suckers	Percentage of individuals that are round body suckers	Round Bodied Suckers are effectively sampled in large rivers, and compromise a significant component of the large river fish fauna. Due to their long life requirements they provide a long term assessment, and their sensitivity to turbidity and marginal to poor water quality results in a sensitivity at the higher end of environmental quality.
% Piscivore	Percentage of individuals that are piscivores	It is only in high quality environments that species occupying the upper trophic levels were able to flourish. Since most piscivores in this region were managed for sport fishing, an upper limit of 30% was used.
% Omnivore	Percentage of individuals that are omnivores	Dominance of omnivores suggests specific components of the food base were less reliable, increasing the success of more opportunistic species. This metric evaluated the intermediate to low categories of environmental quality.
% Insectivore	Percentage of individuals that are insectivores	This metric was intended to respond to a depletion of the benthic macroinvertebrate community. This metric varied inversely with increased environmental degradation.
% Simple Lithophils	Percentage of individuals that are simple lithophilic spawners	This metric used species that have simple spawning behavior that requires clean gravel or cobble for success. This metric detects changes in environmental disturbance, particularly siltation.
% Tolerant Individuals	Percentage of individuals that are tolerant	The presence of tolerant species indicated an increase in degradation of stream quality. This metric detected a decline between stream quality from fair to poor
# Sensitive Species	Number of sensitive species	An absence of sensitive species indicated an anthropogenic stress or loss of habitat. This metric distinguished between streams of the highest quality
# Minnow Species	Number of minnow species	The number of minnow species helps evaluate pool habitat quality; including degradation of rock substrate, instream cover and the associated aquatic macroinvertebrate community. High numbers of minnow species corresponded with higher biological integrity.
# Benthic Insectivores	Number of benthic insectivore species	Benthic insectivore species occupied the same type of niche as darters. This allows a greater degree of sensitivity in evaluating streams that naturally had few darter species An increase in benthic insectivore species was correlated with increased biotic integrity.
Fish per Meter	Average number of fish per meter of stream sampled	Low values indicated that biotic integrity was being compromised, and that the normal trophic relationship of fish communities was being altered. This metric was most sensitive at intermediate to low ends of the sensitivity continuum.

% DELT	Percent of deformities, eroded fins, lesions and tumors anomalies	Presence of these anomalies was an indication of physiological stress due to environmental degradation, chemical pollutants, overcrowding, improper diet, excessive siltation, and other perturbations. This metric was most sensitive in low quality streams.

Table II - 2. Fish metric descriptions for small rivers. PCT implies percent of total sample represented by that taxa or category.

Metric (Abbreviation)	Brief Description	Full Description
QHEI	Qualitative Habitat Assessment	A habitat assessment designed for Ohio streams measuring qualities of the Substrate; Instream Cover; Channel Morphology Riparian Zone and Bank Erosion; Pool and Riffle Quality; and Map Gradient to calculate a combined score for the stream reach.
MSHA	Minnesota Stream Habitat Assessment	A habitat assessment designed for Minnesota streams measuring qualities of the Riparian Zone, Instream Cover and Channel Morphology to calculate a combined score for the stream reach.
Fish IBI	Fish Index of Biotic Integrity	An integrative expression of site condition across multiple metrics. A fish index of biological integrity is often composed of at least seven metrics dealing specifically with the fish sample at that site.
Count of Taxa (TR)	Number of taxa	The total number of species declines as environmental degradation increases. Hybrids, subspecies and exotics are not included in this metric.
Darter Sculpin Noturus (Dart,Sculp,Not)	Number of darter, sculpin and *Noturus* species	The darters, sculpins and madtoms are generally found in higher quality streams. These species are benthic insectivores; they rely on undisturbed benthic habitats to feed and reproduce. The degradation of benthic habitats will cause the species to decline.
Darter (Dart)	Number of darter species	Many darters are considered sensitive to water quality degradation. They require clean coarse substrate material in order to thrive, and tend to disappear in stream affected by siltation or channelization.
Intolerant (Intol)	Number of intolerant species	Intolerant species are those that are known to be sensitive to environmental degradation. They are often the first species to disappear following a disturbance. Their presence in a stream is an indication of a high quality resource.
Tolerant Pct (%Tol)	Percentage of individuals that are tolerant	Tolerant species are those that are known to persist in poor quality streams. They may become the dominant component in streams that have been chemically or physically altered.
Insect (Ins)	Number of invertivore species	Invertivores are specialized feeders that are dependent on a steady invertebrate food base. Disruption in the food base through human disturbance can lead to a decrease in invertivore species. Species classified as tolerant are not included in this metric.
Benthic Insect (Ben Ins)	Number of benthic invertivore species	Benthic invertivores rely on undisturbed benthic habitats to feed and reproduce. Degradation of benthic habitat will cause benthic invertivore species to decline. Species classified as tolerant are not included in this metric.
Omnivore Pct (Omn)	Percentage of individuals that are omnivores	Omnivores have the ability to utilize multiple food sources allows omnivore species to switch to another food source when one is depleted. A fish community dominated by omnivorous species indicates there is an unstable food source.
Omnivore (%Omn)	Number of omnivore species	Omnivores have the ability to utilize multiple food sources allows omnivore species to switch to another food source when one is depleted. A fish community dominated by omnivorous species indicates there is an unstable food source.

Metric (Abbreviation)	Brief Description	Full Description
Piscivore (Pisc)	Number of piscivore species	In moderate size streams and rivers, the presence of viable piscivore population indicates a healthy, trophically diverse fish community.
Piscivore Pct (%Pisc)	Percentage of individuals that are piscivores	In moderate size streams and rivers, the presence of viable piscivore population indicates a healthy, trophically diverse fish community.
Simple Lithophil Pct (%SLith)	Percentage of individuals that are simple lithophilic spawners	Simple lithophilic spawners broadcast their eggs over clean gravel substrates. The metric is inversely correlated with habitat degradation due to siltation.
Number Per Meter	Average number of fish per meter of stream sampled	This metric has been used to identify stream in which sever environmental degradation has occurred. Species classified as tolerant are not included.
Fish DELT Pct	Percent of deformities, eroded fins, lesions and tumors anomalies	This metric has been used to identify stream in which have been severely degraded. In other parts of the Midwest DELT anomalies have been associated with environmental degradation primarily due to industrial pollutants.

Table II - 3. Narrative guidelines for interpreting overall IBI. Modified from Karr (1981), Karr (1986), and Lyons (1992).

Biological Integrity Rating	Overall Upper Miss. River Basin IBI Score	Overall Minnesota River Basin IBI Score	Overall Red River Basin IBI Score	Fish Community Attributes
Excellent	100-80	60-50	51-60	Comparable to the best situations with minimal human disturbance; a full array of age and size classes were represented.
Good	79-60	49-40	41-50	Species richness somewhat below expectations; size/age distributions may show signs of imbalance.
Fair	59-40	39-30	31-40	Decreased species richness; size/age distributions may show signs of imbalance.
Poor	39-20	29-20	21-30	Decreased species richness; size/age distributions may show signs of imbalance; growth rates and condition factors sometimes depressed; hybrids sometimes common.
Very poor	19-0	20-12	12-20	The community is indicative of an environment that is severely modified by human disturbance; few species present. Age/size distributions are abnormal; DELT fish (fish with deformities, eroded fins, lesions, or tumors) may be present in the most severely degraded environments.
	No score	No fish	No score	Thorough sampling finds few or no fish; impossible to calculate IBI.

Table II - 4. Invertebrate metric descriptions for large rivers. Notes drawn from Hilsenhoff (1987).

Metric Abbreviation	Brief Description	Why is this metric used
Invert Taxa # (TR#)	Number of taxa (chironomids identified to family)	Taxa richness is considered a good indicator of environmental quality. In most types of aquatic ecosystems as environmental disturbance increases, taxa richness decreases.
Invert Taxa W Ch# (TR-Ch#)	Number of taxa (chironomids identified to genus)	Taxa richness is considered a good indicator of environmental quality. Identifying chironomid or midge larvae to genus increases the ability of this metric to determine stream condition.
Amphipoda # (Amphi #)	Percentage of individuals that are amphipods	Amphipoda are considered to be tolerant of organic pollution, and can become very abundant in conditions of low dissolved oxygen. Their abundance has been shown to be a good indicator of impairment across a range of stream classes and condition.
Plecoptera # (Plec#)	Number of Plecoptera taxa	Plecoptera, or stoneflies, are among the most sensitive indicator organisms. They occupy the interstitial spaces between rocks, woody debris, and vegetation, and require a relatively high amount of dissolved oxygen in order to survive.
Collect Gather Ch # (Coll-Gath-Ch#)	Number of collector-gatherer taxa (chironomids identified to genus)	The number of collector-gatherer taxa represents the number of different taxa that collect their food by gathering it from the substrate. Diversity within this feeding group indicates abundance and variety of food particles within the stream.
EPT #	Number of Ephemeroptera, Plecoptera, and Trichoptera taxa	One of the more sensitive macroinvertebrate metrics for assessing the condition of streams. Taxa belonging to these orders are sensitive to low oxygen concentrations, sedimentation, and habitat alteration.
Intolerant Ch # (Intol-Ch#)	Number of intolerant taxa (chironomids identified to genus)	Taxa with tolerance values less than or equal to 2. These taxa have been documented as sensitive to organic pollution/low oxygen levels in streams (Hilsenhoff 1987).
Clinger Ch # (Cling-Ch#)	Number of clinger taxa (chironomids identified to genus)	Clinger taxa are organisms that have morphological adaptations that allow them to thrive by attaching to the substrata in fast flowing water. A diverse group of clinger taxa indicate that substrate has not become embedded or covered by fine organic or inorganic material.
Tolerant # (Tol#)	Percentage of individuals that are tolerant	Taxa with tolerance values greater than or equal to 6. Tolerant macroinvertebrates are often found to thrive in areas known to have low dissolved oxygen, high turbidity, or heavy siltation.
Collector-Filterer # (Coll-Filt#)	Number of collector-filterer taxa	The number of collector-filterer taxa represents the number of different taxa that collect their food by filtering it out of the water column. The filtering is typically done one of two ways: 1) by using physical adaptation such as a filamentous antennal structure or 2) by constructing a net which filters the water, gathering filtered material from the net.

Table II - 5. List of sensitive fish species used to calculate % Sensitive Fish Species metric.

Taxon	Common Name	Taxon	Common Name
Petromyzontidae		**Catostomidae**	
Ichthyomyzon fossor	northern brook lamprey	*Carpiodes velifer*	highfin carpsucker
Ichthyomyzon gagei	southern brook lamprey	*Cycleptus elongatus*	blue sucker
Lampetra appendix	American brook lamprey	*Hypentelium nigricans*	northern hogsucker
Petromyzontidae	lamprey ammocoete	*Ictiobus niger*	black buffalo
Petromyzontidae	lampreys	*Minytrema melanops*	spotted sucker
Polyodontidae		*Moxostoma carinatum*	river redhorse
Polyodon spathula	paddlefish	*Moxostoma duquesnei*	black redhorse
Hiodontidae		*Moxostoma valenciennesi*	greater redhorse
Hiodon alosoides	goldeye	**Esocidae**	
Hiodon tergisus	mooneye	*Esox masquinongy*	muskellunge
Hiodontidae	mooneyes	**Salmonidae**	
Cyprinidae		*Salvelinus fontinalis*	brook trout
Clinostomus elongatus	redside dace	**Fundulidae**	
Erimystax x-punctatus	gravel chub	*Fundulus dispar*	starhead topminnow
Hybognathus nuchalis	Mississippi silvery minnow	**Cottidae**	
Hybopsis amnis	pallid shiner	*Cottus bairdii*	mottled sculpin
Macrhybopsis hyostoma	shoal chub	*Cottus cognatus*	slimy sculpin
Nocomis biguttatus	hornyhead chub	*Cottus ricei*	spoonhead sculpin
Notropis anogenus	pugnose shiner	*Cottus*	sculpins
Notropis buchanani	ghost shiner	*Myoxocephalus thompsonii*	deepwater sculpin
Notropis heterodon	blackchin shiner	**Centrarchidae**	
Notropis heterolepis	blacknose shiner	*Ambloplites rupestris*	rock bass
Notropis hudsonius	spottail shiner	*Lepomis megalotis*	longear sunfish
Notropis nubilus	Ozark minnow	*Micropterus dolomieu*	smallmouth bass
Notropis percobromus	carmine shiner	**Percidae**	
Notropis texanus	weed shiner	*Ammocrypta clara*	western sand darter
Notropis topeka	Topeka shiner	*Crystallaria asprella*	crystal darter
Notropis volucellus	mimic shiner	*Etheostoma caeruleum*	rainbow darter
Opsopoeodus emiliae	pugnose minnow	*Etheostoma exile*	Iowa darter
Rhinichthys cataractae	longnose dace	*Etheostoma microperca*	least darter
Ictaluridae		*Etheostoma zonale*	banded darter
Noturus exilis	slender madtom	*Percina evides*	gilt darter
Noturus flavus	stonecat	*Percina phoxocephala*	slenderhead darter

Table II - 6. List of sensitive macroinvertebrate species used to calculate % Sensitive Macroinvertebrate Taxa metric.

GASTROPODA (Snails)	**PLECOPTERA (Stoneflies)**
Viviparidae (River Snails)	**Capniidae (Small Winter Stoneflies)**
Viviparus	*Paracapnia*
EPHEMEROPTERA (Mayflies)	Capniidae
Acanthametropodidae (Acanthametropod Mayflies)	**Chloroperlidae (Green Stoneflies)**
Acanthametropus	Chloroperlidae
Baetidae (Small Minnow Mayflies)	**Leuctridae (Roll-winged Stoneflies)**
Centroptilum	*Paraleuctra*
Heterocloeon	Leuctridae
Ephemerellidae (Spiny Crawler Mayflies)	**Nemouridae (Brown Stoneflies)**
Ephemerella	*Soyedina*
Serratella	Nemouridae
Ephemeridae (Common Burrowing Mayflies)	**Perlidae (Common Stoneflies)**
Ephemera	*Acroneuria*
Heptageniidae (Flathead Mayflies)	*Agnetina*
Epeorus	*Attaneuria*
Heptagenia	*Neoperla*
Leucrocuta	*Paragnetina*
Maccaffertium	*Perlidae*
Nixe	*Perlinella*
Rhithrogena	**Perlodidae (Patterned Stoneflies)**
Stenonema vicarium	*Isogenoides*
Isonychiidae (Brush-legged Mayflies)	*Isoperla*
Isonychia	Perlodidae
Leptophlebiidae (Prong-gilled Mayflies)	**Pteronarcidae (Giant Stoneflies)**
Choroterpes	*Pteronarcys*
Habrophlebia	**Taeniopterygidae (Winter Stoneflies)**
Paraleptophlebia	*Taeniopteryx*
Leptophlebiidae	Taeniopterygidae
Metretopodidae (Cleft-footed Minnow Mayflies)	**HEMIPTERA (True Bugs)**
Siphloplecton	**Veliidae (Broad-shouldered Water Striders)**
Polymitarcyidae (Pale Burrowing Mayflies)	*Microvelia*
Ephoron	*Rhagovelia*
Potamanthidae (Hacklegill Mayflies)	*Veliidae*
Anthopotamus	**COLEOPTERA (Beetles)**
ODONATA (Dragonflies and Damselflies)	**Lampyridae (Fireflies)**
Aeshnidae (Darner Dragonflies)	Lampyridae
Boyeria	**MEGALOPTERA (Fishflies and Alderflies)**
Corduliidae (Green-eyed Skimmers)	**Corydalidae (Fishflies)**
Neurocordulia	*Nigronia*
Somatochlora	
Gomphidae (Club-tail Dragonflies)	
Hagenius	
Ophiogomphus	
Progomphus	
Gomphidae	
Macromiidae (Skimmer Dragonflies)	
Macromia	

Table II - 6 (continued). List of sensitive macroinvertebrate species used to calculate % Sensitive macroinvertebrate Taxa metric.

TRICHOPTERA (Caddisflies)	**LEPIDOPTERA (Moths)**
Brachycentridae (Humpless Case-maker Caddisflies)	**Pyralidae (Aquatic Moths)**
Brachycentrus	*Acentria*
Micrasema	**DIPTERA (True Flies)**
Brachycentridae	**Athericidae (Aquatic Snipe Flies)**
Glossosomatidae (Saddle Case-maker Caddisflies)	*Atherix*
Agapetus	**Chironomidae (Non-biting midges)**
Glossosoma	*Apsectrotanypus*
Glossosomatidae	*Axarus*
Hydropsychidae (Common Net-spinner Caddisflies)	*Heterotrissocladius*
Ceratopsyche	*Hyporhygma*
Diplectrona	*Krenosmittia*
Potamyia	*Microchironomus*
Hydroptilidae (Micro Caddisflies)	*Nilothauma*
Leucotrichia (pictipes	*Pagastia*
Stactobiella	*Pagastiella*
Lepidostomatidae (Lepidostomatid Case-maker Caddisflies)	*Parakiefferiella*
Lepidostoma	*Potthastia*
Lepidostomatidae	*Pseudorthocladius*
Leptoceridae (Long-horned Case-maker Caddisflies)	*Rheosmittia*
Setodes	*Smittia*
Limnephilidae (Northern Case-maker Caddisflies)	*Stempellina*
Apatania	*Stilocladius*
Glyphopsyche	*Synorthocladius*
Goera	*Xenochironomus*
Hydatophylax	*Xylotopus*
Psychoglypha	**Dixidae (Meniscus Midges)**
Philopotamidae (Finger-net Caddisflies)	*Dixa*
Dolophilodes	*Dixella*
Polycentropodidae (Trumpet-net Caddisflies)	Dixidae
Nyctiophylax	**Tipulidae (Crane Flies)**
Psychomyiidae (Tube-making Caddisflies)	*Hesperoconopa*
Lype	*Hexatoma*
Psychomyia	*Pseudolimnophila*
Psychomyiidae	
Rhyacophilidae (Free-living Caddisflies)	
Protoptila	
Rhyacophila	
Sericostomatidae (Sericostomatid Case-maker Caddisflies)	
Agarodes	
Uenoidae (Uenoid Case-maker Caddisflies)	
Uenoidae	

Table II - 7. Fish data for river nutrient study sites. PCT implies percent of individuals in sample and # implies number of taxa, unless otherwise noted. For sites with two samples the mean of the two was used in data analysis. See Table II - 2 for description of abbreviations.

Basin	Field #	Name	Visit	TR	Dart	Ins -Tol	Minn -Tol	Omn	Pisc	Sen	SLith	Tol	%Dart	%Dom2	Ins%	Minn -Tol%	Omn%	Pisc%	Sen%	Slith%	Tol%
MN	00MN001	Blue Earth	8/15/2000	12	0	3	4	4	0	0	1	7	0	59	22	30	53	0	0	3	68
MN	00MN002	Blue Earth	8/15/2000	19	2	9	4	4	2	1	5	6	2	59	27	17	61	2	1	9	69
MN	00MN003	Blue Earth	8/30/2000	16	2	9	3	2	2	2	6	3	3	42	55	34	23	5	11	19	39
MN	00MN004	Blue Earth	8/29/2000	13	1	7	4	3	0	0	2	4	0	80	35	31	60	0	0	3	64
MN	00MN005	Blue Earth	8/30/2000	19	2	11	3	3	3	3	7	4	1	77	87	78	6	5	2	9	7
RD	05RD110	Buffalo	7/26/2006	26	2	13	6	5	3	6	7	8	11	60	25	34	40	1	5	43	46
RD	05RD120	Buffalo	8/21/2006	17	2	10	4	2	3	3	9	3	4	61	86	63	1	4	3	33	4
RD	05RD109	Otter Tail	8/22/2006	16	0	8	2	2	5	2	5	2	0	67	83	55	6	10	4	31	6
RD	05RD129	Red Lake	8/24/2006	14	1	8	3	1	4	3	6	1	1	64	27	59	3	25	32	51	3
RD	05RD121	Red Lake	8/30/2006	21	2	10	3	2	8	4	9	1	14	66	34	2	4	62	61	33	3
RD	05RD115	Wild Rice	7/26/2006	34	3	17	7	5	6	9	11	7	11	35	60	51	5	5	12	50	12
RD	05RD112	Wild Rice	8/23/2006	14	1	8	3	3	2	1	8	2	1	59	80	66	6	3	1	44	5
RN	05RN081	Big Fork	8/3/2005	16	4	8	3	1	3	5	8	3	61	52	74	27	0	4	20	78	2
RN	05RN086	Little Fork	8/30/2005	18	4	13	6	1	2	6	8	1	58	53	86	21	1	3	11	67	1
UM	00UM080	Crow River	7/26/2000	23	3	13	5	2	5	3	8	4	3	45	63	47	23	7	5	19	30
UM	00UM081	Crow River	8/14/2000	16	2	8	3	3	3	3	6	5	14	35	46	21	13	16	6	26	37
UM	99UM010	Crow S. Fork	8/16/1999	20	2	8	3	4	4	0	5	7	1	52	42	26	39	4	0	7	51
UM	00UM026	Crow Wing	7/31/2000	19	2	10	5	1	4	6	8	4	16	36	67	29	6	15	47	43	12
UM	00UM024	Crow Wing	6/13/2000	27	2	15	8	2	5	7	9	5	8	47	41	52	10	10	14	68	12
UM	00UM087	Mississippi	8/22/2000	17	1	11	5	0	4	5	5	1	11	49	83	39	0	7	8	21	1
UM	00UM088	Mississippi	8/22/2000	23	2	13	5	2	6	6	9	3	18	33	77	16	2	17	18	38	3
UM	00UM091	Mississippi	8/24/2000	26	2	11	4	2	9	5	7	5	52	57	73	12	4	17	20	64	6
UM	00UM092	Mississippi	8/29/2000	15	1	7	1	2	5	3	6	3	18	45	40	1	18	41	32	39	19
UM	00UM092	Mississippi	9/14/2000	15	1	6	1	3	6	3	5	3	26	66	44	2	13	44	41	42	13
UM	00UM098	Mississippi	9/13/2000	17	2	10	2	1	4	2	5	3	56	66	84	1	5	10	8	65	6
UM	00UM044	Rum River	9/18/2000	24	2	12	4	2	4	4	6	6	7	42	33	29	4	27	26	16	31
UM	00UM044	Rum River	9/12/2000	22	2	10	3	2	5	4	6	5	12	46	40	31	4	28	27	13	23
UM	00UM066	Rum River	8/28/2000	13	2	7	4	1	3	5	7	2	22	40	58	34	13	21	44	55	14
UM	00UM066	Rum River	7/27/2000	19	3	10	4	2	4	6	8	4	20	41	59	49	12	16	47	58	13

Table II - 7 (continued). Fish data for river nutrient study sites. PCT implies percent of individuals in sample and # implies number of taxa, unless otherwise noted. For sites with two samples the mean of the two was used in data analysis.

Basin	Field #	Name	Drain mi^2	Visit	Fish IBI	QHEI	Report Code	Location
MN	00MN001	Blue Earth	255	8/15/2000	14	43	BE-100	downstream of C.R. 4, 4 mi S. of Blue Earth
MN	00MN002	Blue Earth	804	8/15/2000	24	46	BE-18.2	downstream of C.R. 8, 2 mi N. of Blue Earth
MN	00MN003	Blue Earth	1,371	8/30/2000	24	52	BE-54	upstream of C.R. 12, 1 mi E. of Winnebago
MN	00MN004	Blue Earth	1,395	8/29/2000	22	48	BE-73.2	upstream of Hwy 30
MN	00MN005	Blue Earth	1,528	8/30/2000	40	42	BE-94.3	east of Garden City, upstream of CR 34 bridge
RD	05RD110	Buffalo	336	7/26/2006	40	50	BUFF-10	In Hawley just upstream of the Hwy 10 Bridge
RD	05RD120	Buffalo	1,009	8/21/2006	44	33	BUFF-01	7 mi NE of Moorhead, upstream of CR 94
RD	05RD109	Otter Tail	1,814	8/22/2006	34	67	OT-1	1m E of Breckenridge, ~ 2 m upstream of State Route 9
RD	05RD129	Red Lake	2,157	8/24/2006	36	51	RL-75	6.5m S of Goodridge, downstream of County Route 24
RD	05RD121	Red Lake	3,362	8/30/2006	40	83	RL-1	0.5m S of Thief River Falls, C city Park
RD	05RD115	Wild Rice	921	7/26/2006	44	62	WR-200	NE side of Twin Valley, upstream of County Route 29.
RD	05RD112	Wild Rice	1,567	8/23/2006	32	50	WR-1	0.5 miles E of Hendrum, upstream of County Route 25
RN	05RN081	Big Fork	1,500	8/3/2005		71	BF-46	3.5 miles west of Bigfalls, @ Sturgeon River canoe landing
RN	05RN086	Little Fork	1,691	8/30/2005		50	LF-21	At end of CR 23, just upstream of the town of Little Fork.
UM	00UM080	Crow River	2,633	7/26/2000	65	68	CR-23	downstream of Hwy 55 @ Rockford
UM	00UM081	Crow River	2,751	8/14/2000	55	60	CR-0.2	upstream of Hwy 101, 4 mi. S. of Elk River
UM	99UM010	Crow S. Fork	1,171	8/16/1999	44		CR-44	~1.0 mi. N. of Hwy. 7, .5 mi. W. of Hwy. 25, 2.0 mi. N. of Mayer
UM	00UM026	Crow Wing	939	7/31/2000	80	79	CWR-72	upstream of bridge at county park in Nimrod
UM	00UM024	Crow Wing	2,232	6/13/2000	86	80	CWR-35.5	C.R. 33 at Cass/Todd/Wadena county line, NW of Motley
UM	00UM087	Mississippi	5,838	8/22/2000	68	50	UM-1056	upstream of CR 1 north of Aitkin
UM	00UM088	Mississippi	6,060	8/22/2000	78	59	UM-1029	Hwy. 6 N. of Crosby
UM	00UM091	Mississippi	11,730	8/24/2000	88	68	UM-953.7	downstream of Royalton boat landing
UM	00UM092	Mississippi	14,032	8/29/2000	54	63	UM-895	Public Access @ Monticello
UM	00UM092	Mississippi	14,032	9/14/2000	62	67	UM-895	Public Access @ Monticello
UM	00UM098	Mississippi	19,041	9/13/2000	69	65	UM-872	below 169 bridge - Elm Creek mouth (Miss. Point Park) in Anoka.
UM	00UM044	Rum River	1,273	9/12/2000	69	60	RUM-34	downstream of C.R. 5 in Isanti
UM	00UM044	Rum River	1,273	9/18/2000	69	65	RUM-34	downstream of C.R. 5 in Isanti
UM	00UM066	Rum River	1,325	7/27/2000	77	78	RUM-18	downstream of C.R. 24 in St. Francis
UM	00UM066	Rum River	1,325	8/28/2000	63	77	RUM-18	downstream of C.R. 24 in St. Francis

Table II - 8. Invertebrate data for river nutrient study sites. See Table II - 4 for description of abbreviations.

Basin	Field Num	Name	Chiro	Cling -Ch	Coll -Filt-Ch	Coll -Filt	Coll -Gath	EPT	Intol -Ch	Odo	Tanytar	TR-Ch	Amphi%	Coll -Filt%	Coll -Gath%	Dom2%	Ephem%	HBI	Plec%	Trich%	Vtol%
MN	00MN001	Blue Earth	16	20	5	5	20	20	11	3	1	57	0.2	3.3	66.3	29.3	48.6	5.0	1.2	3.6	2.7
MN	00MN002	Blue Earth	7	10	4	3	9	14	5	1	2	30	0.0	11.6	56.7	24.9	38.6	5.5	0.7	2.9	11.6
MN	00MN003	Blue Earth	8	11	6	5	10	15	7	1	2	32	0.0	45.7	37.9	43.9	34.6	5.0	0.0	37.0	3.6
MN	00MN004	Blue Earth	6	9	4	4	7	11	3	1	1	27	0.0	15.2	55.4	39.1	24.3	5.9	0.4	9.4	11.6
MN	00MN005	Blue Earth	7	8	3	2	9	10	2	0	2	24	0.0	18.6	64.2	44.4	17.2	5.8	0.0	7.1	6.2
RD	05RD120	Buffalo	4	10	5	5	7	9	3	1	0	24	0.0	15.4	43.4	41.3	58.4	5.0	0.0	15.1	2.1
RD	05RD120	Buffalo	6	12	4	4	10	9	3	3	0	31	0.3	22.0	50.7	44.0	54.5	5.1	0.0	21.4	2.1
RD	05RD110	Buffalo	15	13	6	4	16	8	3	2	3	45	0.3	20.8	45.1	37.6	33.5	5.6	0.0	3.2	2.4
RD	05RD109	Otter Tail	9	9	4	4	11	9	2	2	1	40	0.0	24.7	21.6	23.0	33.0	5.3	0.0	16.7	10.3
RD	05RD121	Red Lake	10	20	8	7	13	17	6	2	1	51	5.0	28.0	30.1	19.1	24.9	5.1	0.0	28.5	17.3
RD	05RD112	Wild Rice	6	13	3	3	9	11	5	2	0	32	0.6	3.8	44.0	49.0	63.0	4.4	0.0	9.9	8.2
RD	05RD115	Wild Rice	13	17	9	7	12	14	5	0	2	40	0.0	27.3	35.7	15.9	34.1	4.7	2.2	26.2	2.2
RN	05RN081	Big Fork	20	23	8	6	24	21	11	3	4	59	0.0	53.4	18.6	34.2	21.2	4.8	0.2	34.9	2.4
RN	05RN081	Big Fork	12	21	9	8	21	21	14	3	4	50	0.2	59.2	23.9	40.6	17.9	4.8	0.9	47.7	6.7
RN	05RN086	Little Fork	18	12	5	3	18	13	6	3	3	43	0.0	11.4	46.6	22.0	22.0	5.1	0.6	2.6	8.8
UM	00UM080	Crow	5	11	10	8	5	14	6	0	1	26	0.0	61.6	21.6	42.2	22.8	4.9	0.0	50.0	8.1
UM	00UM081	Crow	5	11	9	8	7	13	4	1	0	27	0.0	43.6	27.1	25.3	35.6	5.1	0.3	26.9	11.4
UM	99UM010	Crow S. Fork	6	6	5	4	7	12	5	1	1	29	0.0	35.3	19.0	39.6	24.5	4.9	0.0	29.0	6.0
UM	00UM026	Crow Wing	5	19	8	8	10	22	12	1	1	42	1.4	19.6	37.3	42.3	29.0	3.6	0.3	45.9	5.0
UM	00UM091	Mississippi	8	19	6	4	10	12	5	6	1	43	0.0	10.6	27.7	70.2	0.0	6.0	0.0	0.0	0.0
UM	00UM066	Rum	9	16	9	8	11	16	8	0	2	36	0.0	39.0	24.5	31.9	6.7	4.8	1.4	36.7	4.5
UM	00UM066	Rum	8	18	11	9	7	18	12	2	1	51	19.1	32.8	33.7	34.6	10.3	5.2	2.2	34.6	23.8

Table II - 9. 2008 Fish data.

	Number	River	# Fish Taxa	Darter	Insect-Tolerant	Minnows-Tolerant	Omnivore	Piscivore	Sensitive	S Lithop	Tolerant
LM	08LM002	S. Branch Root	21	3	11	3	1	5	8	10	4
LM	08LM012	N. Branch Root	28	5	16	6	4	4	12	13	4
LM	08LM014	Bear Creek	25	5	13	5	3	3	10	11	6
LM	08LM114	Vermillion River	14	1	3	1	3	4	0	2	7
LM	08LM127	Wells Creek	10	1	1	0	1	2	0	2	7
MN	08MN003	Maple River	21	3	14	5	3	3	6	10	3
MN	08MN004	Rice Creek	23	3	15	5	4	0	3	8	7
MN	08MN005	Big Cobb	26	3	14	5	5	2	4	10	7
MN	08MN035	Le Sueur	29	4	13	5	5	5	7	11	7
UM	08UM025	Sauk	15	2	7	4	0	4	2	2	3
UM	00UM039	Getchell	16	3	8	7	2	0	3	6	6

	Number	River	Darter %	Dom Two %	Insect %	Minnows-Tolerant %	Omnivore %	Piscivore %	Sensitive %	S Lithop %	Tolerant %
LM	08LM002	S. Branch Root	3.7	53.6	29.6	43.1	15.4	11.2	16.9	80.9	21.0
LM	08LM012	N. Branch Root	1.4	37.9	60.3	29.5	4.6	14.2	52.5	58.7	4.6
LM	08LM014	Bear Creek	9.7	45.1	53.8	15.8	16.0	11.6	36.0	67.7	17.8
LM	08LM114	Vermillion River	3.4	59.0	24.4	14.7	56.8	7.1	0.0	47.4	72.9
LM	08LM127	Wells Creek	6.0	67.2	7.1	0.0	25.3	3.9	0.0	46.1	90.1
MN	08MN003	Maple River	11.4	34.2	85.9	35.4	3.3	7.8	31.8	50.8	6.0
MN	08MN004	Rice Creek	8.4	60.3	79.2	64.1	4.8	0.0	0.4	11.9	20.6
MN	08MN005	Big Cobb	3.2	41.7	59.7	45.3	28.1	0.6	6.5	16.6	35.0
MN	08MN035	Le Sueur	2.4	64.3	86.2	77.3	3.9	2.6	6.5	18.9	7.1
UM	08UM025	Sauk	1.0	47.0	49.4	71.3	0.0	2.6	7.8	23.9	24.8
UM	00UM039	Getchell	3.5	48.8	17.2	50.7	16.3	0.0	10.4	66.8	45.8

C. APPENDIX III WATER QUALITY SITE MAPS FOR 1999 AND 2000, 2000 PERIPHYTON DATA AND 2007 USGS UPPER MIDWEST STUDY SITES AND DATA.

Figure III - 1. Basin and river-specific maps with 1999 and 2000 sample sites: Upper Mississippi Basin and Blue Earth River.

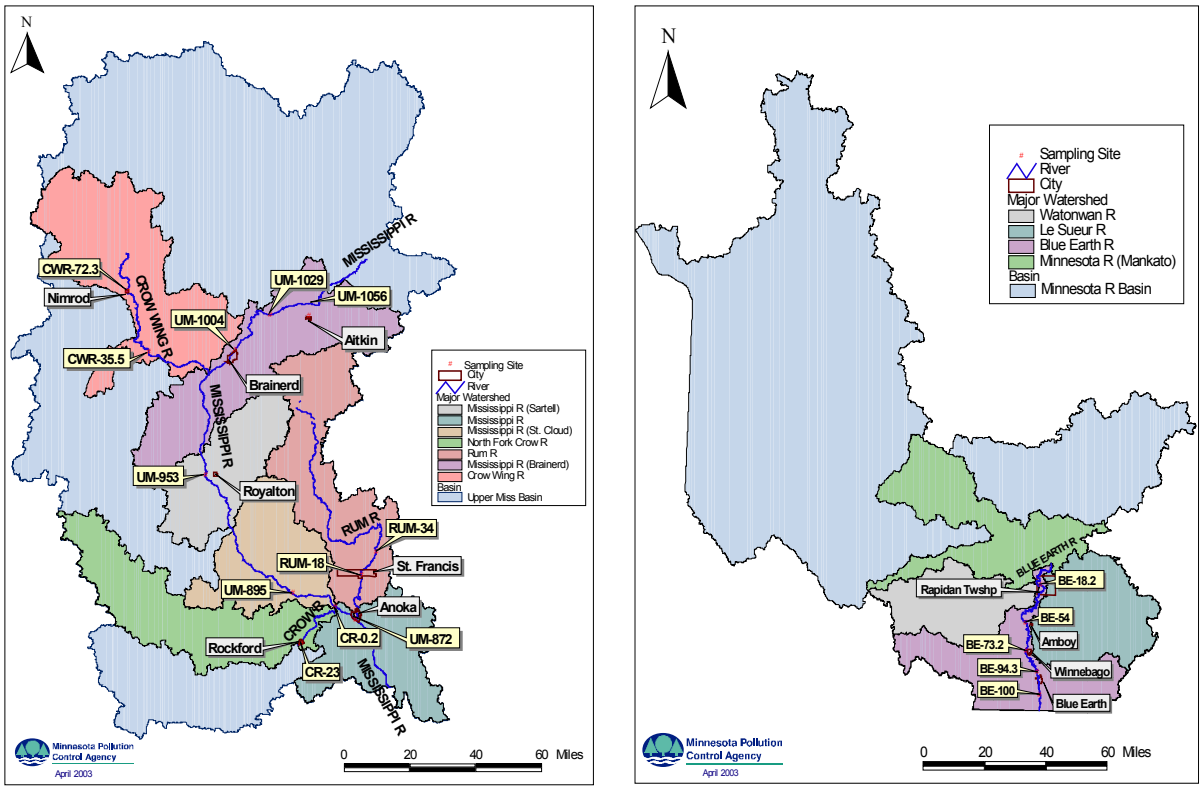
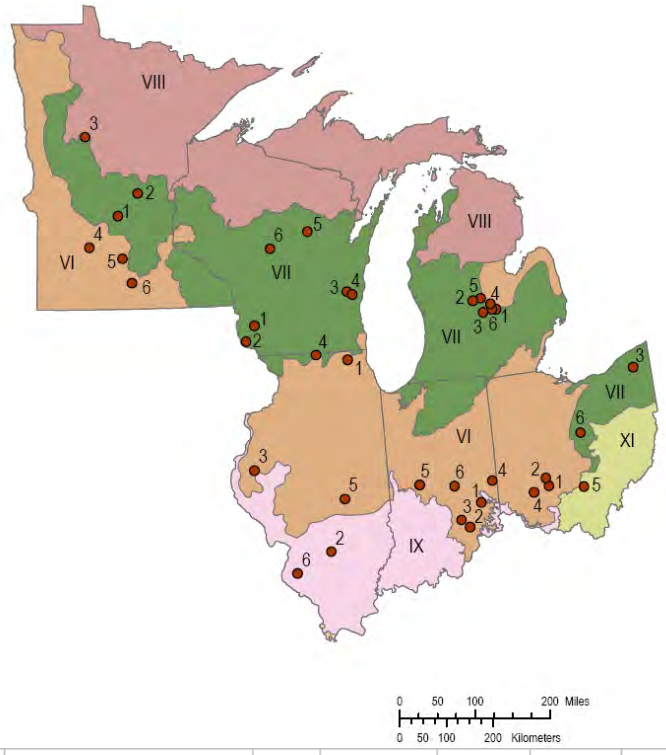


Table III - 1. Water chemistry, seston and periphyton chlorophyll data from MPCA 2000 and USGS 2007 studies. Periphyton Chl-a in mg/m^2 and seston Chl-a, pheophytin, and Chl-T in µg/L.

2000 study	Peri chl-a	Year	TP	TKN	TN	NO3	Chl-a	Pheo	ChlT	BOD	TSS	TSV	TSIN	Turb	T-tube
CWR-72.3	16.9	2000	34	0.78	0.90	0.12	3.4	1.5	4.9	1.2	3	2	1	3	>60
CWR-35.5	25.1	2000	49	1.19	1.41	0.23	3.7	1.7	5.4	1.2	6	3	3	3	>60
UM-1056	9.5	2000	59	0.72	0.79	0.07	4.7	2.1	6.9	1.1	19	3	17	12	53
UM-872	133.5	2000	84	0.93	1.16	0.23	22.7	6.4	29.2	2.1	16	5	11	9	47
RU-18	31.7	2000	133	0.97	1.16	0.18	31.4	10.7	43.9	2.3	11	5	6	6	52
RU-34	54.5	2000	143	0.85	1.13	0.28	20.5	7.2	28.7	1.8	11	4	7	6	56
BE-73.2	10.5	2000	205	1.63	7.18	5.55	87.4	15.4	100.9	5.1	74	15	59	41	17
BE-54	23.8	2000	207	1.60	6.95	5.35	86.7	11.5	96.8	6.3	91	18	73	46	15
RE-536	31.6	2000	208	1.18	1.43	0.25	18.9	8.3	27.2	2.8	55	9	46	27	22
CR-0.2	13.9	2000	284	1.98	3.53	1.56	112.4	26.6	135.7	7.0	64	18	46	32	15
RE-452	32.7	2000	312	1.48	1.73	0.25	23.2	14.5	37.7	2.1	144	19	125	69	14
CR-23	13.5	2000	349	1.94	3.63	1.69	120.3	25.5	142.6	6.6	75	18	57	41	13

Figure III - 2. EPA Region V Upper Midwest 2007 Diel DO study sites and summer-mean data.



Map #	Stream Name	Basin	Sample Date	TP	Ortho-P	TN	NO2+NO3	NO2	NH4-N	Seston Mean CHLa	Periphyton Mean CHLa	Periphyton Mean DO flux
				(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/m2)	(mg/L)
1	Clearwater River	UM	6/18/2007	0.143	0.057	1.583	0.142	0.043	0.178	2.7	198.2	9.2
2	Rum River	UM	6/26/2007	0.144	0.096	0.830	0.097	0.004	0.020	1.5	165.2	4.1
3	Shell River	UM	6/20/2007	0.060	0.020	0.617	0.023	0.002	0.023	0.9	18.6	1.5
4	West Fork Beaver Creek	MN	6/13/2007	0.263	0.148	5.673	4.300	0.058	0.039	17.7	115.7	8.1
5	South Branch Rush River	MN	6/14/2007	0.162	0.076	8.953	7.677	0.049	0.031	44.7	174.4	12.1
6	Little Cobb River	MN	6/12/2007	0.218	0.057	7.617	5.997	0.036	0.081	4.5	30.2	1.6

D. APPENDIX IV. QUANTILE REGRESSION SUMMARIES

Table IV - 1. Raw total BOD_5 threshold concentration values (mg L^{-1}) determined with additive quantile regression smoothing analysis for all stream sizes using STORET data. Abbreviations: T.C. = threshold concentration, MP = midpoint, UBP = upper break point, Fisher's = Fisher's exact test, chi squared = chi squared test.

Region	Group	Metric	Λ	F-test	MP T.C.	MP test	UBP T.C.	UBP test	Final T.C.	Notes
North	Fish	%Sensitive								weak relationship
North	Fish	%Darter								weak relationship
North	Fish	%Simple Lithophils								weak relationship
North	Fish	%Tolerant								weak relationship
North	Fish	%Insectivores								weak relationship
North	Fish	%Piscivores								weak relationship
North	Fish	Taxa Richness								weak relationship
North	Fish	%Intolerant								weak relationship
North	Invert	Taxa Richness								no relationship; small n w/ max BOD_5 1.31
North	Invert	#Collector-Filterer								no relationship; small n w/ max BOD_5 1.31
North	Invert	#Collector-Gatherer								no relationship; small n w/ max BOD_5 1.31
North	Invert	#EPT								no relationship; small n w/ max BOD_5 1.31
North	Invert	#Intolerant								no relationship; small n w/ max BOD_5 1.31
North	Invert	%Tolerant								no relationship; small n w/ max BOD_5 1.31
Central	Fish	%Sensitive	0.5	<0.0001	4.1	0.0670	3.5	1.000		failed Fisher's
Central	Fish	%Darter								weak relationship
Central	Fish	%Simple Lithophils	0.5	<0.0001	3.4	0.2170	2.6	0.143		failed Fisher's
Central	Fish	%Tolerant					3.5	0.149		no lower breakpoint; failed Fisher's
Central	Fish	%Insectivores								weak relationship
Central	Fish	%Piscivores				-	3.5	0.301		no lower breakpoint; failed Fisher's
Central	Fish	Taxa Richness								weak relationship
Central	Fish	%Intolerant	0.6	<0.0001	4.1	0.0130	3.5	0.067	**4.1**	
Central	Invert	Taxa Richness	0.1	<0.0001	2.3	0.0050	2.1	0.0040	**2.1**	Fisher's; use upper breakpoint
Central	Invert	#Collector-Filterer								weak relationship
Central	Invert	#Collector-Gatherer	0.1	0.0139	1.7	0.1000	-	-		failed F-test
Central	Invert	#EPT								weak relationship
Central	Invert	#Intolerant	0.35	0.0129		-	2.1	0.0237	**2.1**	no lower breakpoint; Fisher's; use upper breakpoint
Central	Invert	%Tolerant								weak relationship
South	Fish	%Sensitive	1.5	<0.0001	4.9	0.0230	3.6	0.1400	**4.9**	Fisher's
South	Fish	%Darter	1.95	<0.0001	4.3	0.0220	2.9	0.1820	**4.3**	Fisher's
South	Fish	%Simple Lithophils	4	<0.0001	3.5	0.0030	-	-	**3.5**	Fisher's
South	Fish	%Tolerant	1	0	4.7	0.0470	3.9	0.7440	**4.7**	Fisher's
South	Fish	%Insectivores					3.0	0.1560		failed Fisher's
South	Fish	%Piscivores								weak relationship
South	Fish	Taxa Richness					3.9	0.4560		failed Fisher's
South	Fish	%Intolerant								weak relationship
South	Invert	Taxa Richness	1	<0.0001	1.8	0.0410	-	-	**1.8**	Fisher's
South	Invert	#Collector-Filterer								weak relationship
South	Invert	#Collector-Gatherer	1	<0.0001	1.7	0.0070	-	-	**1.7**	Fisher's
South	Invert	#EPT								weak relationship
South	Invert	#Intolerant	1	<0.0001	3.5	0.0850	3.0	0.0930		failed Fisher's
South	Invert	%Tolerant								weak relationship

Table IV - 2. Raw total BOD_5 threshold concentration values (mg L^{-1}) from regression tree (changepoint) analysis for all stream sizes using STORET data. Abbreviations: T.C. = threshold concentration, L = 90% lower bound, U = 90% upper bound, Fisher's = Fisher's exact test, chi squared = chi squared test.

Region	Group	Metric	Bucket	T.C.	L	U	Test	Final T.C.	Notes
North	Fish	%Sensitive							weak relationship
North	Fish	%Darter							weak relationship
North	Fish	%Simple Lithophils							weak relationship
North	Fish	%Tolerant							weak relationship
North	Fish	%Insectivores							weak relationship
North	Fish	%Piscivores							weak relationship
North	Fish	Taxa Richness							weak relationship
North	Fish	%Intolerant							weak relationship
North	Invert	Taxa Richness							no relationship; small n w/ max BOD_5 1.31
North	Invert	#Collector-Filterer							no relationship; small n w/ max BOD_5 1.31
North	Invert	#Collector-Gatherer							no relationship; small n w/ max BOD_5 1.31
North	Invert	#EPT							no relationship; small n w/ max BOD_5 1.31
North	Invert	#Intolerant							no relationship; small n w/ max BOD_5 1.31
North	Invert	%Tolerant							no relationship; small n w/ max BOD_5 1.31
Central	Fish	%Sensitive	5	3.8	3.2	6.9	0.0126	**3.8**	Fisher's
Central	Fish	%Darter	5				0.2134		failed Fisher's
Central	Fish	%Simple Lithophils	5	1.5	-0.7	2.2	0.0072	**1.5**	Fisher's
Central	Fish	%Tolerant	5	3.8	2.7	6.8	0.0027	**3.8**	Fisher's
Central	Fish	%Insectivores	5				0.1824		failed Fisher's
Central	Fish	%Piscivores	5				0.3752		failed Fisher's
Central	Fish	Taxa Richness	5				0.1069		failed Fisher's
Central	Fish	%Intolerant	5				0.0616		failed Fisher's
Central	Invert	Taxa Richness	5	2.2	1.1	3.2	0.0172	**2.2**	Fisher's
Central	Invert	#Collector-Filterer	5				0.4185		failed Fisher's
Central	Invert	#Collector-Gatherer	5				0.1279		failed Fisher's
Central	Invert	#EPT	5				0.0375		metric response does not match prediction
Central	Invert	#Intolerant	5				0.4279		failed Fisher's
Central	Invert	%Tolerant	5				0.4009		failed Fisher's
South	Fish	%Sensitive	5	2.3	-0.7	2.9	0.0113	**2.3**	Fisher's
South	Fish	%Darter	5	4.4	3.7	6.0	0.0220	**4.4**	Fisher's
South	Fish	%Simple Lithophils	5	4.4	3.4	6.8	0.0003	**4.4**	Fisher's
South	Fish	%Tolerant	5	4.6	3.5	5.9	0.0025	**4.6**	
South	Fish	%Insectivores	5	5.1	3.5	7.8	0.0498	**5.1**	Fisher's
South	Fish	%Piscivores	5				0.1400		not significant
South	Fish	Taxa Richness	5				0.0533		not significant
South	Fish	%Intolerant	5				0.6947		not significant
South	Invert	Taxa Richness	5	4.3	3.1	6.6	0.0133	**4.3**	
South	Invert	#Collector-Filterer	5				0.4429		failed Fisher's
South	Invert	#Collector-Gatherer	5	2.9	1.2	4.4	0.0476	**2.9**	
South	Invert	#EPT	5				0.1589		failed Fisher's
South	Invert	#Intolerant	5	4.3	2.7	5.3	0.0020	**4.3**	
South	Invert	%Tolerant	5				0.8101		failed Fisher's

Table IV - 3. Raw total BOD_5 threshold concentration values (mg L^{-1}) from River Nutrient data using additive quantile regression smoothing analysis. Abbreviations: T.C. = threshold concentration, MP = midpoint, UBP = upper break point, Fisher's = Fisher's exact test.

Region	Group	Metric	Λ	F-test	MP T.C.	MP test	UBP T.C.	UBP test	Final T.C.	Notes
Statewide	Fish	%Sensitive	0.50	<0.0001	2.9	0.1154	-	-		failed Fisher's
Statewide	Fish	%Darter								weak relationship
Statewide	Fish	%Simple Lithophils	1.00	<0.0001	3.7	0.0351	2.1	1.0000	**3.7**	
Statewide	Fish	%Tolerant								weak relationship
Statewide	Fish	%Insectivores								weak relationship
Statewide	Fish	%Piscivores	1.50	<0.0001	2.9	0.6130	-	-		failed Fisher's
Statewide	Fish	Taxa Richness	0.75	<0.0001	1.7	0.0964	-	-		failed Fisher's
Statewide	Fish	%Intolerant	1.20	<0.0001	2.9	0.1348	-	-		failed Fisher's
Statewide	Invert	Taxa Richness								sample size too small to fit AQRS
Statewide	Invert	#Collector-Filterer								sample size too small to fit AQRS
Statewide	Invert	#Collector-Gatherer								sample size too small to fit AQRS
Statewide	Invert	#EPT								sample size too small to fit AQRS
Statewide	Invert	#Intolerant								sample size too small to fit AQRS
Statewide	Invert	%Tolerant								sample size too small to fit AQRS

Table IV - 4. Raw total BOD_5 threshold concentration values (mg L^{-1}) from River Nutrient data using regression tree (changepoint) analysis. Abbreviations: T.C. = threshold concentration, L = 90% lower bound, U = 90% upper bound, Fisher's = Fisher's exact test.

Region	Group	Metric	Bucket	T.C.	L	U	test	Final T.C.	Notes
Statewide	Fish	%Sensitive	5				0.0511		failed Fisher's
Statewide	Fish	%Darter	5				0.3512		failed Fisher's
Statewide	Fish	%Simple Lithophils	5	2.5	1.4	3.7	0.0124	**2.5**	Fisher's
Statewide	Fish	%Tolerant	5	3.9	3.1	6.8	0.0008	**3.9**	Fisher's
Statewide	Fish	%Insectivores	5				0.1353		failed Fisher's
Statewide	Fish	%Piscivores	5				0.2031		failed Fisher's
Statewide	Fish	Taxa Richness	5				0.1827		failed Fisher's
Statewide	Fish	%Intolerant	5				0.1206		failed Fisher's
Statewide	Invert	Taxa Richness	5	2.5	1.5	3.8	0.0090	**2.5**	Fisher's
Statewide	Invert	#Collector-Filterer	5				1.0000		failed Fisher's
Statewide	Invert	#Collector-Gatherer	5	1.9			0.0406	**1.9**	Fisher's
Statewide	Invert	#EPT	5				0.2821		failed Fisher's
Statewide	Invert	#Intolerant	5				1.0000		failed Fisher's
Statewide	Invert	%Tolerant	5				0.5879		failed Fisher's

Table IV - 5. Raw DO flux threshold concentration values (mg L^{-1}) from River Nutrient data using additive quantile regression smoothing analysis. Abbreviations: T.C. = threshold concentration, MP = midpoint, UBP = upper break point, Fisher's = Fisher's exact test.

Region	Group	Metric	Λ	F-test	MP T.C.	MP test	UBP T.C.	UBP test	Final T.C.	Notes
Statewide	Fish	%Sensitive								weak relationship
Statewide	Fish	%Darter								weak relationship
Statewide	Fish	%Simple Lithophils	3	<0.0001	-	-	3.5	0.1052		failed Fisher's
Statewide	Fish	%Tolerant	2	<0.0001	-	-	3.5	0.0119	**3.5**	use upper break
Statewide	Fish	%Insectivores								weak relationship
Statewide	Fish	%Piscivores								weak relationship
Statewide	Fish	Taxa Richness								weak relationship
Statewide	Fish	%Intolerant								weak relationship
Statewide	Invert	Taxa Richness								weak relationship
Statewide	Invert	#Collector-Filterer								weak relationship
Statewide	Invert	#Collector-Gatherer								weak relationship
Statewide	Invert	#EPT								weak relationship
Statewide	Invert	#Intolerant								weak relationship
Statewide	Invert	%Tolerant								weak relationship

Table IV - 6. Raw DO flux threshold concentration values (mg L^{-1}) from River Nutrient data using regression tree (changepoint) analysis. Abbreviations: T.C. = threshold concentration, L = 90% lower bound, U = 90% upper bound, Fisher's = Fisher's exact test, chi squared = chi squared test.

Region	Group	Metric	Bucket	T.C.	L	U	test	Final T.C.	Notes
Statewide	Fish	%Sensitive	5				0.2743		failed Fisher's
Statewide	Fish	%Darter	5				0.2985		failed Fisher's
Statewide	Fish	%Simple Lithophils	5	4.9	4.4	7.1	0.0391	**4.9**	Fisher's
Statewide	Fish	%Tolerant	5	3.1	1.1	3.9	0.0005	**3.1**	Fisher's
Statewide	Fish	%Insectivores	5				0.3252		failed chi squared
Statewide	Fish	%Piscivores	5				0.5968		failed Fisher's
Statewide	Fish	Taxa Richness	5				0.3449		failed Fisher's
Statewide	Fish	%Intolerant	5				0.2985		failed Fisher's
Statewide	Invert	Taxa Richness	5				0.1778		failed Fisher's
Statewide	Invert	#Collector-Filterer	5				0.1748		failed Fisher's
Statewide	Invert	#Collector-Gatherer	5	3.0	-0.4	3.6	0.0166	**3.0**	Fisher's
Statewide	Invert	#EPT	5				0.3698		failed Fisher's
Statewide	Invert	#Intolerant	5				0.3698		failed Fisher's
Statewide	Invert	%Tolerant	5				0.6027		failed Fisher's

Table IV - 7. Raw chlorophyll-a threshold concentration values (µg L^{-1}) from River Nutrient data using additive quantile regression smoothing analysis. Abbreviations: T.C. = threshold concentration, MP = midpoint, UBP = upper break point, Fisher's = Fisher's exact test.

Region	Group	Metric	Λ	F-test	MP T.C.	MP test	UBP T.C.	UBP test	Final T.C.	Notes
Statewide	Fish	%Sensitive	20	<0.0001	21	0.0155	-	-	**21**	Fisher's
Statewide	Fish	%Darter	20	<0.0001	21	0.3944	-	-		failed Fisher's
Statewide	Fish	%Simple Lithophils	20	<0.0001	37	0.0096	-	-	**37**	Fisher's
Statewide	Fish	%Tolerant	15	<0.0001	69	0.0006	50	0.0209	**50**	use upper break
Statewide	Fish	%Insectivores	15	<0.0001	65	0.0585	42	1.0000		failed Fisher's
Statewide	Fish	%Piscivores	15	<0.0001	26	0.2374	-	-		failed Fisher's
Statewide	Fish	Taxa Richness	25	<0.0001	44	0.3786	-	-		failed Fisher's
Statewide	Fish	%Intolerant	15	<0.0001	21	0.0155	-	-	**21**	Fisher's
Statewide	Invert	Taxa Richness	10	<0.0001	26	0.0112	-	-	**26**	Fisher's
Statewide	Invert	#Collector-Filterer								weak relationship
Statewide	Invert	#Collector-Gatherer	10	<0.0001	21	0.3061	-	-		failed Fisher's
Statewide	Invert	#EPT								weak relationship
Statewide	Invert	#Intolerant								weak relationship
Statewide	Invert	%Tolerant								weak relationship

Table IV - 8. Raw chlorophyll-a threshold concentration values (µg L^{-1}) from River Nutrient data using regression tree (changepoint) analysis. Abbreviations: T.C. = threshold concentration, L = 90% lower bound, U = 90% upper bound, Fisher's = Fisher's exact test.

Region	Group	Metric	Bucket	T.C.	L	U	test	Final T.C.	Notes
Statewide	Fish	%Sensitive	5	11	-13	29	0.0069	**11**	Fisher's
Statewide	Fish	%Darter	5				0.0927		failed Fisher's
Statewide	Fish	%Simple Lithophils	5	34	19	63	0.0022	**34**	Fisher's
Statewide	Fish	%Tolerant	5	62	44	110	0.0039	**62**	Fisher's
Statewide	Fish	%Insectivores	5				0.3295		failed Fisher's
Statewide	Fish	%Piscivores	5				0.1086		failed Fisher's
Statewide	Fish	Taxa Richness	5				0.1186		failed Fisher's
Statewide	Fish	%Intolerant	5	11	-11	27	0.0140	**11**	Fisher's
Statewide	Invert	Taxa Richness	5	34	21	55	0.0024	**34**	Fisher's
Statewide	Invert	#Collector-Filterer	5				0.1627		failed Fisher's
Statewide	Invert	#Collector-Gatherer	5	31	-15	78	0.0026	**31**	Fisher's
Statewide	Invert	#EPT	5				0.6447		failed Fisher's
Statewide	Invert	#Intolerant	5				0.2341		failed Fisher's
Statewide	Invert	%Tolerant	5						metric response does not match prediction

Table IV - 9. Raw total phosphorus threshold concentration values (µg L^{-1}) from River Nutrient data using additive quantile regression smoothing analysis. Abbreviations: T.C. = threshold concentration, MP = midpoint, UBP = upper break point, Fisher's = Fisher's exact test.

Region	Group	Metric	Λ	F-test	MP T.C.	MP test	UBP T.C.	UBP test	Final T.C.	Notes
Statewide	Fish	%Sensitive	100	<0.0001	152	0.0464	-	-	**152**	Fisher's
Statewide	Fish	%Darter	100	<0.0001	94	0.0157	-	-	**94**	Fisher's
Statewide	Fish	%Simple Lithophils	150	<0.0001	121	0.0214	-	-	**121**	Fisher's
Statewide	Fish	%Tolerant		<0.0001		-	192	0.0454	**192**	
Statewide	Fish	%Insectivores								weak relationship
Statewide	Fish	%Piscivores	15	<0.0001	112	0.0562	-	-		failed Fisher's
Statewide	Fish	Taxa Richness	50	<0.0001	121	0.6526	-	-		failed Fisher's
Statewide	Fish	%Intolerant	150	<0.0001	106	0.0118	-	-	**106**	Fisher's
Statewide	Invert	Taxa Richness	25	<0.0001	154	0.0007	116	0.0862	**154**	Fisher's
Statewide	Invert	#Collector-Filterer								weak relationship
Statewide	Invert	#Collector-Gatherer	50	<0.0001	233	0.0312	116	0.5485	**233**	failed Fisher's
Statewide	Invert	#EPT								weak relationship
Statewide	Invert	#Intolerant								weak relationship
Statewide	Invert	%Tolerant								weak relationship

Table IV - 10. Raw total phosphorus threshold concentration values (µg L^{-1}) from River Nutrient data using regression tree (changepoint) analysis. Abbreviations: T.C. = threshold concentration, L = 90% lower bound, U = 90% upper bound, Fisher's = Fisher's exact test.

Region	Group	Metric	Bucket	T.C.	L	U	test	Final T.C.	Notes
Statewide	Fish	%Sensitive	5	42	-93	78	0.0274	**42**	Fisher's
Statewide	Fish	%Darter	5	103	70	161	0.0118	**103**	Fisher's
Statewide	Fish	%Simple Lithophils	5	136	96	218	0.0020	**136**	
Statewide	Fish	%Tolerant	5	199	155	273	0.0020	**199**	Fisher's
Statewide	Fish	%Insectivores	5				0.1484		failed Fisher's
Statewide	Fish	%Piscivores	5	81	-1	130	0.0377	**81**	Fisher's
Statewide	Fish	Taxa Richness	5				0.4120		failed Fisher's
Statewide	Fish	%Intolerant	5	81	3	133	0.0007	**81**	
Statewide	Invert	Taxa Richness	5	153	140	166	0.0003	**153**	
Statewide	Invert	#Collector-Filterer	5				0.1206		failed Fisher's
Statewide	Invert	#Collector-Gatherer	5	182	145	258	0.0010	**182**	Fisher's
Statewide	Invert	#EPT	5				0.0730		failed Fisher's
Statewide	Invert	#Intolerant	5				0.6785		failed Fisher's
Statewide	Invert	%Tolerant	5				0.6146		failed Fisher's

Table IV - 11. Total phosphorus threshold concentration values (µg L^{-1}) determined with additive quantile regression smoothing analysis for all stream sizes using biomonitoring data. Abbreviations: T.C. = threshold concentration, MP = midpoint, UBP = upper break point, Fisher's = Fisher's exact test, chi squared = chi squared test.

Region	Group	Metric	Λ	F-test	MP T.C.	MP test	UBP T.C.	UBP test	Final T.C.	Notes
North	Fish	%Sensitive	300	<0.0001	43	<0.001	-	-	**43**	
North	Fish	%Darter	25	<0.0001	100	<0.001	36	0.0450	**36**	use upper break point
North	Fish	%Simple Lithophils								weak relationship
North	Fish	%Tolerant	75	<0.0001	46	<0.001	-	-	**46**	
North	Fish	%Insectivores	175	<0.0001	75	0.0010	-	-	**75**	
North	Fish	%Piscivores	300	<0.0001	121	<0.001	-	-	**121**	
North	Fish	Taxa Richness	500	<0.0001	154	0.0030	-	-	**154**	
North	Fish	%Intolerant	35	<0.0001	48	<0.001	-	-	**48**	
North	Invert	Taxa Richness	50	<0.0001	126	0.0299	56	0.1451	**126**	
North	Invert	#Collector-Filterer	200	<0.0001	87	0.0034	-	-	**87**	
North	Invert	#Collector-Gatherer	75	<0.0001	112	0.0313	58	0.8926	**112**	
North	Invert	#EPT	600	<0.0001	58	0.0018	-	-	**58**	
North	Invert	#Intolerant	25	<0.0001	141	0.0325	87	0.0203	**87**	use upper break point
North	Invert	%Tolerant								weak relationship
Central	Fish	%Sensitive	100	<0.0001	142	<0.001	81	<0.001	**81**	use upper break point
Central	Fish	%Darter	100	<0.0001	158	<0.001	-	-	**158**	
Central	Fish	%Simple Lithophils	100	<0.0001	160	<0.001	118	0.0028	**118**	use upper break point
Central	Fish	%Tolerant	1000	<0.0001	188	<0.001	-	-	**188**	
Central	Fish	%Insectivores								weak relationship
Central	Fish	%Piscivores	500	<0.0001	159	0.3450	-			failed chi squared
Central	Fish	Taxa Richness	300	<0.0001	209	<0.001	-	-	**209**	
Central	Fish	%Intolerant	300	<0.0001	105	<0.001	-	-	**105**	
Central	Invert	Taxa Richness	250	<0.0001	206	0.0126	107	0.0354	**107**	use upper break point
Central	Invert	#Collector-Filterer	100	<0.0001	170	0.0102	128	0.0245	**128**	use upper break point
Central	Invert	#Collector-Gatherer	500	<0.0001	118	0.0038	-	-	**118**	
Central	Invert	#EPT	110	<0.0001	111	0.0175	-	-	**111**	
Central	Invert	#Intolerant	70	<0.0001	152	0.0003	92	0.0020	**92**	use upper break point
Central	Invert	%Tolerant	350	<0.0001	323	0.0902	127	0.3100		failed Fisher's
South	Fish	%Sensitive	600	<0.0001	95	0.0020	-	-	**95**	
South	Fish	%Darter	1500	<0.0001	227	0.1460	-	-		failed chi squared
South	Fish	%Simple Lithophils	100	<0.0001	106	<0.001	-	-	**106**	
South	Fish	%Tolerant	500	<0.0001	383	0.0100	246	0.678	**383**	
South	Fish	%Insectivores								weak relationship
South	Fish	%Piscivores	300	<0.0001	384	0.0760	277	0.752		failed chi squared
South	Fish	Taxa Richness	500	<0.0001	373	0.0020	277	0.145	**373**	
South	Fish	%Intolerant								weak relationship
South	Invert	Taxa Richness	1500	<0.0001	234	0.0465	-	-	**234**	
South	Invert	#Collector-Filterer	625	<0.0001	371	0.3830	-	-		failed chi squared
South	Invert	#Collector-Gatherer	1100	<0.0001	234	0.0465	-	-	**234**	
South	Invert	#EPT								weak relationship
South	Invert	#Intolerant								weak relationship
South	Invert	%Tolerant								weak relationship

Table IV - 12. Total phosphorus threshold concentration values (µg L^{-1}) from regression tree (changepoint) analysis for all stream sizes using biomonitoring data. Abbreviations: T.C. = threshold concentration, L = 90% lower bound, U = 90% upper bound, chi squared = chi squared test.

Region	Group	Metric	Bucket	T.C.	L	U	Test	Final T.C.	Notes
North	Fish	%Sensitive	43	33	5	47	<0.0001	**33**	
North	Fish	%Darter	43	57	-3	82	0.0003	**57**	
North	Fish	%Simple Lithophils	43	39	-	-	0.0162	**39**	
North	Fish	%Tolerant	43	34	11	40	<0.0001	**34**	
North	Fish	%Insectivores	43	53	32	70	<0.0001	**53**	
North	Fish	%Piscivores	43	33	16	46	<0.0001	**33**	
North	Fish	Taxa Richness	43	42	-8	56	0.0025	**42**	
North	Fish	%Intolerant	43	66	60	114	<0.0001	**66**	
North	Invert	Taxa Richness	33	98	78	127	<0.0001	**98**	
North	Invert	#Collector-Filterer	33	74	58	113	<0.0001	**74**	
North	Invert	#Collector-Gatherer	33	102	82	120	<0.0001	**102**	
North	Invert	#EPT	33	91	77	132	0.0010	**91**	
North	Invert	#Intolerant	33	91	66	146	0.0284	**91**	
North	Invert	%Tolerant	33	71	34	100	0.0203	**71**	
Central	Fish	%Sensitive	37	124	89	169	<0.0001	**124**	
Central	Fish	%Darter	37	201	147	245	<0.0001	**201**	
Central	Fish	%Simple Lithophils	37	160	133	202	<0.0001	**160**	
Central	Fish	%Tolerant	37	174	147	260	<0.0001	**174**	
Central	Fish	%Insectivores	37				0.1090		failed chi squared
Central	Fish	%Piscivores	37	85	-114	215	0.0162	**85**	
Central	Fish	Taxa Richness	37	187	138	280	0.0001	**187**	
Central	Fish	%Intolerant	37	86	47	102	<0.0001	**86**	
Central	Invert	Taxa Richness	28	149	74	189	0.0001	**149**	
Central	Invert	#Collector-Filterer	28	142	95	167	<0.0001	**142**	
Central	Invert	#Collector-Gatherer	28	149	92	197	<0.0001	**149**	
Central	Invert	#EPT	28	148	96	177	0.0001	**148**	
Central	Invert	#Intolerant	28	142	106	164	<0.0001	**142**	
Central	Invert	%Tolerant	28	204	174	281	0.0071	**204**	
South	Fish	%Sensitive	28	66	1	113	0.0008	**66**	
South	Fish	%Darter	28	86	9	145	0.0002	**86**	
South	Fish	%Simple Lithophils	28	146	110	188	<0.0001	**146**	
South	Fish	%Tolerant	28	310	209	385	0.0069	**310**	
South	Fish	%Insectivores	28				0.0010		metric response does not match prediction
South	Fish	%Piscivores	28				0.1884		failed chi squared
South	Fish	Taxa Richness	28	395	339	499	<0.0001	**395**	
South	Fish	%Intolerant	28				0.2977		failed chi squared
South	Invert	Taxa Richness	19	337	250	597	0.0018	**337**	
South	Invert	#Collector-Filterer	19	145	-116	266	0.0213	**145**	
South	Invert	#Collector-Gatherer	19	329	266	537	0.0005	**329**	
South	Invert	#EPT	19	329	183	519	0.0122	**329**	
South	Invert	#Intolerant	19	411	321	669	0.0307	**411**	
South	Invert	%Tolerant	19	411	306	677	0.0348	**411**	

Table IV - 13. Total phosphorus threshold concentration values (µg L^{-1}) determined with additive quantile regression smoothing analysis for nonwadeable streams (>500 mi^2) using biomonitoring data. Abbreviations: T.C. = threshold concentration, MP = midpoint, UBP = upper break point, Fisher's = Fisher's exact test, chi squared = chi squared test.

Region	Group	Metric	Λ	F-test	MP T.C.	MP test	UBP T.C.	UBP test	Final T.C.	Notes
North	Fish	%Sensitive								weak relationship
North	Fish	%Darter								weak relationship
North	Fish	%Simple Lithophils								weak relationship
North	Fish	%Tolerant								weak relationship
North	Fish	%Insectivores								weak relationship
North	Fish	%Piscivores	50	<0.0001	32	0.0818	-	-		failed chi squared
North	Fish	Taxa Richness	25	<0.0001	-	-	79	0.1636		failed Fisher's
North	Fish	%Intolerant								weak relationship
North	Invert	Taxa Richness	20	0.0057	-	-	50	0.2293		failed Fisher's
North	Invert	#Collector-Filterer	20	0.1440	-		-			failed F-test
North	Invert	#Collector-Gatherer	20	0.1570	-		-			failed F-test
North	Invert	#EPT	10	0.3270	-		-			failed F-test
North	Invert	#Intolerant	10	0.0004	-		-			metric response does not match prediction
North	Invert	%Tolerant	30	<0.0001	-		-			metric response does not match prediction
Central	Fish	%Sensitive	50	<0.0001	116	0.0435	-	-	**116**	
Central	Fish	%Darter	50	<0.0001	69	0.0650	-	-		failed Fisher's
Central	Fish	%Simple Lithophils	60	<0.0001	-	-	123	0.0408	**123**	Fisher's; use upper break point
Central	Fish	%Tolerant	35	<0.0001	145	<0.0001	110	0.0079	**110**	Fisher's; use upper break point
Central	Fish	%Insectivores								weak relationship
Central	Fish	%Piscivores	20	<0.0001	99	0.0149	82	0.0764	**99**	Fisher's
Central	Fish	Taxa Richness								weak relationship
Central	Fish	%Intolerant	40	<0.0001	131	0.0206	82	1.0000	**131**	
Central	Invert	Taxa Richness	20	<0.0001	123	0.0016	86	0.1041	**123**	Fisher's
Central	Invert	#Collector-Filterer								weak relationship
Central	Invert	#Collector-Gatherer	40	<0.0001	84	0.0491	-	-	**84**	Fisher's
Central	Invert	#EPT	50	<0.0001	144	0.0237	97	0.0527	**144**	Fisher's
Central	Invert	#Intolerant	35	<0.0001	164	0.2100	127	0.2093		failed Fisher's
Central	Invert	%Tolerant								metric response does not match prediction
South	Fish	%Sensitive								weak relationship
South	Fish	%Darter								weak relationship
South	Fish	%Simple Lithophils	150	<0.0001	102	0.0753	-	-		failed Fisher's
South	Fish	%Tolerant	125	<0.0001	-	-	286	1.000		failed Fisher's
South	Fish	%Insectivores	50	<0.0001	131	0.0364	-	-	**131**	Fisher's
South	Fish	%Piscivores								weak relationship
South	Fish	Taxa Richness								weak relationship
South	Fish	%Intolerant								weak relationship
South	Invert	Taxa Richness								weak relationship
South	Invert	#Collector-Filterer								weak relationship
South	Invert	#Collector-Gatherer								weak relationship
South	Invert	#EPT								weak relationship
South	Invert	#Intolerant								weak relationship
South	Invert	%Tolerant								weak relationship

Table IV - 14. Total phosphorus threshold concentration values (µg L^{-1}) from regression tree (changepoint) analysis for nonwadeable streams (>500 mi^2) using biomonitoring data. Abbreviations: T.C. = threshold concentration, L = 90% lower bound, U = 90% upper bound, Fisher's = Fisher's exact test, chi squared = chi squared test.

Region	Group	Metric	Bucket	T.C.	L	U	Test	Final T.C.	Notes
North	Fish	%Sensitive	8	27	-7	44	0.0351	**27**	
North	Fish	%Darter	8				0.1811		failed chi squared
North	Fish	%Simple Lithophils	8				0.5861		failed chi squared
North	Fish	%Tolerant	8				0.5219		failed Fisher's
North	Fish	%Insectivores	8						metric response does not match prediction
North	Fish	%Piscivores	8	29	-7	47	0.0289	**29**	
North	Fish	Taxa Richness	8				0.4413		failed chi squared
North	Fish	%Intolerant	8				0.3157		failed chi squared
North	Invert	Taxa Richness	5	29	8	42	0.0057	**29**	
North	Invert	#Collector-Filterer	5				0.3591		failed chi squared
North	Invert	#Collector-Gatherer	5				0.1727		failed chi squared
North	Invert	#EPT	5				0.4811		failed chi squared
North	Invert	#Intolerant	5				0.0634		failed chi squared
North	Invert	%Tolerant	5				0.1793		failed chi squared
Central	Fish	%Sensitive	5	86	22	118	0.0003	**86**	
Central	Fish	%Darter	5				0.1603		failed chi squared
Central	Fish	%Simple Lithophils	5	75	42	99	<0.0001	**75**	
Central	Fish	%Tolerant	5	86	-5	121	<0.0001	**86**	
Central	Fish	%Insectivores	5				0.4203		failed Fisher's
Central	Fish	%Piscivores	5				0.1126		failed chi squared
Central	Fish	Taxa Richness	5				0.9312		failed chi squared
Central	Fish	%Intolerant	5	86	13	112	0.0007	**86**	
Central	Invert	Taxa Richness	5	102	67	128	0.0003	**102**	
Central	Invert	#Collector-Filterer	5				0.7977		failed chi squared
Central	Invert	#Collector-Gatherer	5	102	82	127	0.0005	**102**	
Central	Invert	#EPT	5				0.0805		failed chi squared
Central	Invert	#Intolerant	5				0.1365		failed chi squared
Central	Invert	%Tolerant	5				0.4461		failed Fisher's
South	Fish	%Sensitive	5				0.9849		failed chi squared
South	Fish	%Darter	5				0.4055		failed Fisher's
South	Fish	%Simple Lithophils	5				0.0773		failed Fisher's
South	Fish	%Tolerant	5				0.0862		failed Fisher's
South	Fish	%Insectivores	5	199	165	265	0.0057	**199**	
South	Fish	%Piscivores	5				0.0100		metric response does not match prediction
South	Fish	Taxa Richness	5				0.3868		failed Fisher's
South	Fish	%Intolerant	5				0.0081		metric response does not match prediction
South	Invert	Taxa Richness	5				0.3889		failed chi squared
South	Invert	#Collector-Filterer	5				1.0000		failed Fisher's
South	Invert	#Collector-Gatherer	5				0.0287		metric response does not match prediction
South	Invert	#EPT	5				0.1378		failed Fisher's
South	Invert	#Intolerant	5				0.6247		failed Fisher's
South	Invert	%Tolerant	5				0.2770		failed chi squared

Table IV - 15. Total phosphorus threshold concentration values (µg L^{-1}) determined with additive quantile regression smoothing analysis for wadeable streams (<500 mi^2) using biomonitoring data. Abbreviations: T.C. = threshold concentration, MP = midpoint, UBP = upper break point, Fisher's = Fisher's exact test, chi squared = chi squared test.

Region	Group	Metric	Λ	F-test	MP T.C.	MP test	UBP T.C.	UBP test	Final T.C.	Notes
North	Fish	%Sensitive	300	<0.0001	43	0.0008	-	-	**43**	
North	Fish	%Darter	50	<0.0001	100	0.0004	36	0.2468	**100**	
North	Fish	%Simple Lithophils								weak breakpoints
North	Fish	%Tolerant	75	<0.0001	49	<0.0001	-	-	**49**	
North	Fish	%Insectivores	75	<0.0001	75	0.0256	-	-	**75**	
North	Fish	%Piscivores	25	<0.0001	52	0.0003	-	-	**52**	
North	Fish	Taxa Richness	25	<0.0001	221	0.0662	145	0.1647		failed Fisher's
North	Fish	%Intolerant	50	<0.0001	48	<0.0001	-	-	**48**	
North	Invert	Taxa Richness	75	<0.0001	126	0.0237	56	0.0831	**126**	
North	Invert	#Collector-Filterer	300	<0.0001	87	0.0079	-	-	**87**	
North	Invert	#Collector-Gatherer	100	<0.0001	111	0.0963	58	0.8947		failed chi squared
North	Invert	#EPT	200	<0.0001	57	0.0077	-	-	**57**	
North	Invert	#Intolerant	25	<0.0001	107	0.0678	-	-		failed chi squared
North	Invert	%Tolerant								weak breakpoints
Central	Fish	%Sensitive	50	<0.0001	141	0.0002	81	0.02170	**81**	use upper break point
Central	Fish	%Darter	100	<0.0001	202	0.0004	109	0.12830	**202**	
Central	Fish	%Simple Lithophils	100	<0.0001	176	0.0002	118	0.00100	**118**	use upper break point
Central	Fish	%Tolerant	1000	<0.0001	154	0.0002	-	-	**154**	
Central	Fish	%Insectivores								weak relationship
Central	Fish	%Piscivores								weak relationship
Central	Fish	Taxa Richness	100	<0.0001	188	0.0012	-	-	**188**	
Central	Fish	%Intolerant	500	<0.0001	111	0.0007	81	0.00560	**81**	use upper break point
Central	Invert	Taxa Richness	50	<0.0001	161	0.0926	121	0.1107		failed chi squared
Central	Invert	#Collector-Filterer	200	<0.0001	290	0.0043	127	0.0064	**127**	use upper break point
Central	Invert	#Collector-Gatherer	100	<0.0001	103	0.0287	-	-	**103**	
Central	Invert	#EPT	60	<0.0001	163	0.0089	92	0.0495	**92**	use upper break point
Central	Invert	#Intolerant	100	<0.0001	162	0.0002	89	0.0409	**89**	use upper break point
Central	Invert	%Tolerant	400	<0.0001	290	0.0261	127	0.1397	**290**	
South	Fish	%Sensitive	100	<0.0001	50	<0.0001	-	-	**50**	Fisher's
South	Fish	%Darter	100	<0.0001	76	0.0464	-	-	**76**	
South	Fish	%Simple Lithophils	100	<0.0001	105	0.0018	-	-	**105**	
South	Fish	%Tolerant	150	<0.0001	252	0.0614	-	-		failed chi squared
South	Fish	%Insectivores								weak relationship
South	Fish	%Piscivores	100	<0.0001	329	0.2596	140	0.9167		failed chi squared
South	Fish	Taxa Richness	300	<0.0001	339	<0.0001	204	0.3905	**339**	
South	Fish	%Intolerant								weak relationship
South	Invert	Taxa Richness	300	<0.0001	277	0.0186	-	-	**277**	
South	Invert	#Collector-Filterer	650	<0.0001	369	0.2552	-	-		failed Fisher's
South	Invert	#Collector-Gatherer	300	<0.0001	277	0.0035	-	-	**277**	
South	Invert	#EPT	300	<0.0001	354	0.0504	226	0.5397		failed Fisher's
South	Invert	#Intolerant	300	<0.0001	337	0.0155	199	0.0071	**199**	use upper break point
South	Invert	%Tolerant								weak relationship

Table IV - 16. Total phosphorus threshold concentration values (µg L^{-1}) from regression tree (changepoint) analysis for wadeable streams (<500 mi^2) using biomonitoring data. Abbreviations: T.C. = Threshold Concentration, T.C. = threshold concentration, L = 90% lower bound, U = 90% upper bound, Fisher's = Fisher's exact test, chi squared = chi squared test.

Region	Group	Metric	Bucket	T.C.	L	U	Test	Final T.C.	Notes
North	Fish	%Sensitive	35	34	13	45	<0.0001	**34**	
North	Fish	%Darter	35	57	4	81	0.0022	**57**	
North	Fish	%Simple Lithophils	35				0.3943		failed chi squared
North	Fish	%Tolerant	35	34	9	44	<0.0001	**34**	
North	Fish	%Insectivores	35	53	30	74	<0.0001	**53**	
North	Fish	%Piscivores	35	33	-2	47	<0.0001	**33**	
North	Fish	Taxa Richness	35	84	57	131	0.0031	**84**	
North	Fish	%Intolerant	35	34	-4	45	<0.0001	**34**	
North	Invert	Taxa Richness	28	98	66	134	<0.0001	**98**	
North	Invert	#Collector-Filterer	28	74	66	110	<0.0001	**74**	
North	Invert	#Collector-Gatherer	28	102	72	123	<0.0001	**102**	
North	Invert	#EPT	28	73	45	105	0.0001	**73**	
North	Invert	#Intolerant	28	75	31	117	0.0143	**75**	
North	Invert	%Tolerant	28	71	25	107	0.0259	**71**	
Central	Fish	%Sensitive	32	122	72	156	<0.0001	**122**	
Central	Fish	%Darter	32	201	154	257	<0.0001	**201**	
Central	Fish	%Simple Lithophils	32	174	143	219	<0.0001	**174**	
Central	Fish	%Tolerant	32	169	135	232	0.0002	**169**	
Central	Fish	%Insectivores	32				0.1381		failed chi squared
Central	Fish	%Piscivores	32				0.2927		failed chi squared
Central	Fish	Taxa Richness	32	159	100	205	0.0001	**159**	
Central	Fish	%Intolerant	32	93	52	110	0.0008	**93**	
Central	Invert	Taxa Richness	25	149	47	201	0.0041	**149**	
Central	Invert	#Collector-Filterer	25	113	33	181	0.0349	**113**	
Central	Invert	#Collector-Gatherer	25	149	71	205	<0.0001	**149**	
Central	Invert	#EPT	25	148	92	175	<0.0001	**148**	
Central	Invert	#Intolerant	25	142	98	165	<0.0001	**142**	
Central	Invert	%Tolerant	25	152	77	183	<0.0001	**152**	
South	Fish	%Sensitive	23	66	-2	115	0.0008	**66**	
South	Fish	%Darter	23	86	-86	216	0.0010	**86**	
South	Fish	%Simple Lithophils	23	145	101	186	<0.0001	**145**	
South	Fish	%Tolerant	23	287	175	451	0.0024	**287**	
South	Fish	%Insectivores							metric response does not match prediction
South	Fish	%Piscivores							metric response does not match prediction
South	Fish	Taxa Richness	23	287	205	323	<0.0001	**287**	
South	Fish	%Intolerant							metric response does not match prediction
South	Invert	Taxa Richness	16	411	351	717	0.0001	**411**	
South	Invert	#Collector-Filterer	16	156	-69	272	0.0064	**156**	
South	Invert	#Collector-Gatherer	16	269	141	399	0.0002	**269**	
South	Invert	#EPT	16	329	235	443	0.0016	**329**	
South	Invert	#Intolerant	16	350	245	481	0.0088	**350**	
South	Invert	%Tolerant	16	350	248	489	0.0041	**350**	

Table IV - 17. Raw total nitrogen threshold concentration values (mg L^{-1}) from River Nutrient data using additive quantile regression smoothing analysis. Abbreviations: T.C. = threshold concentration, MP = midpoint, UBP = upper break point, Fisher's = Fisher's exact test.

Region	Group	Metric	Λ	F-test	MP T.C.	MP test	UBP T.C.	UBP test	Final T.C.	Notes
Statewide	Fish	%Sensitive	0.50	<0.0001	1.9	0.3589	-	-		failed Fisher's
Statewide	Fish	%Darter								weak relationship
Statewide	Fish	%Simple Lithophils	5.5	<0.0001	-	-	3.6	0.3791		failed Fisher's
Statewide	Fish	%Tolerant								weak relationship
Statewide	Fish	%Insectivores								weak relationship
Statewide	Fish	%Piscivores								weak relationship
Statewide	Fish	Taxa Richness								weak relationship
Statewide	Fish	%Intolerant								weak relationship
Statewide	Invert	Taxa Richness	0.5	<0.0001	2.0	0.1904	-	-		failed Fisher's
Statewide	Invert	#Collector-Filterer	1.0	<0.0001	5.5	0.1399	3.3	1.0000		failed Fisher's
Statewide	Invert	#Collector-Gatherer								weak relationship
Statewide	Invert	#EPT								weak relationship
Statewide	Invert	#Intolerant								weak relationship
Statewide	Invert	%Tolerant								weak relationship

Table IV - 18. Raw total nitrogen threshold concentration values (mg L^{-1}) from River Nutrient data using regression tree (changepoint) analysis. Abbreviations: T.C. = threshold concentration, L = 90% lower bound, U = 90% upper bound, Fisher's = Fisher's exact test.

Region	Group	Metric	Bucket	T.C.	L	U	test	Final T.C.	Notes
Statewide	Fish	%Sensitive	5				0.2955		failed Fisher's
Statewide	Fish	%Darter	5				0.2534		failed Fisher's
Statewide	Fish	%Simple Lithophils	5				0.2200		failed Fisher's
Statewide	Fish	%Tolerant	5				1.0000		failed Fisher's
Statewide	Fish	%Insectivores	5				0.0680		failed Fisher's
Statewide	Fish	%Piscivores	5				0.4331		failed Fisher's
Statewide	Fish	Taxa Richness	5				0.1337		failed Fisher's
Statewide	Fish	%Intolerant	5				0.2955		failed Fisher's
Statewide	Invert	Taxa Richness	5	1.4	-1.3	2.9	0.0009	**1.4**	Fisher's
Statewide	Invert	#Collector-Filterer	5	3.6	1.1	6.4	0.0405	**3.6**	Fisher's
Statewide	Invert	#Collector-Gatherer	5				0.1221		failed Fisher's
Statewide	Invert	#EPT	5				0.6882		failed Fisher's
Statewide	Invert	#Intolerant	5				0.6206		failed Fisher's
Statewide	Invert	%Tolerant	5				0.2500		failed Fisher's

Quantile Regression and Changepoint Analysis Figures

(See Methods Section for a description of the datasets used and the methods used to generate these figures)

Page Numbers for Biology/Water Quality Analyses

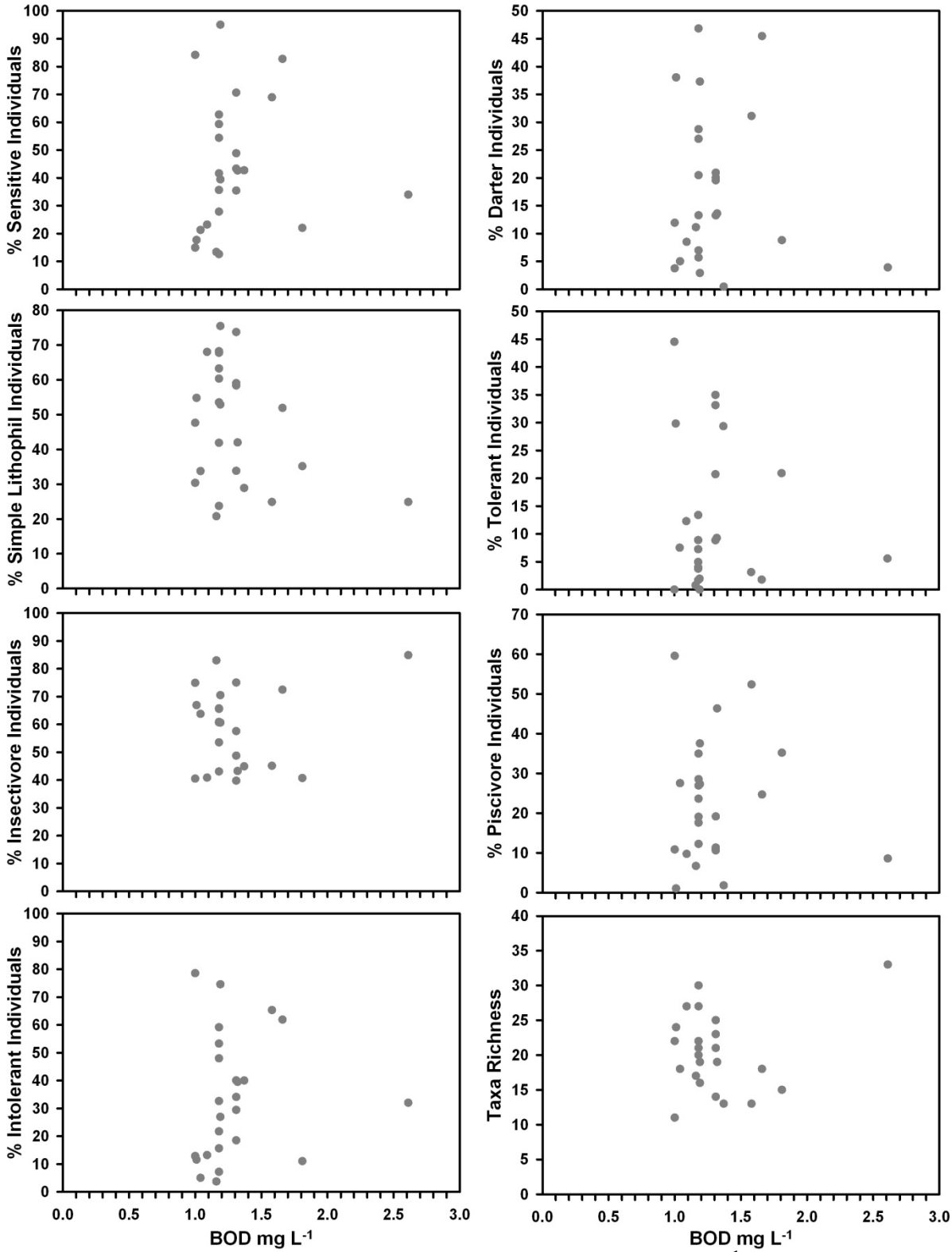


Figure IV - 1. Relationships between Biochemical Oxygen Demand (BOD) mg L^{-1} and fish metrics for all streams in the North Region using STORET Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

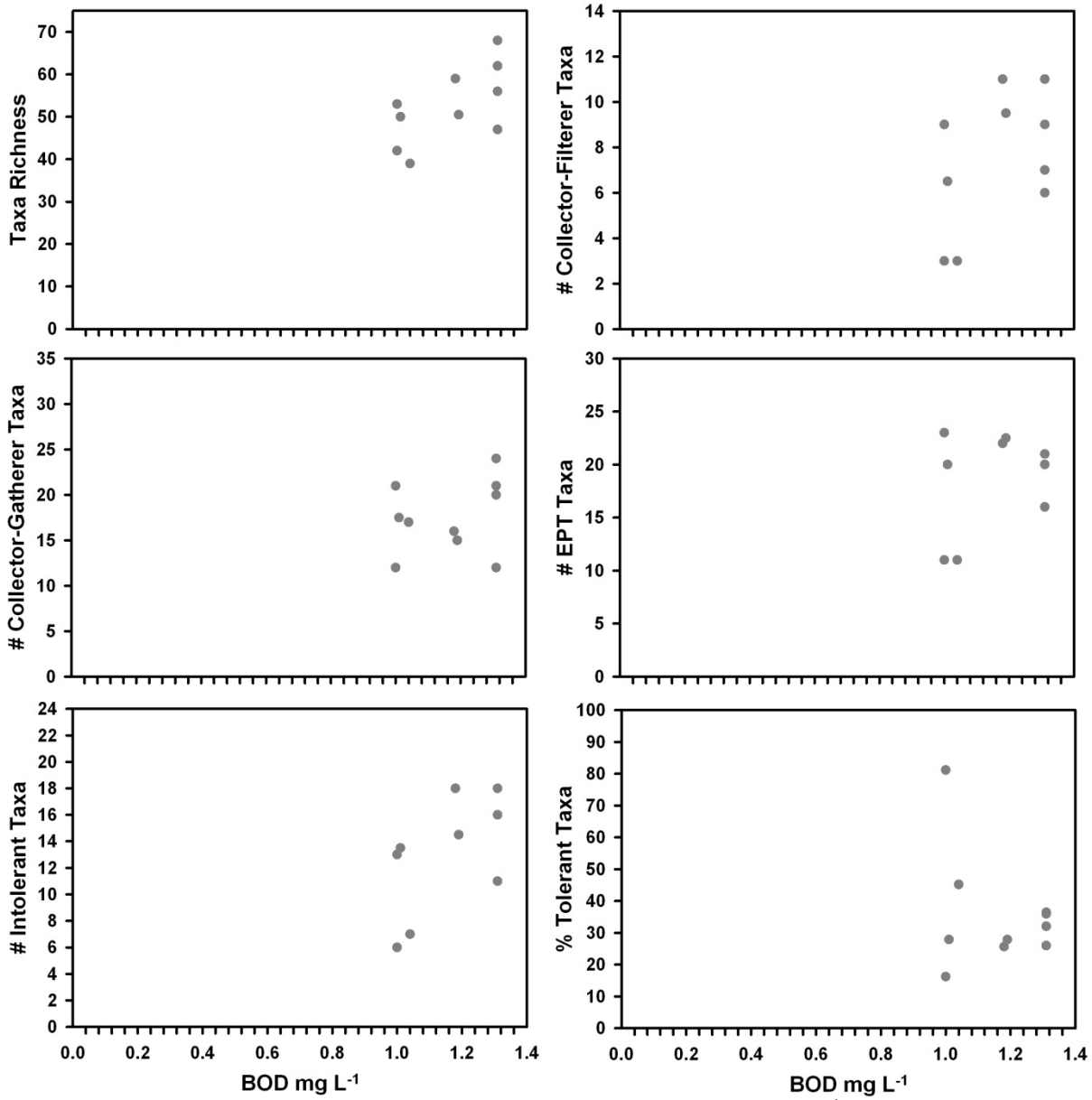


Figure IV - 2. Relationships between Biochemical Oxygen Demand (BOD) mg L^{-1} and macroinvertebrate metrics for all streams in the North Region using STORET Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

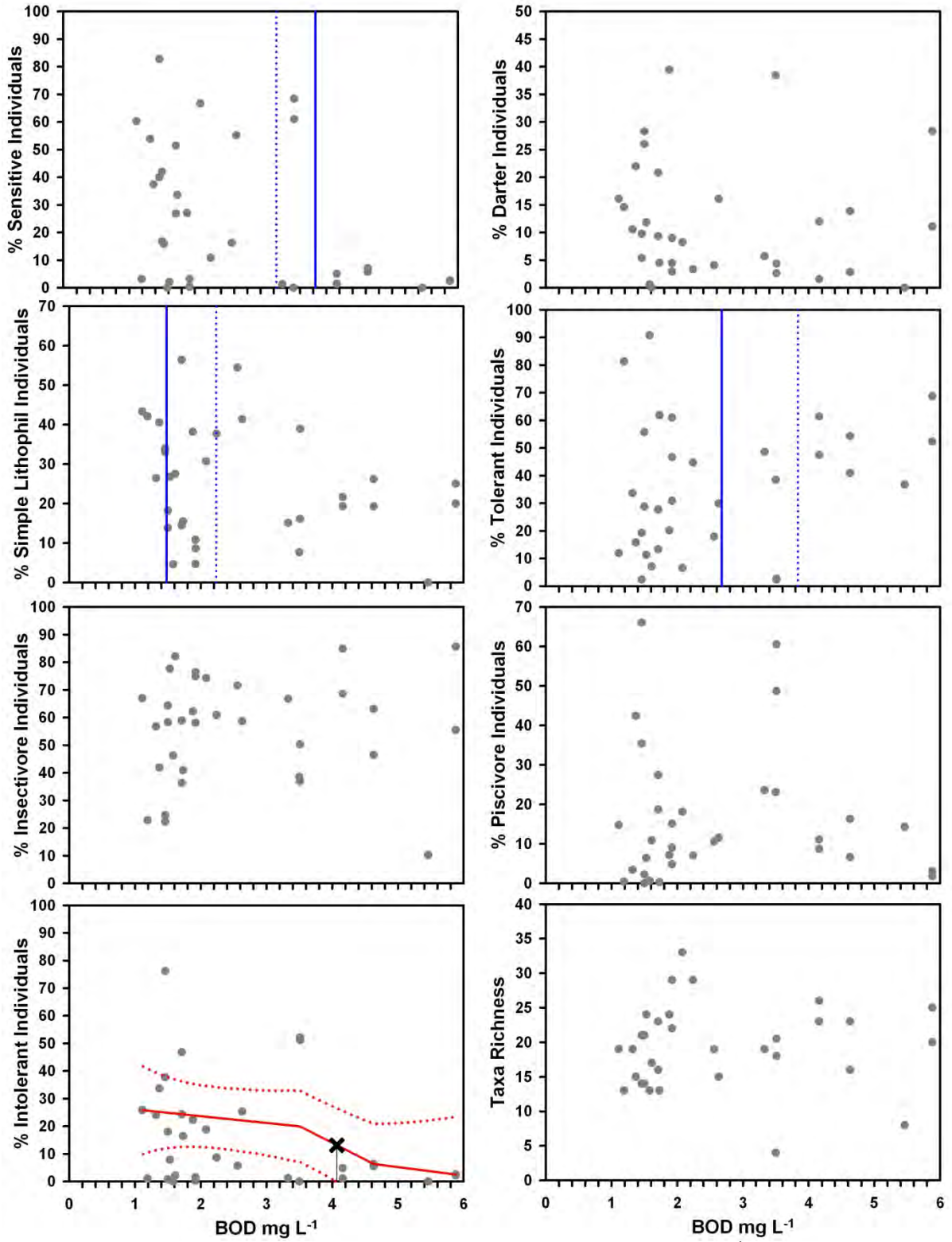


Figure IV - 3. Relationships between Biochemical Oxygen Demand (BOD) mg L^{-1} and fish metrics for all streams in the Central Region using STORET Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

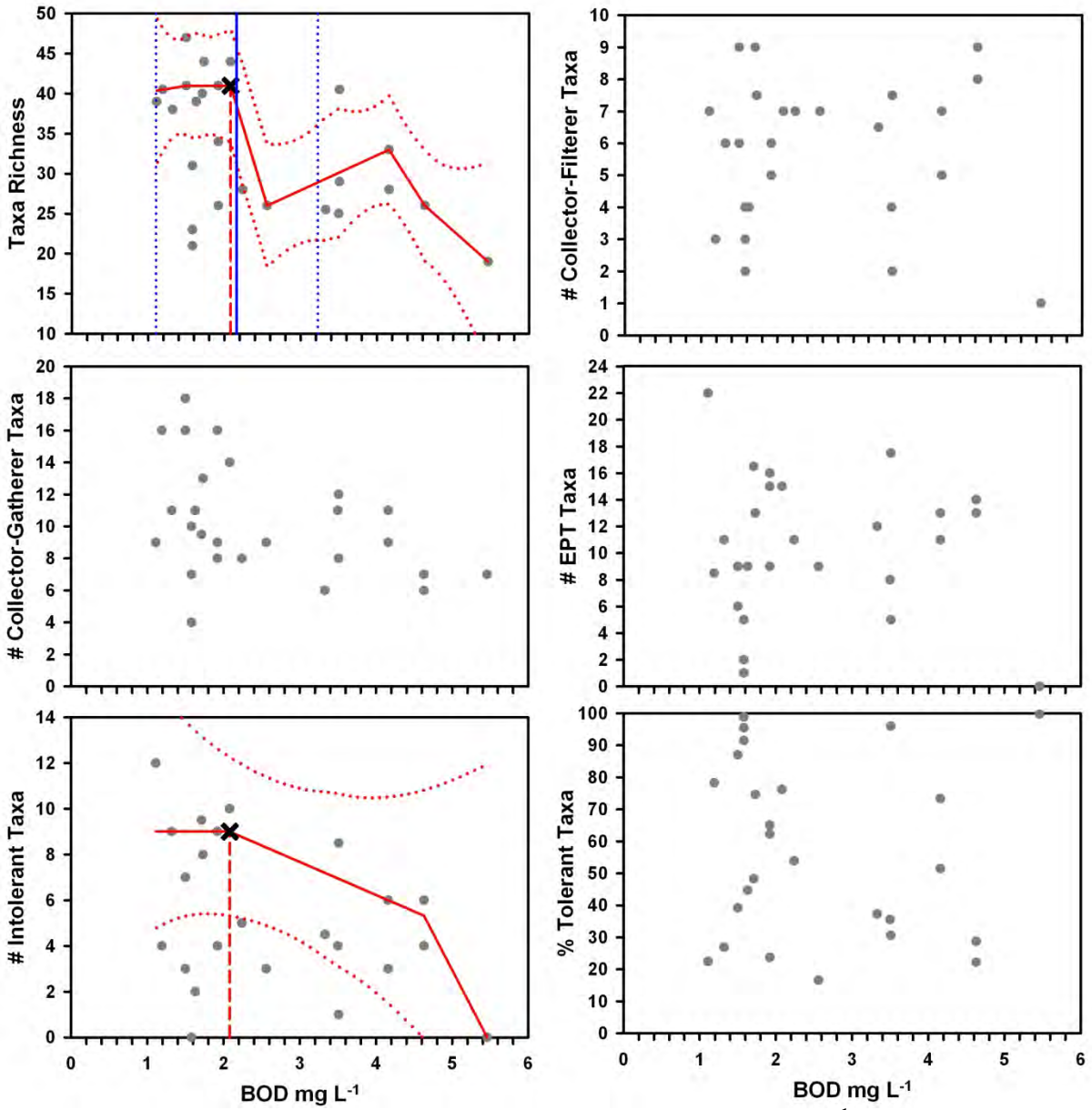


Figure IV - 4. Relationships between Biochemical Oxygen Demand (BOD) mg L^{-1} and macroinvertebrate metrics for all streams in the Central Region using STORET Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

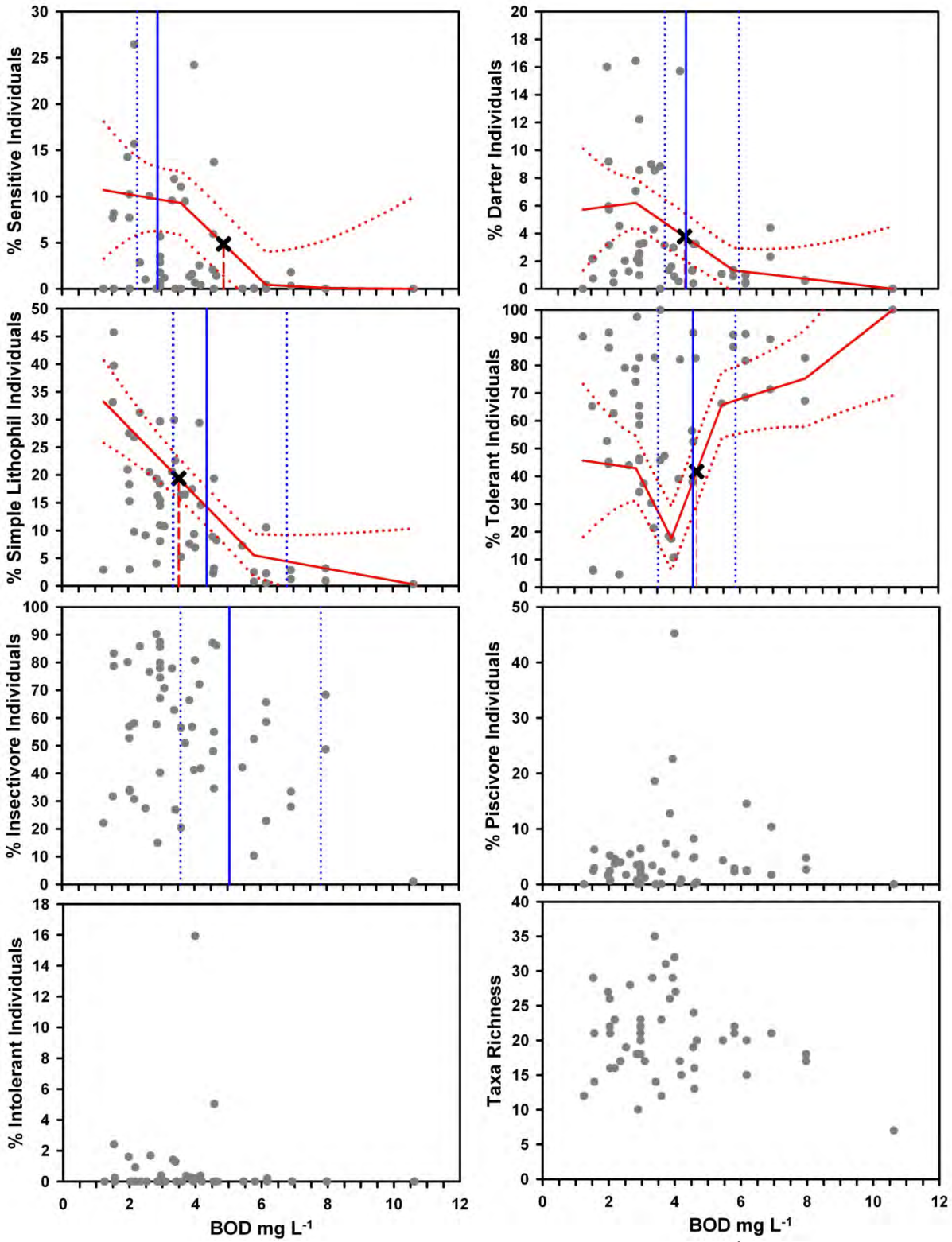


Figure IV - 5. Relationships between Biochemical Oxygen Demand (BOD) mg L^{-1} and fish metrics for all streams in the South Region using STORET Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

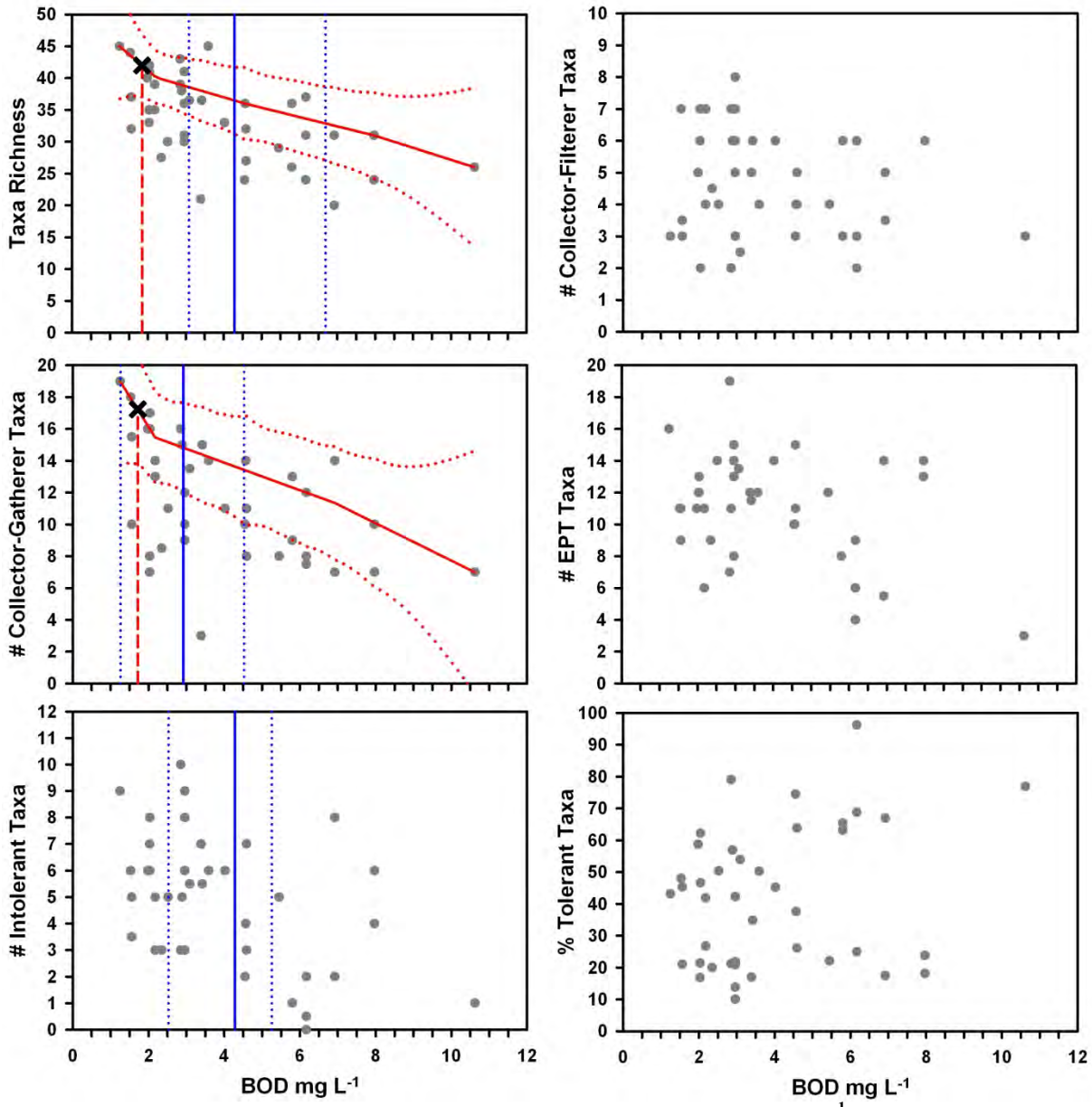


Figure IV - 6. Relationships between Biochemical Oxygen Demand (BOD) mg L^{-1} and macroinvertebrate metrics for all streams in the South Region using STORET Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

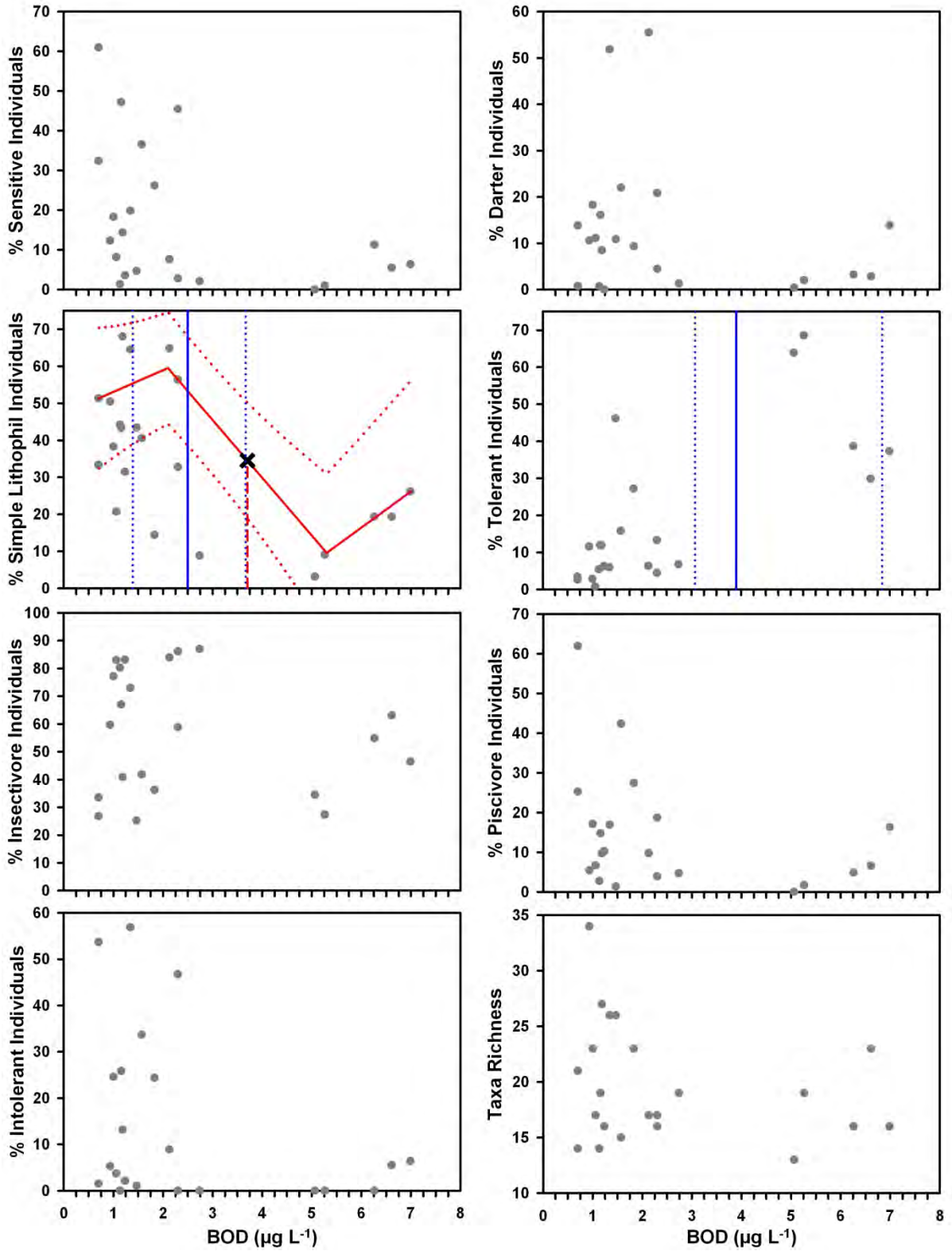


Figure IV - 7. Relationships between Biochemical Oxygen Demand (BOD) mg L^{-1} and fish metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

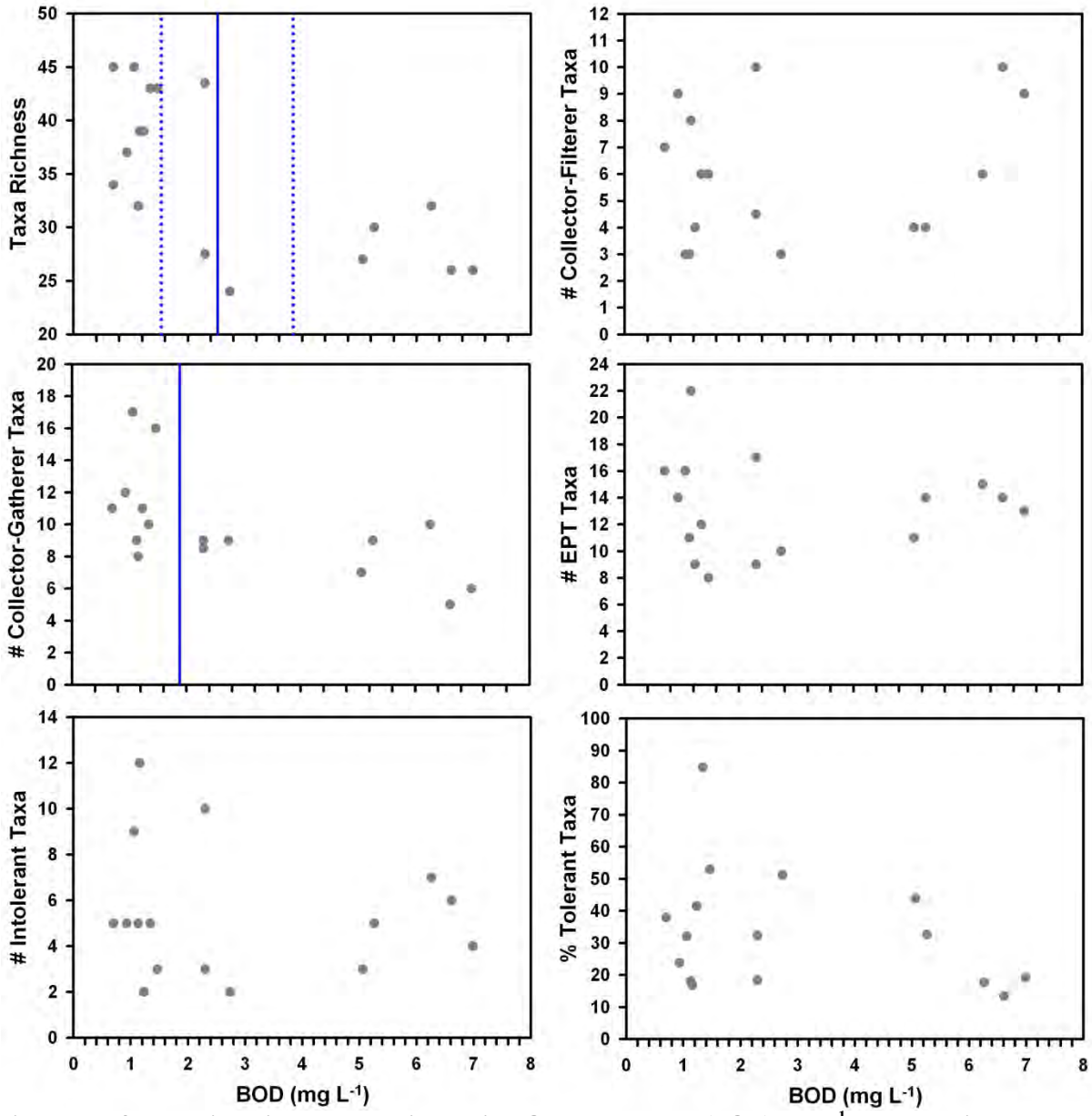


Figure IV - 8. Relationships between Biochemical Oxygen Demand (BOD) mg L^{-1} and macroinvertebrate metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

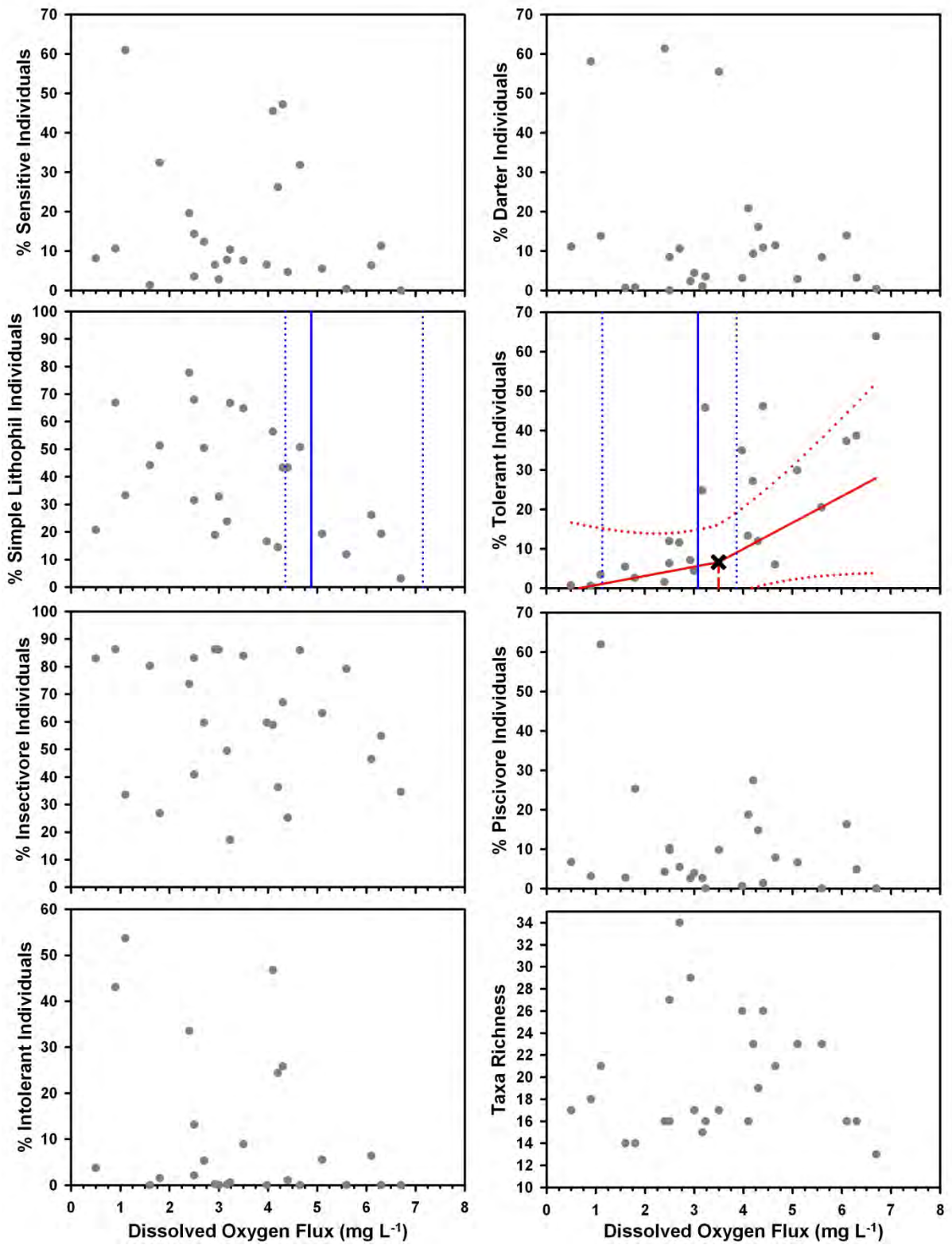


Figure IV - 9. Relationships between Dissolved Oxygen Flux mg L^{-1} and fish metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

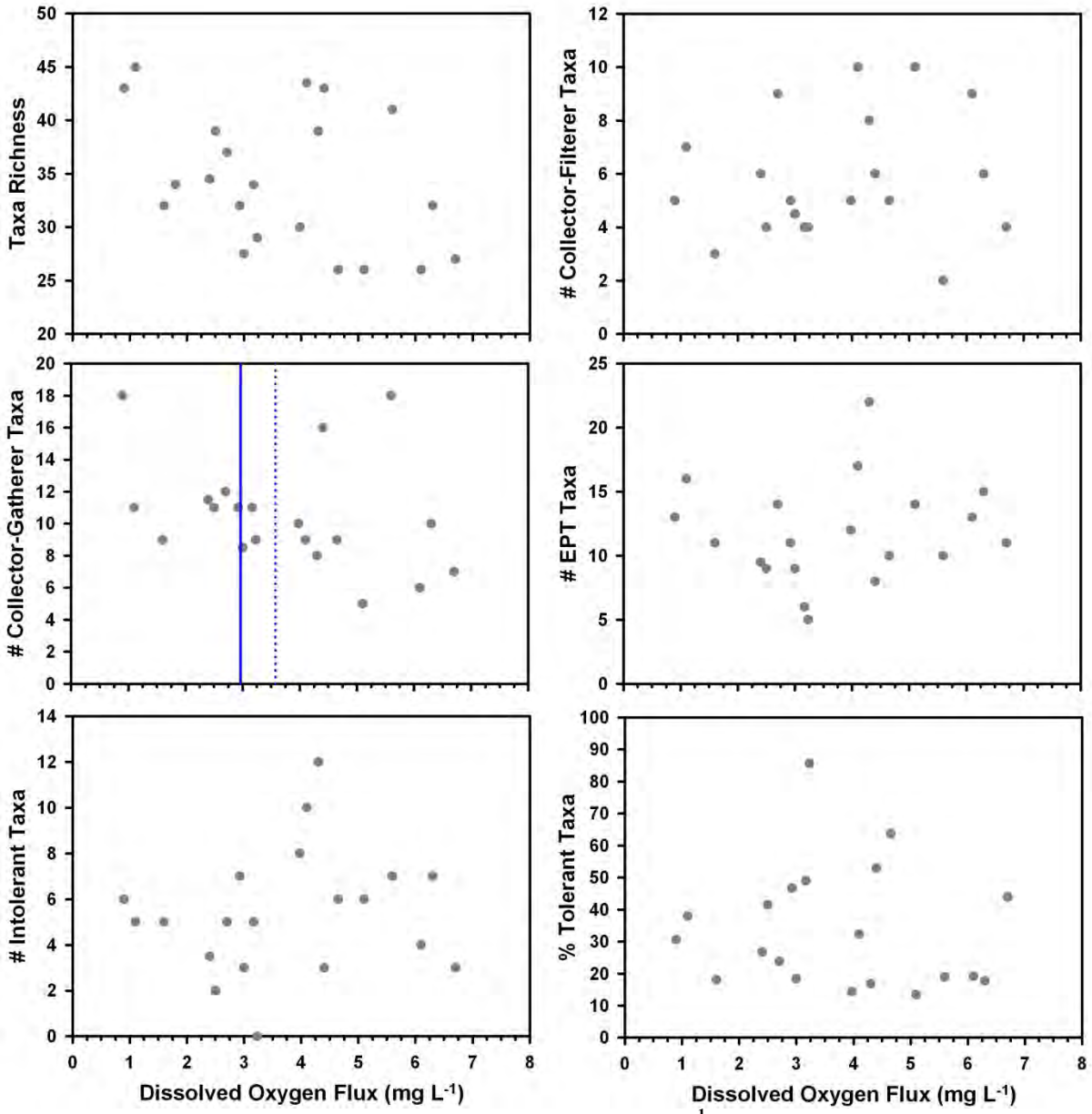


Figure IV - 10. Relationships between Dissolved Oxygen Flux mg L^{-1} and macroinvertebrate metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

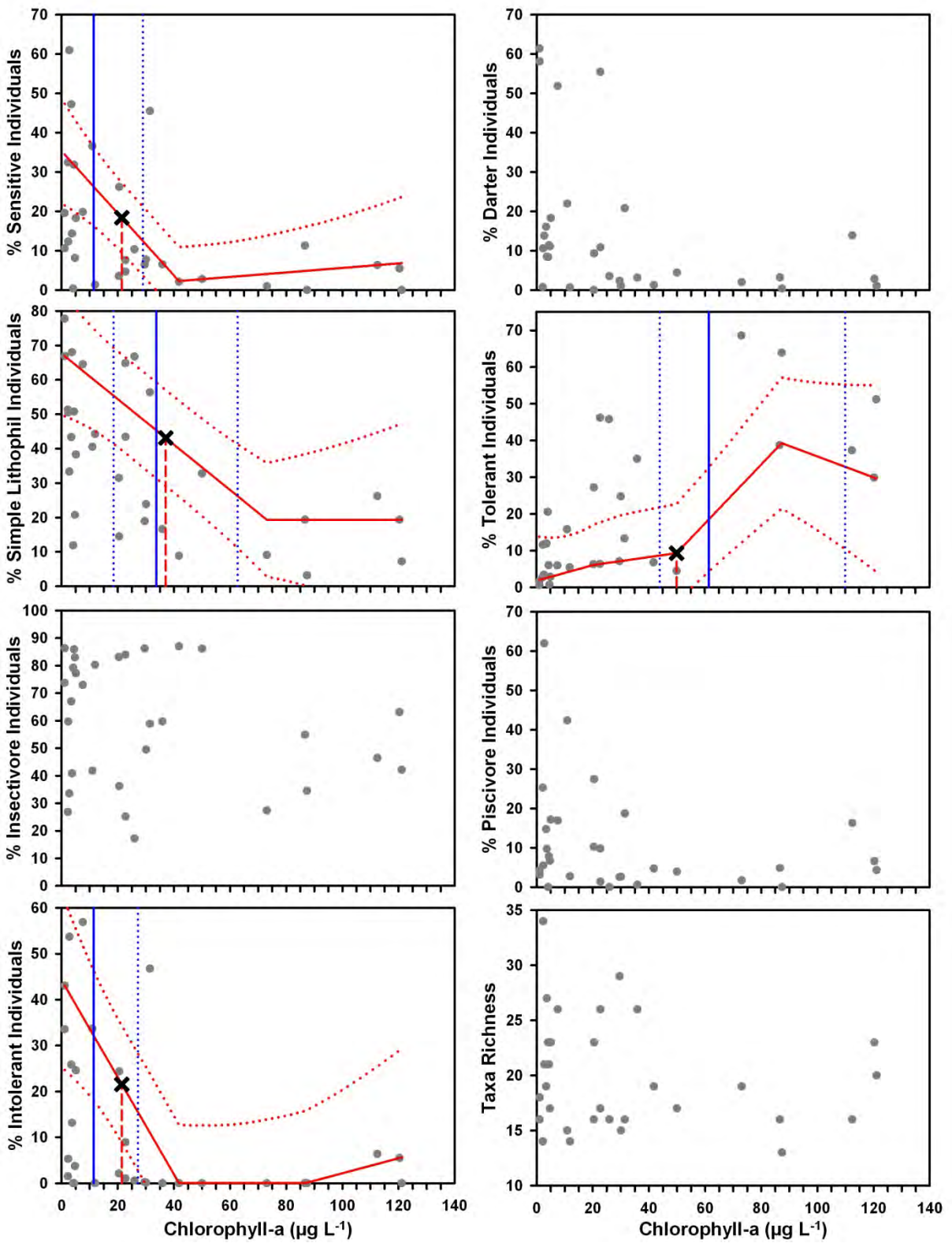


Figure IV - 11. Relationships between Chlorophyll-a µg L^{-1} and fish metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

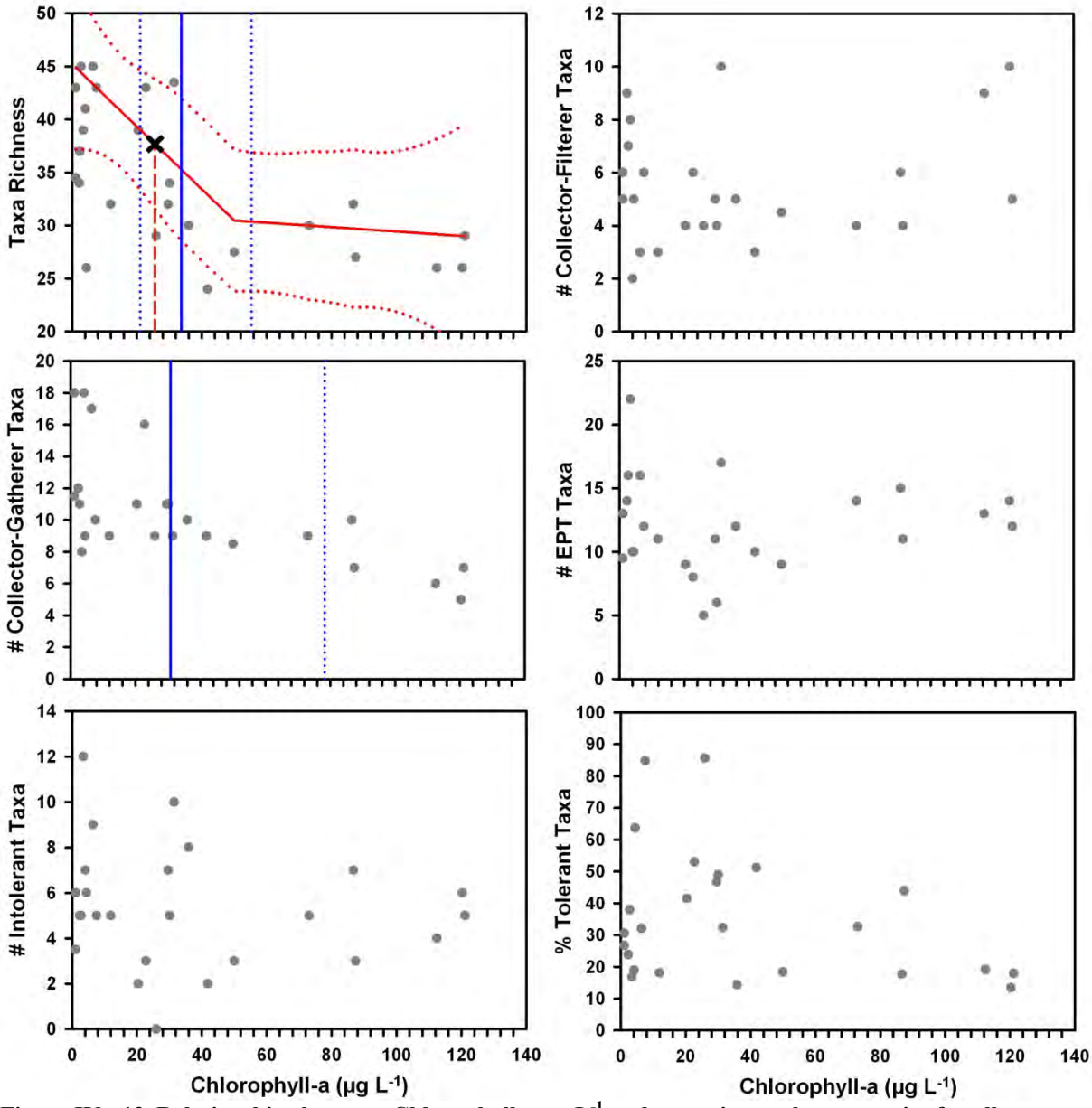


Figure IV - 12. Relationships between Chlorophyll-a µg L^{-1} and macroinvertebrate metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

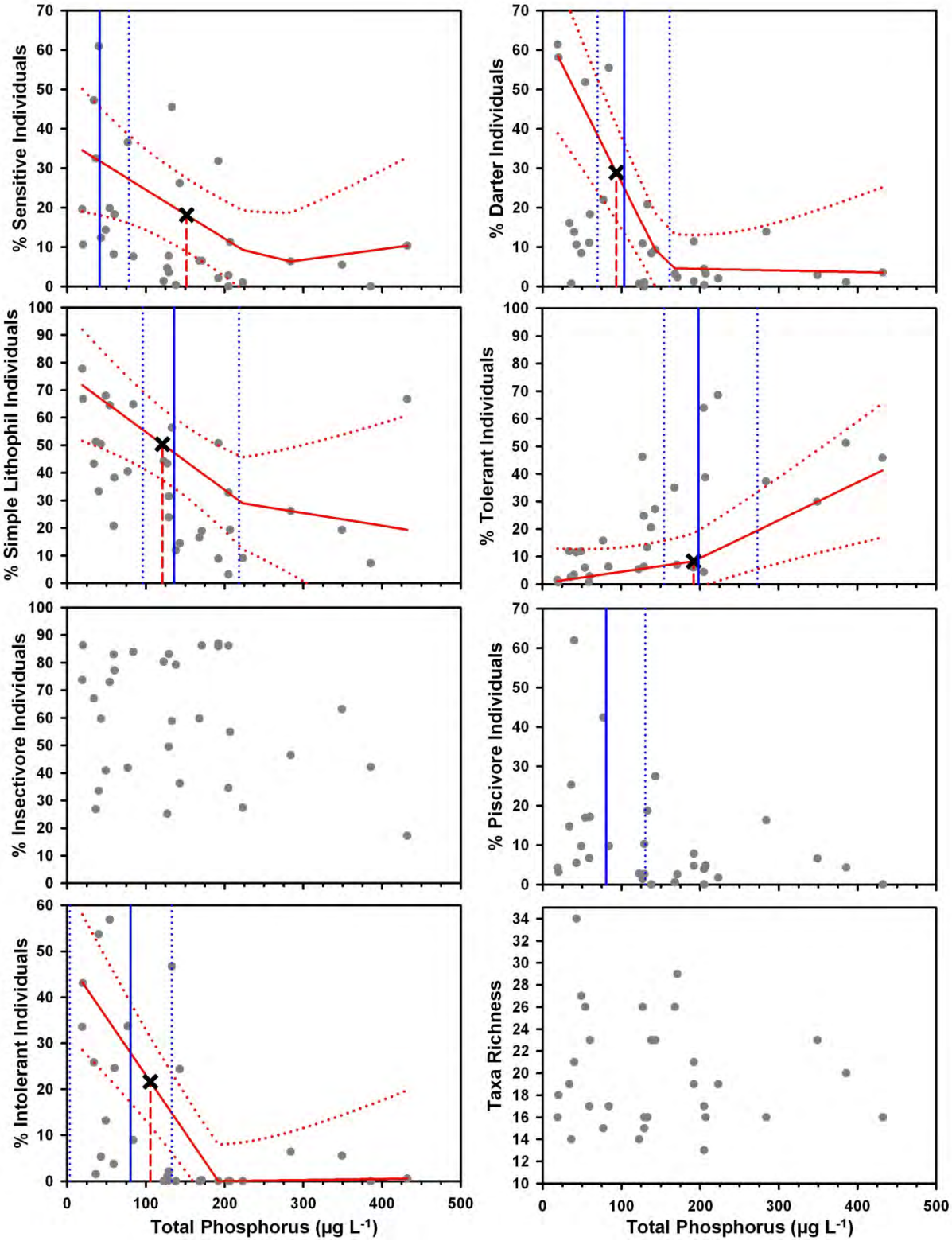


Figure IV - 13. Relationships between Phosphorus µg L^{-1} and fish metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

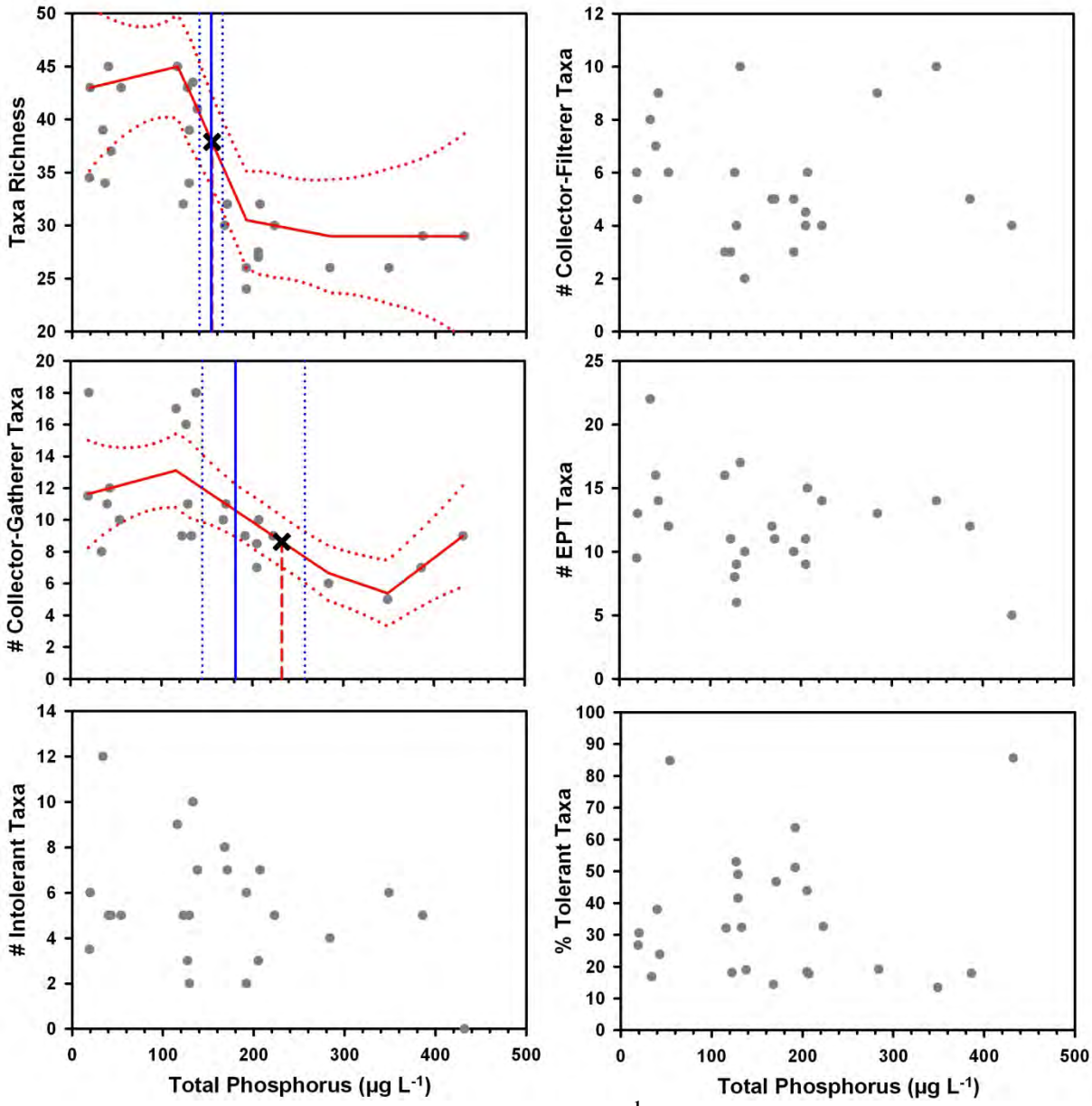


Figure IV - 14. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

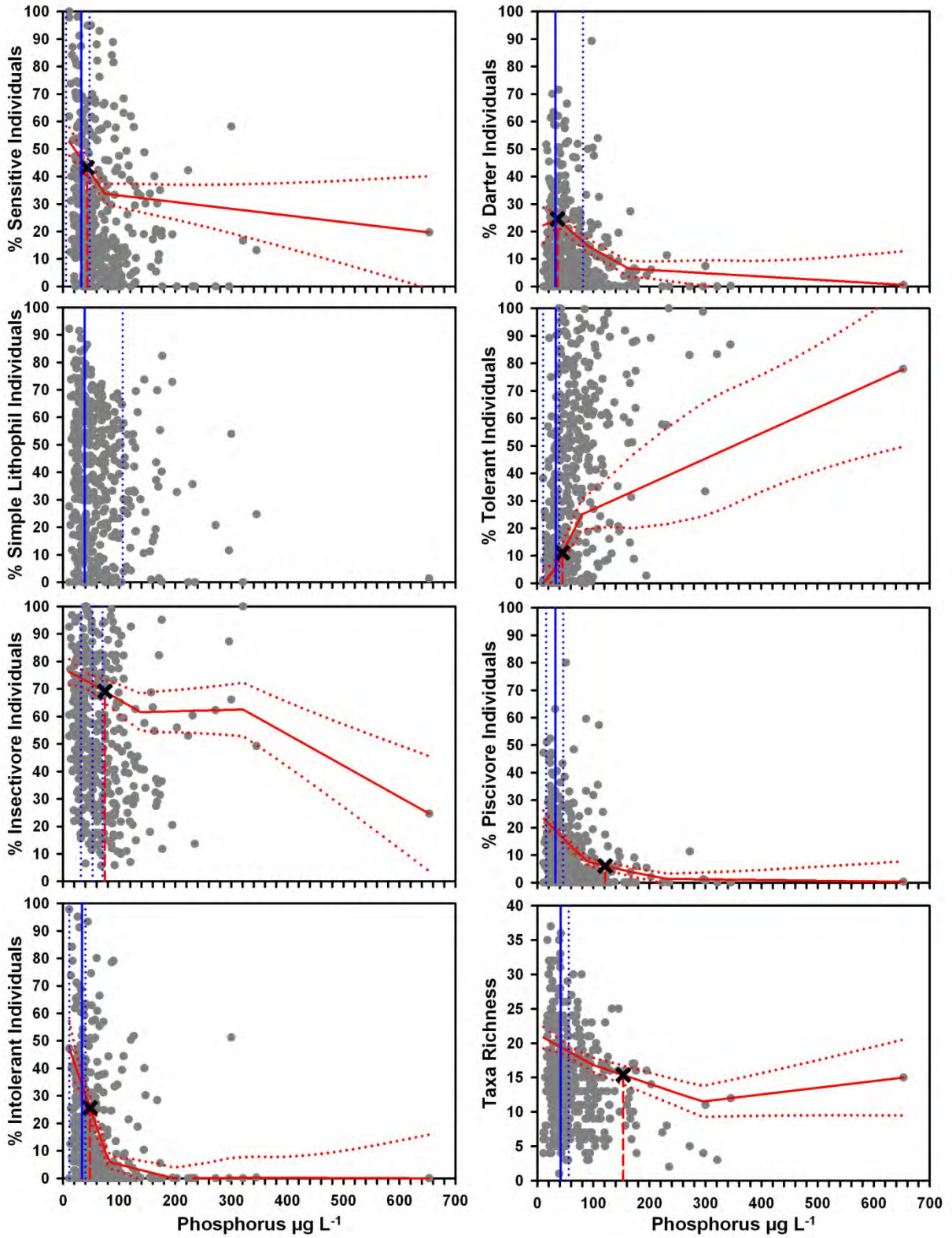


Figure IV - 15. Relationships between Total Phosphorus µg L^{-1} and fish metrics for all streams in the North Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

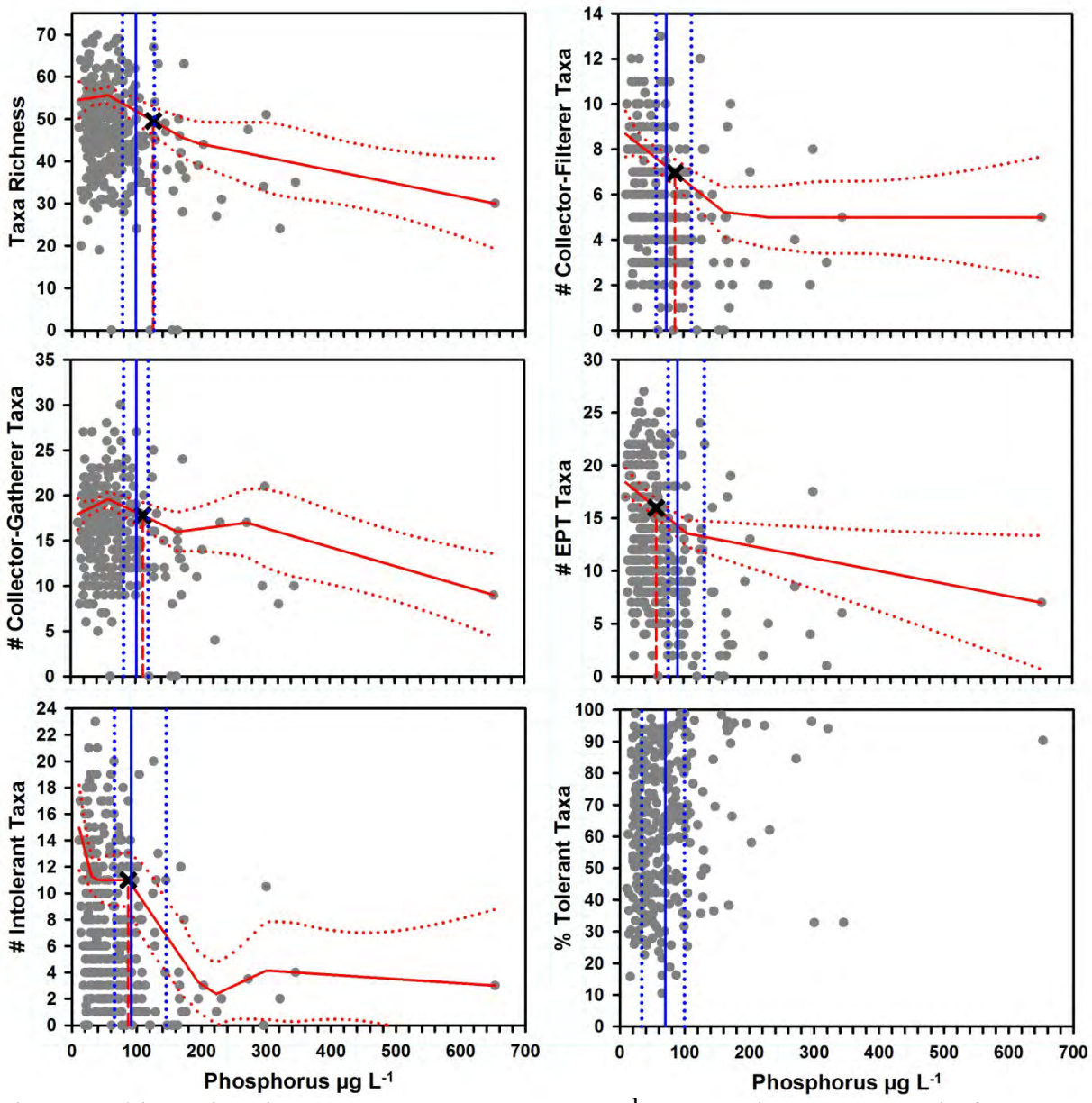


Figure IV - 16. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for all streams in the North Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

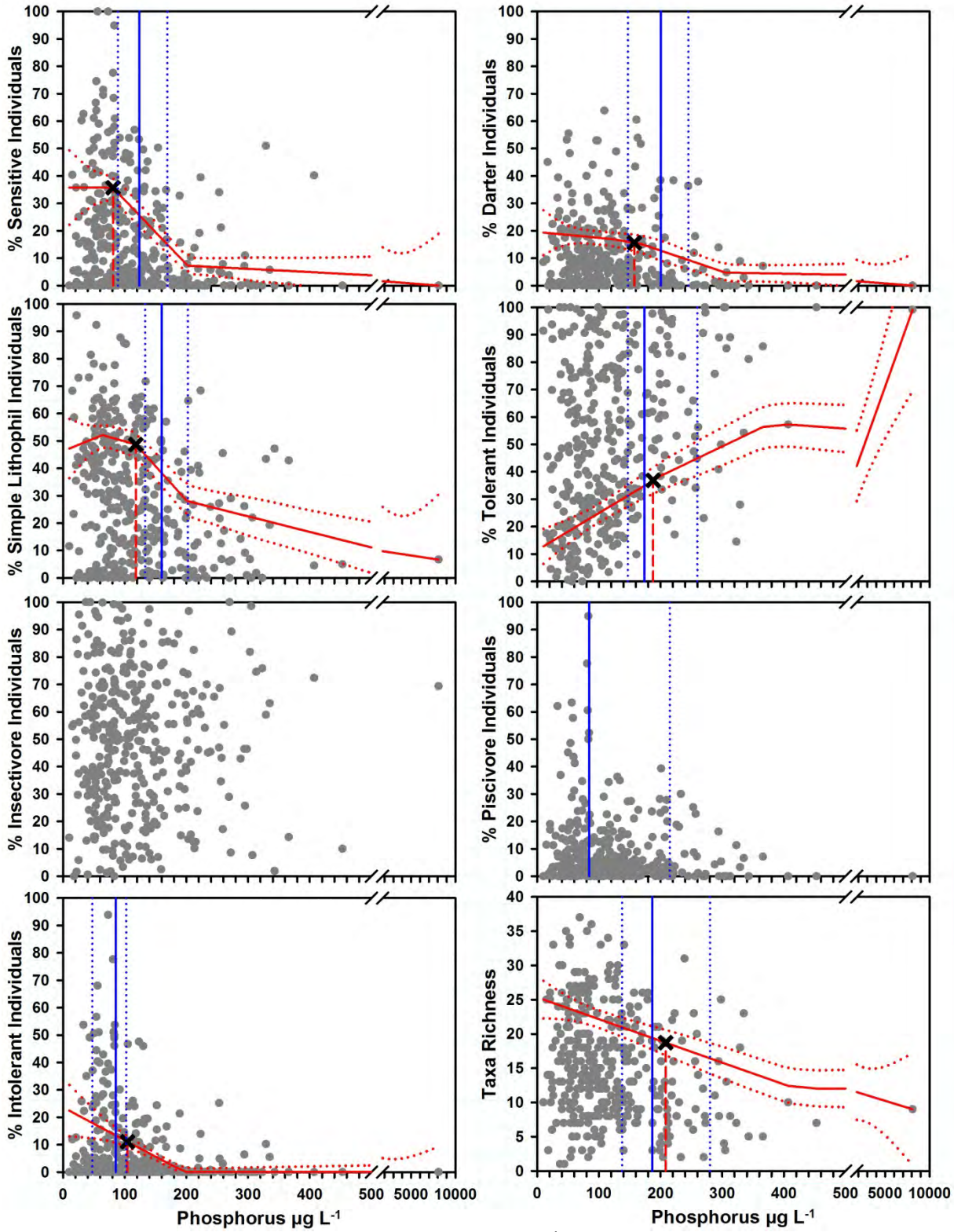


Figure IV - 17. Relationships between Total Phosphorus µg L^{-1} and fish metrics for all streams in the Central Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

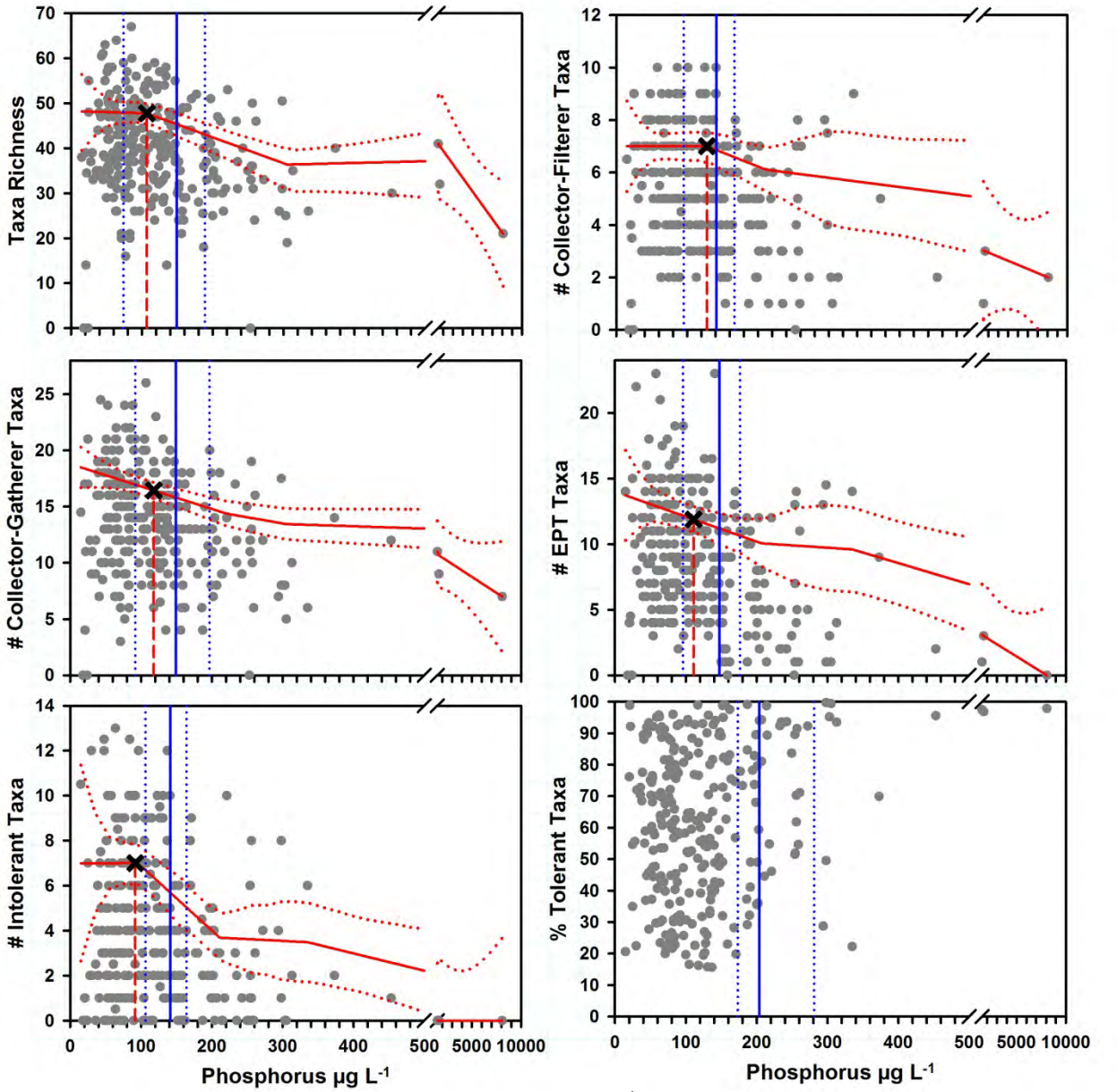


Figure IV - 18. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for all streams in the Central Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

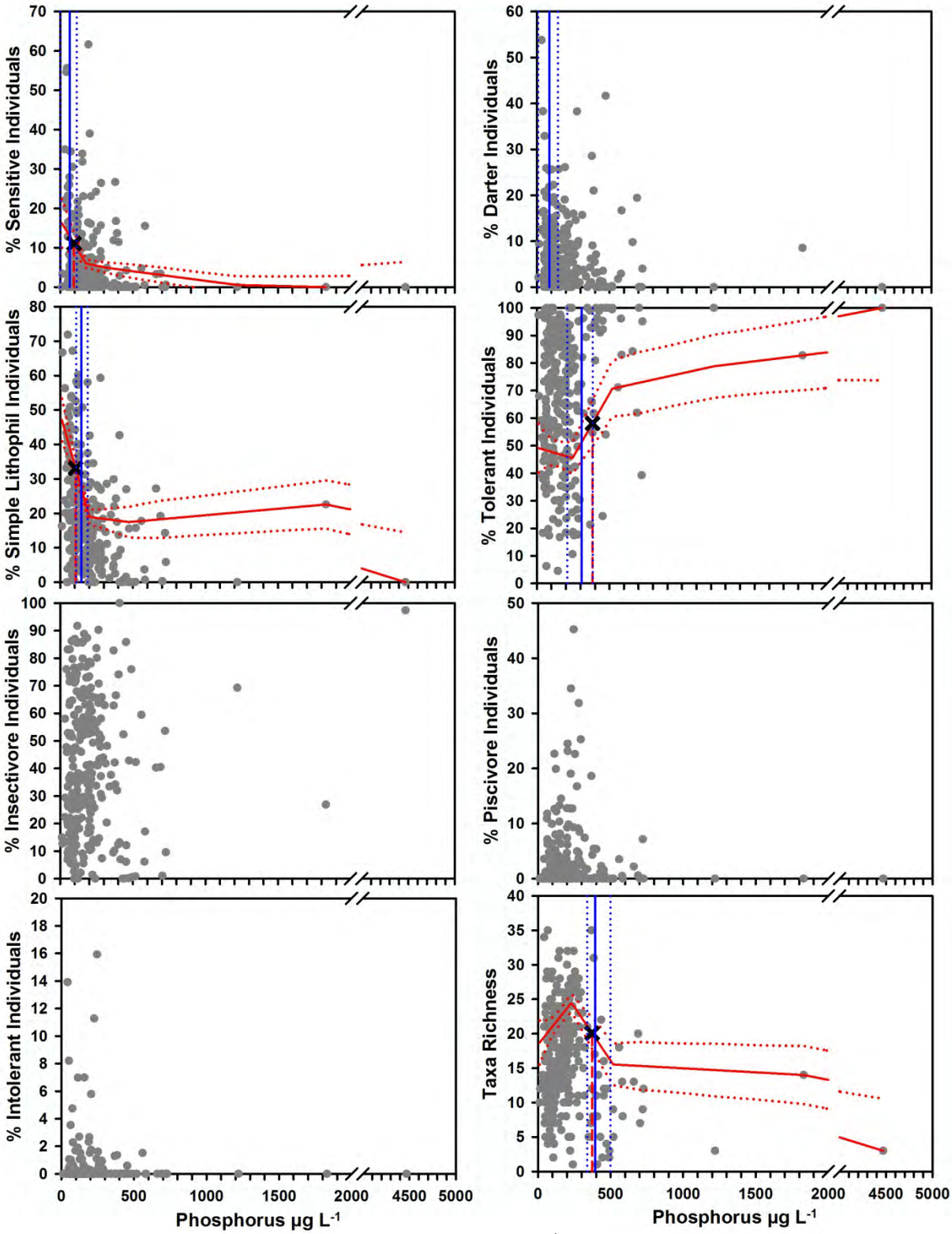


Figure IV - 19. Relationships between Total Phosphorus µg L^{-1} and fish metrics for all streams in the South Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

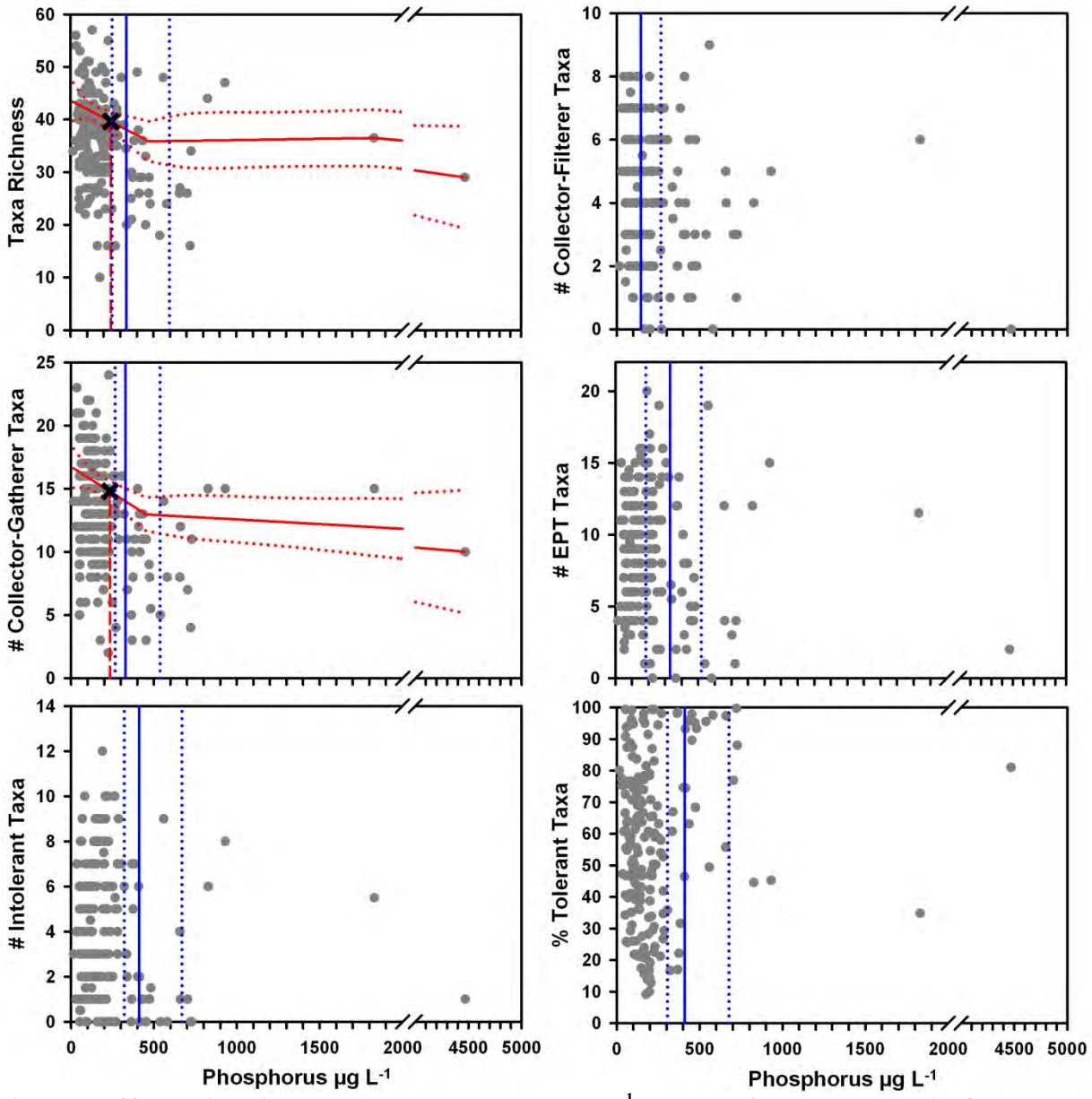


Figure IV - 20. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for all streams in the South Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

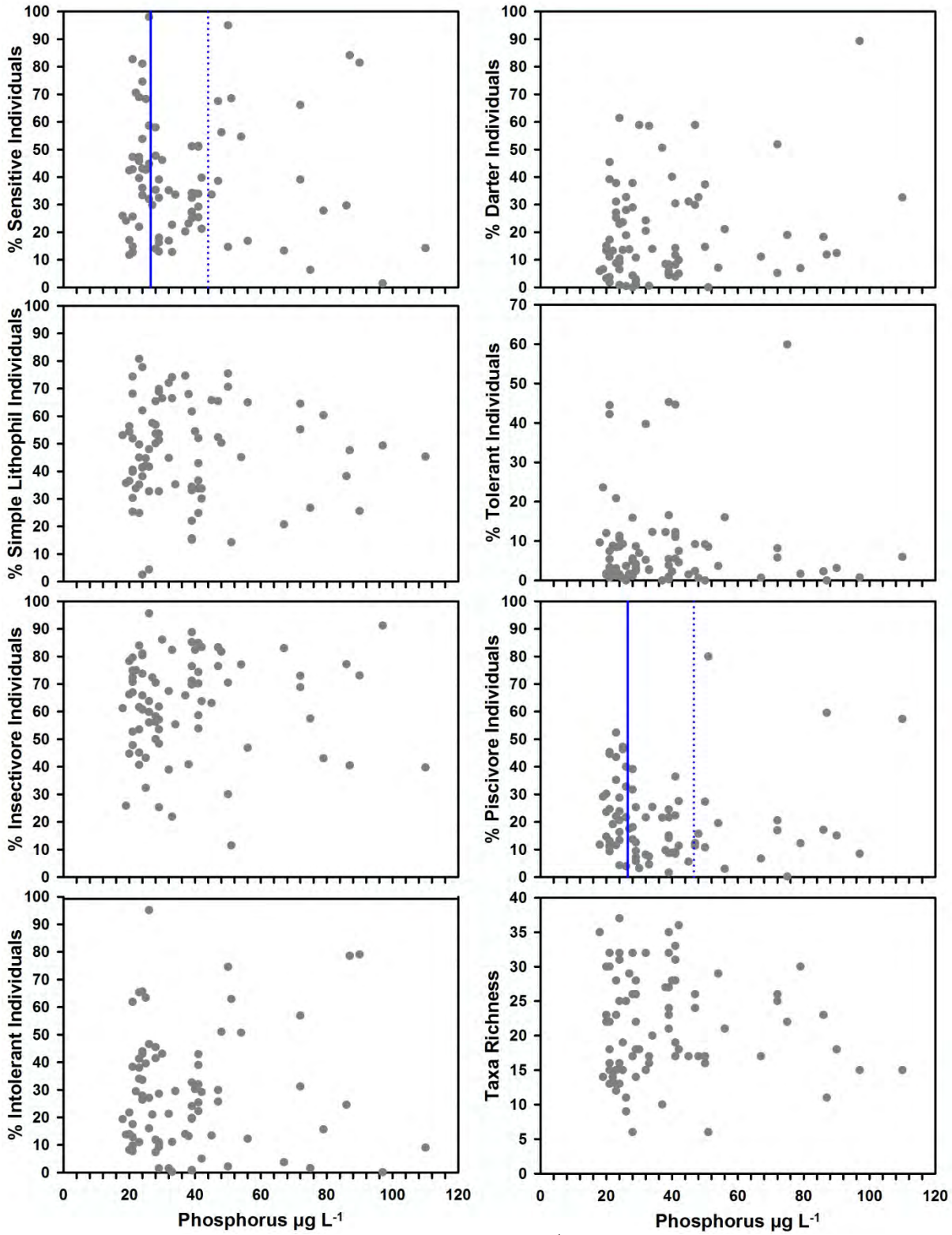


Figure IV - 21. Relationships between Total Phosphorus µg L^{-1} and fish metrics for nonwadeable (>500 mi^2) streams in the North Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

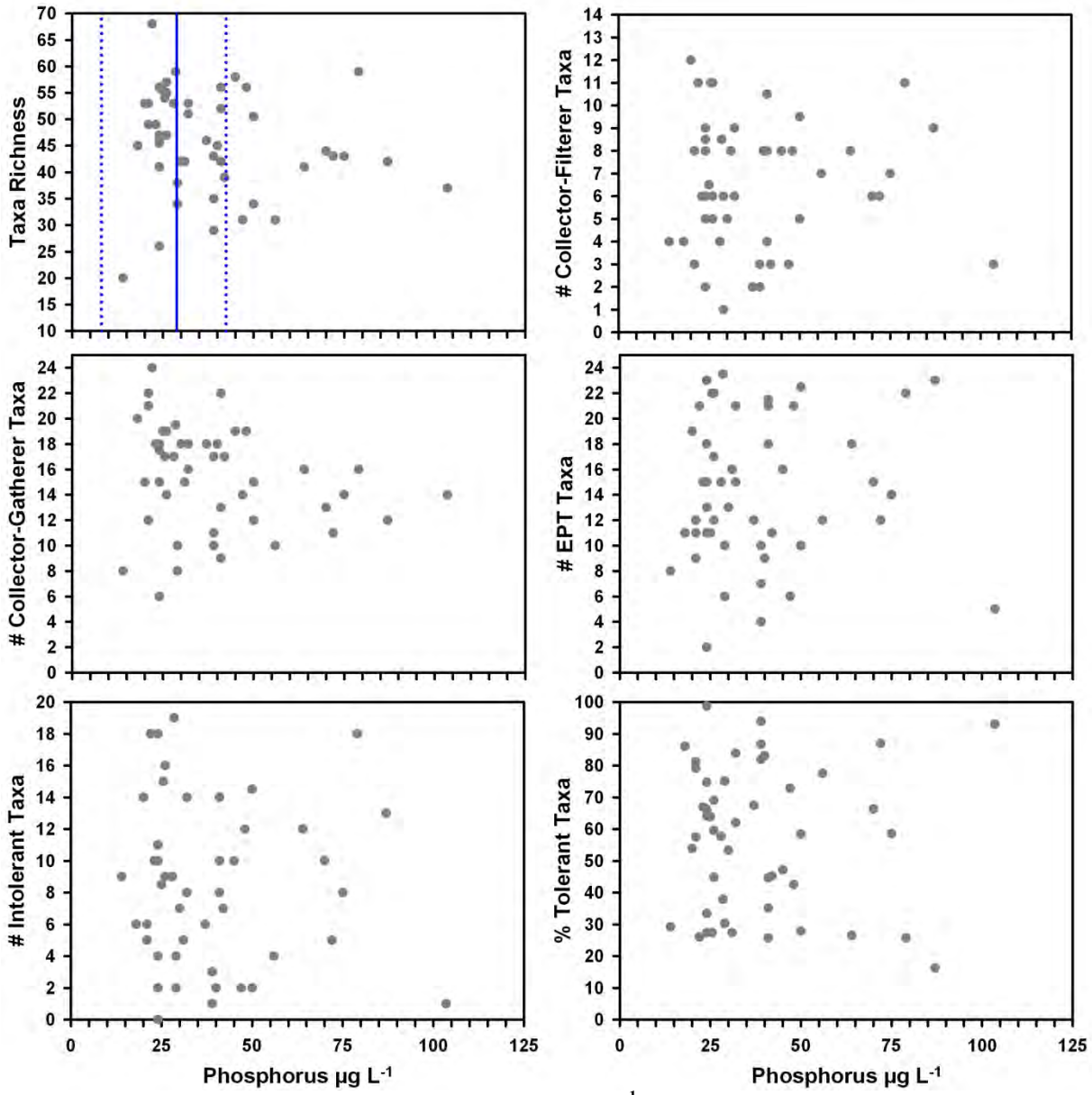


Figure IV - 22. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for nonwadeable (>500 mi^2) streams in the North Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

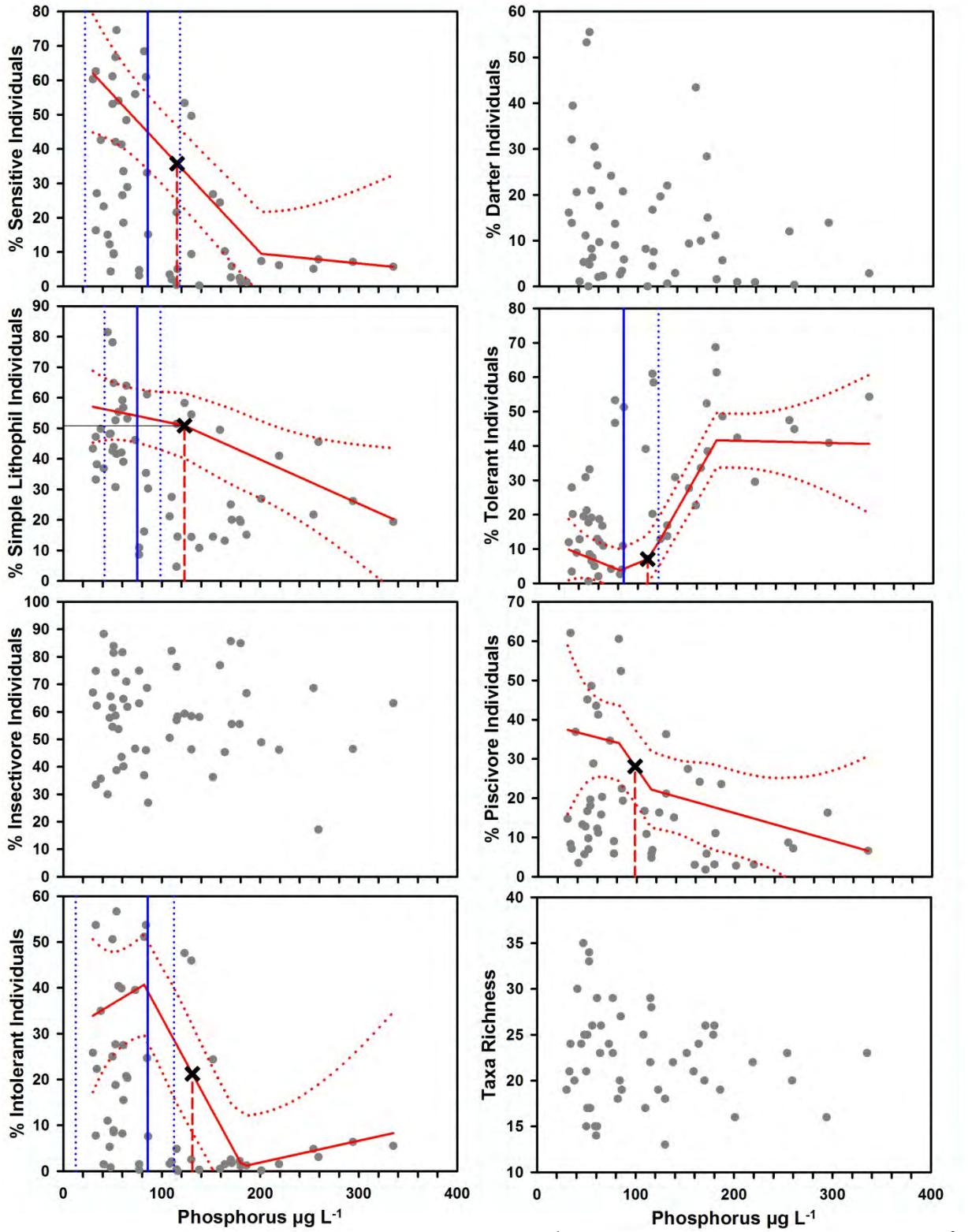


Figure IV - 23. Relationships between Total Phosphorus µg L^{-1} and fish metrics for nonwadeable (>500 mi^2) streams in the Central Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

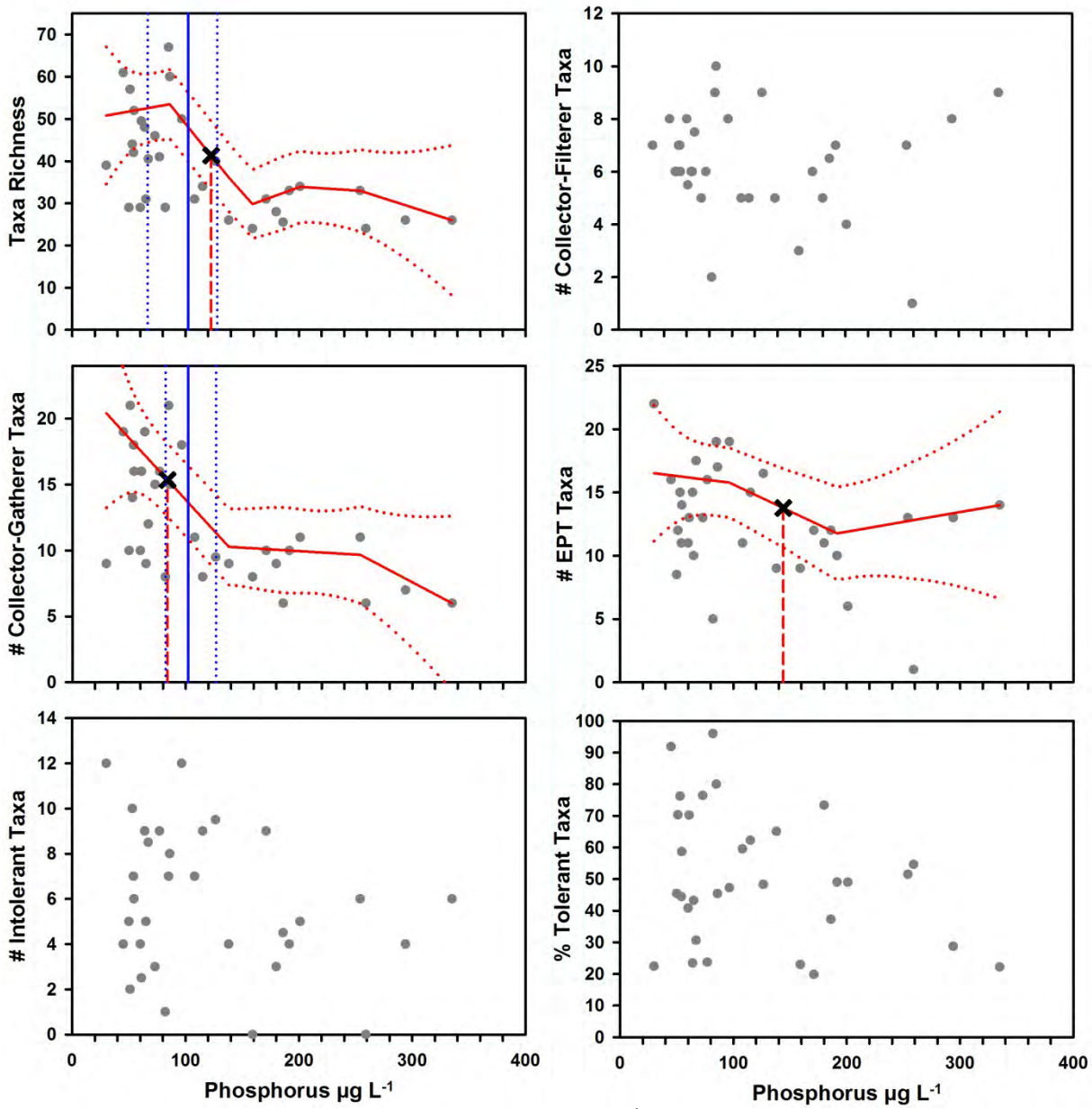


Figure IV - 24. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for nonwadeable (>500 mi^2) streams in the Central Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

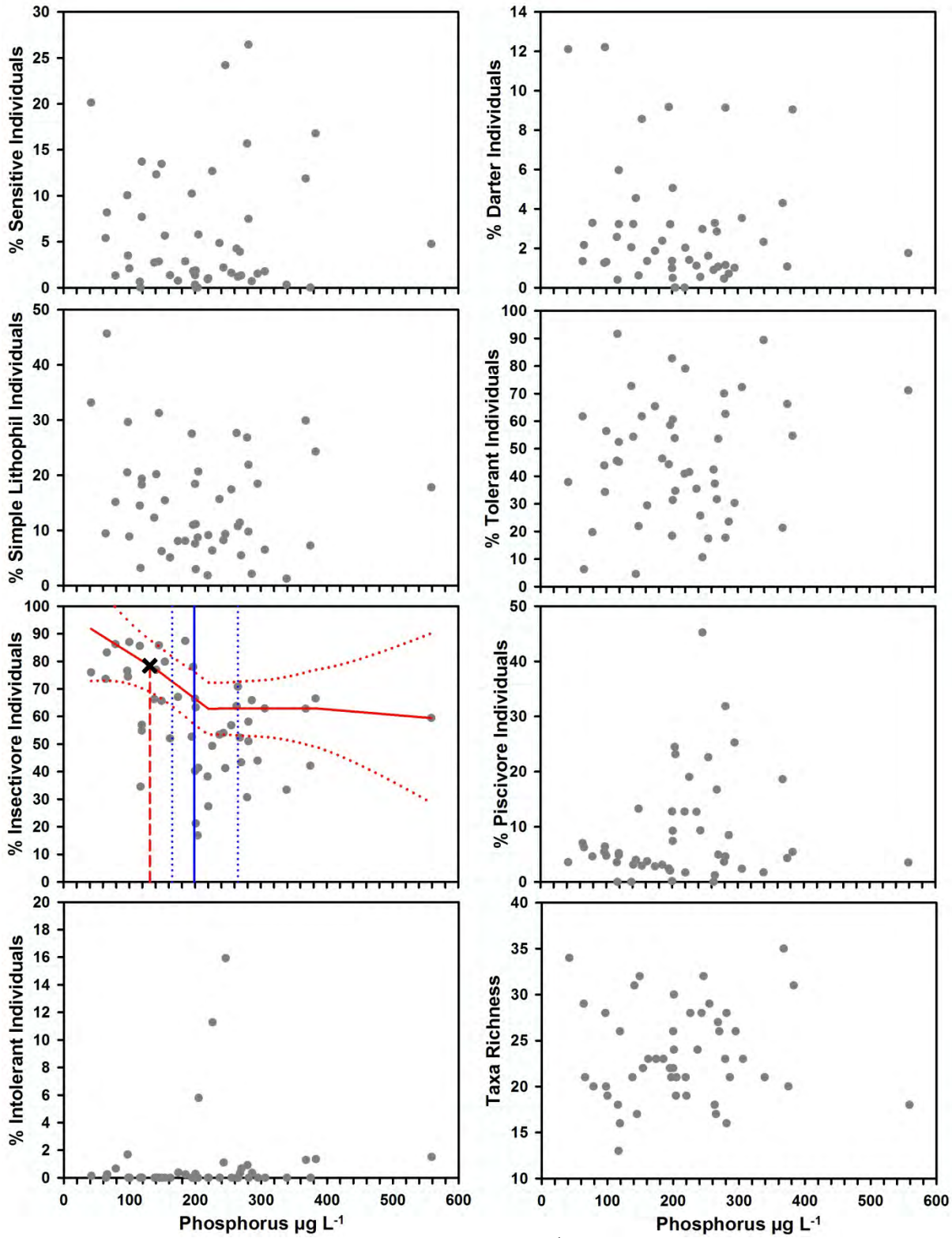


Figure IV - 25. Relationships between Total Phosphorus µg L^{-1} and fish metrics for nonwadeable (>500 mi^2) streams in the South Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

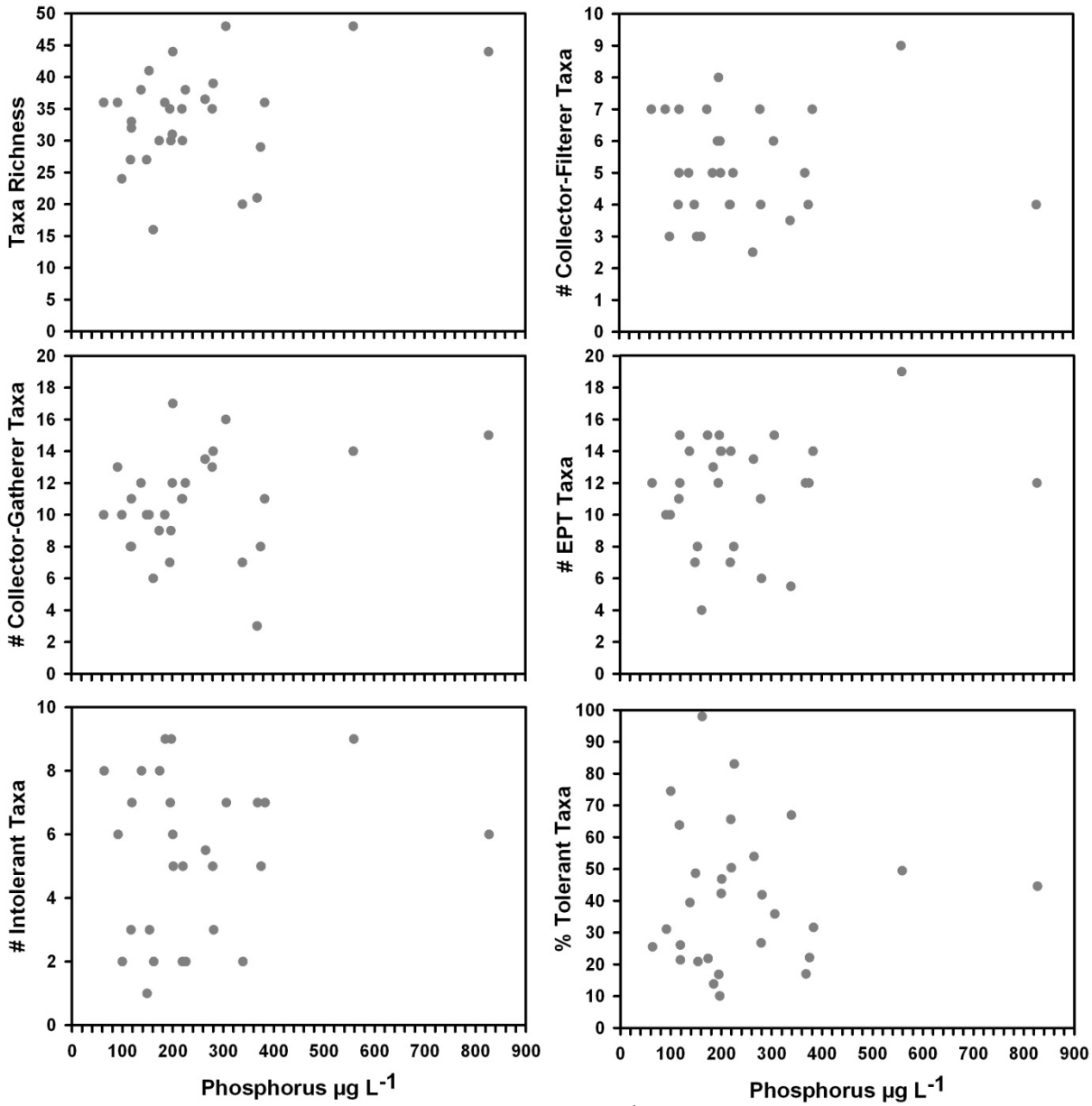


Figure IV - 26. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for nonwadeable (>500 mi^2) streams in the South Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

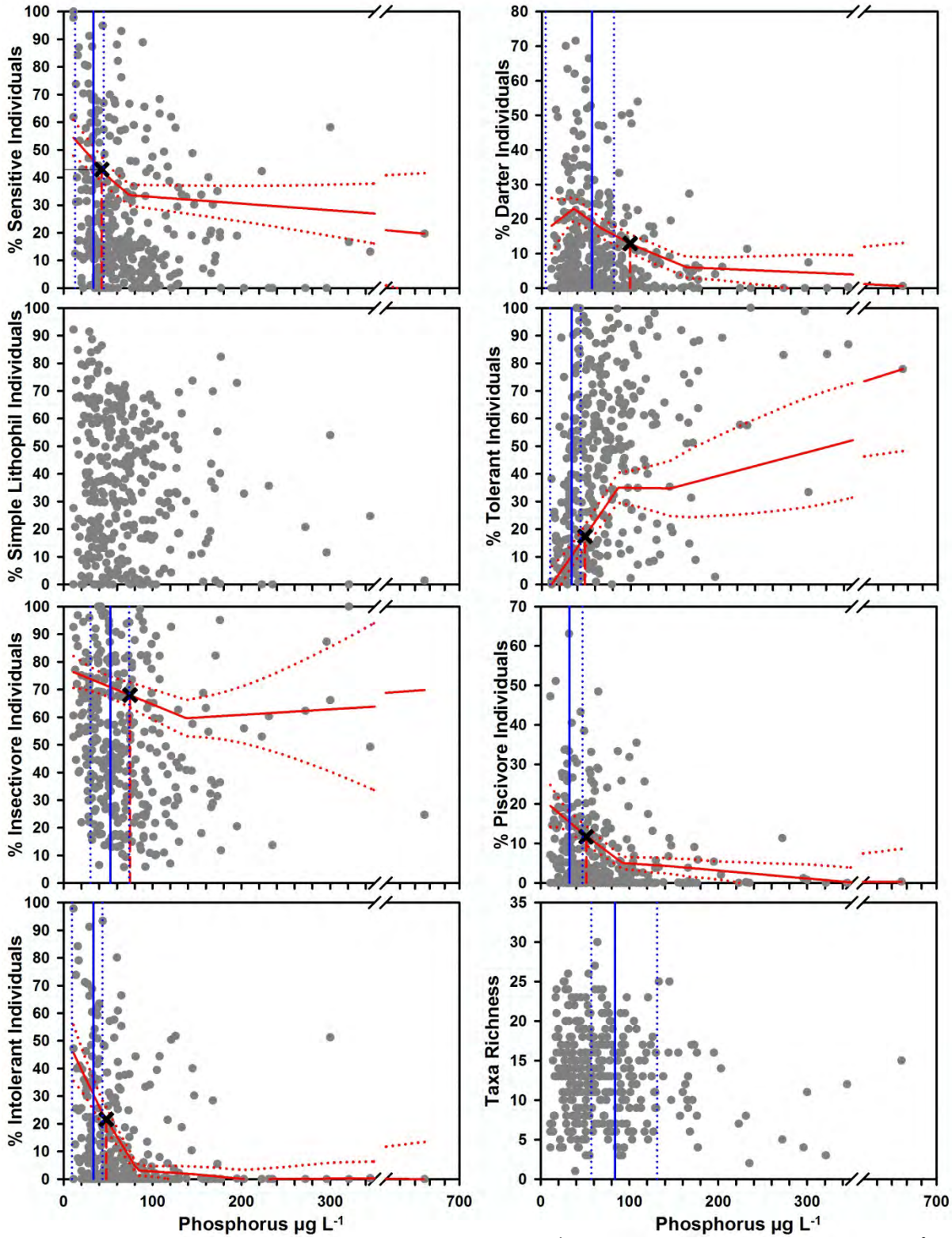


Figure IV - 27. Relationships between Total Phosphorus µg L^{-1} and fish metrics for wadeable (<500 mi^2) streams in the North Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

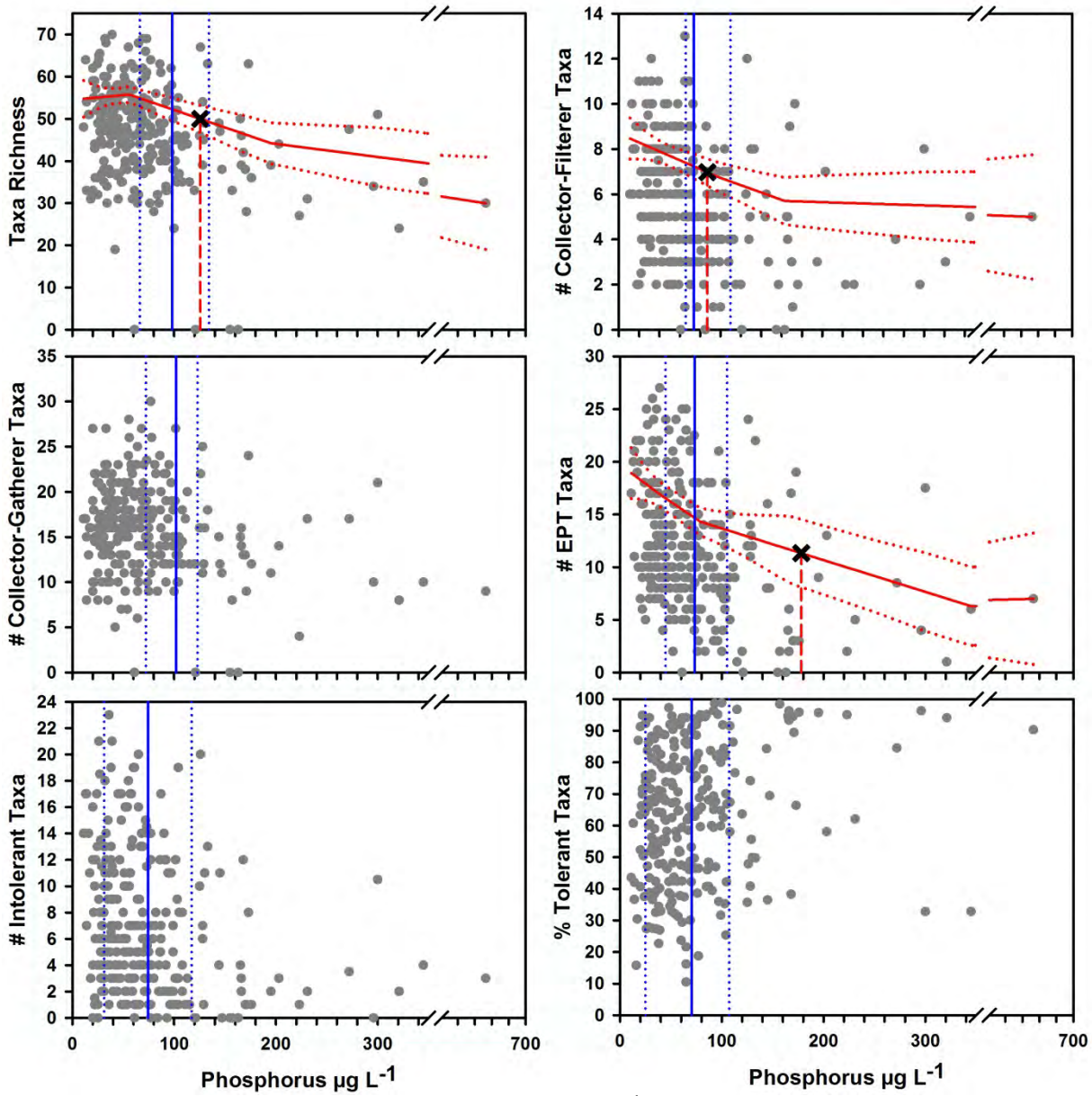


Figure IV - 28. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for wadeable (<500 mi^2) streams in the North Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

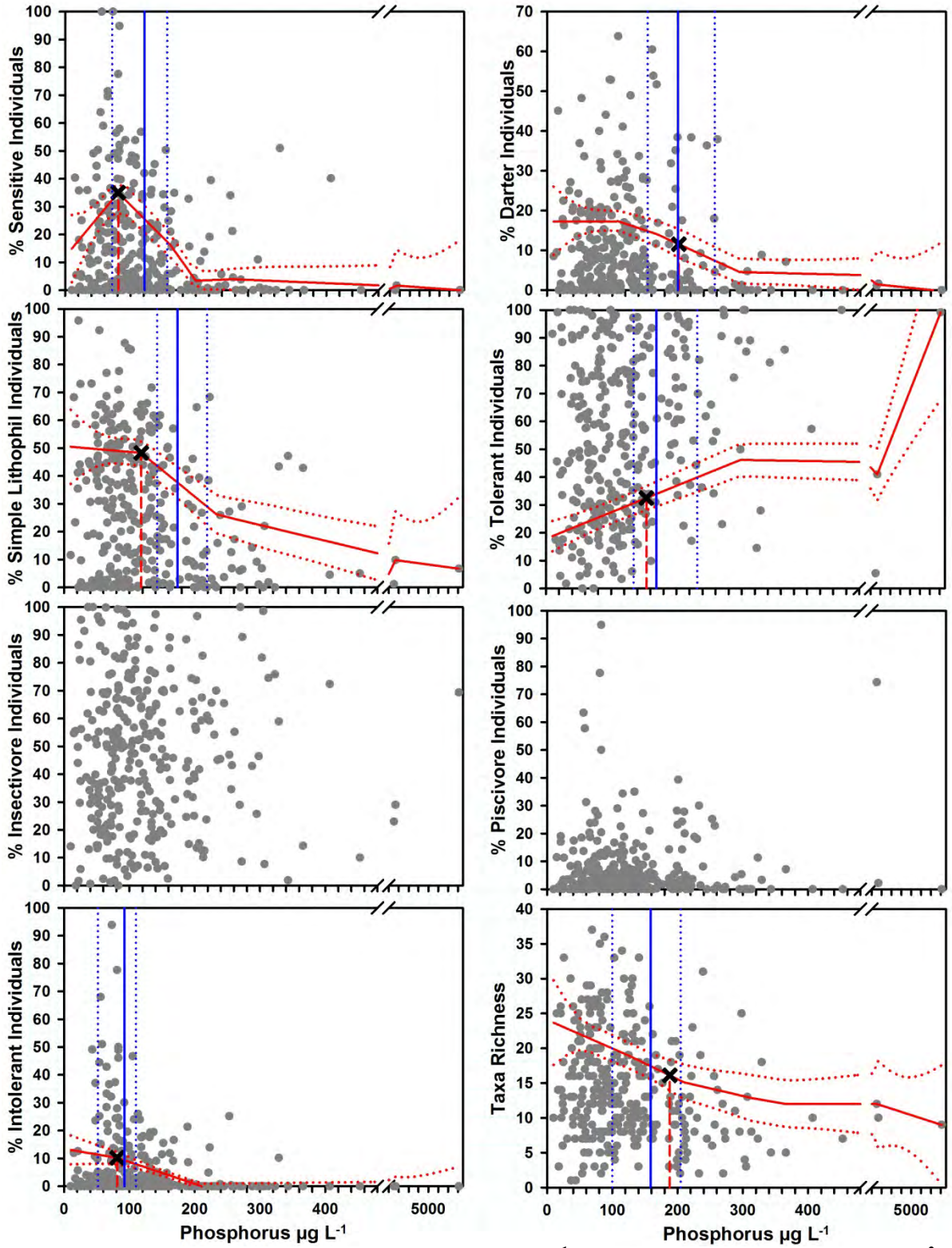


Figure IV - 29. Relationships between Total Phosphorus µg L^{-1} and fish metrics for wadeable (<500 mi^2) streams in the Central Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

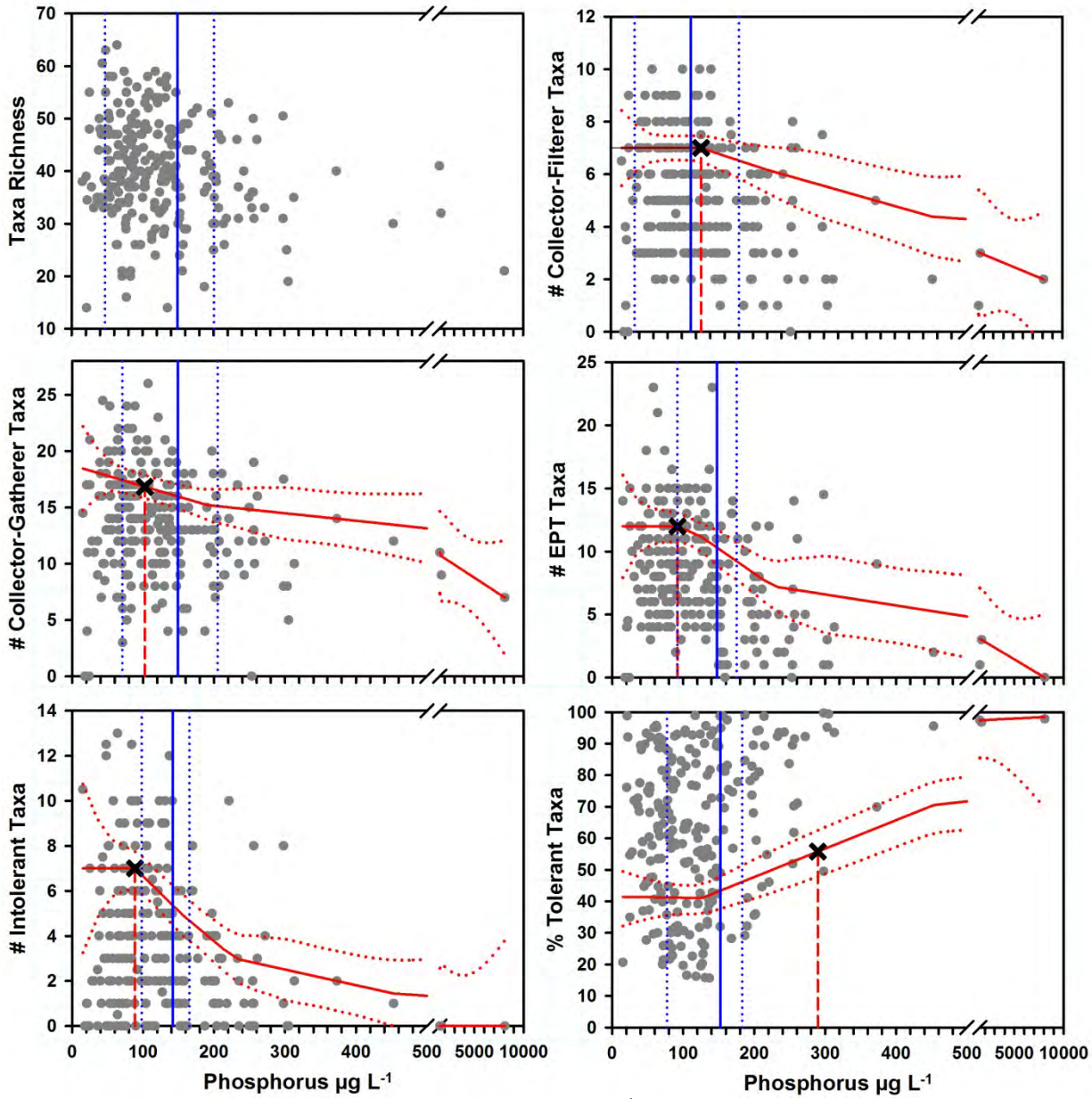


Figure IV - 30. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for wadeable (<500 mi^2) streams in the Central Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

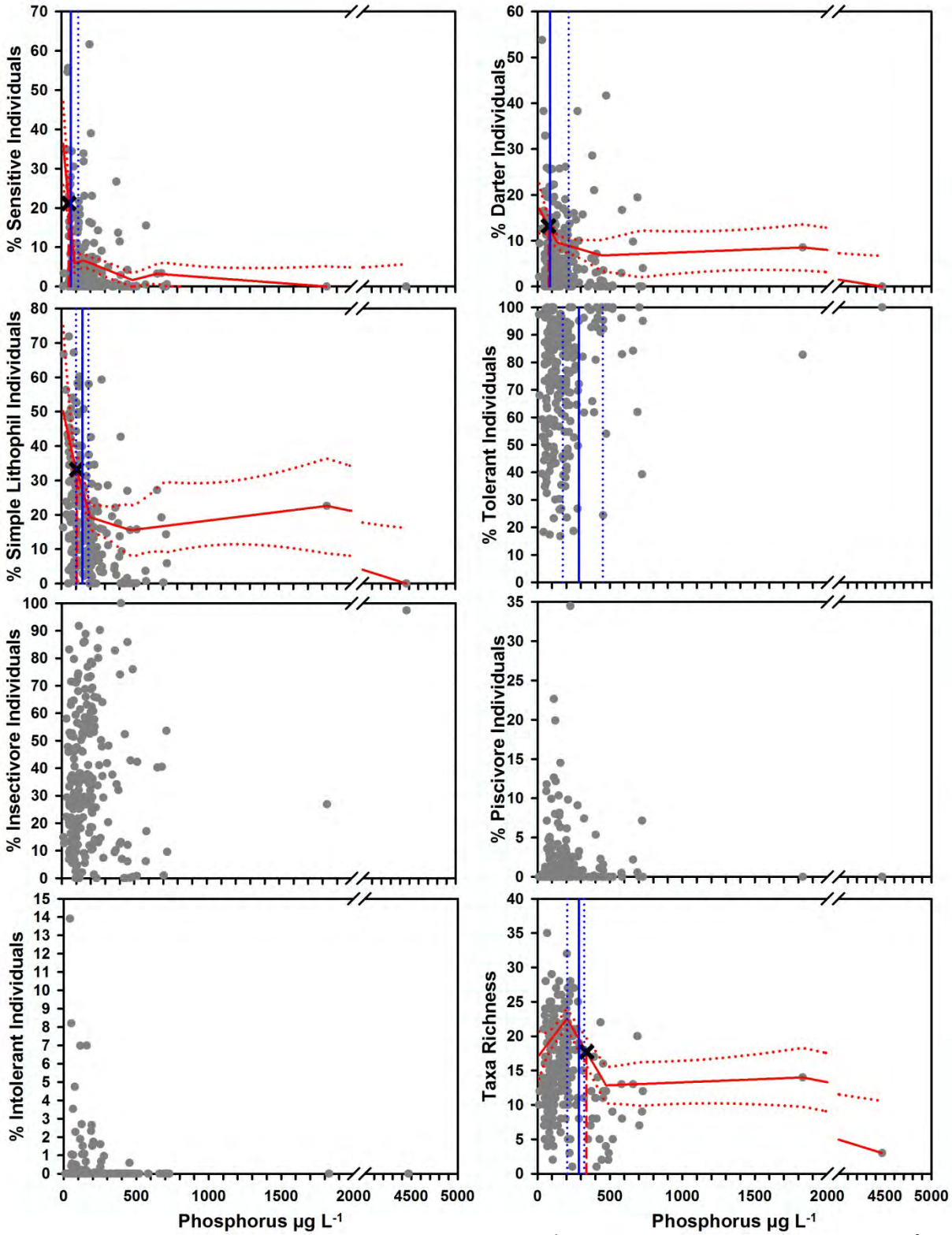


Figure IV - 31. Relationships between Total Phosphorus µg L^{-1} and fish metrics for wadeable (<500 mi^2) streams in the South Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

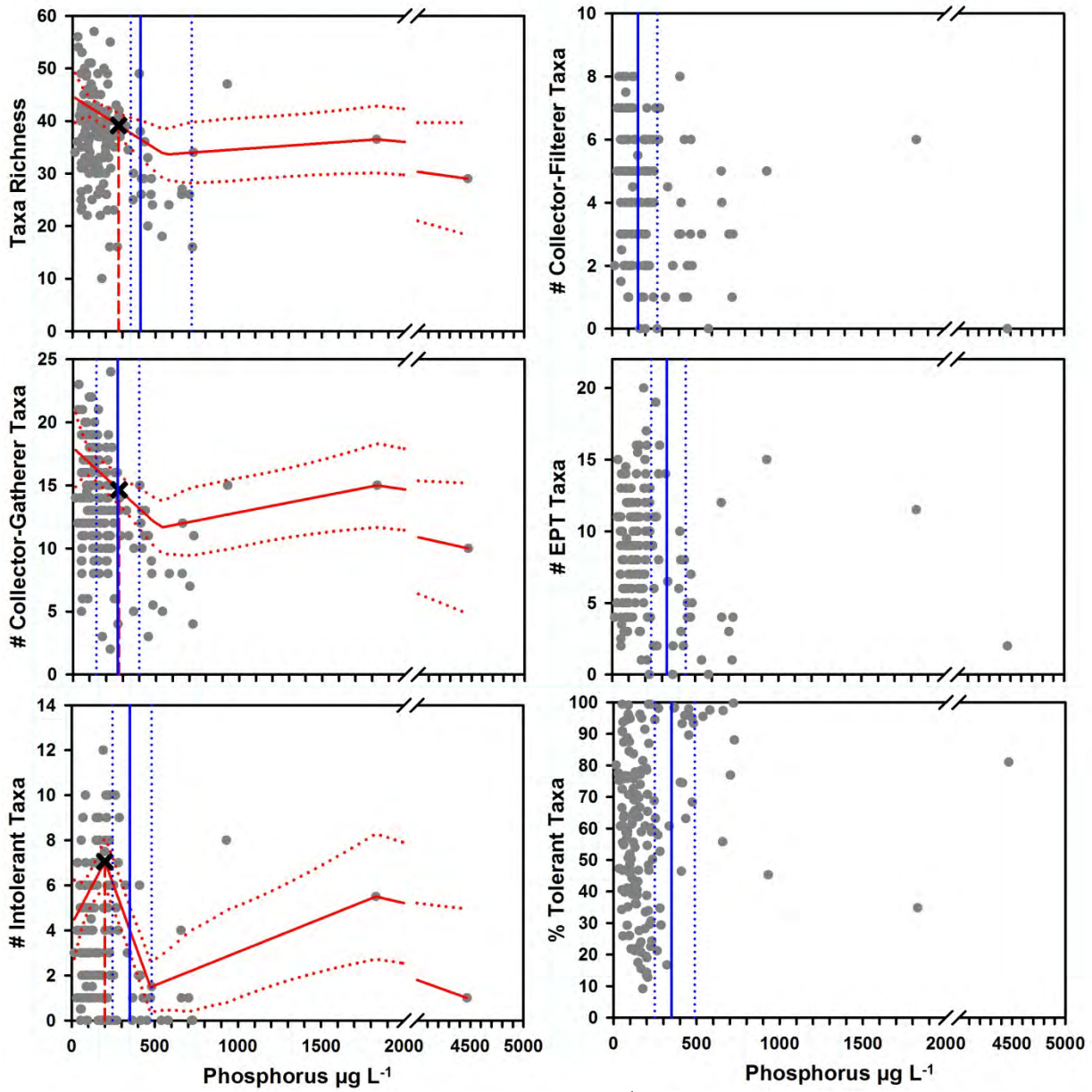


Figure IV - 32. Relationships between Total Phosphorus µg L^{-1} and macroinvertebrate metrics for wadeable (<500 mi^2) streams in the South Region using MPCA Biomonitoring data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

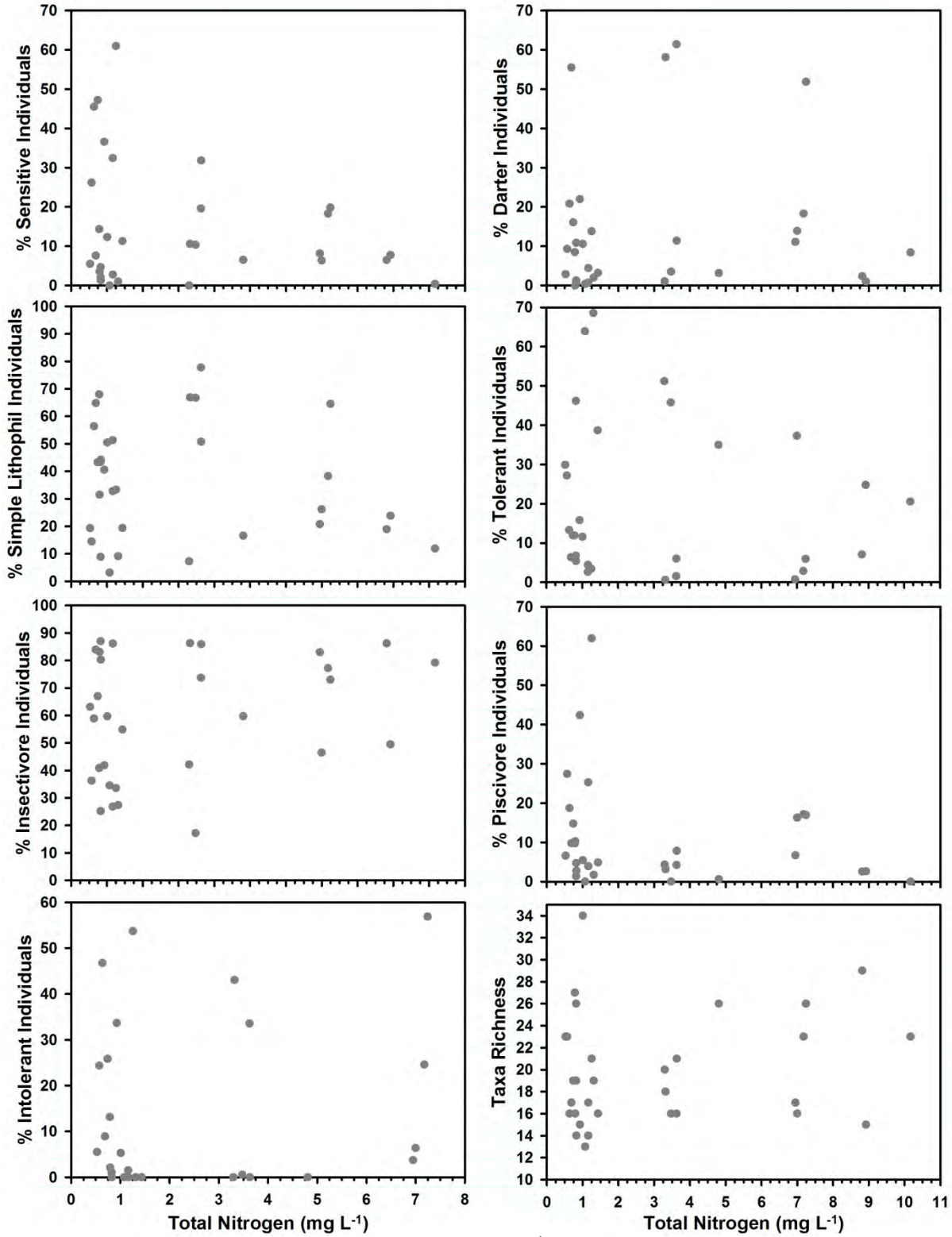


Figure IV - 33. Relationships between Total Nitrogen mg L^{-1} and fish metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).

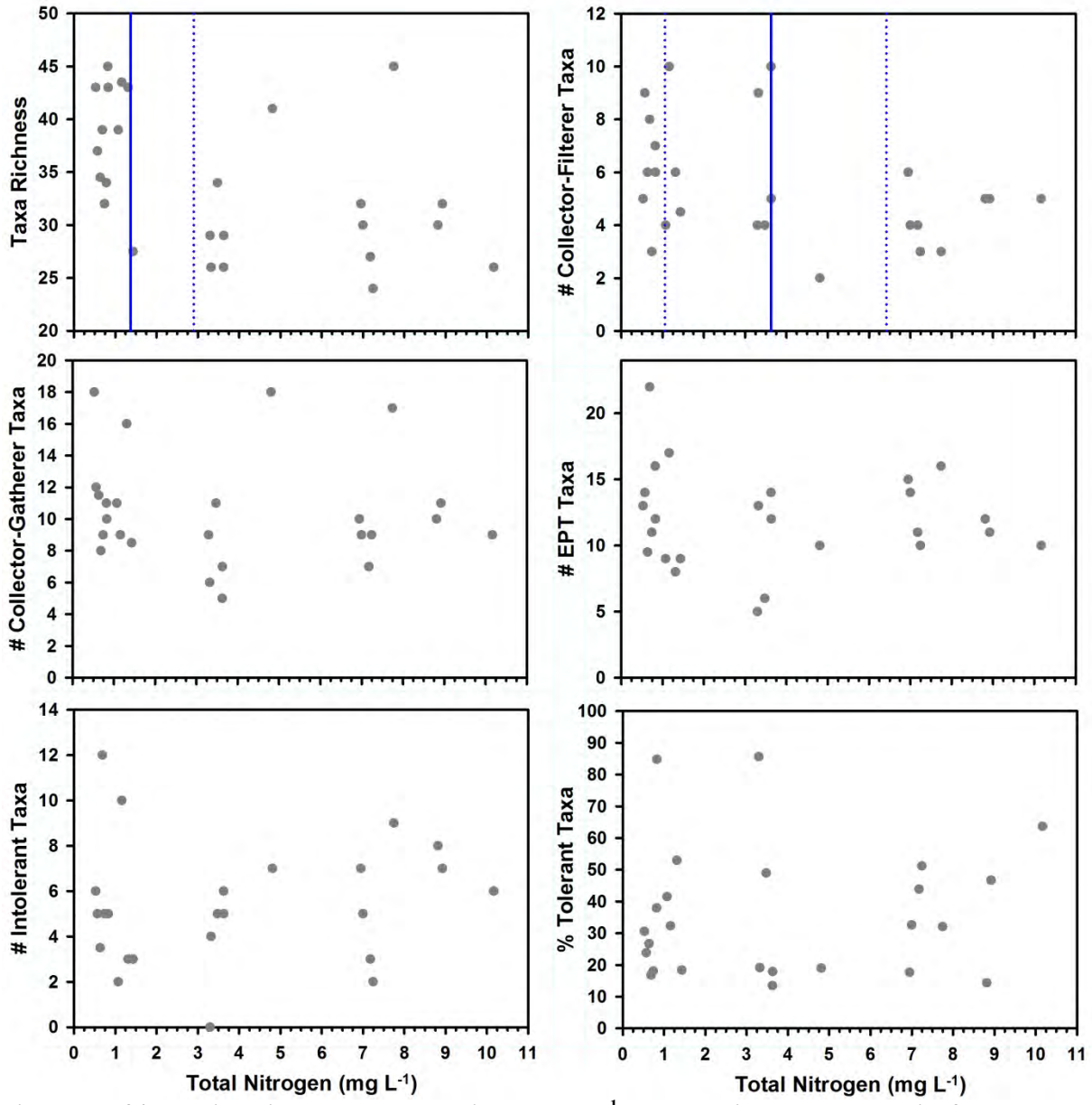


Figure IV - 34. Relationships between Total Nitrogen mg L^{-1} and macroinvertebrate metrics for all streams statewide using River Nutrient Data (red line = additive quantile regression with 90% confidence bands, blue line = changepoint with 90% confidence bands).